



HANDBOOK OF CLINICAL ADULT GENETICS AND GENOMICS

A Practice-Based Approach

Edited by Shweta U. Dhar, Sandesh C.S. Nagamani,
and Tanya N. Eble





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Contents

List of contributors	xv
Foreword	xxi
Preface	xxiii
 Section I The Practice of Adult Genetics	 1
1. Introduction to the practice of adult genetics	3
<i>SHWETA U. DHAR</i>	
Overview of the field of adult genetics	3
Why is it necessary to learn more about this?	3
How are we teaching our medical students?	5
How are we training our genetics residents?	5
Who will impart genetic care to adult patients?	5
Why do we need a book like this?	6
References	6
2. Basic concepts of genetics and genomics	9
<i>JENNIFER E. POSEY</i>	
Introduction	9
Functional elements of the human genome	9
Human genomic variation	12
Modes of inheritance	14
Relevance to clinical care	18
References	19

3. Genetic evaluation of an adult	21
<i>AERON M. SMALL, MONICA GIOVANNI AND MICHAEL F. MURRAY</i>	
Introduction	21
Approach to the patient	21
Understanding the family context	24
How to apply diagnostic DNA technologies	26
Understanding the genetic and genomic contribution to disease	27
Follow-up and management	28
References	29
4. Genetic counseling for adults	31
<i>TANYA N. EBLE, WENDY R. UHLMANN AND LAUREN E. HIPP</i>	
Introduction	31
Finding healthcare providers with genetics expertise	31
Resources for patients and clinicians	32
Principles of genetic counseling in the evaluation of adults	32
Unique considerations for genetics evaluation and testing in adults	37
Case management for adults	39
References	40
5. Genetic testing in adults	43
<i>SANDESH C.S. NAGAMANI AND JILL A. ROSENFELD</i>	
Introduction	43
Basic principles and practical approach to genetic testing in adults	44
Cytogenetic testing	44
Sequencing tests	48
Other tests	50
Variant interpretation	51
Genome sequencing	53
Direct-to-consumer testing	54

Clinical vignettes	54
References	56
6. Transitioning to adult care	59
<i>JOHN CHRISTOPHER BERENS, MARIA BLAZO AND CYNTHIA PEACOCK</i>	
Introduction	59
Psychosocial considerations	60
Education and employment	62
Health insurance	64
Medical decision-making and guardianship	65
Transition planning	66
Conclusion	68
References	68
7. Preconception and prenatal care	71
<i>SANDRA DARILEK, SARAH HUGUENARD AND IGNATIA B. VAN DEN VEYVER</i>	
Introduction	71
Reproductive Carrier Screening	71
Maternal screening for fetal aneuploidy	73
Prenatal diagnostic testing	75
Preimplantation genetic testing	76
Parental genetic disease and reproduction	78
References	80
Section II Genetic disorders in adults	83
8. Cancer syndromes	85
<i>SHWETA U. DHAR AND HUMA RANA</i>	
Introduction	85
Evaluation for a hereditary cancer syndrome	86
<i>BAP1</i> tumor predisposition syndrome	92
Birt Hogg Dube	93

DICER1-related pleuropulmonary blastoma familial tumor and dysplasia syndrome	94
Familial atypical multiple mole melanoma syndrome	95
Hereditary breast and ovarian cancer syndrome	96
Hereditary leiomyomatosis and renal cell carcinoma	100
Hereditary paraganglioma and pheochromocytoma	101
Li–Fraumeni syndrome	102
Prostate cancer syndrome	104
<i>PTEN</i> hamartoma tumor syndrome includes Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome	105
References	108
9. Cardiac disorders	109
<i>JEFFREY ADDISON, LUISA MESTRONI AND MATTHEW R.G. TAYLOR</i>	
Introduction	109
Cardiomyopathies	109
Inherited arrhythmogenic disorders	116
Tranthyretin amyloidosis	122
References	124
10. Hereditary connective tissue disorders	127
<i>DAVID R. MURDOCK, HARRY C. DIETZ III AND GRETCHEN MACCARRICK</i>	
Introduction	127
Conditions with a primary vascular phenotype or aortopathy	128
Conditions with musculoskeletal and skin manifestations	137
Other conditions with phenotypic overlap	143
References	144
11. Disorders that impact reproduction	147
<i>J. SCOTT GABRIELSEN AND DOLORES J. LAMB</i>	
Introduction	147
Disorders that impact male fertility	148

Disorders that impact female fertility	151
Disorders that impact both male and female fertility	153
Disorders of sex development	156
References	161
12. Endocrine disorders	163
<i>DINA WINOGRAD, AVNI MODY, ASHOK BALASUBRAMANYAM AND SUSAN L. SAMSON</i>	
Introduction	163
Monogenic causes of DM	163
Genetic syndromes affecting multiple endocrine glands	168
Genetic disorders of calcium metabolism	174
Genetic disorders caused by Gs-alpha subunit	176
Genetic disorders affecting the adrenal glands	179
References	182
13. Gastrointestinal disorders	185
<i>JENNIFER WEISS, ANNA ZAKAS AND JESSICA HAUSE</i>	
Introduction	185
Diseases of the Colon	185
Diseases of the Stomach	195
Diseases of the Pancreas	199
Diseases of the Liver	205
References	210
14. Hematological disorders	213
<i>SARVARI YELLAPRAGADA AND KATIE LEE BERGSTROM</i>	
Introduction	213
Bleeding disorders	213
Bone marrow failure disorders	218
Thrombophilia	219
Red cell defects	221
Hematological malignancies	227

References	229
15. Immunologic disorders	231
<i>WOJCIECH WISZNIEWSKI</i>	
Introduction	231
Disorders with antibody deficiency	233
Disorders with T-cell deficiency	239
Complement deficiencies	242
Autoinflammatory disorders	244
References	246
16. Intellectual and developmental disabilities	249
<i>JAIME VENGOECHEA AND AIXA GONZALEZ GARCIA</i>	
Introduction	249
Syndromic intellectual disability	252
Nonsyndromic intellectual disability	257
Conclusion	257
References	258
17. Metabolic disorders	261
<i>LINDSAY BURRAGE AND RONIT MAROM</i>	
Introduction	261
Metabolic stroke	267
Neuropsychiatric symptoms	270
Rhabdomyolysis	274
Peripheral neuropathy	276
References	280
18. Mitochondrial disorders	283
<i>JAYA GANESH AND FERNANDO SCAGLIA</i>	
Introduction	283
Evaluation of mitochondrial disorders	285

Genetic testing	294
Genetic counseling	299
References	300
19. Neurologic disorders	305
<i>FUKI M. HISAMA AND THOMAS D. BIRD</i>	
Introduction	305
Neurogenetic disorders	306
Movement disorders	306
Motor neuron and neuromuscular diseases	311
Dementias	316
Paroxysmal disorders	320
References	323
20. Pulmonary disorders	325
<i>SHWETA U. DHAR AND SANDESH C.S. NAGAMANI</i>	
Introduction	325
Obstructive lung disease	325
Restrictive lung disease	330
Spontaneous pneumothorax	332
Vascular disorders	333
Lung cancer	336
References	337
21. Psychiatric disorders	339
<i>MEGAN E. TUCKER</i>	
Introduction	339
Mental health conditions	340
Genetic conditions associated with an increased risk for mental illness	342
References	351

22. Renal disorders	353
<i>MIR REZA BEKHEIRNIA AND NASIM BEKHEIRNIA</i>	
Introduction	353
Cystic renal diseases	353
Inherited glomerulopathies	356
Common genetic disorders with renal involvement	361
Hereditary renal cell carcinoma	363
Genomic medicine in adult nephrology	366
References	366
23. Skeletal disorders	369
<i>SANDESH C.S. NAGAMANI AND BRENDAN LEE</i>	
Introduction	369
GSDs that present with altered BMD	370
GSDs with abnormal bone mineralization	375
<i>FGFR3</i> -related chondrodysplasia	377
References	379
24. Skin disorders	381
<i>KRYSTAL M. JONES AND MOISE L. LEVY</i>	
Introduction	381
Pigmentation disorders	381
Photosensitivity disorders	386
The porphyrias	389
Neurocutaneous disorders	392
Disorders of the epidermis	396
Vascular anomalies	401
References	403

Section III Access to Genetic Services	405
25. Billing and reimbursement	407
<i>DAVID FLANNERY AND ADNAN ALSADAH</i>	
Introduction	407
Referral to a clinical genetic service	407
CPT coding for clinical services	408
Documentation	409
New payment models	411
Billing for clinical services	411
Billing for genetic laboratory services	413
References	416
26. Telegenetics and other care models	417
<i>EDWARD D. ESPLIN, RUTH K. ABRAMSON AND DAVID FLANNERY</i>	
Introduction	417
Use of Telemedicine/Telehealth in adult genetics	418
Equipment and technology for telegenetic services	421
Software applications (commonly known as apps)	421
Use of telegenetic services to support rural health providers	422
E-consults and videoconsults	422
Commercial use of telegenetics	423
Standards and regulatory policy applicable to TM for genetic services	424
Billing and reimbursement for TM services	426
Conclusions	427
References	428
27. Ethical, Legal, and Social Implications	431
<i>CHRISTI J. GUERRINI, AMY L. MCGUIRE AND GABRIEL LAZARO-MUNOZ</i>	
Introduction	431
Decision-making and informed consent	432
Disclosure of results	435

Privacy and discrimination	438
References	441
Section IV Future Directions	443
28. Precision medicine in the genomic era	445
<i>TREVOR D. HADLEY AND SANDESH C.S. NAGAMANI</i>	
Introduction	445
Precision medicine initiative	446
Applicability of precision medicine in the clinic	446
Direct-to-consumer genetic testing and precision medicine	449
Challenges and future directions	450
References	451
29. Pharmacogenomics	453
<i>LILEI ZHANG AND KEVIN E. GLINTON</i>	
Introduction	453
Clinical applications of pharmacogenomics	453
Clinical resources and adaptation of pharmacogenomics	458
Challenges and future directions	459
References	461
30. Gene therapy and gene editing	463
<i>NICOLA BRUNETTI-PIERRI</i>	
Introduction	463
Gene therapy strategies and vectors	463
<i>Ex vivo</i> gene therapy	464
<i>In vivo</i> gene therapy	466
Genome editing	471
Current status, future challenges, and conclusions	473
References	474
Index	479



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Foreword

Having observed the evolution of medical and molecular genetics at Baylor College of Medicine (BCM) over the last 50 years, I am well positioned to comment on the origin of this book. Although the pioneering US genetics programs such as those at Johns Hopkins led by Victor McKusick and at University of Washington led by Arno Motulsky had strong leadership and clinical presence in adult medical genetics, most of the second- and third-generation programs are developed in pediatric environments and have attracted pediatric trainees; but, many early leaders were internists. The program at BCM was typical in this regard with the significant majority of MD trainees having previous training in pediatrics, and nearly 80% of patients were infants and children. Thus, the program at BCM was often perceived as being purely pediatric, and there were no adult genetics clinics for the first 30 years of our program.

The three editors of this book changed all of this for BCM. Over time, we sought out trainees with internal medicine backgrounds and such trainees sought us out. Although a small adult genetics clinic was functioning, it was not until Dr. Dhar's initiative in 2008 that genetics clinics were established at many of the healthcare systems affiliated with BCM. This growth included both increasing inpatient consultation activity and busy outpatient clinics.

As new molecular methods and advances in cytogenetics evolved, genetic diagnosis and counseling services expanded to play a greater role in adult medicine, often having an emphasis on cancer genetic testing with *BRCA1* and *BRCA2* testing providing a paradigm. Fortunately, novel genetic therapies are advancing rapidly, and these interventions are now playing a larger and larger role in adult genetics.

With the very high enthusiasm for individualized medicine, personalized medicine, precision medicine, and related concepts, on the part of patients and healthcare systems, it may seem like every physician will need to be a geneticist. To some extent, lay populations will be increasingly knowledgeable and conversant regarding how genetics impacts their health and reproduction. If populations can become excited about the genetics of their ancestry, they could equally likely become students of their medically relevant genotype. Millions of individual members of the general population are undergoing whole-exome or whole-genome sequencing in multiple countries on multiple continents. It seems only a matter of time, maybe a short time, before all individuals in economically capable societies will have access to whole-genome sequencing as part of routine health care. It will require "genetics literate" physicians across many specialties, genetic specialists, and genetic counselors to meet the growing demand of using genotypes to optimize medical care. It is rapidly becoming essential to merge data from monogenic mutations with genome-wide polygenic risk

scores to adequately understand the risks of individual patients. The future will require more genetic knowledge on the part of all health professionals to meet the expectations of society. Hopefully, this book will further the genetic expertise of a broad range of physicians and many other healthcare professionals when it comes to diagnosing and treating genetic conditions developing primarily in the adult population.

The book is timely because the volume and complexity of genetic testing are growing rapidly, and analysis of the entire genome will become more commonplace replacing the one gene/one disease/one phenotype at a time paradigm. This book seeks to provide a practical guide for implementation of genetics and genomics in routine health care. The authors have many years of clinical experience on which to base their recommendations and are active in national groups debating best practices. The many perspectives brought on by the individual contributors of this book will bring a much needed education and awareness about the role of genetics in the care of adult patients.

Arthur L. Beaudet



Preface

Genetic testing is now increasingly being used for the diagnosis and management of numerous conditions. However, there are few, if any, concise yet comprehensive handbooks on genetic disorders that are commonly encountered in adults. Hence, we developed a convenient and easy-to-read resource for use in the clinics. The *Handbook of Clinical Adult Genetics and Genomics* is a compilation of genetic disorders encountered by healthcare practitioners. We envision that this book will help readers understand the clinical features of genetic disorders that present during adulthood and serve as a practical and useful guide for diagnosis and management. Whereas the field of adult genetics has undergone significant growth and expansion, there is still a dearth in literature regarding this subspecialty of clinical genetics. Likewise, there are few clinics that provide such subspecialty services. This book also offers a practical framework for setting up or expanding adult genetic services by addressing reimbursement, counseling, and use of other care-delivery models.

The book is divided into four sections: Section I focuses on general concepts relevant to medical genetics; Section II is organized by organ systems and review disorders common to each system; Section III discusses the practical considerations for an adult genetic clinical practice including alternative delivery models such as telegenetics; and finally, in Section IV, we conclude with a view into the future with insights into precision medicine, pharmacogenomics, gene editing, and gene therapy.

We express our deepest gratitude to all contributors who have so graciously found time to share their expertise. We are thankful to Elsevier for recognizing the need for and the potential of this book. In particular, we are grateful to Peter Linsley who conceived this project, Samantha Allard who guided us through the process and patiently answered our many questions, and our copy editors and project managers. We thank Chelsea Cook for her administrative assistance and for keeping us on track during this project. Our thanks also go out to our patients—they are the inspiration for all that we do. And of course, we would not have been able to do this without the unswerving support of our spouses (Utpal Dhar, Suman Rajagopalan, and Dan Eble) and children (Arman and Arjun, Suhas and Sanjay, and Elena and Annalise).

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SECTION

I



The Practice of Adult Genetics

Introduction to the practice of adult genetics

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Overview of the field of adult genetics

Clinical genetics has become a key discipline of modern day medicine since the completion of the human genome project. Genetic information is increasingly being used to inform medical care including diagnosis, treatment, and management of patients. A population study of genetic disorders [1] showed that 0.4% of the population have a single gene disorder (Mendelian disease), 0.2% have a chromosomal abnormality, 4.6% have a multifactorial condition, 0.1% have an obvious genetic abnormality of unknown inheritance, and 0.3% have congenital problems that are not genetic. While genetic disorders impact any organ system at any age in a given person's life; historically, the practice of medical genetics has resided in the pediatric realm. With the current advances in technology and medicine, the genetic basis of many adult-onset diseases is being unraveled. We now know that many genetic disorders do not manifest until adulthood and multiple genetic factors may interact with multiple environmental factors over an individual's lifespan to produce a clinical phenotype. Likewise, with earlier diagnosis and improvements in management, many individuals with congenital or childhood-onset conditions are now surviving into adulthood and seeking to transition their genetic care to an adult setting. Hence, the practice of adult genetics has emerged and continues to evolve [2,3]. The number of patients seen in different adult genetics practices has exponentially increased [2–4]. There are undoubtedly many more practices in the nation that are observing the same trends. While there are many centers with dedicated adult genetics clinics, there are others who see adult and pediatric patients in their combined genetic practices. Within these adult genetics practices, many centers have specialty clinics such as cancer genetics, cardiovascular genetics, connective tissue disorders, neurogenetics and inherited disorders of bone, to name a few.

Why is it necessary to learn more about this?

There are a variety of overarching reasons for referral to an adult genetics practice. These include diagnosis of a suspected genetic condition, risk assessment of asymptomatic

individuals with a family history of a known genetic condition, management of childhood-onset conditions in adulthood, or preconception/carrier counseling for estimation of risks in pregnancy. A list of specific examples of indications for referral to adult genetics can be found in the publication by Eble et al. [3]. Genetics can play a role in different categories of disorders such as single gene/Mendelian disorders and multifactorial/complex disorders. More information about application of genetic information in complex disorders can be obtained from Chapters 28 and 29, while a detailed description of different disorders broken down by organ systems can be reviewed in Chapters 8 through 24 of this handbook. It is important to be aware of Mendelian disorders impacting every specialty in adult medicine so that appropriate diagnosis can be made and optimal and timely management is initiated. Table 1–1 lists different genetic disorders encountered by adult practitioners that are covered in this handbook. This is not a complete list but gives an idea of the diversity and variety of genetic disorders seen in adults and that every primary care practitioner as well as specialist may benefit by being aware of these conditions.

It is important to remember that unlike children with genetic disorders, in the diagnosis of a genetic disorder in adults, one needs to sift through common age-related comorbidities that may confound the clinical picture. Patients have often visited with multiple specialties prior to their genetic evaluation and are left with frustration at their diagnostic odyssey. One needs to be sensitive about the challenges these individuals bring to their care. However, it is also essential to not attribute every symptom/sign to the underlying genetic disorder. Adults with genetic conditions can also develop common morbidities just as anyone else, which may merit their own evaluation and workup.

Table 1–1 Common examples of single gene disorders in adults.

Specialty	Genetic disorder
1 Cancer	Hereditary breast and ovarian cancer syndrome, Li–Fraumeni syndrome, PTEN hamartoma syndrome
2 Cardiology	Familial hypercholesterolemia, Long QT syndrome, familial cardiomyopathy
3 Connective tissue disorders	Ehlers–Danlos syndrome, Marfan syndrome, Loeys–Dietz syndrome
4 Endocrinology	Maturity onset diabetes of the young, multiple endocrine neoplasia
5 Gastroenterology	Lynch syndrome, Wilson disease, hereditary hemochromatosis
6 Hematology	Fanconi anemia, hemoglobinopathies
7 Immunology	Common variable immunodeficiency, ataxia-telangiectasia, hereditary angioedema
8 Metabolic disorders	Urea cycle disorders, cobalamin deficiency
9 Neurology	Huntington disease, frontotemporal dementia, muscular dystrophies
10 Pulmonology	Alpha-1 antitrypsin deficiency, cystic fibrosis
11 Psychiatry	Schizophrenia, microdeletion syndromes with psychiatric components
12 Renal	Polycystic kidney disease, Fabry disease
13 Skeletal disorders	Osteogenesis imperfecta, hypophosphatasia
14 Skin disorders	Oculocutaneous albinism, neurofibromatosis type 1, tuberous sclerosis

How are we teaching our medical students?

Medical school lays the grass roots of medical education and practice for our students. If the students are taught clinical genetics within the framework of pediatrics, they will not be able to appreciate the importance of recognizing genetic disorders if they intend to practice adult medicine. Hence, it is imperative that medical school curriculum be balanced between pediatric and adult genetics. Students should be aware of the utility of genetics in adults and be able to recognize genetic conditions in different specialties. Many curricular reforms are underway and national societies such as the Association of Professors of Human and Medical Genetics and the American College of Medical Genetics and Genomics are working hard to ensure that students get a well-rounded education in genetics. Some schools such as Baylor College of Medicine have implemented specialized tracks [5] called the Genetics and Genomics Pathway that provide enrichment in genetic education beyond the core curriculum in genetics.

How are we training our genetics residents?

Genetics residency programs are located in different departments across the country based on the strength of the program and its structure within the academic center. There is a disparity in clinical training of future geneticists based on whether their training program is more focused on pediatric or adult training. Given the paucity of adult trained geneticists, the number of adult genetics focused clinical programs is small. Hence, trainees may be getting a more biased approach to medical genetics that is heavily influenced by pediatric presentations. Establishment of a genetics curriculum in internal medicine residencies [6] as well as combined internal medicine-genetics residency programs [7] may help mitigate some of these limitations and make trainees aware of the expanding world of adult genetic medicine.

Who will impart genetic care to adult patients?

Pediatricians are aware of genetic disorders that are seen in children and can manage them or refer them to genetic specialists. In contrast, the care of the adult patient with a genetic disorder is fragmented among different specialties and adult trained providers may or may not be comfortable managing these conditions. In addition, it is harder to find geneticists that are trained in adult medicine. Hence, it becomes important to train all providers, regardless of specialty, about the impact of genetics in medical care. A cardiologist may need to recognize that a spontaneous aneurysm in a 42-year-old individual with no past medical problems may have resulted from a connective tissue disorder, or a gastroenterologist who sees multiple polyps in a 50-year-old at his first colonoscopy must think that his patient may indeed have a familial polyposis syndrome.

Why do we need a book like this?

The primary objective of this book is to provide a clinical overview of genetic disorders that are commonly encountered in the adult population within different specialties of medicine. A practical approach for diagnosis and management within each organ system will be detailed, thus educating the reader about the clinical aspects of adult genetics. This book will also serve as a basis for learning the challenges and uniqueness about adult genetic practices. Residents and practicing physicians of all subspecialties will find this book to be a handy resource to learn about genetics relevant to their fields of practice. The currently available genetics textbooks are typically large reference books that are most likely relevant only to geneticists and not clinicians of other specialties. This book will serve as a practical handbook that trainees of multiple subspecialties including genetics can carry during “rounds.” In addition, there are no books on the practical approach to the care of an adult with a genetic disorder. This book will use case-based scenarios to help understand diagnostic evaluation and management of patients.

The book is divided into four sections. [Section I](#) outlines the basic concepts of genetics and genomics including genetic testing methodologies and genetic counseling. [Section II](#) is a review of the different genetic disorders relevant to each of the organ systems, and the reader can use the case vignettes to understand clinical scenarios they may experience in practice. [Section III](#) focuses on access to genetic services and here the reader can understand the concepts of billing and reimbursement as well as alternative models for delivery of genetic and genomic care. A chapter on the ethical, legal, and social implications of genetic medicine is also presented. Finally, [Section IV](#) directs the reader to the future with discussions on precision medicine, pharmacogenomics, and gene therapy.

Points to remember

- Adult genetics is an evolving subspecialty of clinical genetics.
- Genetics will impact medical care at all ages and in all subspecialties.
- Every physician needs to be aware of genetic disorders that impact adults so that appropriate diagnosis and timely management can be initiated.

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Basic concepts of genetics and genomics

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Introduction

The last several decades have seen unprecedented growth in our understanding of the genetic and genomic basis of human disease. In many ways, this growth has been driven by a unique combination of clinical insight and technology development, enabling precise detection of rare genetic and genomic variants to be linked directly to clinically observed phenotypes. Completion of the international Human Genome Project in 2003 provided the first draft reference sequence of the human genome, a haploid sequence of $\sim 3 \times 10^9$ base pairs (bp) which led to elucidation of the locations and architectures of $\sim 20,000$ protein-coding genes. The continued development of next-generation sequencing technologies has given clinicians and researchers a means to probe the genome broadly without sacrificing precision.

Unraveling the associations between an individual's genes, genetic variants (their “genotype”), and their clinical presentation (their “phenotype”) will begin—and in some instances has already begun—to form the cornerstone of precision therapeutics. Discoveries involving these “genotype–phenotype” relationships are cataloged in the Online Mendelian Inheritance in Man (OMIM) database, with more than 4000 protein-coding genes (roughly 20% of the estimated 20,000 protein-coding human genes) associated with one or more disease phenotypes (www.OMIM.org, May 30, 2019) [1]. These data illustrate the breadth of discovery to date, as well as the tremendous amount of discovery that remains.

Functional elements of the human genome

To understand how genetic and genomic variations can contribute to genetic conditions in adults, it is helpful to first review the structure of deoxyribonucleic acid (DNA) in the context of the genomic architecture.

Molecular architecture of DNA

The structure of DNA itself is elegant in its relative simplicity as a polymer consisting of only four nucleotides. The precise sequence of the nucleotides is responsible for encoding the instructions needed for an organism to develop and reproduce. Each nucleotide is composed of a purine [adenine (A) or guanine (G)] or pyrimidine [thymine (T) or cytosine (C)] base, as well as one phosphate molecule and one sugar moiety. The molecular architecture of DNA imposes a directionality on each polymer, or “strand.” A DNA strand sequence is classically represented in a 5′-to-3′ direction. In the cell, DNA exists as a double-stranded helix, with the sequence of one strand representing the inverse complement of the sequence of the opposite strand (Fig. 2.1). The strands are held together by hydrogen bonds that form between purine-pyrimidine base pairs: A-T and G-C. This structure enables stable transmission of genetic information from one generation to the next.

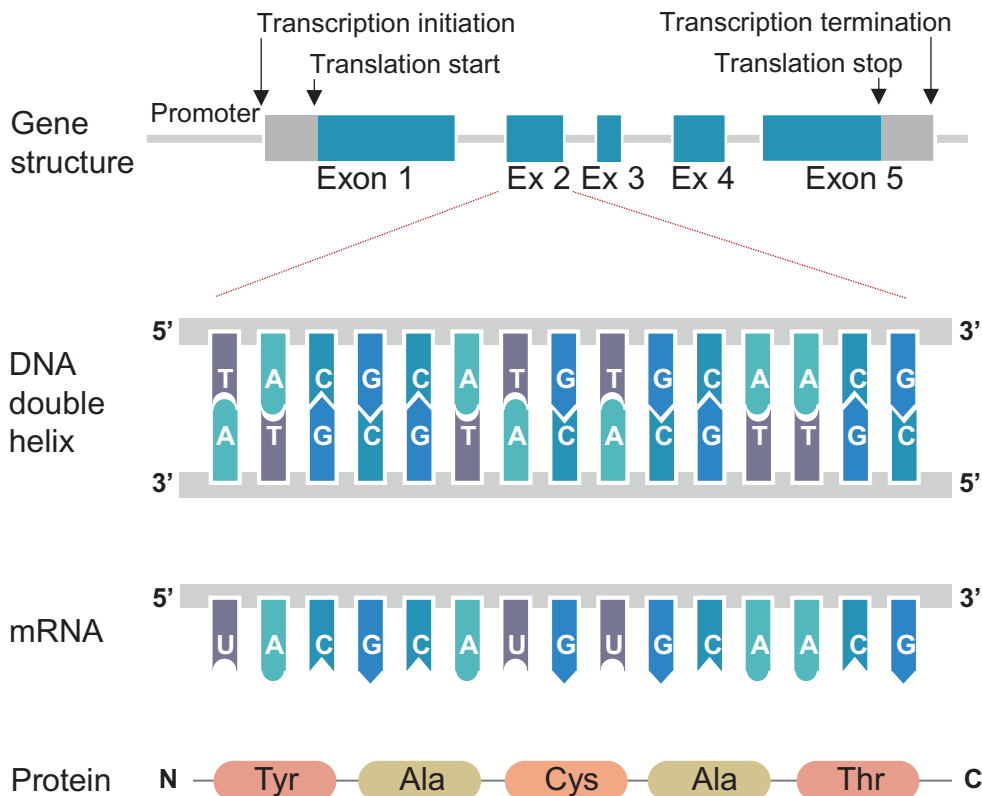


FIGURE 2.1 Gene structure, and the DNA, RNA, and protein molecules involved in transcription and translation. Each gene has a transcription initiation and transcription termination site, which mark the beginning and end of the encoded mRNA. mRNA is single-stranded, and the thymine (T) of DNA is replaced by uracil (U). mRNA codons (groups of three nucleotides) encode the amino acids of a protein.

Nuclear and mitochondrial genomes

If stretched end-to-end, a single cell's nuclear genome would span approximately 2 meters in length. Levels of packaging of the nuclear DNA form chromosomes, imposing a higher-order structure on the nuclear genome and facilitating both cell division and gene expression. Each cell consists of 46 chromosomes: two copies of each autosome (chromosomes 1–22), and two sex chromosomes (either two X chromosomes, or one X and one Y). These chromosomes consist of the DNA as well as a protein family called histones which form 8-protein complexes called octamers around which the DNA double helix is wound. Octamers are spaced about 20–60 bp from each other, and each has nearly two full turns of DNA (~ 140 bp), giving a “beads on a string” appearance to the DNA-histone complex called the nucleosome. Nucleosomes are further organized into cylindrical structures called solenoids, which are bundled into DNA domains measuring approximately 100,000 bp, organized by a nuclear protein scaffold to form chromatin. During cell division, this structure will condense to display the chromosome structure with which readers are most familiar: the 46 paired chromosomes that can be observed by karyotype analysis, each consisting of one short (“p”) arm and one long (“q”) arm joined by a centromere.

In addition to the nuclear genome, every cell contains hundreds, if not thousands, of mitochondria, each harboring multiple copies of the mitochondrial genome. The mitochondrial genome is much smaller than the nuclear genome, consisting of only 37 genes encoded on a single, circular chromosome of 16,569 bp. Proper mitochondrial functioning depends on these 37 genes which encode mitochondrial transfer RNA and components required for oxidative phosphorylation. Many nuclear-encoded genes are also essential for mitochondrial function, and this is one reason why mitochondrial conditions can display very different patterns of inheritance in families.

Gene structure

Each of the approximately 20,000 genes in the human genome has several components. These include both the nucleotide sequence that encodes the amino acid sequence of the protein itself, as well as several regulatory elements that are important for gene expression, including the transcription of DNA into RNA, and the translation of that RNA into an amino acid sequence to form a protein ([Fig. 2.1](#)). “Transcription” describes the process whereby the DNA sequence is copied into a messenger RNA (mRNA) sequence. Subsequently, “translation” is the process by which this mRNA sequence is used to generate an amino acid sequence encoding the intended protein. It is to be noted that only 1%–3% of the genome contains protein-coding genes.

The gene promoter sequence regulates the beginning of RNA transcription at the transcription start site, which is typically the beginning of the first exon. The exons themselves encode the intended amino acid sequence. The exons may be separated by one or more intronic sequences that may contain enhancers or other regulatory elements. These introns are spliced out of the final mRNA product. The 5' portion of the first exon, and the 3' portion

of the final exon also contain untranslated regions (UTRs, one 5'UTR and one 3'UTR) that are transcribed into the mRNA sequence and contain important elements for RNA function and stability, but do not contribute to the final amino acid sequence of the encoded protein.

In the mRNA sequence, an “initiator codon” marks the site at which protein translation will begin and always encodes the amino acid methionine (M, Met); this methionine will become the N-terminus, or amino-terminus of the polypeptide chain, so named for the amino group that is exposed at this end of the protein. From this point, the mRNA sequence consists of a series of consecutive codons, each comprising three bases, which specify the order of particular amino acids within the protein being made. As subsequent sections will discuss, the reading frame established by this triplet code, which defines the position of each codon, is important for accurate protein translation. Translation stops when one of the three “stop codons” is encountered. The final amino acid becomes the C-terminus or carboxy-terminus of the protein named for the carboxyl group that is exposed at this end of the polypeptide chain.

Human genomic variation

An essential foundation for the implementation of individualized, precision medicine is understanding relationships among genes and modes of disease inheritance. Enabled with these data, a clinician can provide individuals and families with key information regarding molecular diagnoses, their implications for current and future health, and recurrence risks for a given family. Although the following sections will detail distinct classifications of variant types and modes of disease inheritance, it is important to consider that for a given gene different variant types and different modes of inheritance may be observed in association with distinct disorders. *LMNA* is one such example, for which rare variants can lead to autosomal dominant (AD) conditions such as dilated cardiomyopathy, Hutchinson–Gilford progeria syndrome, and Emery–Dreifuss muscular dystrophy, or autosomal recessive (AR) conditions such as Charcot-Marie-Tooth disease or, in rare instances, Emery–Dreifuss muscular dystrophy. To date, over 30% of disease genes have been definitively associated with more than one rare disorder (www.OMIM.org).

Types of genetic and genomic variants

Genetic and genomic variants can range in size from a single-nucleotide substitution [a single-nucleotide variant (SNV)] to a small insertion/deletion (indel) of 1–50 bp, to a much larger structural variant (SV) that can impact one or more genes.

Single-nucleotide variant

SNVs that occur within the protein-coding portion of a gene can result in an amino acid residue change in the encoded protein, in which case they are termed “nonsynonymous” or “missense” variants. A change resulting in a stop codon being coded is called a “nonsense” or “stop-gain” variant and will prematurely truncate the protein. Alternatively, because of the

redundancy of the genetic code, with some amino acid residues encoded by more than one codon, some SNVs will result in no amino acid residue change; these are termed “synonymous” variants. Splice site SNVs located near intron/exon boundaries can impact splicing mechanisms, leading to loss of one or more exons, or inclusion of intronic sequence within the final mRNA transcript.

Indel variants

Indel variants will shift the codon reading frame: if the frame is shifted by one or two nucleotides, the codons subsequent to the variant will be “out of frame,” and the variant will be termed a “frameshift” variant (the new frame will encode a new amino acid sequence that ends once a stop codon is encountered within the new frame). In contrast, indel variants that maintain the codon frame (e.g., insertion of three nucleotides, or deletion of six nucleotides) will produce a “non-frameshift” variant.

Large SVs

Large SVs are called so because they impact genomic structure. Depending on their size, they may impact a part of a gene, such as a single or few exons, or a large number of genes. *Copy neutral* SVs, such as inversions, impact the structure of the genome without impacting the copy number of each gene. These may be pathogenic if they disrupt the gene: either the gene sequence or its regulatory elements. Copy number variants (CNVs) alter the number of copies of a particular genomic segment. *Copy number losses* are deletions in which one (if heterozygous) or both (if homozygous) copies of a DNA segment are lost. *Copy number gains* are duplications in which one or more additional copies of a DNA segment are present. These CNVs can cause disease through impacts on gene dosage and can also result in gene interruption depending on the precise location of their breakpoints.

Chromosomal variation

While often thought to present clinically during childhood, chromosomal aneuploidies and translocations are relevant to the practice of adult genetics. *Chromosomal aneuploidies* describe the occurrence of an abnormal number of chromosomes within a cell—technically, this can refer to either one or more absent chromosomes (e.g., Turner syndrome, in which there is only one sex chromosome: 45,X) or one or more extra chromosomes (e.g., trisomy 21, in which there is an extra chromosome 21: 47,XX,+21). The majority of chromosomal aneuploidies are not viable, but individuals with Turner syndrome and trisomy 21 regularly survive into adulthood.

Chromosomal translocations occur when there is a rearrangement of DNA segments between two chromosomes. These may be “balanced” if copy number is maintained, or “unbalanced” if copy number gains and/or losses occur due to the rearrangement. Robertsonian translocation is one type of translocation that involves the joining of the q arms of two acrocentric chromosomes (chromosomes 13, 14, 15, 21, 22). Because the acrocentric chromosomes do not contain protein-coding genes on their p arms, loss of the p arms in the course of these translocations is not considered an unbalanced event; however,

the translocation itself can lead to errors in chromosomal segregation during meiosis and gamete formation, thus increasing the risk of an unbalanced translocation in the next generation.

Uniparental disomy is one additional form of genomic variation that can impact disease expression. The expression of a subset of genes in the human genome is tightly regulated from either the maternally or paternally inherited chromosome, such that biparental chromosome inheritance is essential for normal development. Although a majority of individuals will inherit one set of chromosomes from each parent, and thus have one maternal and one paternal copy of every chromosome, uniparental disomy can occur, in which both copies of a particular chromosome are inherited from the same parent. Depending on the chromosome impacted, consequent errors in gene regulation can impact the development and expression of disease.

Impact of genetic and genomic variation

As one may expect, different variant types can have different impacts on protein function. Scientifically these are classically conceptualized in one of the following five categories: loss-of-function (LoF [amorph]), partial LoF (hypomorph), dominant-negative (antimorph), increase in function (hypermorph), and novel function (neomorph). From a clinical perspective, the classification of variants focuses on whether the impact of the variant can result in expression of the disease: those variants known to be disease-causing are classified as “pathogenic,” and those variants known not to result in disease expression are classified as “benign.” A five-tiered system has been proposed by the American College of Medical Genetics and Genomics (ACMG) to provide a framework for classification of variants: these five tiers are “pathogenic,” “likely pathogenic,” “variant of unknown significance” or VUS, “likely benign,” and “benign [2].”

Modes of inheritance

It is important to remember that modes of inheritance refer not to the segregation of a particular variant, but to the segregation of a disease trait or phenotype observed within a family’s genetic pedigree. The following sections will first describe the more classic and straightforward forms of Mendelian inheritance, followed by several examples of more complex inheritance patterns.

Autosomal Dominant (AD)

AD inheritance is defined by the observation of a disease trait in which male and female offspring of an affected individual each have a 50% chance of being affected. Typical genetic pedigrees will demonstrate transmission of the trait from one generation to the next, and the underlying genetic variant will be present in only one of the two copies (“heterozygous” or “monoallelic”; i.e., one of the two “alleles” or copies) of the relevant gene. The term “autosomal” refers to the observation that AD conditions arise from a variant on an autosome,

defined as any chromosome (Chr 1–22) other than the sex chromosomes (Chr X and Y). Some individuals with an AD condition may have a variant that arose “*de novo*,” meaning that a new mutation occurred around the time of conception and thus is not present in either parent.

Autosomal Recessive (AR)

AR inheritance is defined by the observation of a disease trait that requires the underlying genetic variant(s) to impact both copies (“biallelic” or both alleles) of the relevant gene. Individuals who have an AR disease trait may have either the same variant present on both alleles of the gene (“homozygous”) or two variants, each affecting one copy (“*in trans*”) of the gene (“compound heterozygous”). Parents of individuals who have an AR condition are considered unaffected “carriers” of the condition and are expected to be heterozygous for an etiologic variant. Children born to such a carrier couple will have a one in four chance of inheriting both pathogenic variants (and thus having the AR condition), a two in four chance of inheriting only one of the pathogenic variants (and thus being an unaffected carrier), and a one in four chance of inheriting neither variant.

X-linked (XL)

XL inheritance is defined by the observation of a disease trait that segregates with a gene on the X chromosome. Males and females are usually—but not always—differentially affected by the variants that cause these conditions, as women have two copies of the X chromosome and men have only one copy of the X chromosome (and thus are hemizygous). Classically, XL conditions were considered to be either “dominant” or “recessive” depending on whether heterozygosity in a female was sufficient for disease expression. Some XL conditions indeed affect primarily males and are inherited from unaffected mothers. In contrast, other XL conditions impact males and females similarly, whereas others are observed only in females because the condition in males results in early/embryonic lethality. *Sex-limited traits*, which are distinct from XL conditions, are typically associated with variants in the autosomes but are only clinically observed in one of the two sexes.

Mitochondrial

Mitochondrial inheritance is defined by the observation of a disease trait that segregates with the mitochondrial genome. As mitochondrial genomes are inherited exclusively from the maternal germline, disease traits that result from a variant in the mitochondrial genome are inherited solely from the mother. It is important to note that not all mitochondrial diseases display mitochondrial inheritance. As some genes essential for mitochondrial function are encoded on the nuclear autosomes, and some are encoded within the mitochondrial genome, the class of conditions resulting from mitochondrial dysfunction can display either AR or mitochondrial inheritance. Note that despite this dichotomy, mitochondrial disease associated with a particular gene will display either autosomal or mitochondrial inheritance,

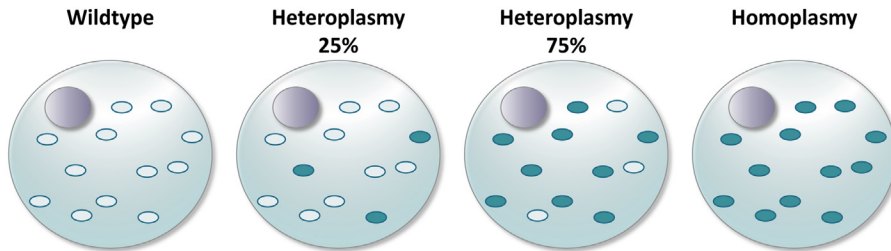


FIGURE 2.2 Mitochondrial heteroplasmy and homoplasmy. Cells will contain one diploid (two copies) set of the nuclear genome, which is present in the nucleus of the cell. However, numerous mitochondria and mitochondrial genomes exist within the cell. For simplicity, the mitochondria have not been shown. Each mitochondrial genome is represented by a small oval: white ovals indicate genomes that do not have a mitochondrial variant, and shaded ovals indicate genomes that do contain a mitochondrial variant. “Heteroplasmy” and “homoplasmy” are used to describe the percentage of mitochondrial genomes that have a given variant.

determined by the location of that particular gene within the genome. Because more than two copies of the mitochondrial genome are present in each cell, variants are not described as heterozygous or homozygous, but as “homoplasmic” (present in all copies of the mitochondrial genome) or as “heteroplasmic” (present in a subset of copies of the mitochondrial genome, [Fig. 2.2](#)). The degree of heteroplasmy (the percentage of mitochondrial genomes that have the variant) can vary from one tissue to the next and can impact the expression and severity of disease.

Imprinting

Rare conditions resulting from imprinting errors (such as Angelman syndrome and Prader-Willi syndrome) can be present when genes that are typically critically expressed from either the maternally or paternally inherited allele of a genomic region are inactive. These conditions will display very distinctive inheritance patterns in families, as expression of disease is determined by whether the variant allele is inherited from the mother or father. For example, individuals with Prader-Willi syndrome are most often missing a segment of the paternal copy of chromosome 15. However, if the same segment of chromosome 15 is absent only on the maternal copy of chromosome 15, the individual in question will not have Prader-Willi syndrome, but will instead develop a distinct condition called Angelman syndrome. Individuals with either condition may simply have a copy number loss (a deletion), or may have uniparental disomy resulting in a neutral copy number of this region of chromosome 15, or a defect in the imprinting machinery itself.

Digenic

Digenic inheritance refers to conditions that require rare pathogenic variants at two distinct loci or genes in order for the disease to be manifest ([Fig. 2.3](#)). The loci themselves are observed to segregate independently within a family (they are typically on distinct

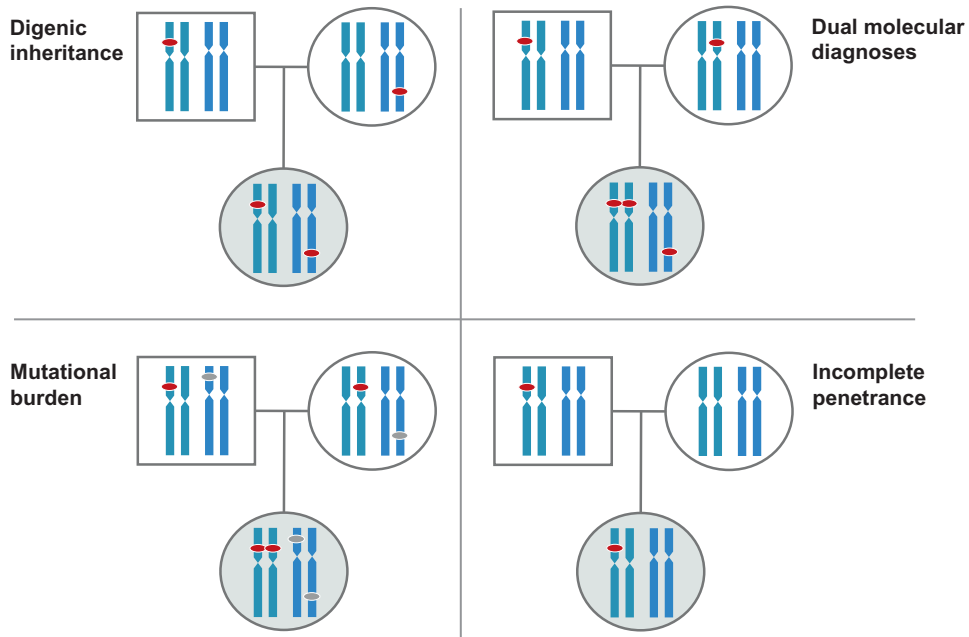


FIGURE 2.3 Complex modes of inheritance. Digenic inheritance involves variants at two loci that are both required for expression of the disease trait. Dual molecular diagnoses involve variants at two loci that each is associated with a disease trait—both disease traits can segregate independently within a family. Shown here is a proband with one recessive condition resulting from biallelic variants, and one dominant condition resulting from a *de novo* variant not present in either parent. Mutational burden describes the phenomenon in which the severity, expression, or age of onset of a trait may be modified by variants at additional loci. Shown here are fully penetrant biallelic variants conferring a recessive condition, as well as two additional non-penetrant variants (which by themselves do not cause disease) that modify the phenotype. Incomplete penetrance can be observed when disease trait manifestation requires one rare LoF allele in combination with a second, often more common, hypomorphic allele, for a disease trait to be manifest. Squares (male) and circles (female) demonstrate the parent-child pedigree structure. Circles filled by light gray background are affected offspring. Two chromosomes are represented with rare, pathogenic variants indicated by red ovals, and hypomorphic variants that do not independently confer a disease trait are indicated by gray ovals.

chromosomes), and the individuals who inherit pathogenic variants at both loci will develop the disease trait. Particular subtypes of Bardet-Biedl syndrome and Usher syndrome are examples of conditions that exhibit digenic inheritance.

Complex inheritance

Many commonly occurring multifactorial disorders such as diabetes, hypertension, and coronary artery disease run in families. The genetic risk in these disorders is typically due to multiple genetic and genomic variants, each with a small effect size. While these conditions tend to cluster in families, the expression of disease is dependent on the interaction between genetic and environmental factors.

Special considerations

Dual molecular diagnoses

Dual molecular diagnoses refer to a scenario in which an individual is found to have not one, but two molecular diagnoses, resulting in two disease traits which are observed clinically as a blended phenotype (Fig. 2.3). When the disease pairs are phenotypically similar, they can be categorized as “overlapping phenotypes” and may appear clinically to be a single condition; in contrast, when the disease pairs are phenotypically different, they can be categorized as “distinct phenotypes” and may appear clinically as an apparently novel condition. In either case, each disease trait will be observed to segregate independently of the other within the family. Dual molecular diagnoses, and multiple molecular diagnoses involving two, three, or even more loci, were once thought to be a relatively rare occurrence. However, they are now well-established to occur in at least 5% of individuals for whom genome-wide clinical testing is diagnostic [3].

Mutational burden

Mutational burden refers to the observation that a given phenotype resulting from a highly penetrant pathogenic variant can be modified (in severity, character, timing of onset) by additional variants that impact other genes acting within the same pathway or functional unit (Fig. 2.3). These additional variants may not be penetrant by themselves, nor segregate perfectly with disease in a family, but can explain the observation of intrafamilial phenotypic variability observed in some conditions; those individuals with more severe phenotypes can be found to have a higher mutational burden, in addition to the primary, etiologic pathogenic variant. Similar modifier effects may result not from genetic or genomic variants, but rather from epigenetic modifiers and/or environmental exposures.

Incomplete penetrance

Incomplete penetrance of a condition refers to the observation that not all individuals who have an etiologic variant for a particular disease trait will actually develop that disease trait (Fig. 2.3). This phenomenon may explain the apparent skipping of generations in an AD pedigree.

Relevance to clinical care

Basic concepts of genetic and genomic variation and inheritance establishes a framework for understanding the clinical impact of a particular variant or set of variants on disease manifestation. This genotype–phenotype relationship can inform molecular diagnoses for individuals and guide medical treatment and expectant management, while often providing the “why” that patients so often seek. This enables a precision molecular diagnosis, guiding clinical management of disease for the individual and their family [4].

Points to remember

- Protein-coding genes comprise less than 3% of the genome.
- Genetic and genomic variation can be categorized as SNVs, indels, CNVs, and copy neutral variants.
- Understanding the pattern of inheritance is critical in identifying a genetic disorder and assessing risk for family members.

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Genetic evaluation of an adult

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Introduction

Most adult care providers have limited experience in incorporating DNA-based testing into the clinical care of their patients. Twenty-first century standards of care will increasingly use DNA-based testing to inform disease risk identification, disease diagnosis, and long-term management. The incorporation of DNA-based testing into adult care includes three categories of genetic or genomic disorders: classical genetic disorders (e.g., rare monogenic or chromosomal syndromes), monogenic instances of common disease (e.g., familial hypercholesterolemia and *BRCA1/2*-associated cancer), and polygenic risk for common diseases.

The approach to diagnosis and management of genetic disorders in adult patients requires careful consideration of clinical features (including a history of presenting symptoms, a careful physical examination, and review of available laboratory and imaging data), family history, cost and efficacy of genetic diagnostic testing, and attention to the implications of testing for the management of a patient's disease. Here, we review a basic approach to the evaluation of an adult who presents with concern for a genetic disorder. In this chapter, we provide an overview of common presentations and discuss the importance of family context, available diagnostic DNA technologies, genetic and genomic contributions to disease, and approaches for follow-up and management.

Approach to the patient

The clinical consideration of the genetic contributions to the entries on a patient's "problem list" may come to light in two different ways. There is the longstanding manner in which consideration of a genetic contribution comes into the process, that is, where providers develop a differential diagnosis that includes consideration of a genetic contribution based on presenting signs, symptoms, or medical history (personal or family history). This approach, using clinical clues, or indications, to drive consideration of incorporating genetics, is referred to as "phenome-first." The indications for a genetic evaluation may either

reflect the patient's personal medical history or family history. Genetic evaluation may be indicated for individuals who have multiple organ systems affected, an earlier age of onset of disease than is typical, a diagnosis in a less often affected gender (e.g., male breast cancer), multifocal or bilateral disease, or disease in the absence of obvious risk factors. Family history may be suspicious for a hereditary condition when there are multiple affected family members with the same or related disorders, consanguinity, intellectual disability, a known or suspected genetic condition, or a history of recurrent spontaneous abortions. Patients may also seek evaluation due to infertility. Individuals who are healthy may seek evaluation because of an interest in universal or ethnicity-based carrier screening, or because they lack family history information and want to better understand their future risks.

Occasionally, DNA results precede clinical evaluation by an adult geneticist. There is now a growing use of DNA-based testing in both individuals and populations without a clinical prompt; such testing can reveal evidence of genetic risk in people without any clinical clues. The use of screening tests to identify pre-symptomatic disease risk has many precedents in clinical care (e.g., routine blood pressure checks). The use of DNA-based screening tests to identify pre-symptomatic disease risk is referred to as “genome-first” and is a rapidly evolving area of medical care. The relationship of a positive genetic finding to the diagnosis of a genetic or genomic syndrome is distinct in phenome-first versus genome-first approaches. In the phenome-first approach, the DNA test often confirms a diagnosis toward the end of a workup, while in genome-first care the DNA test often initiates a workup (see Fig. 3.1). In genome-first management, the clinical diagnosis of the genetic disorder may not ever be achieved. This phenomenon is known as *nonpenetrance*. It is essential that adult care providers and patients come to appreciate the applied concept of nonpenetrance in long-term care management.

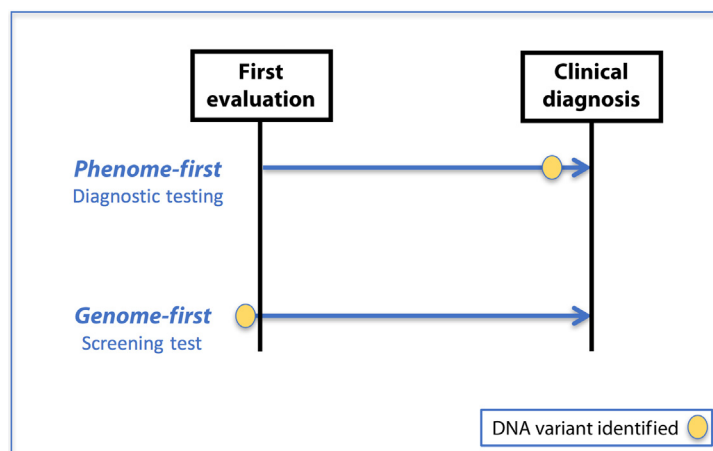


FIGURE 3.1 Differences in timing of DNA-based testing. An important distinction between the path to the diagnosis of a genomic condition in phenome-first and genome-first approaches is that a DNA finding often concludes the process in phenome-first, while it initiates the process in many genome-first cases.

Phenome-first approach

When individuals are considered for a genetic diagnosis it is often in the context of a clinical complaint or family history suspicious for a hereditary condition. In adult patients, a clinical concern may have already been evaluated by a previous medical provider and can take the form of laboratory results, exam findings, imaging results, or a symptom complex. The genetic differential diagnosis can include both nonsyndromic diagnoses and syndromic diagnoses depending on whether a finding is isolated or part of a multisystem disease. For example, a thoracic aneurysm can be nonsyndromic and not associated with the involvement of other organ systems or can be syndromic and linked to the genes that cause Marfan or Loeys–Dietz syndromes.

Misattribution of disease etiology, specifically assuming nongenetic etiology in the setting of genetic disease, can occur in adult medicine. The extent to which misattribution of cases of monogenic forms of diseases that are attributed to “sporadic disease” is unclear, but is an area of active investigation. One scenario is when providers fail to recognize syndromic patterns. This occurs for instance in familial cancer syndrome diagnosis when providers overlook the association between cancers such as endometrial cancer and colorectal cancer in Lynch syndrome. There are also instances when a lack of associated findings should prompt consideration of genetic etiology. For instance, practicing clinicians should consider genetic testing to exclude monogenic etiologies for disease when associated nongenetic findings are not present, for example, left ventricular hypertrophy without corresponding hypertension or valvular disease.

A review of the appropriate data and patient history should be based on the differential diagnosis associated with the presenting concern. For example, oncologic diagnoses may require a review of imaging, pathology results from biopsy specimens, or discussion of previous treatments and goals of testing. For cardiovascular diseases, a review of relevant testing might include electrocardiograms, echocardiography, or other cardiac structural tests including magnetic resonance imaging. A wide range of genetic diagnoses to explain a clinical presentation include many different kinds of disorders and could require input from genetic specialists. Genetic professionals include certified genetic counselors who are master’s degree level specialists with board certification through the American Board of Genetic Counseling (ABGC) and clinical geneticists who are physicians with specialty training in genetics. Clinical geneticists are board-certified through the American Board of Medical Genetics and Genomics (ABMGG). In addition to these professionals, there are physician specialists with expertise related to certain groups of genetic diseases, such as cancer geneticists and cardiovascular geneticists.

Within each medical subspecialty, recommendations for genetic testing vary by the clinical syndrome. For example, current recommendations for genetic testing in cardiovascular disease include atherosclerotic diseases, arrhythmias (testing is performed to assess for long QT or Brugada syndromes) [1], cardiomyopathies (testing is performed to assess for hypertrophic cardiomyopathy, dilated cardiomyopathy, or arrhythmogenic right ventricular cardiomyopathy), and sudden cardiac death. Care should be taken to characterize the presenting

constellation of symptoms and determine whether these make sense in the context of a genetic condition. Appropriate clinical and genetic testing guidelines from professional societies and peer-reviewed sources should be consulted when available.

Genome-first approach

Providers should expect that patients will increasingly present to them with a report of a DNA finding in hand. These reports may be the patient's and received from a direct-to-consumer testing company, a population screening program, a test ordered by their primary care physician or another specialist. Alternatively, the results may be of a relative of the patient who was recently diagnosed with a genetic disorder and your patient is seeking familial testing in themselves. Lastly, a patient may present with concern for an "incidental finding" or secondary finding discovered on genetic testing for another condition.

Incidental or secondary findings come about in the setting of next-generation sequencing (NGS) which generates a vast amount of genetic data and can include genetic variants with clinical effects that are unknown. The American College of Medical Genetics and Genomics (ACMG) currently recommends that laboratories only screen a small number of genes that are known to be medically actionable, meaning that a mutation in these genes changes medical management. The ACMG recently revised the terminology for incidental findings on targeted genetic testing to "secondary findings," to reflect that genes were intentionally assessed [2]. Consent for genetic testing requires that patients be informed that they will be tested for select medically actionable genes and allows patients to opt out of testing if desired.

Similar to the approach outlined for "phenotype first" patient presentations, a thorough history and review of data should be performed for all patients presenting with a new DNA finding, with particular emphasis on the clinical features and laboratory or imaging data relevant to the patient's hypothesized genetic disorder. [3] Occasionally a patient may present with a variant of concern that has uncertain clinical significance. The ACMG has developed a standardized system of reporting the clinical significance of genetic variants, and categorizes variants into either "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," or "benign" [4]. Variants are categorized into these groups using a number of data sources including population databases (e.g., Exome Aggregation Consortium, 1000 Genomes Project), variant databases [e.g., ClinVar, the Online Mendelian Inheritance in Man (OMIM)], as well as a review of primary literature. For variants with less certain clinical significance (variants of uncertain significance), patients should be informed of the limited data available to guide decision-making. Follow-up should be discussed, as the discovery of new data may inform future clinical management.

Understanding the family context

The genetic family health history is essential in the clinical assessment of genetic disorders. The family health history, defined as the description of the genetic relationships and medical history of a family, is an important risk factor that reflects inherited genetic susceptibility,

shared environment, and common behaviors. Accurate family history can help determine the mode of inheritance of a monogenic disease, whether it be autosomal dominant, autosomal recessive, X-linked, or mitochondrial as well as previously unrecognized disease risk in a family. Even with advances in our knowledge of the human genome and our increased ability to perform genetic testing, a family history continues to play a crucial role in the assessment and management of patients in the healthcare setting. Traditionally used as a means to diagnose rare Mendelian disorders, today's family health history can also provide clinical information about the diagnosis and management of more common complex diseases, including cancers, type 2 diabetes, cardiovascular disease, and stroke.

Family history should be recorded using the medical pedigree, a standardized graphical notation to document family history and genetic relationships. The Pedigree Standardization Task Force developed a proposal in 1995, later updated in 2008, for a set of symbols to be used in the medical pedigree, which has since become an international standard [5]. Typical nomenclature involves men designated with a square, women designated with a circle, and unknown sex with a diamond (Fig. 3.2). Relationships between individuals are designated with lines; a married couple, for example, will have a horizontal line connecting the two

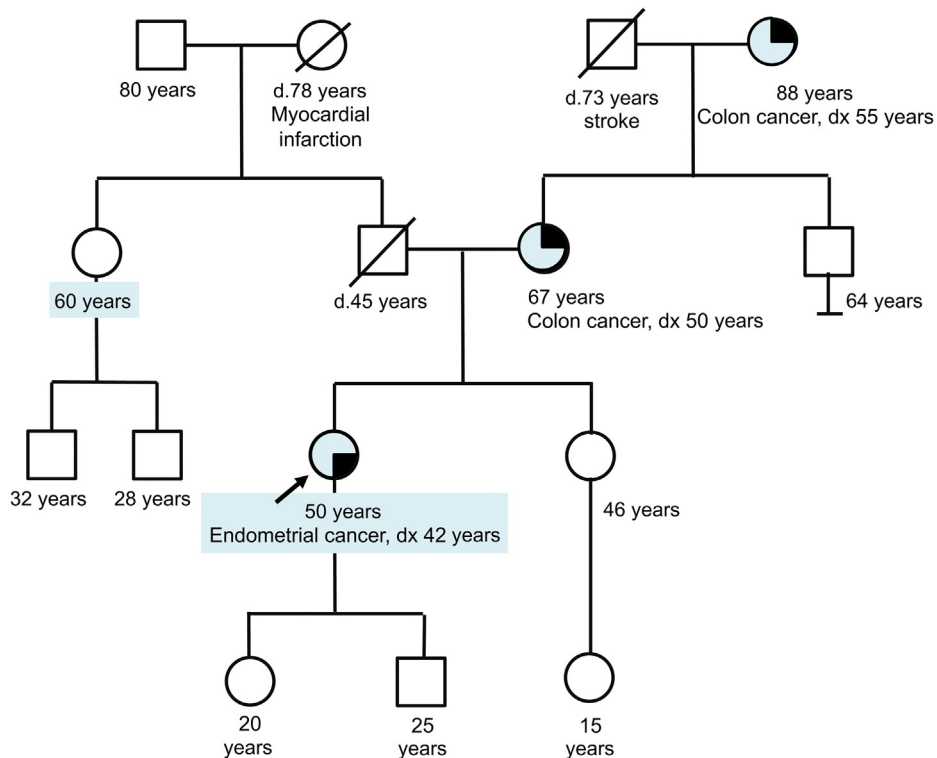


FIGURE 3.2 An example of medical pedigree.

individuals. Offspring are designated with a vertical line of descent, and siblings are generally connected with a horizontal line. Deceased individuals are qualified with a diagonal line, and individuals affected with a suspected disease are shaded. A typical pedigree should be recorded for at least three to four generations in order to inform accurate assessments of disease risk.

In this era of big data, it is easy to assume that genomic sequencing will provide all the information necessary to provide truly personalized care; however, the utility of a patient's self-reported family history should not be overlooked. Family history remains one of the most powerful predictors of disease, and the collection of such information has been demonstrated to identify patients that would benefit from further follow-up. In the absence of other genetic information, a comprehensive family history can serve as a strong predictor for individual disease risk. A comprehensive family health history is simple and cost-effective, and while newer technologies and integrated electronic health records may quickly generate useful data to guide patient care, family history remains a first-line predictor for familial disease risk. The necessity for healthcare providers to obtain and analyze family history information to provide personalized risk assessments will remain for the foreseeable future.

It is also important to recognize that not all patients may have access to family history information. Genetic evaluation may provide insight regarding genetic risk that is otherwise uncertain due to limited family history information.

Finally, it is necessary to consider possible explanations for negative family history in the context of a proband with a genetic condition. *De novo* rates, incomplete penetrance, and variable expressivity may explain the apparent absence of the disease in the family. Other explanations such as nonpaternity or an incomplete knowledge of the family history may also be factors.

How to apply diagnostic DNA technologies

The approach to characterizing genetic disorders has largely evolved with the advent of new sequencing technologies. Traditional family and twin studies gave way to Sanger sequencing in the 1980s, and more recently next-generation sequencing (NGS) technologies have enabled cost-effective and high-throughput sequencing to be performed on large populations. The current assessment of genetic disorders takes advantage of several key diagnostic DNA technologies, including chromosomal analysis, fluorescence in situ hybridization (FISH), chromosomal microarray, and whole exome/genome sequencing

Clinical cytogenetics and chromosomal analysis

Clinical cytogenetic analysis is useful for the detection of large chromosomal anomalies and copy number variant testing. In a traditional chromosomal analysis, cellular division is arrested and metaphase chromosomes are stained and observed under the microscope. Karyotype analysis is useful to determine whether there are large chromosomal deletions, duplications, or rearrangements, which in adult patients may be helpful for the identification

of somatic mutations in cancer tumor cells and for the confirmation of aneuploidies and evaluation for balanced chromosomal rearrangements in the setting of recurrent spontaneous abortions.

Chromosomal microarray and FISH are alternative cytogenetic technologies that make use of DNA labeling using fluorescent molecules. These technologies are helpful to clarify copy number variants, which are not easily identified with traditional sequencing technologies.

DNA sequencing

Traditional Sanger sequencing technologies relied on the incorporation of fluorescent chain-terminating nucleotides and were limited by cost and throughput. Newer sequencing technologies, termed “next-generation sequencing,” work by fragmenting the genome and sequencing multiple fragments simultaneously to allow for high-throughput and cost-effective sequencing [6]. NGS can take place for the entire genome (whole genome sequencing), or can cover only coding regions (whole exome sequencing), or be targeted towards specific conditions (panel testing).

Choosing the right test

It is important when considering diagnostic testing in a patient presenting phenotype-first, to select a technology that is capable of detecting the genomic variation that causes the condition of interest. All methodologies have limitations; there is no single genomic test capable of detecting every known genomic condition. Likewise, for patients who present genome-first, it is critical to be cognizant of what variation was not interrogated by the methodology to avoid falsely reassuring a patient. Depending on the indication, whole genome/exome, panel testing, or more targeted testing for a pathogenic variant previously identified in the patient’s family may be appropriate.

Understanding the genetic and genomic contribution to disease

Monogenic disease

Historically, genetic discoveries focused on single genetic variants with large and observable phenotypic effects. In medicine, these single gene to disease relationships are described as Mendelian or monogenic. True monogenic diseases are typically rare and are caused by highly penetrant genetic variants (or by one of several genetic variants modulating a given gene). Examples of classical monogenic disorders include sickle cell anemia, cystic fibrosis, and muscular dystrophy. It is notable that single alleles may also impact conditions traditionally thought of as polygenic (monogenic instances of common diseases). For example, coronary artery disease is a traditionally polygenic disease; however, a variety of single, rare genetic variants (e.g., low-density lipoprotein receptor mutations in familial hypercholesterolemia) can independently increase the risk for the disease up to threefold. Another example of a

single genetic variant impacting an otherwise polygenic disease includes *BRCA1* and *BRCA2* variants in breast cancer.

Over the last several decades, thousands of Mendelian disorders have been discovered and catalogued. The OMIM database provides a growing database of monogenic diseases.

Polygenic disease

Many common diseases are polygenic, meaning that they are caused by a large number of common genetic variants, each contributing a small role in the pathogenesis of the disease. Polygenic disease architecture has been described for many common diseases through genome-wide association studies (GWAS), a research technique in which single variant-disease associations are screened across the genome within a large population of individuals with and without the condition of interest. Within the last decade, several large biobanks (collections of biological samples for research purposes) with genetic data have been established, permitting the discovery of many polygenic disease loci for a variety of common diseases including coronary artery disease, heart failure, and diabetes.

In contrast to genetic variants in monogenic disease, a single genetic variant in polygenic disease typically confers only a modest increase in disease risk. Recently, through the development of polygenic risk scores (PRS), it is possible to identify individuals with a risk of polygenic disease equivalent to those seen for rare monogenic mutations. PRS uses summary statistics from large GWASs, and generates a score from several million disease-causing genetic variants. The development of PRSs has successfully enabled the identification of large numbers of individuals with greater than a threefold increased risk for common conditions such as coronary artery disease, atrial fibrillation, type 2 diabetes, inflammatory bowel disease, and breast cancer.

Follow-up and management

In adulthood, the long-term management team for most genetic diagnoses includes a clinical subspecialist and a primary care provider. The inclusion of genetic specialists, such as medical geneticists (physicians) and genetic counselors (nonphysicians), is variable and depends on the condition and the practice setting.

When a diagnosis is initially made it is prudent to engage genetic professionals to confirm that comprehensive phenotyping, laboratory testing, family history, and potential for cascade testing of at-risk family members is appropriately pursued.

A genetic counselor is a nonphysician specialist who has training in medical genetics and counseling and can interpret genetic testing as well as guide individuals regarding the implications of their inherited condition with respect to further genetic testing or inheritance by other family members. A genetic counselor can help patients understand the personal and familial implications of a genetic diagnosis.

Communication between the various members of the healthcare team is essential. By working cooperatively, the genetic professionals and other providers can develop a well-coordinated care plan for the patient.

Points to remember

- Patients may present with a personal or family history of a genetic condition and the evaluation may be phenome-first or genome-first.
- Awareness of current professional society practice guidelines and peer-reviewed literature can inform diagnosis and management.
- Genetic care of the adult patient does not occur in a vacuum. Comorbidities should be taken into consideration and communication between geneticists and other members of the care team is recommended.

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Genetic counseling for adults

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Introduction

Genetic counseling, as defined by the National Society of Genetic Counselors (NSGC), is “the process of helping people understand and adapt to the medical, psychological, and familial implications of genetic contributions to disease. This process integrates the following:

- Interpretation of family and medical histories to assess the chance of disease occurrence or recurrence.
- Education about inheritance, testing, management, prevention, resources, and research.
- Counseling to promote informed choices and adaptation to the risk or condition” [1].

While genetic counselors provide information, support, and sometimes even guidance, respecting patients’ autonomy and helping them arrive at a decision that is consistent with their values and beliefs are key to providing genetic counseling and are emphasized in the NSGC Code of Ethics [2]. The respect for patients’ autonomy extends to prenatal testing and testing of children for adult-onset conditions. For adult-onset conditions where screening in childhood is not indicated, testing should be deferred until the legal age of consent (18 years) to allow the child to exercise their autonomy surrounding whether or not to be tested. This tenet extends to prenatal testing in instances where results will not impact the parents’ decision to continue or terminate a pregnancy [3–6].

Finding healthcare providers with genetics expertise

Clinical geneticists are physicians who have completed a residency or fellowship in genetics. Genetic counselors have a master’s degree from a graduate program in genetic counseling, and the curriculum is based on attaining the practice-based competencies delineated by the

Accreditation Council for Genetic Counseling [7]. Many states now have licensure for genetic counselors. Board certification for clinical geneticists is through the American Board of Medical Genetics and Genomics (ABMGG) and for genetic counselors, the American Board of Genetic Counseling (ABGC). Both the ABMGG and the ABGC have directories at their websites to find the certified providers. The NSGC and the American College of Medical Genetics and Genomics also have online directories of genetics providers and genetics clinics as does the National Cancer Institute. Some nurses have advanced training in genetics and certifications as well; the International Society of Nurses in Genetics has 350+ members. Genetics providers in other countries can be identified through Orphanet, Transnational Alliance for Genetic Counseling, International Federation of Human Genetics Societies, and searches of specific countries' genetics organizations.

Nationally, there are a limited number of genetics providers, and many genetics clinics are based in academic medical centers. This means that patients in less populated and rural areas may be hours away from a genetics clinic. To increase access, some genetics clinics offer satellite clinics periodically in other locations. Few specialized adult genetics clinics exist because there are few clinicians with dual certification in internal medicine and clinical genetics. Therefore, adults are generally seen in other specialty clinics or may be evaluated by a pediatric geneticist in a general genetics clinic or if their child is evaluated for a genetic condition.

Genetic counselors are increasingly working in non-genetics clinic settings including oncology, cardiology, neurology, and ophthalmology. While the traditional genetic counseling model was in-person, this is expanding to include phone genetic counseling and telemedicine to meet increasing patient volumes and geographical challenges. There are also companies, searchable online, which offer phone genetic counseling by certified genetic counselors.

Resources for patients and clinicians

A key component of genetic counseling is providing patients with information about their condition in understandable terms and linking patients to supportive resources. [Table 4.1](#) includes resources for patients and clinicians, and an overview of genetics internet resources for clinicians can be found in Uhlmann and Gutmacher's article (2008) [8].

Principles of genetic counseling in the evaluation of adults

Vignette 4.1

A 30-year-old man is referred by his local neurologist for a medical genetics evaluation to rule out an underlying genetic syndrome. Referral indication specifies generalized dystonia, hypokinesia, and hypotonia. He presents to the evaluation with his parents and older, unaffected sister. During contracting, your patient shares his desire to find an explanation for his ongoing health concerns. The family hopes that the genetics evaluation provides information on

Table 4.1 Genetics resources for patients and clinicians.

Information about genetic conditions	
MedGen	A portal with information about genetic conditions and phenotypes maintained by the National Center for Biotechnology Information (NCBI)
GeneReviews	Peer-reviewed articles about genetic diagnoses with a focus on diagnosis and management
Online Mendelian Inheritance in Man (OMIM)	Provides detailed summaries of genes and phenotypes
Genetics Home Reference	Patient-friendly information about genes and genetic conditions
Genetic and Rare Diseases Information Center (GARD)	Patient-friendly information about rare or genetic diseases in English and Spanish; phone line listed on the website for additional patient information
Information about genetic testing	
Genetic Testing Registry	Resource with information about genetic conditions and a registry of laboratories offering genetic testing and banking
Eurogentest	Provides information for international providers and patients about genetic testing in general and the utility of tests. Information about inheritance patterns and testing is available in several languages
Information about genetic support and advocacy	
Genetic Alliance	A nonprofit advocacy organization with a focus on genetic and genomic research
GINAHELP	Provides information regarding the Genetic Information Nondiscrimination Act
National Organization for Rare Disorders (NORD)	A patient advocacy resource for individuals with rare or genetic conditions
Orphanet	A resource, originating in Europe, that provides information about rare diseases and orphan drugs

prognosis and therapeutic options for his symptoms. The older sister reports that she is considering family planning and would like information to inform her own future reproductive risks.

Contracting

In every clinic visit, contracting can better shape the goals and expectations. Both the clinician and the patient have a role in contracting. The clinician should acknowledge the reason for the referral and elicit from the patient their understanding of the reason for their visit, expectations, and goals for seeking a genetic evaluation. The counselor should provide an overview of the clinic visit and the genetic evaluation process.

Approaches to history taking

Another important element of the genetic evaluation involves obtaining an accurate history on which to base the risk assessment. Explaining the purpose of obtaining the history can help focus the patient to convey relevant information.

Personal history—special considerations in adults

In general, the adult patient will have a more extensive personal medical history than the average child. It can be challenging to keep a patient with a long and complicated medical history focused on the medical complaints relevant to the genetic evaluation. Furthermore, adults may have a wider variety of exposures and lifestyle factors that affect their risk, compared to the pediatric patient.

A particular challenge in obtaining a personal medical history is to assess which features of the history are associated with the underlying genetic etiology and which are unrelated comorbidities. While a genetic condition can affect multiple organ systems, not all medical problems will be the direct result of the diagnosed syndrome; some medical conditions can occur in adults because they are common in the general population. When obtaining the history, it is important to ascertain the results of any prior genetic testing.

Elements of a pedigree

In a genetic evaluation, assessment of family history is of critical value. The decision to obtain a focused versus comprehensive family history will depend on the clinical indication for the visit. Typically, the practitioner should aim to obtain a multiple generation history. Appropriate pedigree standards and nomenclature can be found in Standardized Human Pedigree Nomenclature: Update and Assessment of the Recommendations of the NSGC [9]. Pedigrees should include the patient's first- (children, siblings, parents), second- (aunts/uncles, nieces/nephews, grandparents, grandchildren), and third-degree (first cousins) relatives.

Information to gather regarding each family member should include current age or age at death (cause if known), any diagnoses relevant to the indication for the evaluation or other genetic conditions, and age at the time of diagnosis. Generally, it should be noted on the pedigree if any family member has a congenital anomaly, intellectual disability/developmental delay, a history of sudden death, or a history of pregnancy loss. It is also important to ask about consanguinity and maternal and paternal ancestry, as there are genetic conditions that may be more common in specific ethnic groups.

It is essential to remember that negative history may be as relevant as a positive history in the overall assessment of the patient's risk. Having multiple unaffected family members who are older than the typical age of onset and unaffected may lessen suspicion for a hereditary condition. If the family members are younger than the typical age of onset, their unaffected status would not significantly reduce suspicion for a hereditary condition. Regardless of family history, having a young onset of symptoms is concerning for a genetic condition.

Incomplete family history information

Many adult patients may lack information about prior generations and may not be in contact with extended family members. In some families, discussions about health may be taboo, limiting the patient's awareness of their family history of disease. Depending on condition and inheritance, whether a patient has a positive, negative, or unknown family history will potentially impact risk assessment and can impact decisions about genetic testing and who to test.

Vignette 4.1 (contd.)

During the patient's initial genetics visit, you obtain a four-generation family history. There is no other family history of neurologic or neuromuscular conditions. No family history of birth defects, intellectual disability, or recurrent pregnancy losses. Maternal and paternal ancestry is reported to be Indian/East Asian. Consanguinity is denied. Given the patient is the only family member affected and his early age of onset of symptoms, you suspect autosomal recessive inheritance. Given the wide differential diagnosis, whole-exome sequencing (WES) is recommended as the next step in the patient's evaluation. He gives his informed consent to proceed with testing. Following the completion of insurance prior authorization and testing, the patient returns with his sister. Results from WES are returned with the identification of compound heterozygous TH gene mutations, consistent with a diagnosis of tyrosine hydroxylase deficient dopa-responsive dystonia. Inheritance of the condition is autosomal recessive, consistent with the patient's reported family history. Results are actionable as affected individuals are known to have a complete and sustained response to levodopa treatment. The patient is also found to have a pathogenic variant in a gene associated with Lynch syndrome (hereditary colon cancer syndrome) and he is counseled regarding this diagnosis and informed of screening guidelines for this condition. The patient's sister seeks to test for these two known pathogenic variants and is relieved to find out she is not a carrier for either.

Patient education and counseling

Health literacy has been defined as “the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions” [10]. The level of health literacy varies among individuals and may be affected by their general level of education, language, and culture as well as by a variety of socioeconomic and health/psychological factors. Moreover, genetic information may be complicated, especially given terminology and concepts, and unfamiliar.

It is important for clinicians to assess the patient's understanding and level of familiarity with genetic concepts and to tailor their explanations to the level most appropriate for their patient. Assessing understanding can be done by asking patients directly if they understand the information provided or by less direct methods such as asking a patient how they would explain their condition and inheritance to a family member. The clinician should also watch for visual clues, such as the patient's expressions and body language. It is important not to assume that a patient's nodding and/or lack of questions equals understanding. Assessing understanding should occur throughout the conversation. When possible, the use of visual aids and metaphors can facilitate communication of information. Communication should include use of layman terms and avoidance of unnecessary jargon. Providing patients with links to supportive resources and a copy of the clinic visit note can help patients understand their condition and facilitate sharing relevant information with other healthcare providers and family members.

Helping patients to understand risk, particularly as numerical estimates, can be a challenge. It may be useful to describe risk in multiple ways, such as giving both percentages and fractions. Patients may also benefit from hearing not only the chance that something will occur, but conversely, the chance that it will not occur. For example, there is a 25% chance that your child will be affected; but there is a 75% chance that your child will not be affected. It can also be helpful to compare the patient's risk to the general population risk.

How patients perceive risk is also highly subjective. While two patients may both understand their risk to be 10%, one individual may consider that to be a negligible risk, while the other may believe this is a very high risk. The clinician should discuss with the patient their individual perception of risk to understand the patient's perspective and identify opportunities for education. This can help the clinician and the patient make the most informed decisions regarding testing and future management.

The information imparted to patients will differ based on the indication and individual patient needs, but in general, common topics include a review of the natural history of the disease, basic genetic concepts, inheritance pattern, recurrence risk, genetic testing options, management, and supportive resources.

The natural history information may include information about the comorbidities associated with the diagnosis and age of onset of the symptoms. Signs and symptoms that may occur can also be addressed. Concurrently, informing patients about screening, surveillance, and management options can potentially lead to earlier detection and reduce complications.

When discussing risk, the clinician may provide information regarding the likelihood that a patient has a hereditary condition. The risk may be calculated based on a known or suspected mode(s) of inheritance or be estimated based on the published empiric data. Accurate risk assessment is critical since it can impact provided recurrence risk figures and decisions about genetic testing. If multiple genetic conditions are under consideration, the clinician may explore the possible differential diagnoses with the patient and the rationale for suggesting further testing.

Obtaining informed consent is essential when genetic testing is indicated and in some states even legally required. The risks, benefits, and limitations of testing should be reviewed. The possible results and implications of each type of result should also be discussed. This should include a discussion of the possibility of identifying secondary (incidental) findings or other information unrelated to the evaluation indication (e.g., unexpected pathogenic variants, consanguinity, nonpaternity). Psychosocial and ethical implications of testing, both for the patient and for their family members, should be considered and reviewed with the patient or the legal representative providing consent. These discussions are particularly important to have when a patient is having predictive genetic testing (testing an asymptomatic patient) since results could potentially impact their insurance coverage. The Genetic Information Nondiscrimination Act (GINA) passed in 2008 generally offers protection that extends to most health insurance plans but does not provide protection for life, long-term disability, or long-term care insurance.

The decision to proceed with genetic testing is the patient's choice. Patients will make different testing decisions depending on their condition/risk status, timing/what else they are

handling, personal values, priorities, and finances. Information about testing should be provided in an unbiased manner with an emphasis on patient-centered decision-making and autonomy.

Once a genetic diagnosis is known, genetic counseling should be comprehensive and could include a review of the disease and implications for the patient and family including management, recurrence risk, and cascade testing. Genetic test results can eliminate the need for additional investigation into the etiology of the patient's condition, direct medical and surgical management, and provide support for insurance coverage of the same. A genetic diagnosis can also inform about the potential risk to other family members and patients should be informed about cascade testing, and when appropriate, reproductive options. Cascade testing refers to testing close family members for a known hereditary condition, generally by testing for the pathogenic variant identified in the proband, and then successively testing other more extended family members as additional carriers of the genetic condition are identified.

Provision of genetic counseling may include any or all of the topics discussed above, sometimes repeated at multiple points during the patient–provider relationship as changes in family and medical histories occur and/or as genetic advances are made. A common model includes having pretest and posttest counseling, but sometimes patients may first present after already having genetic testing with another provider. Genetic counseling may also occur for patients in the absence of genetic testing, either because genetic testing is unavailable, available but limited in test sensitivity and/or costly, not indicated, or declined by the patient. Regardless of the duration of the relationship, the goal is to provide information and support with a patient-centered approach.

Unique considerations for genetics evaluation and testing in adults

Pursuance of genetic testing continues to increase as genes are identified for human diseases, more tests are available, and the cost decreases. Insurance coverage, requirements for prior authorizations, and eligibility criteria for testing will vary between specific insurance companies, plans, and even within plans may be test or syndrome specific. Although this is not unique to the medical genetics field, the clinician should be aware of the nuances and special circumstances that arise when coordinating genetic testing for an adult patient. Some genetic testing laboratories will look into whether insurance will cover the cost of genetic testing and/or may have specific out-of-pocket limits for patients.

Any genetic test ordered should be supported with clear documentation of medical necessity and clinical utility. One may argue for coverage of genetic testing to confirm a diagnosis that cannot be established based on the personal history or physical examination alone; specifically, if that confirmation would impact treatment, allow for intervention to prevent future syndrome-specific health complications, or would be used for reproductive management. Genetic testing to assist in the evaluation, risk assessment, or care of other family

members is often not viewed as a valid reason for insurance coverage if it does not impact the care of the individual policy holder. This may ultimately limit the evaluation of an unaffected individual at risk for a familial condition if the affected family member, who is the best candidate for initial testing, does not have the insurance coverage required to identify the causative gene mutation or genetic syndrome in the family. Documentation of medical necessity may also be challenging when testing is considered for a new or rare condition for which there is limited data on the genetic test, the natural history of the condition, or when there are little to no published guidelines for the medical management of that condition.

Many adult genetics patients have undergone previous evaluations in childhood when options for genetic testing were limited or unavailable. Patients presenting for re-evaluation as adults may still face issues with access given policy exclusions for testing of pediatric conditions, even if lifelong management for their condition is required. Newly available tests may also be denied if felt to be “experimental” or “investigational” by an insurer.

Some insurance companies may have policies that require genetic counseling by a board-certified or board-eligible genetic provider prior to authorization of testing.

When insurance coverage is denied, options to explore include self-pay, availability of financial assistance policies, and payment plans. For rare genetic conditions, it may be possible to provide patients with contact information for research studies, clinical trials, or laboratory programs that offer complimentary testing to a patient’s family members.

Testing coordination

Beyond issues of insurance coverage and eligibility, the clinician faces additional challenges in the coordination of genetic testing for the adult patient. A number of genetic conditions result in an intellectual or a physical disability that may require a family member or other court-appointed advocate or durable power of attorney to give consent for evaluation or testing. When possible, assent for testing should be obtained from the patient. Given that results of genetic testing for an affected individual may have implications for the medical care and reproductive decision-making of other relatives, there may be competing motivations for testing among family members and conflicting opinions as to whether genetic information should be obtained and shared between relatives. When obtaining informed consent for genetic testing, the clinician should be aware of these family dynamics to ensure that consent is obtained from the authorized party and that it is being pursued free from coercion.

Adding another degree of complexity, family members may be called upon to assist in the process of interpreting genetic testing and results. Certain testing options, such as whole-exome sequencing, may use samples obtained from parents or other relatives to filter gene variants identified during testing. Samples from other affected relatives may be used to determine whether a specific gene variant tracks with a disease in a family or may be used as positive controls during testing of unaffected family members. The clinician often serves as the point of contact, not only between family members but also when relaying information between the family and testing laboratory. Release of information forms should be obtained as needed in accordance with the Health Insurance Portability and Accountability Act (HIPAA) laws.

Case management for adults

Disclosure of genetic test results can bring a variety of emotions and need for follow-up counseling, both within the clinic and with other licensed mental health practitioners in the patient's community. Patients receiving a positive result and confirmation of a genetic diagnosis may experience anger regarding their disease status, depression, and fear of future health risk, as well as guilt surrounding passing a genetic condition on to children. However, for some families, a positive result brings relief from a long diagnostic odyssey, hope for future screening and treatment, and a sense of control by providing the option of predictive and prenatal testing for other family members. A negative result may be accompanied by relief and reassurance in some families, while for others, it may cause frustration, confusion, survivor guilt, and/or disappointment. The genetics clinician often builds a relationship with families that allows them to anticipate these emotions and to convey results in a manner consistent with a family's needs and expectations. The genetics clinician is often in a unique position to appreciate the patient's diagnostic journey and to provide psychosocial support.

The management of a patient's genetics evaluation may not end with disclosure of results. Referrals to other specialists and additional tests/evaluations may be needed, regular follow-up and identifying resources for patient advocacy and psychosocial support for the patient and family members.

In the event of a positive result, the genetics clinician should also consider the implications of the patient's results to the patient's relatives and may offer guidance and support regarding informing at-risk family members. In some instances, the clinician may even be asked to assist the patient in disclosing these results to other family members. In some clinics, this may involve providing a summary letter of the patient's results, a copy of the progress note, or other written documentation. A genetics clinician may identify resources and genetics clinics local to other family members wishing to establish care for their own evaluations. The initial counseling on inheritance and risk to other relatives should be documented in the medical record, and the provider should ensure that the required release of information forms are signed before any discussions with other family members so that all interactions are compliant with HIPAA laws.

Patients should be advised to re-contact their genetics providers with any changes to their personal or family history, as this may alter the assessment of a patient's risk, considerations for diagnostic testing, and the recommendations for future follow-up. Furthermore, our understanding of the genetic contributions to human disease, as well as technological advances in genetic testing, continues to evolve, and additional genetic testing options may be available in the future. It is also possible that genetic test results, particularly variants of uncertain significance, may be reinterpreted as more is learned.

For some patients, the end of evaluation may coincide with the end of life. This may be a stressful time for a family as they coordinate end-of-life care for their relative, grapple with emotions surrounding the impending loss of a loved one, while also navigating the process of disclosing genetic testing and results. Families with a nondiagnostic evaluation

may wish to consider the option of DNA banking. DNA banking involves the collection and storage of genetic material, often a blood sample, from an affected family member for the use of future genetic testing. Genetic testing is most informative when completed for an affected family member, and thus, DNA banking preserves this option for the future when new genetic tests may be available but an affected relative may not be alive. The process of DNA banking often includes appointing family delegates with authorized future access to the sample and may involve coordination of sample collection prior to shipment to the laboratory designated for future storage. This process may be logistically challenging, and thus, DNA banking should be discussed early in the genetics evaluation, especially when anticipating the end of life.

While clinicians of all specialties can and should educate patients, they can also refer to a genetics provider for additional counseling and education. It has been shown that patients have improved information recall and psychological well-being when they have had genetic counseling [11]. Likewise, collaboration with other specialists is important for the management of patients with genetic conditions.

Points to remember

- Genetic counseling is intended to provide information and support to patients.
- Patients should be provided with information at an appropriate level for their comprehension.
- Pretest and posttest counseling are important for genetic testing.
- A variety of factors affect patient decision-making, and it is important to respect patient autonomy.

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Genetic testing in adults

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Introduction

Understanding of human genetics and its connection to disease has been bolstered by many great discoveries, starting with Gregor Mendel's laws of inheritance in 1865, progressing to the first establishment of mutant proteins causing disease with Linus Pauling's work on sickle cell anemia in 1949, Watson and Crick's discovery of the structure of DNA in 1953, definition of the human chromosome number as 46 by Tjio and Levan in 1956, development of technology to sequence DNA by Fredrick Sanger, Allan Maxam, and Walter Gilbert in 1977, and the completion of the Human Genome Project in 2003. Comprehension of the principles of genetics and establishment of the "normal" human genetic makeup has allowed for the creation of testing modalities to identify variations that cause human disease. The earlier tests could only examine chromosome number, but improvements in cell culturing and chromosome banding techniques later allowed for identification of structural chromosomal abnormalities [1,2]. Advancements in molecular cytogenetic techniques and DNA sequencing carried genetic testing beyond the microscope; as the sequencing techniques have evolved to be faster, larger, and cheaper [the so-called next-generation sequencing (NGS)], sequencing of multiple genes, and even the whole exome or genome, has become more commonplace in the diagnostic genetic evaluation [3]. This has led to rapid discovery of new disease gene associations, and currently over 4100 of the ~20,000 human genes have variants associated with phenotypes [4]. As clinical genetic tests are available for many of these genes, and as multiple and varying testing methodologies exist to assay for different types of genetic alterations, the options in ordering genetic tests are numerous and can often be daunting.

Whereas providing comprehensive information on all of the technologies used for clinical genetic testing and discussing the nuances of interpretation of genetic variants are beyond the scope of this chapter, here, we introduce the basic methodologies, application, utility, and limitations of the commonly used genetic testing modalities. Additionally, we present the future of genetic testing for Mendelian (caused by variants in a single gene) and common disorders (which may be influenced by variants in a larger number of genes, as well as the environment).

Basic principles and practical approach to genetic testing in adults

In an adult patient, genetic testing may be considered for reproductive risks in preconceptional and prenatal settings, for predictive, presymptomatic, or diagnostic purposes, and for therapeutic purposes. The type of genetic testing to be ordered depends not only on the specific condition but also on the type of genetic variants that cause the condition.

Simplistically, genetic changes that can cause human diseases can be grouped into one of the following categories: (1) alterations in the number of chromosomes (e.g., missing or extra chromosomes like 47,XXY in Klinefelter syndrome and 45,X in Turner syndrome), (2) alterations in the structure of chromosomes (e.g., chromosomal translocation in a woman with recurrent miscarriages), (3) copy-number variation (e.g., deletion or extra copies of segments of DNA like a duplication of a small segment of chromosome 17p12 in Charcot–Marie–Tooth disease, type 1A), (4) copy-neutral variation (e.g., inversion of intron 22 in *F8* in hemophilia A), (4) single-nucleotide variants (SNVs; e.g., pathogenic missense variant in *FBN1* in Marfan syndrome), (5) insertion/deletion (indels) of nucleotides (e.g., 185delAG and 5382insC in *BRCA1* in individuals with breast cancer predisposition), (6) nucleotide repeat expansions (e.g., CAG trinucleotide expansion in *SCA1* in spinocerebellar ataxia type 1), (7) mitochondrial DNA (mtDNA) mutations (e.g., m.3243A > G pathogenic variant in the mitochondrial gene *MT-TL1* in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), and (8) changes that alter expression of genes (e.g., methylation of *MLH1* in colon cancer and maternal uniparental disomy of chromosome 14). As expected, no single test can reliably detect all genetic changes that can cause human disease. [Fig. 5.1](#) illustrates some of the types of genetic variations and the genetic testing modalities that can be used to detect these variations.

Genetic testing can be performed on DNA obtained from blood, saliva, skin fibroblast culture, bone marrow, tissue samples, and tumors. The type of specimen used for testing can vary depending on the indication for testing, the type of genetic test, and the preference of the diagnostic laboratory. The most common specimen used for genetic testing is DNA extracted from the white blood cells from peripheral blood. For sequencing tests and molecular tests, most laboratories accept 3–6 mL of blood collected in an ethylenediaminetetraacetic acid tube (purple top). The sample is shipped at room temperature and should arrive in the diagnostic laboratory typically within 3 days of collection. For karyotype analysis, which requires culturing of the leukocytes, blood is typically collected in sodium heparin tubes (green top) and shipped at room temperature so that they arrive in the diagnostic laboratory within 2 days of collection.

Cytogenetic testing

Cytogenetic testing examines the number and structure of chromosomes. While large chromosome abnormalities, including monosomies (an entire missing chromosome) and

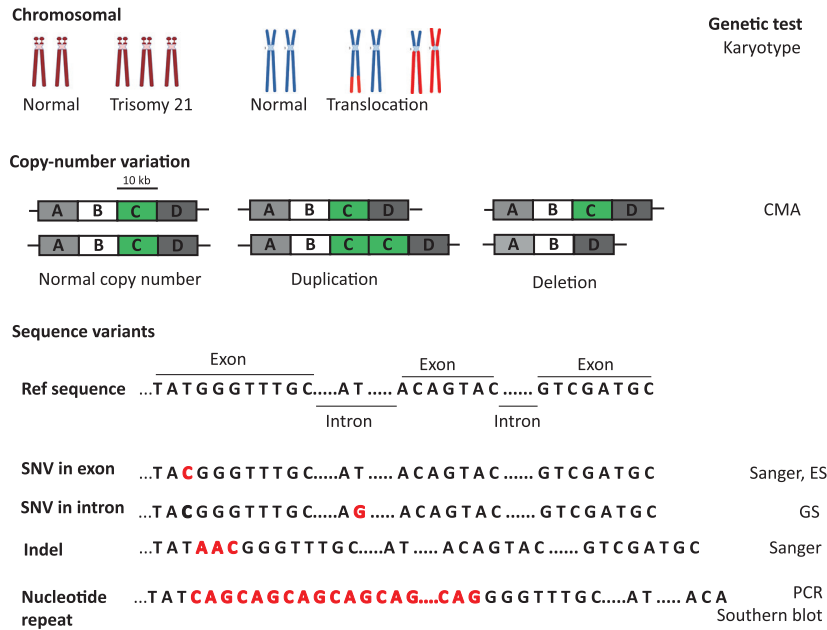


FIGURE 5.1 Examples of genetic variants that can cause human disease and the genetic testing modalities that can be used to detect such variants. CMA - chromosomal microarray analysis; ES - exome sequencing, GS - genome sequencing; PCR - polymerase chain reaction.

trisomies (a third copy of an entire chromosome), may be either nonviable or result in syndromes with multiple congenital anomalies [e.g., trisomy 13, 18, and 21 (Down syndrome)], some sex chromosome abnormalities (e.g., Klinefelter syndrome) and smaller chromosomal imbalances may remain undiagnosed into adulthood. The phenotypes caused by a chromosome abnormality will depend on the gene content of the imbalance and/or disruption of regulation of nearby genes. Testing methodologies for chromosome abnormalities include traditional cytogenetic studies (karyotyping), which can detect large abnormalities visible under the light microscope, including balanced rearrangements, and molecular cytogenetic techniques [fluorescence in situ hybridization (FISH) and chromosomal microarrays (CMAs)], which use locus-specific probes to detect abnormalities that may be too small to be detected by traditional cytogenetics [1,2,5].

Karyotyping

A karyotype is an arrangement of the chromosome pairs, assembled from microscopic images of the cell nucleus. To make the chromosomes identifiable, a patient's cells are cultured in hypotonic solutions, arrested during metaphase when the chromosomes are condensed and visible, and stained with chemicals that differentially mark heterochromatin (tightly wound sections of DNA typically associated with "inactive genes") and euchromatin

(more loosely wound sections of DNA typically associated with “active genes”), resulting in a chromosome-specific banding pattern. Banding patterns help in identification of the chromosomes, in addition to allowing the recognition of any large deletions, duplications, or rearrangements. The resolution of a karyotype will depend on the banding level achieved, with higher banding levels showing longer, more spread out chromosomes that allow for the detection of smaller chromosome abnormalities. An average karyotype from a blood sample will typically allow detection of abnormalities ~5–10 megabases (Mb, million DNA base pairs) in size. The resolution achievable will also depend on the sample type; some cells are not amenable to techniques used to increase resolution, such as many cancer and tumor samples [1,2]. While karyotyping is commonly performed on blood because of its accessibility, there may be reasons to perform karyotyping on other tissue types, such as skin fibroblasts. If a patient has undergone a blood transfusion in the past 30 days or bone marrow transplant, a sample other than blood is needed to test that person’s constitutional chromosomal makeup. Additionally, if there is suspicion for mosaicism (meaning some cells carry a genetic abnormality, while other cells are normal), another sample type other than blood, particularly a sample from an affected area, if possible (e.g., an area of skin with a different pigmentation or a biopsy from an affected organ), should be tested. Of note, such sample type considerations are not unique to cytogenetic techniques; they are relevant for all types of genetic testing. While buccal swabs provide another accessible sample type, they do not provide the dividing cells necessary for karyotyping; buccal samples may therefore only be used for molecular assays [5].

Indications for karyotyping include multiple congenital anomalies, dysmorphic features, and/or intellectual disability/autism spectrum disorders [6]. Signs that may indicate a sex chromosome abnormality, such as infertility, gynecomastia, or amenorrhea, are also indications [2]. As karyotyping can detect balanced rearrangements, which can lead to infertility and/or multiple miscarriages, traditional chromosome analysis is recommended in individuals with these symptoms, as molecular techniques such as CMA testing cannot detect balanced rearrangements [7].

Fluorescence in situ hybridization

FISH is a molecular cytogenetic technique that uses probes specific to a chromosomal region to test for its copy number and chromosomal location. Fluorescent probes are applied to metaphase or interphase chromosomes, and then cells are examined under a microscope to evaluate the number and location of probes that have adhered to the chromosomes. This can allow for the detection of deletions or duplications that may be too small to see on karyotype. If the test is performed on metaphase chromosomes, it can detect the chromosomal location of the signals, thus also allowing identification of rearrangements. This test is objective, as opposed to karyotyping, in which identification of subtle chromosomal abnormalities may be more subjective. Also, unlike karyotyping, which can be performed even without a suspicion of a locus-specific diagnosis, FISH probes need to be selected in advance, making this a targeted test. For example, 22q11.21 FISH (for DiGeorge syndrome or the reciprocal

microduplication syndrome) or 7q11.23 FISH (for Williams syndrome or the reciprocal microduplication syndrome) may be requested. FISH panels, such as subtelomeric FISH, which employ a larger number of probes in one test, have also been used in some instances for less specific phenotypes, as submicroscopic deletions and duplications of the ends of the chromosomes (called the telomeres) may result in these types of clinical presentations; however, such testing has largely been replaced by CMA testing, which is a molecular cytogenetic test on a genome-wide scale [1,2,5].

Chromosomal microarray

CMA combines the objectivity and increased resolution of a molecular assay with a genome-wide approach that negates the pretest requirement for a suspected diagnosis. The technology uses thousands to millions of locus-specific probes in one assay to assess for missing or extra segments of DNA [deletions or duplications, also called copy number variants (CNVs)]. While karyotype standardly can detect abnormalities $\sim 5\text{--}10$ Mb in size, and FISH can detect submicroscopic abnormalities of $\sim 50\text{--}250$ kilobases (kb, 1000 DNA base pairs), the resolution of CMA may be as small as just a few kb. The resolution of a specific CMA platform will depend on the number and chromosomal location of the probes used; probes derived from closely spaced genomic loci can detect smaller CNVs than probes from more distantly spaced loci [2,5]. While some early CMA platforms only used probes at specific, targeted chromosomal locations, most currently used CMA platforms are genome-wide. Currently, some arrays use probes from equally spaced loci across the genome, but most clinical arrays have probes concentrated in clinically relevant genes and loci, with “backbone” (less dense) coverage throughout the rest of the genome. This allows for detection of smaller CNVs within disease-associated regions [8].

There are two types of CMAs: microarray-based comparative genomic hybridization (aCGH) and single-nucleotide polymorphism (SNP)-based microarrays. With aCGH, DNA isolated from a test (patient) sample and a sex-matched reference sample are labeled with different fluorescent dyes, mixed together in equal amounts, and hybridized to the array. Alterations in the ratio of the two fluorescent dyes indicate a different quantity of DNA in the test sample as compared to the control, and therefore these alterations correspond to locations of CNVs. SNP arrays use probes containing polymorphic nucleotides. A reference sample is not used in the experiment; computational algorithms translate signal intensity into information on copy number, as well as SNP genotype(s) present in the sample. Because SNP-based arrays provide data on genotypes as well as DNA copy number, they may detect certain DNA copy number-neutral abnormalities. Stretches where there is absence of heterozygosity (AOH) can indicate consanguineous parents (with AOH spread throughout the genome, making recessive conditions more likely to occur), or segments of uniparental isodisomy (when two copies of one chromosome come from one parent and none from the other parent), which can be disease-causing if an imprinted chromosome is involved or if a recessive disease allele is present within the segment of AOH [2,5].

Given its genome-wide approach, CMA is a useful test when clinical features may be nonspecific and the differential diagnosis is broad. CMA testing is recommended as a first-tier test for individuals with developmental delay, intellectual disability, autism spectrum disorders, or multiple congenital anomalies [8,9]. It is important to remember that CMA testing cannot detect balanced karyotypic abnormalities, such as reciprocal translocations, which could be of clinical significance if they disrupt a critical gene. However, many apparently balanced rearrangements identified on karyotype turn out to have sub-microscopic imbalances at the breakpoints, so at least a subset of such rearrangements may be identified using molecular techniques [5]. However, if there is suspicion for a balanced chromosomal rearrangement (e.g., due to a history of recurrent pregnancy loss), traditional karyotyping is clinically indicated [7]. CMA is also a test that may need to be repeated for a specific patient, based on the test resolution; if a previous CMA was on a lower resolution platform, rerunning the test using a higher resolution platform may identify a small, clinically relevant CNV, and the addition of SNP analysis may allow identification of AOH.

Sequencing tests

Single-gene testing

One of the first major breakthroughs in DNA sequencing technology was the development of dideoxy sequencing by Sanger in 1977; a version of this methodology is still used today and is called Sanger sequencing. This technology uses labeled dideoxynucleotides, which can be incorporated into a growing strand of DNA but lack the 3' hydroxyl group needed for the attachment of the next nucleotide, thus stopping the elongation. Use of electrophoresis allows for the detection of the label and length of each terminated DNA fragment, providing a readout of the sequence of the template DNA fragment under study. Sanger sequencing is highly accurate and therefore used in the setting of clinical genetic testing [5]. Single-gene tests frequently use Sanger sequencing. Indications for single-gene testing include confirmation of a clinical diagnosis where all or a majority of cases are caused by variants in one gene (e.g., cystic fibrosis and *CFTR* or neurofibromatosis type 1 and *NF1*). It is important to note that Sanger sequencing will only detect alterations in the sequence of the DNA; larger deletions or duplications of parts or entire copies of the gene (larger than ~25–60 base pairs) can be missed by this technology [5]. Therefore, if deletions or duplications may be a possible cause of the condition under consideration, it may be worthwhile to seek testing that not only uses sequencing technologies but also performs a deletion and duplication analysis for a higher detection rate.

Panel testing

Further revolutions in genetic testing technology have come with the development of massively parallel sequencing (MPS), also known as NGS. MPS allows for sequencing millions to

billions of DNA fragments at the same time, thus greatly increasing the amount of sequencing information that can be generated while reducing the cost of testing. Testing laboratories have taken advantage of NGS to develop an increasing number of gene panels, containing more genes than may have been previously offered using only Sanger sequencing. NGS gene panels start with a “capture” step, where the genes of interest are first amplified from the patient sample, and then that amplified DNA is sequenced [3,5].

Genetic testing panels are focused on groups of genes that cause related phenotypes. For example, testing may cover a panel of genes that cause cardiomyopathy, neuropathy, epilepsy, or cancer predisposition. Therefore, panel testing is useful for patients with diseases falling into a specific category, where this disease category may be caused by any of a number of genes. Depending on the phenotype, the genetic testing panel may have a few to a few hundred genes. Advantages of using panel testing come from its phenotype-focused nature, which can simplify interpretation of variants identified, as opposed to the broader exome sequencing (ES), which may identify pathogenic genetic changes unrelated to the disease category. Smaller gene panels may also allow more complete and in-depth sequencing of the targeted genes than large gene panels or ES, thus decreasing the chances of false negative results. When using genetic testing panels, it is important to consider the possibility of variants that cannot be detected reliably by NGS (including deletions or duplications larger than ~8–20 base pairs or repeat expansions) [3,5]. In situations where such types of variants may be present, testing laboratories typically include supplemental assays to detect such changes, but this is important to verify when selecting a gene panel.

Exome sequencing

Development of NGS has also brought ES to the forefront of clinical genetic testing. Similar to the use of NGS in gene panels, ES starts with a “capture” step of amplification of genes, although in this case all ~20,000 human protein-coding genes are amplified, followed by sequencing. However, the “capture” step is not perfect, so ES cannot provide 100% coverage of the entire exome. Even if the total amount of sequencing is increased (at increased cost), certain poorly captured regions of the exome still will not be sequenced. Typically, ES sufficiently covers >90% of the exome; specific coverage metrics are typically provided by the testing laboratory, either in their educational materials or upon request. Also, certain types of genetic changes, such as large deletions or duplications and triplet repeat expansions, are best detected by other testing modalities. As ES results in a large amount of data, the majority of effort in completion of the testing goes into bioinformatics and personnel required to analyze and interpret the data. This also increases the turnaround time and cost of testing as compared to gene panels [3,5].

Despite some of its limitations, ES has proven to be a powerful diagnostic tool, providing diagnoses for ~17.5% of adults referred for testing across all indications at one diagnostic center. A trio-based approach (concurrent ES of a patient along with both parents) can simplify interpretation (allowing identification of *de novo* and compound heterozygous variants), decreasing turnaround time and providing a slightly increased diagnostic yield [10]. ES is a

useful test for patients in whom a genetic etiology is suspected but do not clearly fall into a diagnostic category associated with a gene panel. Additionally, some symptoms, such as intellectual disability or autism spectrum disorder, are not specific enough to provide for a manageable gene panel, making ES a better genetic test. ES does offer some advantages over gene panels, as ES may provide coverage of newly discovered disease genes that are not yet included on panels. It also provides the opportunity for disease gene discovery or diagnosis in the future, as the testing generates exome-wide data that can be reanalyzed at a later time, at which point a diagnosis could be made based on new publications [3,5,11].

Because ES is a genome-wide testing technology, pathogenic variants in multiple genes that could each explain a part of the clinical phenotype or in genes associated with other human diseases unrelated to the patient's phenotype may be uncovered. The possibility of these so-called dual diagnoses and incidental findings should be discussed with patients prior to sequencing. In some cases, individuals can opt out of receiving such results. The American College of Medical Genetics and Genomics (ACMG) provides a list of genes that should be examined for pathogenic variants; the list contains genes primarily associated with cancer predisposition, cardiomyopathies, and aortopathies. These genes have been selected for reporting due to the ability to take actions to either screen for or prevent associated complications. The ACMG estimates that ~1% of individuals undergoing ES will have one of these medically actionable incidental findings [12].

Other tests

Certain types of genetic variants require specialized testing technologies for detection. Nucleotide repeat expansions, which cause conditions including many ataxias (e.g., spinocerebellar ataxias and Friedreich ataxia), neurodegenerative disorders (e.g., Huntington disease, *C9orf72*-related amyotrophic lateral sclerosis and frontotemporal dementia, and fragile X-associated tremor/ataxia syndrome), and myopathies (e.g., myotonic dystrophy type 1), are not detectable by ES [13]. The diagnosis of such nucleotide repeat disorders requires specialized tests employing polymerase chain reactions or Southern blotting to detect the abnormally expanded alleles. Another specialized case is *SMN1*, the gene associated with spinal muscular atrophy, where gene deletion is the major cause of disease, but due to the existence of another, almost identical gene nearby, CMA cannot detect the deletion, and the gene cannot be captured and accurately sequenced by ES. Other disease-causing abnormalities, such as disorders involving imprinted chromosomes (caused by uniparental disomy or alterations in the methylation patterns of the chromosome), may not be associated with any DNA sequence-level changes, and therefore methylation-based studies are indicated to achieve maximum diagnostic utility [1,2].

Testing of the mitochondrial genome may also require special consideration. While NGS can be used to sequence mtDNA, only some ES tests will include mtDNA in the capture. It is therefore important to understand the coverage by ES if mitochondrial disease caused by mutations in the mitochondrial genome is a diagnostic consideration. NGS of mtDNA may

be ordered as a stand-alone test to identify sequence variants and deletions/duplications; additional quantitative testing for mtDNA depletion may also be indicated. Given that heteroplasmy (a mixture of normal and variant mtDNA within an individual) is common, and the mutation load (the level at which a variant is present) can vary across tissue types and determine if that tissue is affected, sample type for mitochondrial testing is another important consideration. A pathogenic variant may be absent or present at a low level in blood, while it is present at higher, pathogenic levels in affected tissue, such as muscle, gut, or brain. While the affected tissue type is not always available for testing, testing of an alternative sample type, such as muscle or skin biopsy, may help increase the likelihood of detecting a pathogenic variant [14]. As with any type of testing, the decision to pursue testing on a secondary tissue type should be governed by the degree of clinical suspicion of a related diagnosis.

A resource such as GeneReviews (<https://www.ncbi.nlm.nih.gov/books/NBK1116/>) may be consulted to understand the frequencies in which different types of genetic alterations cause a specific syndrome and the corresponding yield of different testing technologies. In some cases where multiple types of genetic changes may cause a given condition, multiple testing modalities may be indicated to rule out a diagnosis. However, testing strategy may be tiered in some cases, with the initial test performed for identification of the most common cause(s) of the condition, followed by lower yield testing technologies if clinical suspicion of the condition is high. Table 5.1 provides a summary of the utility and limitations of the various genetic testing modalities.

Variant interpretation

Similar to other types of laboratory tests, genetic testing results do not always have obvious clinical interpretations. The variants detected on genetic testing are classified as benign, likely benign, variants of uncertain significance (VUS), likely pathogenic, and pathogenic. The chance of finding a VUS increases as the amount of DNA studied increases. Genome-wide testing technologies such as CMA and ES uncover a lot of variation (hundreds to thousands of variants in the case of ES), and the vast majority of changes compared to reference sequence is benign or likely benign and reflects background genetic variation. In patients undergoing clinical genetic testing, however, the prior probability that they carry a disease-causing variant can be high, and identification of a disease-causing variant among rare polymorphisms can be difficult. The ACMG has developed guidelines for the interpretation and reporting of sequence variants, and frequently, multiple lines of evidence are required to allow classification of a variant as any category other than uncertain. Certain lines of evidence (e.g., loss-of-function variants in genes where loss of function is known to cause disease, the previous finding of the same variant in an affected individual, and well-established functional studies) are weighed more heavily than other evidence (e.g., computer predictions of variant pathogenicity and similarity between patient phenotype and the disease caused by the gene). Population databases that catalog variants in a large number of individuals from the general population

Table 5.1 Methodologies available for genetic testing.

Methodology		Variations detected	Limitations	Example(s) of disorders that can be detected
Cytogenetic testing	Karyotype	Chromosomal aneuploidy, large chromosomal deletions, duplications, and rearrangements	Cannot detect small deletions and duplications	Trisomy 21
	Chromosomal microarray	Chromosomal aneuploidy, and CNVs including small chromosomal deletions, duplications, and complex genomic rearrangements	Cannot detect SNVs, balanced translocations, and inversions	22q11.2 deletion syndrome, structural variants associated with autism
	Fluorescence in situ hybridization	Targeted CNVs	Cannot detect all aneuploidies, deletions, duplications	22q11.2 deletion
DNA sequencing	Sanger sequencing (usually offered for a single gene)	SNVs, frameshift alterations in gene(s) of interest (up to ~25-60 bp)	Cannot detect chromosomal variation, deletions, duplications	Specific Mendelian disorders
	Next-generation sequencing-based panel tests	SNVs, frameshift alterations in gene(s) of interest (up to ~8 to 20 bp)	Cannot detect chromosomal variation, CNVs may be detected based on read outs of genes	Mendelian disorders with specific phenotypes like “hypertrophic cardiomyopathy”
	Exome sequencing	SNVs in coding sequence	Typically, cannot detect chromosomal variation, deletions, duplications, or noncoding variants	Any Mendelian disorders
	Genome sequencing	SNVs in coding and noncoding sequence, CNVs	More expensive than exome sequencing and limited availability	Any Mendelian disorder
	DNA genotyping microarray	Targeted assessment of specific variations in the genome	Data do not include many important rare variants nor any novel variants	Polygenic risk scoring and pharmacogenomic testing
Others	Polymerase chain reaction and Southern blots	Repeat expansions		Repeat expansion disorders like myotonic dystrophy

bp, basepairs; *CNV*, copy number variant; *SNV*, single-nucleotide variant.

(such as the Exome Aggregation Consortium and the related Genome Aggregation Database) have provided another useful tool to determine if variants may be present in individuals in the general population and therefore less likely to cause disease [15].

For a specific patient with a VUS identified, results of familial studies may be considered to aid in interpretation [16]. When parental samples are supplied at the time of ES, variant segregation within a family may be incorporated in the variant interpretation algorithm. Upon receipt of results, testing of additional family members (e.g., healthy siblings in the case of compound heterozygous VUS, maternal male relatives in the case of X-linked VUS in men, or additional affected family members if the phenotype is present in multiple individuals) may provide additional evidence for or against pathogenicity. It is important to remember that while *de novo* (new in the proband) variants are more likely to be pathogenic, and inherited variants (in the case of a dominant disease model) are more likely to be benign, this is not a hard-and-fast rule. This has been well demonstrated in the case of some CNVs that are considered to be risk factors for the development of disease. These CNVs can show reduced penetrance; they may be present in a healthy parent. However, population-based studies have clearly shown that these CNVs are enriched in disease populations, confirming their pathogenicity. Additional factors, both environmental and genetic, likely influence the manifestation of symptoms in individuals carrying such risk factors [17]. As ES and genome sequencing (GS) continue to be used in more research and clinical settings, some of these additional genetic factors influencing disease severity may be identified, but such findings will likely remain cryptic in many cases. The future of genetic sequencing may also hold discovery of SNVs similar to the risk factor CNVs—variants that increase the likelihood of development of a certain phenotype but will not cause disease in all carriers. Such examples are already known, including the *APOE4* allele and its association with Alzheimer disease [18].

Genome sequencing

The protein-coding portion of the human genome (the “exome”) is only ~1.5% of the total DNA sequence. Variants outside of the exome, including deep intronic variants and variants in untranslated and regulatory regions of genes, may alter gene splicing or expression patterns and cause disease. Partly because of this possibility, GS is now being offered as a clinical genetic test. Unlike gene panels and ES, this NGS test does not require a “capture” step; genomic DNA is isolated from a patient sample and directly sequenced. This provides some advantages, including more even coverage of the genome; portions of genes that fail capture in ES may be covered by GS [3]. If specialized bioinformatics tools are employed, GS permits detection of types of variants other than sequence variants, such as CNVs and chromosomal rearrangements (through the identification of junction of chromosomal segments that normally do not adjoin), as well as nucleotide repeat expansions. Due to these additional functionalities, the future of genetic testing likely lies in GS, as a single test can take the place of multiple testing methodologies with minimal sacrifice of detection rate. However, there are

still limitations to GS. There is typically an increased cost, requiring more total sequencing and more data storage and processing. Despite the additional sequencing, a given locus is likely to have less coverage (number of times a section of DNA is sequenced in a given test), which makes the test less sensitive to detection of mosaic variants (variants not present in every cell of an individual). Interpretation of variants outside of the coding regions remains difficult, as it is hard to predict what type of impact these variants may have. In the future, additional technologies such as RNA sequencing may be used in conjunction with a test like GS, as it can provide a functional readout of how splicing or expression may or may not be altered in a gene with a noncoding VUS.

Direct-to-consumer testing

Direct-to-consumer (DTC) testing, which allows individuals to have access to some form of information about their genetic make-up, has been available since 1996. Previously, DTC “genetic testing” results typically contained information about one’s ancestry and information about SNVs that were associated with risk for many common disorders. In 2013 the United States Food and Drug Administration (FDA) regulated the marketing and genomic information that could be reported from such tests. Currently, the FDA allows DTC companies to report on certain genetic information. One such DTC provider reports on risk for a limited number of diseases, carrier status for certain recessive disorders, three common *BRCA1* and *BRCA2* mutations found in individuals of Ashkenazi Jewish heritage, and two particular mutations in *MUTYH*. Most recently, diagnostic laboratories that are accredited by Clinical Laboratory Improvement Amendments and College of American Pathologists are considering offering DTC genetic testing for a range of disorders in an effort to make such testing available to individuals who may not have access to clinical geneticists or genetic counseling services.

Clinical vignettes

Vignette 5.1

A young couple undergoes evaluation for infertility. The husband, who is 26 years of age, is much taller than the expected mid-parental height, has minimal facial hair, and has gynecomastia. Semen analysis in this gentleman reveals azoospermia. Which would be an appropriate genetic test in this man?

The phenotype in this gentleman is classical for Klinefelter syndrome, a chromosomal disorder caused by the presence of an extra X chromosome in a man (47,XXY). As the genetic abnormality is the presence of an entire X chromosome, karyotyping would be the first test of choice. Note that if one had ordered a CMA (though not typical practice), this testing would have also shown increase in the copy number of multiple probes on the entire X chromosome and thus

would have also diagnosed the condition. However, a genome-wide sequencing technique like ES would not have picked up this particular genetic change.

Vignette 5.2

A 30-year-old man who is 6 ft and 6 in. tall and has skeletal abnormalities including scoliosis, pectus excavatum, hypermobility, and arachnodactyly comes to the clinic for genetic evaluation. He states that he had to undergo replacement of his proximal aorta due to a dissection of the ascending aorta. He states that his father, his paternal aunt, and a cousin through this aunt have also had aortic dissections. He also shows a genetic testing report, which states that sequencing of FBN1 was performed with no pathogenic variants identified. What testing strategies would be the next step in the diagnosis?

This individual presents with a heritable form of connective tissue disorder that presents with aortopathy of which Marfan syndrome and Loeys–Dietz syndrome are classic examples. The heritable aortopathies can be caused by mutation in many but a small number of genes. He has only had sequencing of FBN1, which was normal. One possibility is that there is a deletion of a part or all of FBN1. One approach would be to use a test that would detect deletions or duplications involving FBN1, such as CMA or targeted deletion/duplication testing [e.g., using multiplex ligation-dependent probe amplification]. If such testing is negative, then testing for all of the heritable aortopathy disorders with a gene panel focused on such disorders would be the next step. Alternatively, one could consider ordering the gene panel test as the first-line test. Most of the gene panel tests also complement their sequencing with assays for deletions and duplications involving the target genes. If the gene panel testing is unrevealing, given that there are four affected family members, testing with ES or GS may reveal a novel genetic cause for the phenotype in the family.

Vignette 5.3

A 20-year-old man with intellectual disability, scoliosis, ventricular septal defect, and renal anomalies is referred for genetic evaluation. What diagnostic strategies would be appropriate for evaluation?

The multiple organ systems involved in this individual point toward a genetic etiology; however, many of the clinical features are nonspecific and thus the differential remains broad. A genome-wide approach for testing would be most appropriate. The testing strategy should include CMA and ES, and GS if the former two are unrevealing. Note that a “panel-based” testing would not be appropriate because the phenotype is not restricted to one or a few specific conditions.

Vignette 5.4

A 70-year-old man presents with ataxia and tremor that have been ongoing for 3 years. He has a daughter who is 35 years old and is in good health; however, her son, who is 5 years of

age, has some dysmorphic features including long face, prominent forehead, and large ears, and significant developmental delay. What testing methodology is most likely to detect the genetic change in this family?

This family history is suggestive of FMR1-related disorder. The child likely has fragile X syndrome due to a CGG trinucleotide expansion to a “full mutation” length in FMR1. The mother and grandfather of this child likely have a shorter “premutation” expansion, and the grandfather likely has fragile X-associated tremor/ataxia syndrome. Neither sequencing of FMR1, CMA, nor ES would be able to detect the trinucleotide repeat and thus would be nondiagnostic. The appropriate test to order would be a polymerase chain reaction/Southern blot to assess for the repeat size in the FMR1 gene.

Points to remember

- Genetic testing has become faster, simpler, and cheaper with advancing technologies.
- Genetic testing can either be targeted or performed on a genome-wide scale.
- Healthcare providers should be cognizant of the advantages and limitations of each methodology prior to ordering genetic testing.

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Transitioning to adult care

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Introduction

Healthcare transition is defined as the intentional, planned process of transferring care from a pediatric- to adult-based healthcare setting. There are nearly five million youth with special healthcare needs aged 12–17 years who will soon transition to adult health care [1], though it is difficult to quantify the proportion of these individuals that have a primary genetic diagnosis. Despite consensus from multiple medical societies that transition is a healthcare priority, nationwide survey data indicate that the vast majority of transitioning youth do not receive the transition preparation and support that they need [1]. Numerous barriers hinder successful transitions that exist at the level of the patient, medical provider, and medical system. These barriers, combined with the developmental challenges normally faced by adolescents and young adults make this a high-risk period that is often associated with negative health outcomes [1,2].

It is important for medical providers to be knowledgeable about the issues faced in transitioning youth with special healthcare needs to the adult healthcare setting. While there will be some discussion of medical issues associated with specific genetic diagnoses, this chapter will primarily focus on important general considerations that are applicable to most transitioning individuals who have chronic disease and/or disability [3]. Please see Table 6.1 for resources on how to find additional information about specific genetic diagnoses.

Vignette 6.1

AA, a 19-year-old girl with Noonan syndrome, comes to a family practice clinic to establish care. She is accompanied by her mother. She has a known history of short stature, bicuspid aortic valve, and pulmonary valvular stenosis requiring stenting in childhood. She was followed by pediatric cardiology until she became pregnant at age 17, and now has an 18-month-old girl who also has a congenital heart defect. AA is 142 cm tall and weighs 49 kg [body mass index (BMI) 24.2kg/m²]; she did not receive growth hormone. She also has a

Table 6.1 Additional information and support for specific genetic conditions.

Resource	Description	Location
GeneReviews	Peer-reviewed information on diagnosis, management, and genetic counseling for many genetic conditions	https://www.ncbi.nlm.nih.gov/books/NBK1116/
NIH Genetics Home Reference	Patient-facing information on genetics and genetic conditions	https://ghr.nlm.nih.gov/
Genetic Alliance	Advocacy organization seeking improved healthcare delivery, education, and community engagement for all genetic diagnoses	http://geneticalliance.org/
Genetic and Rare Diseases Information Center	Information for patients and medical professionals on rare (including genetic) conditions	https://rarediseases.info.nih.gov/
Online Mendelian Inheritance in Man	Comprehensive database of all known mendelian disorders intended for scientists and medical professionals	https://www.omim.org/

Examples of existing resources that provide information and treatment considerations for specific genetic diagnoses, as well as support and advocacy for patients and families. Consensus-based care recommendations for adults with genetic conditions are often available through disease-specific advocacy groups.

history of learning difficulties in school but obtained her high school general education diploma. Her primary concern at the visit is her daughter, whose cardiologist has requested genetic testing and follow-up. She has become very anxious about her own health and that of her daughter.

Noonan syndrome is a recognizable genetic condition known to cause short stature, congenital heart defects, and distinct skeletal and craniofacial features. It is among the most common genetic disorders with an estimated incidence of one in 1000–2500 individuals. While most adults with Noonan syndrome have a normal cognitive function, learning and behavioral issues are prevalent, and up to one-third of adults with Noonan syndrome have a mild intellectual disability [4]. AA has several health issues associated with Noonan syndrome that require multidisciplinary evaluation and care transition, including congenital heart disease, increased bleeding tendency, and reproductive recurrence risk. In addition to these considerations, she expresses worsening anxiety. The following section will focus on psychosocial elements to consider in the transition from pediatric- to adult-based health care for individuals with a genetic diagnosis.

Psychosocial considerations

Independent of chronic medical conditions, adolescence and young adulthood can be an incredibly challenging developmental period. A thoughtful, systematic approach to taking a psychosocial history is important to identify the barriers faced by these individuals that may hinder optimal self-care. While there are several standardized approaches to collecting the history, most include elements of a person’s living situation, social support structure,

interests and vocations, screening for high-risk behaviors, and evaluation for depression and other mental illness. One such standardized approach is the HEADSSS exam (*H*ome environment, *E*ducation and employment, *E*ating, *A*ctivities, *D*rugs, *S*exuality, *S*uicide/depression, and *S*afety) [5]. When the patient is accompanied by a guardian or caregiver, it is also important to give the patient an opportunity to speak with the physician independently to foster autonomy and allow the individual to discuss sensitive issues that may otherwise be overlooked. An independent exam may not be possible if there are severe cognitive, communicative, and/or behavioral challenges.

An individual's social support structure may include family, friends, classmates, colleagues, and caregivers. In addition to these typical supports, many individuals belong to groups for their particular genetic diagnosis, made easier by the rise of social media. These groups can provide a community of peers that is often lacking for those with a rare diagnosis, though privacy is often a concern depending on the platform [6]. Understanding an individual's support system can be an important factor in effective care delivery depending on an individual's medical diagnoses and level of independence. Social support is also a known protective factor for mental health, which is particularly important given higher rates of depressive symptoms for individuals with chronic illness that is also more likely to worsen during the transition period [7,8]. Additionally, having a chronic illness or condition, particularly one with readily visible features, is associated with higher rates of being bullied [9]. This context underscores the need for consistent screening for depression as recommended in the general adult population by the US Preventive Services Task Force [10].

Another common feature of adolescence and young adulthood is experimentation with potentially high-risk behaviors, such as drugs, alcohol, and certain sexual practices. One study suggests that adolescents with chronic conditions are more likely to perform certain risky behaviors [11], highlighting the need to discuss these issues within the medical encounter. There may be additional considerations when interviewing and counseling the transitioning patient with a genetic diagnosis. In regard to drug and alcohol use, patients should be counseled on the potential interactions these substances may have with medications, such as interfering with the metabolism of anti-seizure medications or having an additive effect with benzodiazepines or baclofen (e.g., used for spasticity management). Certain substances can also have amplified risk depending on a patient's underlying diagnoses. Examples include a higher risk of subsequent lung disease for persons with cystic fibrosis using inhaled drugs (vaping, cigarettes, etc.) or an increased risk for breakthrough seizures when someone with a seizure disorder consumes alcohol.

Having frank discussions around sexual health is important for all patients, regardless of diagnosis or ability. The position statement on sexuality from The Arc (www.thearc.org), a national advocacy group for individuals with intellectual and developmental disabilities, emphasizes the inherent right of all people to “exercise choices regarding sexual expression and social relationships” [12]. In practice, these discussions often include education on sexual health, appropriate behaviors, consent, and potentially the desire to become a parent. Regardless of one's desire to rear a child, questions may arise regarding fertility and the likelihood of passing on a particular genetic diagnosis. For more information on this topic,

please see Chapter 7, Preconception and Prenatal care. It is also important for providers to consider and discuss related safety issues, as available data demonstrates that young people with a disability or serious chronic condition are at significantly higher risk of sexual, physical, and emotional abuse [13].

Vignette 6.2

GB, a 20-year-old man with Down syndrome, comes to the clinic for his first adult-based healthcare appointment. He has a known history of hypothyroidism for which he takes daily levothyroxine, as well as a history of atrial septal defect that spontaneously closed in childhood. His BMI today is 31kg/m², up from 29 a year ago. GB's only concern is that over the last 6 months, he has felt sleepier during the day and takes naps more often. He is here with his parents, who live with GB and provide occasional assistance with his activities of daily living and completely manage his finances and other affairs. GB receives Supplemental Security Income (SSI) and is on a managed Medicaid plan. He is currently taking life skills courses at his high school and is interested in finding work when he graduates next year.

Down syndrome is one of the most common occurring chromosomal disorders, with life expectancy now reaching nearly 60 years. GB has several medical issues commonly seen with Down syndrome, including a history of congenital heart disease and hypothyroidism. He is also reporting symptoms that suggest undiagnosed obstructive sleep apnea. The prevalence of sleep apnea in adults with Down syndrome is at least 80%, though typical self-reported sleep measures may miss many individuals with the disease [14]. His BMI falls into class one obesity. Although the prevalence of overweight and obesity is much higher in individuals with Down syndrome, related metabolic syndrome and atherosclerotic disease is disproportionately lower [15]. Regardless, a healthy diet and lifestyle counseling would be appropriate to discuss. For additional medical considerations, please refer to available medical guidelines for adults with Down syndrome [16].

This vignette brings up several other domains to consider during the transition process, including employment and education, health insurance, and medical decision-making. Where appropriate, considerations for individuals with intellectual disabilities will be addressed, but please refer to Chapter 16 for additional information on intellectual and developmental disabilities.

Education and employment

Understanding an individual's goals and aspirations upon finishing secondary school is an important element of a transition-focused social history. For young adults who choose post-secondary education, location is a key factor in making a medical plan; if an educational institution is remote, it may be necessary to collaborate with a local physician and to dedicate more time to develop a portable medical summary. While it is always a good practice to evaluate patient skills related to healthcare independence—such as managing medications, making clinic appointments, and understanding one's health conditions—it is especially prudent to

assess these abilities and ensure that adequate supports are in place prior to patient relocation. College-bound youth can also benefit from information and empowerment in seeking out necessary accommodations, which typically must be requested by the individual. Please see [Table 6.2](#) for additional resources on education and related disability rights.

GB will remain in high school through age 21. In most states, individuals with intellectual disability have the option to extend schooling past age 18, oftentimes with opportunities to focus on life skills and/or employment-specific skills. Upon graduation, individuals with more severe disabilities may have the option to attend daytime habilitation (dayhab) programs that offer developmentally appropriate activities and peer interactions and serve as caregiver respite. Some individuals, like GB, will be interested in employment opportunities. It is important for healthcare providers to be familiar with local vocational rehabilitation

Table 6.2 Resources for healthcare transition.

Resource	Description	Location
Association of University Centers on Disabilities, postsecondary education webpage	List of resources on postsecondary education and relevant national policies	https://www.aucd.org/template/page.cfm?id=509
Employer Assistance and Resource Network on Disability Inclusion	List of state-based vocational rehabilitation agencies	http://www.askearn.org/state-vocational-rehabilitation-agencies/
GotTransition	Provides structured processes and resources surrounding healthcare transition geared toward patients and medical providers, including reimbursement strategies	http://www.gottransition.org/
Medicaid Waivers	Link to state-based Medicaid waiver programs	http://medicaidwaiver.org/
National Resource Center for Supported Decision-Making	Resource hub and state-based list of supported decision-making options	http://supporteddecisionmaking.org/
Supplemental Security Income	Additional information regarding Supplemental Security Income benefits	https://www.ssa.gov/benefits/ssi/
The Arc	A national advocacy organization that serves people with intellectual and developmental disabilities and their families	https://www.thearc.org
Think College	Search and comparison tools for postsecondary education for students with intellectual disabilities	https://thinkcollege.net/college-search
Ticket to Work	Federal program facilitating employment for those receiving disability benefits; includes information on retaining Medicaid while working	https://choosework.ssa.gov/index.html
TransCen	A national organization providing resources and support in finding employment for individuals with disabilities	https://www.transcen.org/

Assortment of resources relevant to issues commonly encountered during the transition from pediatric- to adult-based health care.

programs; [Table 6.2](#) includes references for state-based and national agencies. These organizations may provide services such as counseling, job placement, and transportation accommodations. They are available to individuals with physical and mental disabilities that impair their ability to find employment, as long as the disability is not so severe that employment would not be possible even with assistance.

It is important to understand how employment might affect another area critical to consider for the transitioning adolescent or young adult: health insurance.

Health insurance

There are often substantial changes to health insurance coverage as one transitions from pediatric to adult health care and adult-based health insurance, particularly for ongoing services, such as physical therapy, and for durable medical equipment (DME). It is important for providers to be familiar with these changes to appropriately counsel families. Individuals who receive SSI—supplemental income provided to individuals with disabilities—should also be aware that there is a re-qualification process at age 18 for these benefits and the standards can be more difficult to meet. Parental income is no longer a factor in the determination; as referenced earlier, income earned by the individual can affect eligibility for these benefits, so careful planning and counseling is essential to avoid lapses in benefits.

Young adults have several options for health insurance. They can:

1. Stay on their parents' plan through age 26. Most states also allow an indefinite extension if an individual is unable to care for themselves and are claimed as a dependent. This designation requires physician certification.
2. Apply for Medicaid. This option is available to adults below a specified income threshold in states that have expanded Medicaid. In all states, individuals who receive SSI are automatically eligible. It is also possible to continue receiving Medicaid after SSI benefits cease due to earned income. Please refer to [Table 6.2](#) for additional information.
3. Purchase health insurance on a publically available exchange that was established through the Affordable Care Act in 2010.
4. Receive Medicare if (1) their parents receive it and the individual is already on SSI, or (b) if they qualify for Social Security Disability Insurance and have received these benefits for at least 2 years.
5. Apply for COBRA coverage for a limited period following a qualifying event.

Medicaid waiver programs are another important part of comprehensive coverage. Waivers are designed to provide services to individuals living in the community who would otherwise require care in a nursing facility or state-supported living center. Each state has different waiver programs and eligibility criteria, but a waiver should be considered for individuals with significant disease burden, disability, and/or large DME or nursing needs. Waiver benefits may pay for services not typically covered by health insurance, such as ongoing therapies, dental care, and DME. It should be noted that the wait time to receive waiver

services can often be greater than 5–10 years depending on the state of residence. Please see [Table 6.2](#) for more information on waivers.

Medical decision-making and guardianship

Once an individual turns 18 years old, he or she is legally an adult with the right to independently make decisions. For individuals who have an intellectual disability, this transition can be especially difficult, as parents and caregivers are often accustomed to being the primary decision maker. Legal options exist to support individuals with intellectual disability who do not have the capacity to make their own medical decisions. It is important to note that capacity—which is a temporary clinical assessment of a person’s ability to understand one’s medical condition, the consequences of his or her treatment options, and the ability to consistently communicate medical decisions—is different from competency, which is the legal definition of someone’s baseline ability to perform these tasks.

In most states, various degrees of support exist. The most comprehensive and restrictive form of assisted decision-making is guardianship, where a person (usually a family member or close friend) is court-appointed to make decisions on behalf of an individual who is unable to do so. Typically, this designation can apply to all decisions (total) or only apply to specific domains (partial), such as medical or financial. In some cases, a less restrictive option, “shared decision-making,” is more appropriate; it does not take away the legal rights of an individual but officially recognizes the support provided by a designated confidant to help a person make decisions in one or more areas. The legal backing of such a designation varies by state, but it remains an important consideration when available. In all cases, it is imperative to identify the least restrictive means of supported decision-making that will maintain an individual’s autonomy while ensuring there is an adequate level of support. Please see [Table 6.2](#) for additional resources on this topic.

The process of applying for guardianship can be costly and time-intensive. The physician role is often twofold: (1) act as a source of up-to-date information regarding the available options for supported decision-making and (2) assist in completing the necessary paperwork, which typically consists of a capacity evaluation as it relates to an individual’s medical conditions. Some medical institutions have medical–legal partnerships that can be a source of assistance in the application process.

Vignette 6.3

An 18-year-old young woman, LJ, presents for her annual physical examination and vaccinations prior to attending a local community college following high school graduation. She and her father come to the visit with questions about her diagnosis and family history of myotonic dystrophy, type 1 (DM1). She has not had genetic testing, but her mother had a known molecular diagnosis of DM1 and died last year following respiratory complications of anesthesia. LJ has an individualized education plan with adaptations for mobility and mild learning

disabilities. She plans to live independently when starting college. She otherwise feels well today, but on review of systems reports worsening fatigue and trouble staying awake during class over the last several months.

DM1 is a congenital muscular dystrophy characterized by myotonia (sustained muscle contraction). Symptom severity can vary, but DM1 classically causes muscle wasting and weakness, cataracts, and cardiac conduction abnormalities. Inheritance is autosomal-dominant (through a trinucleotide repeat expansion in the noncoding region of the *DMPK* gene) with anticipation, so subsequent generations may experience earlier-onset and more severe disease. LJ has several manifestations worth noting, including muscle involvement that will affect her mobility and endurance, cognitive impairment that could impact her independence, and fatigue and daytime sleepiness that requires additional evaluation for sleep-disordered breathing and cardiac dysfunction. For additional medical considerations, please refer to available medical guidelines for adults with DM1 [17].

Other considerations for this visit include discussing genetic testing, which in general may be recommended to (1) confirm a diagnosis to eliminate the need for further diagnostic testing and reduce anxiety surrounding symptom etiology, (2) apply established guidelines to aid medical management and anticipatory care, and (3) provide counseling regarding family planning. Lastly, given LJ's age, it is important to discuss the transition from pediatric- to adult-based medical care, though ideally conversations and transition readiness assessment should begin in early adolescence. The following section will focus on the framework, evidence-based practices, and relevant outcomes to consider when creating transition planning practices and processes.

Transition planning

The transition from pediatric to adult-based health care requires considerable planning and benefits from having dedicated infrastructure and processes in place. It is best thought of as a three-tiered process: (1) preparation, which typically takes place in a pediatric environment and ideally spans several years; (2) transfer, which is the actual act of switching from pediatric to adult-based health care; and (3) integration, which is the ongoing effort to assimilate into the adult healthcare system. While discussions on transition typically consider two totally separate healthcare systems, the same process and philosophy also applies when there is no physical transfer, such as an individual continuing to see the same family physician as he or she becomes an adult.

One of the most widely known sources to describe the key components of transition is GotTransition [18], which describes “six core elements” as illustrated in Fig. 6.1 and described below:

1. Establish a clear *transition policy* that is known by the healthcare providers and readily available to families. Ideally, discussing transition begins as early as age 14.
2. Identify youth who will be transitioning and utilize a *registry to track* progress.

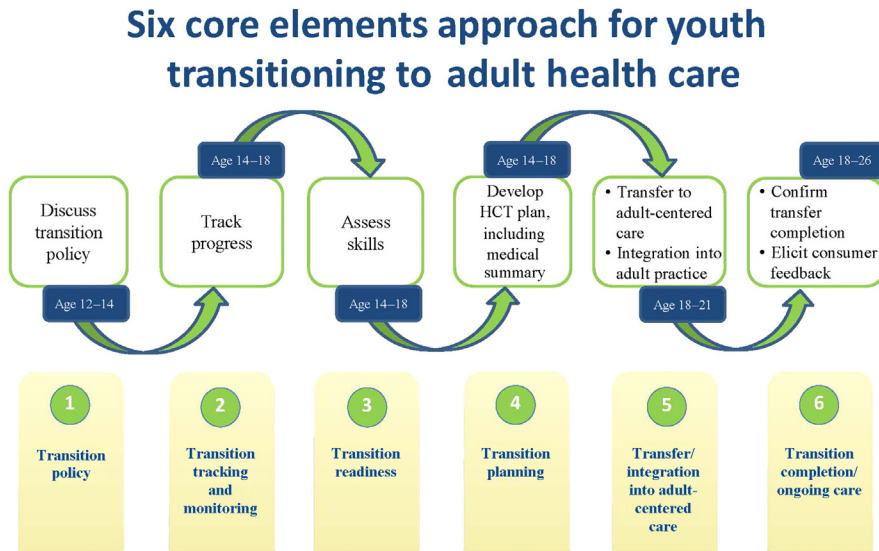


FIGURE 6.1 Model for health care transition developed by GotTransition that breaks down the transition process into six primary elements and lists a typical time frame for each element

3. *Assess skills* that are necessary for healthcare independence (e.g., making a follow-up visit, obtaining a medication refill) that helps identify knowledge gaps and informs developmentally appropriate goal setting.
4. *Make a transition plan* that includes identifying adult physicians, creating a medical summary, and considers the specific issues identified elsewhere in this chapter, such as medical decision-making and changes to health insurance.
5. *Transfer of care* to the adult medical system, which ideally includes communication between the pediatric and adult healthcare providers before the initial visit.
6. Finally, ensuring the *transfer is complete* by facilitating communication between adult and pediatric providers and answering any questions that remain.

These guiding principles may result in very distinct structures and processes depending on the clinical environment and local circumstances. One systematic review suggests that establishing a structured transition process leads to improved outcomes, while the specific intervention (such as using a care coordinator or increasing transition preparation) may be less important [19]. When evaluating the merits of a given transition process, various outcome measures may be considered, including population health/disease-specific guidelines (e.g., improved Hemoglobin A1c in diabetes), the experience of care (e.g., improved patient satisfaction), and cost/utilization measures (e.g., decreased emergency room visits). It is not surprising that establishing and maintaining these processes often requires considerable time and resources. There are billing strategies for enhanced reimbursement for some of these services, but many healthcare systems have identified alternative methods to enhance transition planning. Two examples include a pediatric consult team that plans and executes

transitions for medically complex patients [20] and an adult primary care clinic that provides care to individuals with neurodevelopmental conditions and facilitates the transition for remaining specialists and other needs [21].

Conclusion

Transition from pediatric- to adult-based care is a complex period, particularly for individuals with chronic disease and disability. In addition to relevant medical concerns, there are numerous other dimensions that can be equally important to a person's health and wellness. This chapter illustrates several of these issues, including medical systems challenges, changes to health insurance, psychosocial matters, vocation planning, and medical decision-making. Through familiarization of these issues and the available resources, it is possible to create processes to improve this difficult transition and in doing so, improve the health and well-being of countless adolescents and young adults.

Points to remember

- The transition from pediatric- to adult-based health care is often challenging for countless adolescents and young adults with chronic illness and/or disabilities. In caring for adolescents and young adults with genetic conditions, an interdisciplinary approach to transition is essential.
- A structured psychosocial history is an important part of a medical interview that can mitigate risky behaviors, identify supports and strengths, and provide opportunities to educate individuals on how to lead healthier lives.
- There are many different opportunities for education, employment, and enrichment following high school graduation and resources exist to help individuals explore these options.
- Many changes take place to health insurance during the transition period and there are several options available to young persons to help pay for medical care.
- For individuals with cognitive disabilities, it is important to consider assisted decision-making and partner with family members to promote self-advocacy, ideally prior to transitioning to adult care.
- Structured processes to facilitate transition are important to improve outcomes related to disease control, resource utilization, and patient experience.

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Preconception and prenatal care

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Introduction

Adults who are diagnosed with a genetic condition or who have family members diagnosed with a genetic condition may desire to learn about the risk of this condition to the health of their current or future children. Young adults who were diagnosed with a genetic disorder during childhood and are transitioning their care from pediatric to adult genetics providers may have similar questions as they contemplate starting their own families. These individuals can be referred to genetics professionals who have expertise in reproductive and prenatal genetic counseling or can be counseled regarding reproductive risks by an adult genetics service. Various genetic screens and tests are available before and during a pregnancy to determine the risk for offspring to be affected with genetic conditions, including conditions that are not ascertainable through family history, such as aneuploidy in offspring and carrier status for recessive conditions common in the general population. Depending on the indication for screening or the reason for parental concern, a provider may offer preconception or prenatal carrier screening, prenatal maternal serum screening or diagnostic testing, or *in vitro* fertilization with preimplantation genetic testing (PGT).

In addition to reproductive risks, certain genetic conditions in adults affect fertility and pregnancy outcomes and occasionally affect the accuracy of routine genetic screens and tests offered to pregnant women. Pregnancy itself can also influence the progression or severity of genetic disorders. While there are many examples of such conditions, we will highlight the more common ones with recommendations for their management related to reproduction and pregnancy.

Reproductive Carrier Screening

Carrier screening is typically defined as genetic screening performed on an asymptomatic individual to determine whether they carry a pathogenic variant that puts a current or future

pregnancy at increased risk of being affected with a genetic disorder [1]. This allows for informed reproductive decision-making and use of targeted genetic testing either prior to pregnancy to identify affected embryos before transfer into the uterus or during pregnancy to identify an affected fetus.

Carrier screening focuses on identifying carriers of select autosomal recessive and X-linked genetic disorders. The conditions for which screening is offered depends on the screening approach. The two most common approaches to carrier screening are targeted carrier screening, which is largely ethnicity-based, and pan-ethnic expanded carrier screening. In targeted carrier screening, the number of conditions included in the screening panel is limited, and the conditions are selected based on a combination of population carrier frequency and ethnic background. A limitation of this approach is that some individuals have limited or inaccurate knowledge about their ethnic background or have mixed ancestry. An array of different pan-ethnic expanded carrier screening panels are available that vary in content but have in common that they test for pathogenic and likely pathogenic variants in many genes (up to several hundred) simultaneously and can be offered to individuals of all ethnicities. The incorporated genes typically include those that are associated with childhood-onset disorders that are characterized by significant morbidity and reduced lifespan. Carrier screening can be offered sequentially, whereby one member of a couple is screened first and the partner undergoes testing only if the first individual has positive results, or simultaneously wherein both members of a couple are screened at the same time. Sequential carrier screening is cost saving but requires more time to fully determine a couple's reproductive risk.

When there is a family history of a known autosomal recessive or X-linked condition, at-risk individuals are offered specific targeted carrier testing (not "screening") for the familial pathogenic variant, if it is known, or can be offered sequencing of the associated disease gene. Such familial carrier testing does not replace but complements carrier screening with multigene panels.

Assays used for carrier screening

The two most commonly used methods for carrier screening are genotyping and sequence analysis. Genotyping assesses only well-described variants known to be associated with the conditions of interest. This works well for particular conditions in specific ethnic populations but generally has a lower detection rate compared to sequencing of all the genes of interest. Thus, many of the newer expanded carrier screening assays are done by sequencing of the entire coding regions of genes of interest. In such testing, all pathogenic or likely pathogenic variants are reported [2]. One caveat of this approach is that over time some variants may be reclassified as benign variants or variants of uncertain significance. For some genetic disorders, assays other than sequencing may be required. These include copy number analysis for exon 7 of the *SMN1* gene for spinal muscular atrophy, polymerase chain reaction (PCR) and/or Southern blotting to determine CGG repeat sizes in the *FMRI* gene for fragile X syndrome,

hexosaminidase A enzyme activity for Tay–Sachs disease, and a complete blood count with red blood cell indices and hemoglobin electrophoresis for hemoglobinopathies.

Practical considerations and limitations of carrier screening

Current recommendations support offering both targeted ethnicity-based carrier screening and expanded carrier screening as valid approaches but encourage that providers are consistent in what they offer [3]. Carrier screening is best performed prior to conception, if possible, since more options are available for management if the result indicates an increased risk to offspring.

It is important that individuals undergoing carrier screening receive pretest counseling. This should include general information about the test and counseling that expanded carrier screening panels do not include all inherited conditions. It should also be explained that carrier screening does not provide information about genetic disorders that are caused by different mechanisms, such as chromosomal abnormalities, and that while negative carrier screening results significantly reduce risk, they do not completely eliminate the risk for screened disorders. If paternity is inaccurate or the reproductive partner of an individual is not available for testing, carrier screening can be of limited benefit. A special circumstance is screening in adopted individuals who have no information about their biological relatives; expanded carrier screening is the preferred approach for these individuals.

Maternal screening for fetal aneuploidy

The goal of maternal serum screening (with or without ultrasound) during pregnancy is to identify pregnancies at increased risk for conditions that are usually not inherited. Such screening does not “rule in” or “rule out” certain conditions but rather adjusts the a priori risk. Most screens target risk for fetal aneuploidies (trisomies 21 and 18) for which risk increases with maternal age [4]. However, because aneuploidy is possible in any pregnancy, some form of aneuploidy screening is offered to all pregnant women. Clinicians consider a priori risk and clinical circumstances to offer the most appropriate screen for a particular woman. *A positive screening result is an indication to offer diagnostic testing.*

Multiple marker screening

First trimester screening

First trimester screening utilizes an algorithm that incorporates maternal age-related risk, gestational age, ultrasound measurement of fetal nuchal translucency, and maternal serum levels of free β -human chorionic gonadotropin (β -hCG) and plasma-associated protein of pregnancy-A (PAPP-A) to determine the risks for trisomy 21 and trisomy 18. Some labs will calculate a combined risk for trisomy 18 and trisomy 13. First trimester screening is performed between 11 weeks and 13 weeks 6 days of gestation. A positive result indicates that

the risk for that condition exceeds a laboratory-determined threshold. The sensitivity of first trimester screening is approximately 82%–87% for trisomy 21 for a 5% screen-positive rate.

Second trimester serum screening

Quadruple screening is performed between 15 and 22 weeks of gestation. The algorithm incorporates maternal age-related risk, gestational age, ethnicity, maternal serum levels of hCG, unconjugated estriol (uE3), dimeric inhibin A (DIA), and maternal serum alpha-fetoprotein (MSAFP) to determine risks for trisomy 21, trisomy 18, and open neural tube defects (ONTDs). A positive result indicates that the risk for that condition exceeds a laboratory-determined threshold. The sensitivity of quadruple screening is approximately 81% for trisomy 21 and 85% for ONTD. Of note, only MSAFP (not the other serum markers) is used to infer risk for ONTD, which can be used as a stand-alone screen for ONTD in the second trimester if a woman has already had other screening for aneuploidy. Additional anomalies such as abdominal wall defects or fetal renal disease can also cause elevated MSAFP. When elevated MSAFP is unexplained by the afore-mentioned conditions, there may be increased risk for pregnancy complications. Rarely MSAFP can be elevated because of maternal disease, such as a liver tumor.

Sequential and integrated screening

Sequential and integrated screening combine maternal serum analytes from the first trimester and second trimester in different paradigms to more precisely assess the risk for chromosome aneuploidy. They have a greater sensitivity and specificity than first trimester screening or quadruple screening alone, but the drawback is that women have to wait until the second trimester for the final result.

Cell-free DNA screening

Cell-free DNA (cfDNA) screening, also known as noninvasive prenatal testing (NIPT) or non-invasive prenatal screening (NIPS), can evaluate the risk for fetal aneuploidy as early as 10 weeks of gestation. It is done by analyzing cfDNA fragments in maternal plasma that originate from the placenta and make up about 10%–20% of all circulating cfDNA, referred to as the “fetal fraction (FF).” Most currently available cfDNA tests focus on detecting fetal aneuploidy of chromosomes 21, 18, 13, X, and Y using one of the two methodologies. The first is a single nucleotide polymorphism (SNP)-based analysis, which examines relative allele frequencies of SNPs located along the target chromosomes. The second uses massively parallel shotgun sequencing with counting of sequenced fragments to assess for small deviations from the expected number originating from target chromosomes to indicate increased risk for an additional fetal chromosome. The metrics of clinical utility of cfDNA screening (i.e., how well it works in practice) are the positive predictive value (PPV) and negative predictive value (NPV), which estimate the likelihood of a positive result being a “true” positive and a negative result being a “true” negative. Because PPV and NPV are dependent on the

prevalence of a condition, cfDNA screening works best for trisomy 21 (the most common trisomy in liveborns) in women of advanced maternal age who are at higher risk, but it is not a diagnostic test. The PPV of cfDNA screening is also superior to that of multiple marker screening in average and low-risk women. Because trisomy 13 and trisomy 18 are much rarer, the PPV for these conditions is lower, resulting in more false-positive results. *A positive cfDNA screen must therefore always be followed by a recommendation for a diagnostic confirmatory test.* Some laboratories offer cfDNA screening for other rare trisomies and specific microdeletion conditions, such as 22q11.2 deletion syndrome, but these conditions are even more rare and thus, as expected, the PPV and NPV values are low. Recently, cfDNA screening for select single gene disorders that often arise *de novo*, such as osteogenesis imperfecta and Noonan syndrome, has also become available, but data on the clinical validity are still limited.

Practical considerations and limitations of cfDNA screening

Before undergoing cfDNA screening, women should be informed that it is a screening test and not a diagnostic test, and that all positive (high-risk) results should be confirmed by diagnostic testing. While negative (low-risk) results are reassuring because NPVs are typically >99%, they cannot guarantee absence of a condition. cfDNA screening performs better in singleton pregnancies than in multiple pregnancies. Because cfDNA originates from the placenta, an abnormal result may reflect confined placental mosaicism (CPM) where the abnormality is only present in the placenta and not in the fetus. When there is concern for CPM, amniocentesis is a better follow-up diagnostic test than chorionic villus sampling (CVS). There are other limitations to cfDNA screening. Low FF, typically <4%, may lead to an unreportable result. Low FF is associated with increasing maternal body weight but by itself increases the risk for aneuploidy. In rare cases, cfDNA screening may yield unexpected results because of maternal health conditions such as fibroids, cancer, or maternal chromosome abnormalities like a previously unknown low level 45, X mosaicism.

Prenatal diagnostic testing

Prenatal diagnostic genetic testing is done via invasive procedures such as CVS and amniocentesis. Because these procedures yield pure (unmixed) fetal or placental DNA, an array of different genetic tests can be performed.

CVS, amniocentesis, and other procedures

CVS, typically performed between 10 and 14 weeks of gestation, involves obtaining 30–40 mg of placental villi, either by transabdominal needle aspiration or by transcervical catheter aspiration. The risk of procedure-related pregnancy loss from CVS is 1:450. Other more common complications include bleeding, cramping, and very rarely infection or membrane rupture. Amniocentesis can be performed from 15 weeks onwards (usually it is done

after 16 weeks) by transabdominal needle aspiration of approximately 20–40 mL amniotic fluid (AF) that contains fetal cells. The procedure-related risk for pregnancy loss from amniocentesis is 0.1%–0.3%. Other possible complications include temporary cramping, leakage of fluid, or, more rarely, frank rupture of membranes and bleeding. Percutaneous umbilical cord blood sampling may be utilized to obtain fetal cells from an umbilical vein if amniocentesis is impossible, for example, when there is anhydramnios.

Genetic testing modalities

Genetic tests are performed on DNA directly extracted from the cells in the AF and CVS samples or from cultures of those cells. Karyotyping to test for aneuploidy or larger chromosomal rearrangements is the only test that always requires cultures in order to obtain metaphase spreads, and it typically takes 2 weeks to obtain results. When a CVS is performed, there is a 1%–2% risk to detect CPM for one of the common aneuploidies. To obtain a fast result (within 48–72 hours), rapid analysis for common aneuploidies can be done by quantitative fluorescence PCR (a procedure that is rarely performed in the United States) or by fluorescence in situ hybridization (FISH). However, FISH results should always be confirmed by karyotype or CMA. Chromosomal microarray analysis (CMA) detects aneuploidy and copy number variants (CNVs). After a normal karyotype, 6% of pregnancies with a fetus with structural anomalies and 1.7% of pregnancies with a fetus without structural anomalies are diagnosed with a clinically significant CNV, which is significantly higher than the age-related risk for aneuploidy. This justifies offering CMA to all women; although this is not common practice. There is a 1% chance for detecting a CNV of uncertain significance and occasionally there can be incidental findings, which must be addressed in pretest counseling. Single gene testing for known familial pathogenic variants, multigene panel testing, or, more recently, whole exome testing are also available if standard testing is unrevealing or the suspicion of a single gene disorder is high [5].

Preimplantation genetic testing

PGT is done to test embryos for an inherited genetic condition or chromosomal aneuploidy [6,7]. PGT entails ovarian stimulation, egg retrieval, in vitro fertilization likely with intracytoplasmic sperm injection, embryo culture, and ultimately transfer of an embryo to the woman's uterus. For PGT, five to ten cells are biopsied from the trophectoderm of embryos on day 5 of culture followed by DNA extraction, whole-genome amplification, and genetic testing. Embryos are frozen until genetic testing results are available and unaffected or (in some cases of PGT for monogenic disorders) carrier embryos are selected for transfer.

Forms of preimplantation genetic testing

PGT-A for aneuploidy and PGT-SR for structural rearrangements

PGT-A is used to identify embryos with aneuploidy (A) and can improve pregnancy rate, in particular for women of advanced reproductive age, or with a history of recurrent

pregnancy loss or previously failed IVF cycles [8]. PGT-SR is used to identify embryos with an unbalanced structural rearrangement (SR) when one parent carries either a balanced chromosomal translocation or inversion. Until recently, PGT-A and PGT-SR were performed by FISH or microarray analysis, but currently most testing is performed by next-generation sequencing (NGS) technologies, which allows for detection of whole chromosome loss or gain (trisomy, monosomy), large segmental loss or gain (deletions and duplications), and potentially mosaicism.

PGT-M for monogenic disorders

PGT-M is used when a parent carries a known pathogenic variant associated with an autosomal dominant monogenic (M) condition, both parents are carriers of an autosomal recessive condition, or a mother is a carrier of an X-linked condition [9]. The molecular assay is done by targeted haplotyping of one or more closely linked polymorphic markers, direct targeted mutation detection, or a combination of both.

Practical considerations and limitations

Pretest counseling for PGT should address the risk for misdiagnosis with PGT, which is approximately 1%–5% due to possible allele drop-out, mosaicism, or contamination. The resolution of PGT-A is lower than prenatal or postnatal chromosomal-based testing, and it cannot detect most chromosomal microdeletions or microduplications. For these reasons, a follow-up diagnostic test (CVS or amniocentesis) is offered if pregnancy is achieved. Abnormal PGT-A results may not be fully representative of the chromosomal makeup of an embryo due to mosaicism, and the trophoctoderm may not be representative of the inner cell mass, which becomes the fetus. While PGT-SR can detect unbalanced forms of a chromosomal translocation or inversion, it cannot differentiate between embryos carrying a balanced chromosomal rearrangement and those with no chromosomal rearrangement. The size of the chromosomal translocation and inversion affects how or if PGT-SR can be performed.

PGT-M is offered for a known familial pathogenic variant. Before PGT-M is done, the laboratory will use DNA samples from relevant family members to set up a specific assay for the familial variant(s). PGT assay designs for a *de novo* pathogenic variant in a prior affected child (to address the possibility of germline mosaicism) are much more challenging because the parental samples typically do not have the variant. When PGT-M is performed for an autosomal recessive disorder, both parents, and for an X-linked disorder the mother, must have their carrier status confirmed molecularly. Sometimes carrier testing of additional family members is required to develop the PGT-M test. For diseases caused by a triplet repeat expansion (such as Fragile X syndrome), inversion (as in hemophilia A), or a large gene deletion, testing must be performed by haplotyping as the mutation itself is difficult to detect through PGT. When an affected family member has been found to have a variant of uncertain significance, the ability to perform PGT-M will be at the discretion of the testing laboratory. PGT is not offered for certain disorders, such as conditions caused by mutations in

mitochondrial DNA. Finally, especially if only limited numbers of embryos can be tested, a couple may be left with no unaffected embryos for transfer after PGT.

Parental genetic disease and reproduction

More children with genetic disorders are reaching adulthood and other genetic conditions only become symptomatic in adults. Some genetic disorders negatively affect gamete development or function resulting in infertility or reduced fertility. Maternal genetic disorders and their treatments can also affect pregnancy outcome, and conversely, pregnancy can alter the course of genetic conditions. Thus, all adults with a genetic disorder who reach reproductive age should not only be counseled about the recurrence risk for their future children, but also should be informed about options to optimize fertility, and women should be informed about the risks of pregnancy to their health and that of the fetus. This is best initiated prior to pregnancy and often requires a multidisciplinary approach by a team of adult geneticists and genetic counselors, fertility specialists, maternal – fetal medicine specialists, and appropriate consulting services with expertise in treatment of affected organ systems. Although details are beyond the scope of this text, a few pertinent examples with key associated issues are provided below.

Examples of genetic disorders that affect maternal and fetal outcomes

Turner syndrome

Women with Turner syndrome have infertility because of streak ovaries, but a proportion of these women are mosaic (45, X/46, XX) and may be fertile or can become pregnant with their own cryopreserved oocytes. With assisted reproductive technologies, some patients with Turner syndrome achieve pregnancy with donor oocytes, but these pregnancies are at increased risk for complications. Women with Turner syndrome can have (corrected) congenital heart disease and aortic root dilatation, placing them at increased risk for serious cardiovascular complications and aortic rupture during pregnancy.

Marfan syndrome, other aortopathies, and collagen disorders

The leading cause for mortality in Marfan syndrome is aortic dissection, the risk for which is increased in pregnancy due to altered hemodynamics. It is recommended that pregnancy be avoided if the aortic root diameter is above 40 mm. Marfan syndrome is caused by pathogenic variants in *FBNI*, but there are other conditions with risk for aortic dilatation and rupture, such as Loey–Dietz syndrome caused by pathogenic variants in genes encoding proteins of the TGF- β signaling pathway. Some collagen disorders, in particular vascular-type Ehlers–Danlos syndrome (vEDS), can weaken the smooth muscle wall of blood vessels and hollow organs, including the uterus. vEDS is associated with increased risk for uterine rupture and vascular or bleeding complications during pregnancy.

Phenylketonuria and other inborn errors of metabolism

Phenylketonuria is caused by phenylalanine hydroxylase deficiency, which converts phenylalanine to tyrosine. The resulting hyperphenylalaninemia causes neurodevelopmental disability, which is preventable by dietary restriction of phenylalanine. If women who do not adhere to a restricted diet become pregnant, elevated phenylalanine levels can cause intellectual disability, microcephaly, and congenital heart disease in the offspring.

Women who are carriers of X-linked ornithine transcarbamylase (OTC) deficiency, especially when they have unfavorable X-inactivation skewing, may experience hyperammonemia in periods of catabolic stress. Their ammonia levels should be monitored in the peripartum period. Fetal fatty acid oxidation disorders (FAODs) increase the risk for acute fatty liver of pregnancy (AFLP), hemolysis, elevated liver enzymes and low platelet syndrome. A diagnosis of AFLP should trigger testing for an FAOD in the offspring.

Myotonic dystrophy

Myotonic dystrophy (DM) is caused by a CTG triplet repeat amplification of the 3'-untranslated region of the *DMPK* gene, which can expand when maternally transmitted. Women with mild DM can be undiagnosed until they have an affected fetus with congenital DM, with akinesia and polyhydramnios. These pregnancies are at increased risk for preterm labor and hypertension. It should be kept in mind that treatment with magnesium sulfate puts these women at risk for possible life-threatening respiratory depression.

Skeletal dysplasias

Women with skeletal dysplasias or other conditions that cause deformities of the spine, such as severe scoliosis, may have reduced respiratory capacity. Pelvic deformities may not allow for vaginal delivery. These women are at increased risk for reduced respiratory capacity and preterm birth and often have dystocia that requires elective cesarean section for delivery. They may have increased anesthesia risks related to airway access and limited access for spinal or epidural anesthesia.

Cystic fibrosis

Individuals with cystic fibrosis (CF) regularly survive into adulthood. While CF causes male infertility due to bilateral absence of the vas deferens, most women with CF are fertile even though they have thickened cervical mucus. Many women with CF can have successful pregnancies, but they are at increased risk for gestational diabetes, preterm birth, fetal growth restriction, hypertension, and exacerbation of respiratory and digestive symptoms. They require multidisciplinary management from before conception to optimize respiratory and nutritional status and manage potential complications.

Vignette 7.1

RT is a 35-year-old woman, who is seen for prenatal genetic counseling at 8 weeks gestation after expanded carrier screening reveals that she is a carrier of OTC deficiency, an X-linked disorder with a severe neonatal-onset phenotype in males. Female carriers can be asymptomatic but have an increased risk for catabolism-related hyperammonemia during delivery and the postpartum period (see above). A referral to maternal – fetal medicine and metabolic genetics is made for management and counseling about pregnancy-related risks and monitoring for hyperammonemia in the peripartum period.

The patient is counseled about OTC deficiency and that 50% of male offspring will be affected and 50% of female offspring will carry the abnormal OTC gene and may have symptoms although typically less severe than in males.

Prenatal testing through CVS or amniocentesis should be offered. Alternatively, cfDNA screening can be offered to determine fetal sex, and if the fetus is male, the option of CVS or amniocentesis can then be offered. If the fetus is affected, the patient should be counseled about pregnancy options including continuing the pregnancy and recommended management of the fetus after delivery versus termination of pregnancy.

Preconceptionally, the option of PGT-M should also be discussed with the patient. Carrier testing of other family members may need to be coordinated in order for PGT-M to be performed. Should PGT-M be pursued, the recommendation of prenatal testing through CVS or amniocentesis or, at a minimum, postnatal confirmation testing should be discussed.

Points to remember

- Some carrier statuses carry associated health risks for the carrier individual. In some cases, these are exacerbated by pregnancy.
- PGT-M reduces but does not eliminate risk for an inherited condition and as a result does not replace prenatal diagnostic testing.
- Certain genetic conditions affect fertility and pregnancy outcomes and occasionally affect the accuracy of routine genetic screens and tests offered to pregnant women. Moreover, pregnancy itself can also influence the progression or severity of genetic disorders.

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Genetic disorders in adults

Cancer syndromes

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Introduction

Sporadic versus inherited

Cancer can present in anyone, oftentimes irrespective of gender or ethnicity, with age being an important risk factor for all. For instance, colon cancer is the third most common cancer worldwide in individuals over 60 years of age, and breast cancer is the most common cancer in postmenopausal women over the age of 50 years. These are considered to be sporadic cancers with multiple factors leading to its etiology. However, when a young individual, such as a 30-year-old woman, develops breast cancer, a hereditary cancer syndrome should be suspected. Confirmation of such a diagnosis has the potential to impact not only that individual's management as they may be at risk for developing other cancers but also close family members who could seek out preventive strategies to reduce their risk of cancer. A comprehensive review [1] has identified 54 hereditary cancer syndromes, and this number continues to rise as newer cancer predisposing genes are identified. A majority of the hereditary cancer syndromes are autosomal dominant leading to a 50% risk to each offspring of an affected individual.

Personal and family history features suggestive of a hereditary cancer syndrome

- Age of onset of cancer is younger than typically seen for that cancer (usually <50 years in adults)
- Cancer occurring in the sex not typically affected (e.g., breast cancer in a man)
- Bilateral cancers (in paired organs)
- Multiple tumors in the same organ or multifocal tumors
- Synchronous/metachronous primary tumors in the same individual
- Constellation of cancers in the same patient or in multiple members of the same family (e.g., breast and ovarian or breast and thyroid)

- Uncommon or rare type of cancer
- Cancers spanning multiple generations that are suggestive of a common cancer syndrome (e.g., multiple family members with the same cancer or family members with different cancers that can occur in a syndrome, such as colon and uterine cancer).

Evaluation for a hereditary cancer syndrome

The diagnosis of a hereditary cancer syndrome is highly dependent on the accuracy of the personal and family history data. Several key points of information need to be collected from the patient to determine the familial nature of the cancer.

1. *Tumor characteristics:* This includes the type or pathology of cancer; special characteristics of the tumor (e.g., mucinous signet ring cells in colonic adenocarcinoma is more often found in Lynch syndrome); immunohistochemical staining (e.g., ER/PR/Her2 negative tumors are more likely to be associated with pathogenic variants in *BRCA1*).
2. *Family history:* A detailed three- to four-generation pedigree should be obtained from the patient. Ask about ages of onset of cancer in relatives, known risk factors (e.g., relative with liver cancer who had a significant history of ethanol abuse), the type of cancer in affected relatives (if patients are not sure, ask more information about the types of treatment received such as chemotherapy, radiation, or surgery). If patients do not have enough information, offer them the opportunity to call someone who may know more or have them return to you after they have gathered more information. Negative family history should be noted as it may help to rule out certain diagnoses. For example, if a patient's mother had breast cancer at the age of 50 years, but she had five older sisters without a history of cancer, and no history of cancer in maternal grandparents, then the mother's cancer may be less likely to be hereditary. Also ask about genetic testing in family members. Remember that the risk is independent of each side of the family (maternal versus paternal).
3. *Scoring tools:* Many online software tools are available to determine the risk of finding a genetic variant such as BRCAPRO, PREMM, and IBIS. These are often based on the personal and family history information. Some of these tools may require additional licenses. Also, some can be time consuming and challenging to implement in a busy clinical practice.
4. *Diagnostic testing:* Once a pretest clinical assessment for hereditary cancer syndromes has been conducted, genetic testing can be ordered. This can include a single gene or multiple genes. Current standard of care for evaluation of hereditary cancer syndromes is testing multigene panels, unless single site testing is pursued for known familial variants.

According to one study, two-thirds of adult genetics practice are related to personal and/or family history of cancer [2]. This could be explained by the heightened awareness among oncologists, primary care physicians, and patients about the role genetics plays in cancer-related health. Table 8.1 lists a variety of cancers commonly associated with cancer syndromes.

Table 8.1 List of select cancers with their associated genes and cancer syndromes.

Organ	Key points [3]	Associated genes	Hereditary cancer syndrome	Where to find this topic?
Adrenal	15% of adrenal cortex tumors are inherited and are more common in children	<i>TP53, CDKN1C, H19, IGF2, KCNQ1OT1</i> <i>MEN, APC</i>	Li – Fraumeni, Beckwith Wiedemann, MEN1, FAP	This chapter, Chapter 12 (endo), Chapter 13 (GI) BWS is a childhood cancer syndrome and is beyond the scope of this book
Basal cell	Most common type of skin cancer; comprise 80% of all skin cancers; develop in sun-exposed areas	<i>XPC, ERCC2, POLH, PTCH1</i>	Xeroderma pigmentosum, basal cell nevus syndrome (Gorlin syndrome)	Chapter 24 (skin)
Bladder	Most bladder cancers are sporadic with average age at diagnosis of 73 years, M > F	<i>MLH1, MSH2, MSH6, PMS2, EPCAM, RB1, PTEN</i>	Lynch, retinoblastoma, Cowden	This chapter, Chapter 13 (GI)
Bone	Most bone cancers are not inherited; >40% are chondrosarcomas with average age at diagnosis of 51 years; osteosarcomas are most often seen in children and teens	<i>TP53, RB1, EXT1, EXT2, EXT3, TSC1/2</i>	Li – Fraumeni, retinoblastoma, multiple osteochondromas, tuberous sclerosis	This chapter, Chapter 24 (skin)
Breast	5%–10% of breast cancers are hereditary	<i>BRCA1, BRCA2, CDH1, NF1 PTEN, STK11</i> <i>TP53, ATM, BARD1, CHEK2, MRE11A, NBN, PALB2</i>	Hereditary breast and ovarian cancer Hereditary diffuse gastric cancer, Neurofibromatosis Type 1, PTEN hamartoma/Cowden, Peutz – Jeghers, Li–Fraumeni	This chapter, Chapter 13 (GI), Chapter 24 (skin)
Central nervous system (brain/spinal cord)	Most often not familial, lifetime risk for malignant tumors of brain/spinal cord is <1%	<i>APC, MLH1, MSH2, MSH6, PMS2, EPCAM, VHL, MEN</i>	Turcot Lynch, Von Hippel Lindau Multiple endocrine neoplasia	Chapter 12 (endo), Chapter 13 (GI), Chapter 22 (renal)

(Continued)

Table 8.1 (Continued)

Organ	Key points [3]	Associated genes	Hereditary cancer syndrome	Where to find this topic?
Cervix	Most often diagnosed in women 35–44 years of age, HPV infection is the most important risk factor	Not genetic, but may run in families due to inherited predisposition of reduced ability to fight against HPV	N/A	Beyond the scope of this book
Colon	Third most common cancer worldwide; 96% of colorectal cancers are adenocarcinomas while carcinoid, GIST, lymphoma, and sarcoma are less common	<i>Polyposis</i> <i>APC, GREM1</i> <i>MUTYH, MSH3, NTHL1</i> <i>Nonpolyposis</i> <i>ATM, MUTYH, MLH1, MSH2, MSH6, PMS2, EPCAM, CHEK2</i> <i>Hamartomatous</i> <i>STK11, PTEN</i> <i>BMPR1A</i>	Familial adenomatous polyposis, MUTYH-associated polyposis, Lynch, Peutz–Jeghers PTEN hamartoma/ Cowden, Juvenile polyposis	This chapter, Chapter 13 (GI)
Endometrium (uterus)	Average age at the diagnosis of sporadic cancer is 60 years	<i>MLH1, MSH2, MSH6, PMS2, EPCAM, FH, RB1, FLCN</i>	Lynch syndrome, HLRCC (Reed), retinoblastoma, Birt Hogg Dube	This chapter, Chapter 13 (GI)
Esophageal	Adenocarcinoma is the most common in Whites, while SCC is more common in AA	<i>MLH1, MSH2, MSH6, PMS2, EPCAM</i>	Lynch syndrome	Chapter 13 (GI)
Gastric (stomach)	Average age of diagnosis of sporadic cancer is 68 years	<i>CDH1</i> <i>CTNNA1</i> <i>MLH1, MSH2, MSH6, PMS2, EPCAM, APC, TP53, STK11</i>	Hereditary diffuse gastric cancer, Lynch, FAP, Li–Fraumeni, PJS	Chapter 13 (GI)
Hepatobiliary (hepatocellular, gallbladder, intra, and extrahepatic cholangiocarcinoma)	Most common is HCC with chronic hepatitis B/C being the leading cause	<i>MLH1, MSH2, MSH6, PMS2, EPCAM</i>	Lynch syndrome	Chapter 13 (GI)

Kidney cancer	Majority are renal cell carcinomas, which can be clear cell (most common), papillary, chromophobe, and other rare types (medullary, multilocular cystic, collecting duct). 5%–10% are transitional cell carcinomas (urothelial). Other rare types include Wilms tumor and renal sarcoma	<i>VHL</i> <i>SDHx</i>	Von Hippel Lindau syndrome Hereditary paraganglioma and pheochromocytoma	Chapter 22 (renal) This chapter
		<i>MLH1, MSH2, MSH6, PMS2, EPCAM, FH</i> <i>MET</i>	Lynch syndrome (urothelial) HLRCC Hereditary papillary renal carcinoma (HPRC)	Chapter 13 (GI) This chapter This chapter
Leukemia	Accounts for 2% of all cancers. Genetic factors are more likely to be implicated in chronic lymphocytic leukemia than in acute leukemias or chronic myeloid leukemias. Familial clustering is often observed in leukemias due to shared environmental exposures	<i>FLCN</i> <i>CEBPA, DDX41, RUNX1, ANKRD26, ETV6, GATA2, TP53, PAX5, SH2B3</i>	Birt Hogg Dube (BHD) Myeloid neoplasms with germline predispositions; genetic disorders associated with leukemia (ataxia telangiectasia, bone marrow failure syndromes, immune deficiency, incontinentia pigmenti, Kostmann syndrome, Li–Fraumeni syndrome, NF1, Seckel syndrome, Trisomy 21, Wiskott–Aldrich syndrome)	Chapter 14 (heme)
Lung cancer	About 85% of lung cancer are nonsmall cell (squamous, adenocarcinoma, large cell); 10%–15% are small cell while <5% are lung carcinoid tumors. Leading cause of lung cancer is smoking; genetics plays a small part in the etiology of lung cancer and familial clustering may be seen due to shared environmental exposures	<i>EGFR</i>	No familial cancer syndromes known	N/A
Lymphoma	Hodgkin's and Non-Hodgkin's lymphoma are most often caused by acquired somatic variants in genes	<i>Not genetic</i>	N/A	Chapter 14 (heme)
Melanoma	Melanoma accounts for 1% of all skin cancers; 20 times more common in Whites than African Americans; average age of diagnosis is in the early 60s	<i>CDKN2A/CDK4</i> <i>BAP1</i> <i>POT1, TERT, ACD, TERF21P</i> <i>MC1R</i> <i>BRCA2</i> <i>CHEK2</i>	FAMMM	This chapter

(Continued)

Table 8.1 (Continued)

Organ	Key points [3]	Associated genes	Hereditary cancer syndrome	Where to find this topic?
Multiple myeloma	Relatively uncommon	<i>None</i>	No hereditary genes implicated	
Myelodysplastic syndrome	Most commonly diagnosed in people in their 70s	<i>RUNX1, ANKRD26, ETV6, SAMD9, SAMD9L</i>	Familial syndromes that are more likely to develop MDS such as bone marrow failure syndromes, familial platelet disorder with a propensity to myeloid malignancy, severe congenital neutropenia, dyskeratosis congenita	Chapter 14 (heme)
Neuroendocrine tumors	Most commonly develop in the GIT, lung, pancreas, and adrenals; majority are sporadic and not inherited	<i>MEN1, MEN2, VHL, NF1, TSC1/TSC2</i>	Multiple endocrine neoplasia, Von Hippel Lindau, neurofibromatosis type 1, tuberous sclerosis	This chapter, Chapter 12 (endo)
Ovarian cancer	Mainly develops in older women in their 60s, most often after menopause; about 5%–10% are part of a hereditary cancer syndrome	<i>BRCA1, BRCA2, DICER1, BRIP1, RAD51C, RAD51D, MLH1, MSH2, MSH6, PMS2, EPCAM, SMARCA4</i>	Hereditary breast and ovarian cancer syndrome Hereditary ovarian cancer? Lynch syndrome Predisposes to small cell carcinoma of the ovary, hypercalcemic type, and rhabdoid tumor predisposition syndrome type 2	This chapter Chapter 13 (GI)
Pancreatic cancer	Most common type is exocrine, of which 95% are adenocarcinomas; slightly more common in men than women; 25% caused by smoking, while 10% are caused by inherited genetic syndromes	<i>STK11, CDKN2A, BRCA1, BRCA2, PALB2, PRSS1, SPINK1, MLH1, MSH2, MSH6, PMS2, EPCAM</i>	Peutz – Jeghers syndrome FAMMM Hereditary breast and ovarian cancer syndrome Hereditary breast cancer syndrome Hereditary pancreatitis Lynch syndrome	This chapter Chapter 13 (GI)
Prostate cancer	Fifth most common malignancy in the world; develops mainly in older men (> 66 years of age) and more commonly in African Americans	<i>STK11, BRCA2, CHEK2, ATM, HOXB13, MLH1, MSH2, MSH6, PMS2, EPCAM</i>	Peutz – Jeghers syndrome Hereditary breast and ovarian cancer syndrome Lynch syndrome	This chapter, Chapter 13 (GI)

Renal cell carcinoma	Average age of diagnosis is 64 years	<i>See kidney cancer</i>		Chapter 22 (renal)
Sarcoma	Soft tissue sarcomas include undifferentiated pleomorphic, liposarcoma, and leiomyosarcoma; radiation treatments are a common cause of sarcomas and occur 10 years after treatment	<i>TP53</i> <i>NF1</i> <i>APC</i> <i>RB1</i> <i>PTCH1</i> <i>TSC1/TSC2</i>	Li–Fraumeni syndrome Neurofibromatosis type 1 Gardner (FAP) Retinoblastoma Gorlin syndrome Tuberous sclerosis Klinefelter syndrome	This chapter, Chapter 24 (skin)
Testicular cancer	Average age of diagnosis is 33 years; most cases are sporadic but can be familial	<i>47, XXY</i>		Chapter 11 (repro)
Thyroid cancer	Usually diagnosed at a younger age than most other cancers (40–50 years); three times more common in women than in men; most often sporadic but can be associated with some hereditary conditions	<i>RET</i> <i>PTEN</i> <i>APC</i> <i>PRKAR1A</i>	MEN—medullary thyroid cancer Cowden syndrome, Familial adenomatous polyposis Carney complex	Chapter 12 (endo), Chapter 13 (GI)

BWS, Beckwith-Wiedemann syndrome; *EGFR*, epidermal growth factor receptor; *FAMMM*, familial atypical multiple mole melanoma; *FAP*, familial adenomatous polyposis; *GI*, gastrointestinal; *GIT*, gastrointestinal tract; *HLRCC*, hereditary leiomyomatosis and renal cell carcinoma.

BAP1 tumor predisposition syndrome

Overview

BAP1 tumor predisposition syndrome, also known as COMMON syndrome (cutaneous/ocular melanoma, atypical melanocytic proliferations, and other internal neoplasms) is a hereditary cancer syndrome that includes cutaneous and ocular/uveal melanoma, mesothelioma (peritoneal or pleural), and renal cell carcinoma. The hallmark tumor is the atypical melanoma, also known as BAPoma, which has features of atypical Spitz nevi and melanoma [4].

Evaluation

Individuals with mesothelioma, uveal/ocular melanoma, or BAPoma should be offered testing. Any tumor demonstrating loss of staining for the *BAP1* protein on immunohistochemistry also warrants germline evaluation. Personal or family history of asbestos exposure in the setting of a mesothelioma does not preclude the presence of a germline *BAP1* variant.

BAP1 tumor predisposition syndrome should be suspected in an individual if either of the following criteria are met:

1. Two or more confirmed *BAP1*-associated tumors (atypical Spitz tumors, uveal melanoma, malignant mesothelioma, cutaneous melanoma, clear-cell renal cell carcinoma, basal cell carcinoma)
2. One *BAP1*-associated tumor AND a family member (first- or second-degree relative) with a confirmed *BAP1*-associated tumor

Genetic testing

Panel testing is recommended given the other genes associated with the risk for melanoma, the potential for misreporting of peritoneal mesothelioma as ovarian cancer or vice versa and the numerous genes associated with renal cell carcinoma.

Management

Periodic screening for the associated tumors is recommended.

1. Annual dilated eye examinations beginning at the age of 11 years.
2. Annual full-body dermatologic examinations beginning around the age of 20 years.
3. Annual abdominal ultrasound examination with an abdominal magnetic resonance imaging (MRI) every 2 years.
4. Consider annual urinalysis.

No evidence-based screening modalities exist for mesothelioma.

Genetic counseling

This is an autosomal dominant disorder. The *de novo* mutation rate is unknown. Assisted reproductive technologies including preimplantation genetic screening is an option if the pathogenic variant is known in the family.

Birt Hogg Dube

Overview

Birt Hogg Dube (BHD) syndrome affects various organ systems including the skin, lungs, and kidneys and is characterized by facial fibrofolliculomas, spontaneous pneumothoraces, and risk for renal tumors. Nonspecific cutaneous findings associated with BHD include trichodiscomas, angiofibromas, acrochordons, and perifollicular fibromas. About 5% of renal masses in BHD are oncocytomas, which are benign eosinophilic tumors arising from type B intercalated cells of the collecting ducts within the renal cortex. About 50% of renal masses are hybrid chromophobe renal cell carcinoma (RCC)-oncocytomas (so-called oncocyctic hybrid tumor, which is a hallmark of BHD) while 9% are clear-cell RCC [5].

Evaluation

Physical exam for facial fibrofolliculomas should be undertaken. These skin lesions begin to appear from the age of 20–40 years and grow in multitude over time. Skin-punch biopsy is needed for confirmation. Spontaneous pneumothorax occurs in less than a quarter of individuals with BHD. However, lung cysts are quite common and are more prevalent in the periacinar region and tend to be bilateral and multifocal. Parotid oncocytoma and oral papules have been reported. Diagnostic criteria for BHD are met when an individual has one major or two minor criteria [5].

Major criteria (any one)

- At least five fibrofolliculomas or trichodiscomas, at least one histologically confirmed, of adult onset
- Pathogenic variant in *FLCN*

Minor criteria (any two)

- Multiple lung cysts: Bilateral basal cysts of no clear etiology, with or without spontaneous primary pneumothorax
- Renal cancer of early onset (<50 years) or multifocal or bilateral or oncocyctic hybrid tumor
- First-degree relative with known diagnosis of BHD

Genetic testing

BHD is associated with pathogenic variants in the *FLCN* (also known as *BHD*) gene. Sequence variants are found in 88%, while deletions are found in 3%–5% of families with BHD. Thus, a pathogenic variant is not detected in 7%–9% of individuals who meet the diagnostic criteria. The *FLCN* gene should be examined in the context of panel-based testing for families with

RCC. Even one pathologically confirmed facial or truncal fibrofolliculoma warrants genetic testing. Individuals with multiple or bilateral chromophobe, oncocytic, or hybrid renal tumors or individuals with one renal tumor plus family history of RCC should be tested.

Management

Pneumothorax should be treated with standard-of-care emergency management. Renal tumors are treated by nephron-sparing surgery. There are no consensus guidelines for screening, but the following may be considered:

1. Renal tumor: Annual MRI of kidneys or alternatively computed tomography (CT) scan with contrast.
2. Melanoma: Full-body skin examination can be undertaken annually due to potential risk of melanoma in these individuals.
3. Lung cysts: CT chest should be done at baseline/diagnosis to quantify and evaluate for lung cysts or blebs that may increase risk for pneumothorax. Affected individuals should be advised to refrain from smoking, high ambient pressures, and radiation exposure.

Genetic counseling

This is an autosomal dominant disorder with variable penetrance.

DICER1-related pleuropulmonary blastoma familial tumor and dysplasia syndrome

Overview

The *DICER1* gene is the only known genetic cause of pleuropulmonary blastomas (PPBs) and is also a cause of cystic nephromas in childhood. While most of the tumors associated with *DICER1* variants have a pediatric age of onset, such as renal sarcoma, embryonal rhabdomyosarcoma, Wilms tumor, nasal chondromesenchymal hamartoma, ciliary body medulloepithelioma, pineoblastoma and pituitary blastoma; ovarian sex cord-stromal tumors, particularly Sertoli–Leydig cell tumor is also seen and hence *DICER1* is included in all panels testing for hereditary ovarian cancer syndromes. Other features include multinodular goiter due to nodular hyperplasia and thyroid carcinoma, with thyroid nodules being the most penetrant feature. Small intestinal polyps have also been reported. The tumors seen with *DICER1* are part of the pleuropulmonary familial tumor and dysplasia syndrome.

Evaluation

Regressed PPBs (type Ir) have been identified in asymptomatic adults with germline *DICER1* variants. In some cases, individuals can present with shortness of breath or respiratory distress due to pneumothorax, which may be secondary to PPB cyst rupture. Consideration of BHD syndrome should be made as it can give a similar presentation due to spontaneous pneumothorax, which is seen in these individuals.

Genetic testing

DICER1 testing is recommended for individuals with component tumors; the majority of individuals with PPB have inherited loss of function mutations in the RNase IIIb domain, but intronic, splice site, and large deletions have been described. Tumor-specific hotspot mutations are well described. This syndrome is characterized by reduced penetrance and some adults with the disease are found to have regressed PPB on screening done after the genetic diagnosis has come to light. Testing is recommended for children and adults at risk of having a *DICER1* pathogenic variant as there are published screening guidelines.

Management

American Association for Cancer Research (AACR) guidelines include:

1. Baseline chest CT has been recommended at the age of 3–6 months with repeat at the age of 2.5–3 years if normal. Baseline chest CT at the time of diagnosis if older when identified. Intensive lung imaging beginning with chest X-ray from birth and every 4–6 months until 8 years of age. From age 8 to 12 years, annual chest X-ray.
2. Baseline thyroid ultrasound by age 8 and then every 2–3 years if asymptomatic or no nodules.
3. Renal screening with ultrasound every 6 months until age 8 and annually thereafter until age 12. Baseline renal ultrasound if mutation identified after age 12.
4. Annual dilated eye exam from 3 to 10 years of age. For women, abdominal and pelvic ultrasound at least annually after age 8 with education regarding symptoms of virilization.

Genetic counseling

This is an autosomal dominant condition and the *de novo* mutation rate is estimated to be 10%–20%. Assisted reproductive technologies including preimplantation genetic screening are an option.

Familial atypical multiple mole melanoma syndrome

Overview

Familial atypical multiple mole melanoma syndrome is an autosomal dominant cancer predisposition syndrome characterized by multiple melanocytic nevi and a family history of melanoma. It has reduced penetrance and variable expressivity and is associated primarily with pathogenic variants in the *CDKN2A* gene. Moreover, the risk for malignant melanoma and other cancer risks include pancreatic cancer. Norris reported the first case as early as in 1820 [6]; however, the name familial atypical multiple mole melanoma (FAMMM) was proposed by Lynch in 1978 [7].

Evaluation

Diagnostic criteria include:

1. Malignant melanoma in one or more first- or second-degree relatives

2. High total body nevi count (> 50)
3. Nevi with certain histological characteristics on microscopy (e.g., lentiginous pattern, nuclear atypia)

Genetic testing

FAMMM is primarily associated with pathogenic variants in *CDKN2A* (in 40% of cases) and rarely with *CDK4*. In 60% of the cases, a molecular etiology is not identified. *CDKN2A* is a tumor suppressor gene involved in cell cycle inhibition.

Management

Screening for melanoma begins at 10 years of age with a baseline total body skin exam (including scalp, oral mucosa, genital area, and nails). Observe nevi for asymmetry, border irregularity, color variation, diameter > 6 mm, evolution/elevation (ABCDE) for the development of melanoma. While there are no routine screening protocols for pancreatic cancer, endoscopic ultrasound can be initiated at age 50 or 10 years younger than the earliest age at diagnosis in a family member.

Hereditary breast and ovarian cancer syndrome

Overview

Breast cancer is the most common cause of cancer in women, worldwide, with an annual incidence of 1.38 million new cases reported each year [8]. One in eight women will be diagnosed with breast cancer each year in the United States. Most breast cancers are sporadic and occur in postmenopausal women as a result of multiple factors, including gender ($F > M$), age (> 55 years), race (Caucasians), early menarche (< 12 years), late menopause (> 55 years), having first child at an older age (> 30 years), nulliparity as well as environmental and lifestyle factors. However, 5%–10% of breast cancer is associated with inherited pathogenic variants in several different genes. Lynch and Krush first described the genetics of breast and ovarian cancer in 1971 [9]. This inherited breast cancer predisposition syndrome is now widely known as the hereditary breast and ovarian cancer syndrome (HBOC) and is characterized by a high risk of breast and ovarian cancers along with other cancers such as male breast, prostate, pancreatic, and melanoma. The two genes associated with HBOC are *BRCA1* and *BRCA2*, which are tumor suppressor genes involved in DNA repair. The prevalence of *BRCA1/2* mutation carriers in the general population is 1 in 500–1 in 1000 and is considerably increased to 1 in 40 in individuals of Ashkenazi Jewish ancestry. HBOC is inherited in an autosomal dominant manner but is incompletely penetrant. The risk of cancers associated with HBOC is shown in [Table 8.2](#):

Evaluation

A diagnosis of HBOC should be suspected in the following scenarios:

1. One or more women in the family with a diagnosis of breast cancer at age 45 or younger.

Table 8.2 Risk of cancers associated with hereditary breast and ovarian cancer syndrome as compared to the general population.

Cancer	General population risk	Risk because of <i>BRCA1</i> variants	Risk because of <i>BRCA2</i> variants
Female breast	12.3%	46%–87%	38%–84%
Male breast	<1%	1.2%	8.9%
Ovarian	1.6%	39%–63%	16.5%–27%
Pancreatic	0.5%	1%–3%	2%–7%
Prostate	11%	8.6% (by age 65)	15% (by age 65)
Melanoma	1.6%	No increase	Increased

2. One or more women in the family diagnosed before age 50 with additional family members with diagnosis of prostate, melanoma, or pancreatic cancer.
3. Breast and/or ovarian cancers in multiple generations on the same side of the family.
4. Bilateral breast cancer or breast and ovarian cancer in the same individual with a personal or family history of male breast cancer.

There are no specific features on physical examination suggestive of HBOC. Detailed information on the pathology of the cancer, including hormone receptor (estrogen/progesterone/Her2) status is helpful, whenever available. *BRCA1/2* variants are associated with invasive intraductal carcinoma. Invasive lobular carcinoma of the breast is associated with variants in *CDH1* gene leading to hereditary diffuse gastric cancer. *BRCA1* related cancers are more likely to be triple negative (ER – /PR – /Her2 –) [10]. The pathology of epithelial ovarian cancers, typically seen in *BRCA* carriers, is serous adenocarcinomas as well as non-mucinous types. Nonepithelial ovarian cancers (germ cell and sex cord-stromal tumors) are not associated with *BRCA1/2* variants. Ovarian low malignant potential (i.e., borderline epithelial ovarian tumors) and tubal intraepithelial carcinomas are also seen in *BRCA* variant carriers as are tumors of the fallopian tube and primary peritoneal tumors. There is also a twofold to sixfold increased risk of prostate cancer in *BRCA2* carriers [11], and these cancers have a more aggressive phenotype (Gleason ≥ 8), nodal involvement, and distant metastases. A higher risk of pancreatic cancer and melanoma, particularly ocular melanoma, is seen in *BRCA* carriers.

Genetic testing

Single gene testing for the *BRCA1/2* genes has been now largely replaced with panel-based multigene testing, with the advent of NGS technologies. Since there are no typical features distinguishing cancers associated with different genes, it is prudent to test a panel of genes that include the genes associated with the cancers found in the proband and his or her family. Different laboratories group genes in slightly different panels based on their requirements, and it is up to the ordering provider to determine which panel would be most appropriate based on the patient's personal and family history. Individuals with a known pathogenic variant in the family generally only need to be tested only for the familial variant, unless the family

history is suggestive of a possible risk for a hereditary cancer syndrome coming from the other side of the family as well. Individuals from Ashkenazi Jewish ancestries with a known familial variant should also be tested for the founder variants (187delAG and 5385insC in *BRCA1* and 6174delT in *BRCA2*). Whenever possible, testing an affected family member is optimal as they have the highest likelihood of carrying a pathogenic variant. Online tools such as Tyrer–Cuzick model, BRCAPRO, and the BOADICEA model can be used to determine likelihood of finding a pathogenic variant in the *BRCA1/2* genes and determining the risk of cancer.

Indications for genetic testing [12]:

- A. Personal history of breast cancer AND
 1. Diagnosed at the age of 45 years or younger
 2. Diagnosed with at least two breast cancer primaries (bilateral or two separate unilateral tumors, the first at the age of 50 years or younger)
 3. Diagnosed at the age of 50 years or younger AND one or more close relatives with breast cancer at any age, or pancreatic cancer or prostate cancer (Gleason ≥ 7 or metastases)
 4. Diagnosed with triple-negative breast cancer at the age of 60 years or younger
 5. Diagnosed at any age AND one or more close relatives with breast cancer at the age of 50 years or younger
 6. Diagnosed at any age AND two or more close relatives with breast cancer, pancreatic, prostate (Gleason ≥ 7) at any age
 7. Diagnosed at any age AND one or more close relatives with ovarian cancer at any age
 8. Close male relative with breast cancer at any age
- B. Personal history of ovarian cancer at any age
- C. Male breast cancer at any age
- D. Personal history of high-grade prostate cancer (Gleason ≥ 7) at any age with additional family history of at least one relative with ovarian cancer at any age, breast cancer at younger than 50 years, or two relatives with breast, pancreatic, or prostate cancer at any age.

Vignette 8.1

A 37-year-old Caucasian woman, JH, was recently diagnosed with invasive ductal carcinoma, ER + /PR + /Her2 – . She is referred for a genetics evaluation to determine optimal surgical management including bilateral mastectomy if found to have a genetic predisposition. On taking a detailed family history, you learn that her mother is alive at age 63 with no history of cancer (mother had TAH + BSO at age 45 because of endometriosis), her maternal aunt died from bilateral breast cancer at age 49, her maternal uncle had pancreatic cancer at age 70 and died a year later (he was also a heavy smoker), and her maternal grandfather had prostate cancer at age 63 and died at age 65 from metastatic disease. Her father is alive at age 65 and has a recent diagnosis of colon cancer and her paternal aunt died from ovarian cancer at age 69. Her paternal grandparents died of older ages and two of her paternal uncles are alive with no cancer. All of her cousins are alive and well with no history of cancer, as far as she knows. Do you think her history is suspicious for an inherited cancer syndrome?

JH meets National Comprehensive Cancer Network (NCCN) guidelines for genetic testing because of her personal history of breast cancer at a young age. Other factors to consider are as follows:

- *History of bilateral breast cancer, pancreatic cancer, and prostate cancer in maternal relatives is highly suspicious for HBOC (note: smoking is a risk factor for pancreatic cancer and the maternal uncle with pancreatic cancer could be a phenocopy; prostate cancer is the most common cancer in men, but the high-grade cancer at age 63 in her maternal grandfather is suspicious for a hereditary type of cancer).*
- *Mother does not have cancer, which could be due to reduced penetrance or modifying effects of bilateral oophorectomy and protection against breast cancer).*
- *Her father's history of colon cancer could be sporadic given that colon cancer is the third most common cancer worldwide but in the context of ovarian cancer in her paternal aunt, is now suspicious for Lynch syndrome. It is also possible that her father is a phenocopy and the ovarian cancer in the aunt is associated with HBOC. As her father only had one sister, there are not enough women in that family to express the disease.*

Genetic testing should include a panel of genes (BRCA1/2 for HBOC, pancreatic, TP53, Lynch syndrome genes, ovarian cancer genes, and prostate cancer genes).

Management

Screening for breast cancer is recommended and begins at an earlier age than recommended for the general population.

- Monthly breast exams should be started at age 18 with semiannual clinical breast exams from age 25. Annual breast MRI should begin at age 25 with annual mammogram from age 30. Mammograms and breast MRI should be done 6 months apart to ensure that imaging of the breast is done every 6 months. After age 75, management is considered on an individual basis. Men with *BRCA1/2* variants should also be screened with annual clinical breast exam from age 35 along with monthly self-breast exams. Regularly scheduled mammograms are not recommended due to lack of supportive data.
- Prostate cancer screening should begin at age 45.

Risk-reducing surgeries are recommended to reduce the risk of breast and ovarian cancer in women with HBOC. Bilateral risk-reducing mastectomy (RRM) reduces risk of breast cancer by at least 90%, while risk-reducing salpingo-oophorectomy (RRSO) reduces risk of ovarian cancer by 80%–90% and of breast cancer by 50% [13]. RRSO is recommended at age 35–40 in *BRCA1* carriers and at age 40–45 in *BRCA2* carriers since ovarian cancer onset tends to be later in women with *BRCA2*. Many genetic ovarian cancers appear to start in the fallopian tubes. Thus, there have been some recent trials of women with *BRCA1* and *BRCA2* variants having only the tubes removed and leaving the ovaries until a later date to avoid menopausal side effects. It remains to be seen if this will become a common practice [14]. Chemopreventive strategies include use of selective estrogen receptor modulators

(tamoxifen, raloxifene) and aromatase inhibitors, which are also known to reduce the risk of breast cancer by about 50%.

Genetic counseling

Once a diagnosis of HBOC is made in the family, other family members can be tested for the known familial variant.

Hereditary leiomyomatosis and renal cell carcinoma

Overview

Hereditary leiomyomatosis and renal cell carcinoma (HLRCC), also known as Reed syndrome, is a rare genodermatosis characterized by multiple cutaneous leiomyomas and uterine leiomyomas and leiomyosarcomas. It is also associated with an increased risk of renal cell carcinoma. Flesh-colored or pink-brown crops of papules or nodules appear on the skin of the trunk, extremities, and face in individuals in their 20s–40s. Biopsy confirms the diagnosis of leiomyomas. Rarely, these cutaneous leiomyomas may transform into leiomyosarcomas. Uterine leiomyomas, commonly known as fibroids, are present in the most women at a median age of 30 years and can cause dysmenorrhea, menstrual irregularities, and menorrhagia. About 15% of individuals with Reed syndrome develop papillary type renal cell carcinoma usually at a median age of 44 years.

Evaluation

Clinical diagnosis of HLRCC is likely when an individual meets the major criteria, which includes histopathologically confirmed multiple cutaneous leiomyomas. HLRCC may be suspected in individuals who have at least two minor criteria including symptomatic and/or multiple uterine leiomyomas before age 40; type 2 papillary renal cell carcinoma before age 40 and a first-degree relative who meets one of the above criteria. Differential diagnosis for renal cell cancers includes hereditary papillary renal cell cancer syndrome, Von Hippel Lindau syndrome and BHD syndrome. A definitive diagnosis is given when an individual has a positive germline *FH* variant [15].

Genetic testing

HLRCC is associated with pathogenic variants in the *FH* gene that encodes fumarate hydratase enzyme. Testing of the FH enzyme activity can be done in individuals who have an atypical presentation and an undetectable *FH* variant.

Management

There is no consensus on clinical surveillance, but the following can be considered in individuals with a known *FH* pathogenic variant:

1. Skin: Full skin examination by dermatology every 1–2 years.
2. Uterus: Annual gynecologic exam to evaluate uterine fibroids and its transformation to leiomyosarcomas.

3. Renal: Annual thin slice MRI of the kidneys.

Several studies have shown promise in the treatment of renal cancers with anti-vascular endothelial growth factor and novel tyrosine kinase inhibitor treatment.

Genetic counseling

HLRCC is an autosomal dominant disorder with reduced penetrance. Partner testing of *FH* for childbearing individuals is recommended as biallelic mutations in *FH* cause an inborn error of metabolism, fumarase deficiency/fumarate hydratase deficiency/fumaric aciduria associated with microcephaly, hypotonia, seizures developmental delay, and premature death.

Hereditary paraganglioma and pheochromocytoma

Overview

Tumors arising in the adrenal medulla are called pheochromocytomas (PCCs) while tumors arising in neuroendocrine tissues outside the adrenal medulla are called paragangliomas (PGLs). PCCs may occur at any age but are most commonly seen in the 40- to 50-year age group. PCCs presenting in young patients are more likely to be hereditary. PGLs can be parasympathetic, which occur predominantly in the head and neck or sympathetic, which are found mainly below the diaphragm. It is now recognized that 30%–40% of PCCs and PGLs are hereditary in nature. Germline variants in the genes encoding succinate dehydrogenase (SDH) enzymes are associated with hereditary paraganglioma and PCC syndrome. SDHx variants (*SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*) are also associated with renal cell carcinoma, gastrointestinal stromal tumors (GISTs), and pituitary adenoma. *MAX* variants are associated with predisposition to bilateral PCC with malignant potential. Variants in *RET*, *NF1*, and *VHL* are associated with risk for PCC, but all have numerous other manifestations and are addressed elsewhere.

Individuals with Carney triad (pulmonary chondroma, GIST, PGL) usually have somatic silencing of the *SDHC* gene through promoter hypermethylation, rather than a germline variant. Somatic variants in *EPAS1* are associated with PGL.

Evaluation

PCC and PGL can be functional or nonfunctional depending on whether they secrete catecholamines or not. Functional tumors that produce catecholamines lead to the classic triad of sweating, palpitations, and headache. It is now recommended that all patients with PGL/PCC undergo genetic evaluation and testing. The differential diagnosis includes multiple endocrine neoplasia, neurofibromatosis type 1, and von Hippel Lindau syndrome. Each of these disorders has specific characteristics, which can help in evaluating the patient and reaching a diagnosis.

Genetic testing

Testing is completed in the context of multigene panels with comprehensive evaluation of *MAX*, *SDHA*, *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, or *TMEM127* due to the multitude of genes associated with PGL/PCC/GIST.

Management

Every 2-year surveillance with full-body MRI (skull base through pelvis) and every 2-year biochemical screening (plasma metanephrines) are part of the evaluation for both nonsecreting and secreting PGL/PCC. There is no standardized evaluation for GIST, but there should be a low threshold for endoscopy and colonoscopy for iron deficiency anemia or gastrointestinal complaints.

Genetic counseling

While most of these are autosomal dominant disorders, *SDHD* is associated with a parent-of-origin effect and paternally inherited variants are largely associated with PGL/PCC predisposition. *SDHAF2* and *MAX* are suspected to exhibit similar parent-of-origin effects.

Biallelic mutations in *SDHx* complex genes are a rare cause of pediatric mitochondrial disease specifically isolated mitochondrial complex II deficiency. Due to the risk for a severe autosomal recessive disease in the case of the *SDH* complex genes, carrier testing in partners is recommended to inform reproductive risk. Tumor screening for at-risk individuals begins at the of age 6–8 years. Assisted reproductive technologies including preimplantation genetic screening is an option.

Li–Fraumeni syndrome

Overview

First described in 1969 by Drs. Fred Li and Joseph Fraumeni Jr. after review of medical records of kindreds affected by pediatric rhabdomyosarcoma, Li–Fraumeni syndrome (LFS) is an autosomal dominant, highly penetrant pleiotropic cancer susceptibility syndrome. In 1990 *TP53* variants were identified as the cause of LFS. LFS is classically characterized by exceptionally early age of onset of cancers and association with rare tumors including choroid plexus carcinomas, adrenocortical carcinomas, soft tissue and osteosarcomas, certain hematologic malignancies (hypodiploid acute lymphoblastic leukemia) as well as brain and breast cancers.

Evaluation

Individuals who meet all of the following criteria are said to have classic LFS:

1. Personal history of sarcoma diagnosed before age 45
2. A first-degree relative with any cancer before age 45
3. A first- or second-degree relative with any cancer before age 45 or a sarcoma at any age

While classic LFS is associated with high penetrance, there is greater recognition that this cancer susceptibility syndrome has variable expressivity and some families are

characterized by attenuated LFS phenotypes. The revised Chompret criteria include the following:

1. History of LFS associated tumor (e.g., soft tissue sarcoma, osteosarcoma, brain tumor, premenopausal breast cancer, adrenocortical carcinoma, leukemia, lung bronchoalveolar cancer) before age 46 AND at least one first-/second-degree relative with an LFS tumor before age 56 or with multiple tumors OR
2. History of multiple tumors (except multiple breast cancers), two of which belong to the LFS tumor spectrum and one of them occurred before age 46 OR
3. History of adrenocortical carcinoma or choroid plexus tumor, regardless of family history.

Cancer penetrance is lower in men than in women with LFS and thus individuals with a paternally inherited *TP53* variant may not demonstrate a typical family history. Due to the rare types of cancers associated with LFS, individuals are likely to misreport tumor types that would suggest LFS (e.g., sarcoma or ACC), rendering it difficult to make a diagnosis. In childhood, there are higher risks in boys, however; in early adulthood, risks for women are higher owing largely to the significant risk for breast cancer. Triple-positive breast cancers occur more frequently (*ER* + /*PR* + /*Her2* +) than in women without LFS.

Genetic testing

LFS is associated with pathogenic variants in the gene *TP53*. Loss of function variants, splice alterations, deletions, duplications, and missense variants have all been reported in LFS. *TP53* is on all cancer panel tests and should be evaluated in the context of a panel because it has overlapping features with a number of other hereditary cancer syndromes. The *de novo* variant rate for *TP53* is estimated to be 10%–20%. *TP53* results can be disproportionately affected by mosaicism due to clonal hematopoiesis of indeterminate significance (CHIP) especially in older individuals and those who have previously received systemic chemotherapy.

Management

Extensive surveillance protocols for LFS have been published by the AACR and NCCN. Cancer surveillance in LFS begins at birth.

- Birth: Annual whole-body MRI is recommended (including brain MRI). In nonrandomized studies, this has demonstrated a survival benefit.
- Childhood: Biochemical screening and imaging with ultrasound for ACC are recommended from birth and continued every 3–4 months.
- Adulthood: Annual whole-body MRI, annual physical exam with neurological exam, gastrointestinal surveillance with EGD and colonoscopy every 2–5 years beginning at age 25 and an annual skin exam with a dermatologist.
- For adult women, the following are recommended: breast awareness from 18 years old, annual breast MRI beginning at age 20–25, alternating with mammogram at 6-month

intervals from the age of 30 to 75 years, and consideration of bilateral preventive mastectomies.

- The utility of screening for ACC and for acute hematologic malignancies through blood work is unclear in adults. Therapeutic radiation therapy should be avoided when possible.

Genetic counseling

LFS is inherited in an autosomal dominant manner. Testing is recommended for everyone, irrespective of age as there are life-long implications that begin in early infancy. Psychosocial support is recommended, given the classically high cancer risks associated with LFS. Some families with LFS are characterized by significant loss, grief, pediatric illness, and feelings of guilt related to transmitting a pathogenic variant to their children. Assisted reproductive technologies including preimplantation genetic screening is an option for childbearing adults with LFS who are interested in reducing the risk of transmission.

Prostate cancer syndrome

Overview

Prostate cancer is the fifth most common malignancy in the world with a typical age of onset of over 65 years. Prostate cancer has always been thought to result from multiple factors including genetic and environment. With recent advances in NGS technologies, it has become evident that families with an increased predisposition to prostate cancer and individuals with advanced disease may have germline pathogenic variants. About 90% of metastatic castration-resistant prostate cancer contain a genetic alteration of which about 8%–12% changes are in the germline, leading to hereditary prostate cancer [16].

Evaluation

There are no consensus criteria for what defines a possible hereditary prostate cancer syndrome. Generally, any individual with age of onset of less than 55 years should be considered for genetic evaluation for a hereditary predisposition. American College of Medical Genetics & Genomics guidelines recommend that individuals who meet any one of the following criteria should be referred for genetic testing:

1. Three or more first-degree relatives with prostate cancer
2. Two or more first-degree relatives with prostate cancer diagnosed before age 55
3. Gleason grade more than 7 and family history of more than or equal to two individuals with breast, ovarian, or pancreatic cancer.

In addition to these criteria, NCCN recommends genetic evaluation for the following individuals as well:

1. History of Gleason grade more than or equal to 7 regardless of age and one or more close relative with breast cancer (onset at or less than age 50) and/or ovarian cancer
2. Personal history of metastatic prostate cancer

Genetic testing

Multigene panels are available from various laboratories that include the currently known causally related genes. These include *BRCA2*, *ATM*, *CHEK2*, *BRCA1*, *HOXB13*, *RAD51D*, *PALB2*, *ATR*, *NBN*, *PMS2*, *GEN1*, *MSH2*, *MSH6*, *RAD51C*, *MRE11A*, *BRIP1*, and *FAM175A*. Newer genes with preliminary evidence of prostate cancer predisposition continue to be discovered. Recently, several landmark studies have demonstrated that specific somatic alterations may predict response to chemotherapy in prostate cancer. Hence, experts recommend routine testing for all men with prostate cancer for germline and somatic variants [17].

Management

Screening for other associated cancers is recommended based on the gene identified to have a pathogenic variant. Screening guidelines are listed for those individual hereditary cancer syndromes associated with prostate cancer (e.g., Lynch syndrome in Chapter 13: Gastrointestinal disorders and Hereditary breast and ovarian cancer syndrome in this chapter).

PTEN hamartoma tumor syndrome includes Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome

Overview

Cowden syndrome (CS) is named after Rachel Cowden, the first patient who was reported to have multiple tumors including fibrocystic breast disease, a large multinodular goiter, oral papillomas, brain abnormalities, and dysmorphic facies [18]. It is characterized by numerous benign and malignant tumors of the thyroid, breast, endometrium, skin, and gastrointestinal tract and is associated with pathogenic variants in the *PTEN* gene. As several different phenotypes were found to be associated with *PTEN*, CS became a part of the *PTEN* hamartoma syndrome, which also includes Bannayan–Riley–Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome. Benign manifestations include macrocephaly, trichilemmomas, oropharyngeal papillomatous papules, intestinal ganglioneuromas, and esophageal glycogenic acanthosis. BRRS is a pediatric disorder characterized by macrocephaly, lipomas, hamartomatous GI polyps, vascular malformations, Hashimoto's thyroiditis, and penile freckling [19]. PS is an overgrowth syndrome characterized by multiple hamartomas, epidermal nevi, and hyperostoses. Proteus-like syndrome has some clinical features similar to PS, but these individuals do not meet the diagnostic criteria for PS.

Evaluation

Patients with multiple cancer diagnosis particularly breast and thyroid cancer or thyroid and endometrial cancer, in the same individual or within the same family, should prompt evaluation of CS. The diagnosis is not readily recognized in patients who mostly have the benign features. A strong suspicion for CS should arise in the presence of the following [20]:

1. Children with extreme macrocephaly ($> 5SD$) or macrocephaly and developmental delay or autism.
2. Endometrial cancer before age 30
3. Hamartomatous gastrointestinal polyposis
4. Glycogenic acanthosis
5. Lhermitte–Duclos disease (dysplastic cerebellar gangliocytoma)
6. Oral mucosal papillomatosis
7. Penile freckling

Physical exam should include head circumference ($> 97\%$) for age is considered macrocephaly (> 57 cm for female adults and > 60 cm for male adults) and investigate the presence of dolichocephaly (increased A-P diameter/length, best assessed by having patient turn head to the side), mucocutaneous stigmata (papillomatous papules), acral and plantar keratoses, and penile freckling. Differential diagnosis includes Peutz–Jeghers syndrome, Juvenile polyposis, and other overgrowth syndromes. The Cleveland Clinic PTEN Risk Calculation Tool can be used to calculate the individual's a priori risk to have a pathogenic variant in PTEN (<http://www.lerner.ccf.org/gmi/ccscore/>).

Genetic testing

Based on the patient's presentation and family history, a panel of genes is usually ordered that would include the *PTEN* gene. 10% of individuals with a Cowden phenotype do not have an identifiable *PTEN* sequence variant. While large deletions have been reported in individuals with CS, the exact prevalence is unknown. Hence, it becomes important to evaluate if the patient meets clinical diagnostic criteria.

Revised PTEN hamartoma tumor syndrome clinical diagnostic criteria (NCCN; [Table 8.3](#))

Operational diagnosis of CS

1. Three or more major criteria, but one must include macrocephaly, Lhermitte–Duclos, or GI hamartomas OR
2. Two major and three minor criteria

Management

Children with CS or family history of CS should have a neurodevelopmental evaluation. Management for adults with predisposition to different cancers includes enhanced screening with the goal of cancer prevention through prophylactic surgery or identifying tumors at early stages when they are most treatable. Prophylactic hysterectomy and mastectomy should be considered in women with PTEN variants once childbearing is complete. Screening recommendations for cancer include the following:

1. Breast cancer: Annual mammogram at age 30, alternate at 6-month intervals with annual breast MRI
2. Thyroid cancer: Annual ultrasound starting at age at diagnosis
3. Renal cancer: Renal imaging (CT/MRI) every 2 years

Table 8.3 Major and minor criteria for PTEN hamartoma syndrome.

Major criteria	Minor criteria
Breast cancer	Autism spectrum disorder
Endometrial cancer (epithelial)	Colon cancer
Thyroid cancer (follicular)	Esophageal glycogenic acanthoses (≥ 3)
GI hamartomas (≥ 3)	Lipomas (≥ 3)
Lhermitte–Duclos disease (adult)	Intellectual disability
Macrocephaly (≥ 97 th percentile—58 cm for women and 60 cm for men)	Renal cell carcinoma
Macular pigmentation of the glans penis	Testicular lipomatosis
Multiple mucocutaneous lesions (any of the following)	Thyroid cancer
• Multiple trichilemmomas (≥ 3)	
• Acral keratoses (≥ 3)	
• Mucocutaneous neuromas (≥ 3)	
• Oral papillomas (tongue, gingiva) (≥ 3)	
	Thyroid disease (adenoma, multinodular goiter)
	Vascular anomalies (multiple intracranial developmental venous anomalies)

4. Endometrial cancer: Annual endometrial biopsy or transvaginal ultrasound starting at age 30
5. Colorectal cancer: Colonoscopy starting at age 35–40 with frequency depending on polyp burden
6. Melanoma: Annual dermatologic exam starting from age at diagnosis

Genetic counseling

CS is inherited in an autosomal dominant pattern. The *de novo* mutation rate is unknown. Assisted reproductive technologies including preimplantation genetic screening is an option.

Points to remember

- Know when to suspect a hereditary cancer syndrome.
- Accurate family history information is key to identifying hereditary predisposition; negative family history is equally important as positive family history of cancer.
- Genetic testing involves multigene panels. It is important to have a differential diagnosis for different hereditary cancer syndromes so that genes associated with those syndromes are included in the panel.
- Management of hereditary cancer syndromes includes chemoprevention, prophylactic surgeries, and aggressive screening regimens.
- Diagnosis of a hereditary cancer syndrome is not only important for the management of the individual but also helps in identifying at-risk family members.

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Cardiac disorders

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Introduction

The field of cardiovascular genetics continues to experience rapid change as more genes that either directly cause or confer significant risk for cardiovascular disease are identified. Gene discoveries are then rapidly translated into clinically available genetic testing that advances the diagnosis of these conditions for patients and their at-risk relatives. Already, there are multiple examples of diseases where genetic testing leads to a diagnosis of specific genetic disorders, which in turn reveal prognostic information that may change medical management and help guide disease-directed therapies. For both the cardiologist and the generalist, these advances elevate the importance of understanding genetics, family history taking, genetic counseling, and genetic test interpretation as aspects of regular medical care. This chapter covers some of the more common and better understood cardiovascular genetic conditions and the lessons learned from these conditions. In each condition, a genetic evaluation that includes a multigeneration pedigree analysis and genetic counseling is typically recommended.

Cardiomyopathies

The traditional definition of cardiomyopathy is that it is a primary “disease of the heart muscle,” which occurs in the absence of any secondary causes. The European Society of Cardiology definition specifically excludes coronary artery disease (CAD), hypertension, valvular heart disease, and congenital heart disease that could independently explain myocardial impairment. The term “idiopathic cardiomyopathy” is still used to refer to primary cardiac muscle diseases although we now understand that a large proportion of these ‘idiopathic’ cases are actually due to underlying genetic variants.

Hypertrophic cardiomyopathy

Overview

Hypertrophic cardiomyopathy (HCM) is a well-known genetic cardiomyopathy, frequently linked to arrhythmia and sudden cardiac death (SCD) events in athletes. It is characterized by hypertrophy of the left ventricle (LV), usually in an asymmetric pattern, most commonly in the interventricular septum that is not otherwise explained by pressure or volume overload on the ventricle. The prevalence is 1 in 500 people [1], and approximately 50%–60% of HCM is caused by identifiable genetic mutations, most frequently in genes that encode various cardiac sarcomere proteins [2]. Less commonly, HCM occurs due to pathogenic variants in genes responsible for metabolic disorders (e.g., Anderson–Fabry disease, Danon disease), mitochondrial disorders, neuromuscular disease (Friedrich ataxia), and malformation syndromes (e.g., Noonan syndrome, Costello syndrome, Noonan syndrome with multiple lentiginos). Often, these latter disorders and syndromes present in childhood or adolescence although adult-onset presentations are also well described.

Evaluation

Although the classic presentation is that of syncope or SCD with exercise, many individuals with HCM are asymptomatic. Symptoms, when present, can include fatigue, angina, dyspnea, and palpitations. Syncope can result from arrhythmias or left ventricular outflow tract (LVOT) obstruction. The classic physical finding is a systolic ejection murmur that is heard best over the left sternal border and increases in amplitude with maneuvers that decrease preload such as Valsalva maneuver or standing up from a supine position. Electrocardiogram (ECG) findings, which may precede overt echocardiographic changes, typically show left-axis deviation of QRS complex, left ventricular hypertrophy, left atrial enlargement, or Q waves in the inferior-lateral leads. Giant T wave inversions in leads V2–V4 are suggestive of apical HCM [1]. A definitive diagnosis of HCM is most commonly made using transthoracic echocardiography (TTE) although cardiac magnetic resonance imaging (cMRI) is increasingly being used to confirm or stage the diagnosis. A LV wall thickness of >15 mm *that is not explained by pressure or volume overload conditions* such as hypertension or valvular heart disease is diagnostic of HCM [1]. An important distinction that can sometimes lead to clinical confusion is physiologic LV hypertrophy sometimes known as “athlete’s heart.” This is characterized by usually more symmetric LV hypertrophy that is typically <12 mm, whereas HCM tends to be more focal to certain areas of the LV, most often the septum.

Genetic testing

The majority of cases of HCM are inherited in an autosomal dominant manner with notable exceptions being Anderson–Fabry disease and Danon disease, both of which are inherited in an X-linked manner. A careful interpretation of a three- to four-generation family history can be instrumental in identifying inheritance patterns. The most common causative genes include *MYH7*, which codes for beta-myosin heavy chain, and *MYBPC3*, which codes

for myosin binding protein C; both inherited and *de novo* variants in these genes have been reported [3]. Genes that have been less commonly implicated in causing HCM include *CSRP3*, *ACTN2*, *TCAP*, *ANKRD1*, and *JPH2* [3]. Histologically, pathogenic variants that cause HCM lead to myocyte disarray and fibrosis [2]. Genetic testing is recommended to confirm a molecular diagnosis and provide an opportunity to screen family members. In some instances, diagnostic genetic testing reveals treatment options (e.g., enzyme replacement therapy for Anderson–Fabry disease or Pompe disease) and, increasingly, access to clinical trials. If an affected individual has an identified disease-causing variant, then genetic testing is typically offered to living first-degree relatives. Older children and adults found to carry a pathogenic variant should then be referred for genetic and cardiac evaluations including ECG and TTE. When genetic testing is unrevealing (or not performed), living at-risk relatives (typically first-degree relatives) are recommended to undergo ECG and TTE testing. Relatives who have normal studies and remain at risk of developing disease are typically advised to undergo serial screening every 1–5 years depending on the age of the relatives and according to the published guidelines. For instance, one set of current recommendations is that at-risk children be re-screened every 12–18 months and that at-risk adults be re-screened every 5 years; decisions on excluding at-risk patients from competitive sports and implantation of cardioverter–defibrillator are best made on an individualized basis [4].

Vignette 9.1

RT is an otherwise very healthy 21-year-old male college football player, without any known significant medical history, who collapsed on the field during practice and was found to be pulseless. Luckily cardiopulmonary resuscitation and cardioversion were initiated promptly, which restored the player's pulse. Following admission to an intensive care unit, he was fortunate enough to wake up with normal neurologic function and was deemed medically stable. Echocardiographic studies diagnosed HCM with a septal wall thickness of 16 mm and a family history analysis noted a maternal aunt who died unexpectedly before the age of 30 years while hiking.

Management

Medical management is best coordinated by a cardiologist who is familiar with HCM and includes beta-blocker therapy when hemodynamically significant (>50 mmHg) LVOT obstruction is present. Verapamil and disopyramide are second- and third-line options, respectively. For patients with persistent heart failure or syncope despite maximally tolerated medical therapy, invasive options such as septal ablation or myectomy can be considered. HCM can be complicated by congestive heart failure as well as atrial fibrillation. An important consideration in all patients with HCM is prevention of SCD. It is strongly recommended that any individual who carries this diagnosis abstains from all competitive sports and intense physical activity. Any patient with a history of cardiac arrest or unstable ventricular arrhythmia such as ventricular tachycardia (VT) or ventricular fibrillation (VF) should be

considered for the placement of an implantable cardiac defibrillator (ICD; secondary prevention). The decision on whether to place an ICD for primary prevention is more complicated. A HCM-risk SCD calculator, developed by the European Society of Cardiology, exists to help clinicians risk-stratify patients (<http://www.doc2do.com/hcm/webHCM.html>).

Dilated cardiomyopathy

Overview

Genetic forms of dilated cardiomyopathy (DCM) are characterized by dilation of the LV and progressive systolic dysfunction. Early studies estimated the prevalence to be 1 in 2500 to 1 in 12,000; however, this is thought to be an underestimation, and more current analyses suggest a prevalence between 1 in 250 and 1 in 500 [5]. Several nongenetic conditions can also generate a similar clinical phenotype, most notably, viral infection, autoimmune conditions, toxins (such as cocaine, amphetamines, and chemotherapy drugs), prolonged tachycardia, and nutritional deficiencies, among others.

Evaluation

The clinical presentation of genetic forms of DCM includes symptoms of heart failure and/or arrhythmias in a younger individual without obvious risk factors. Physical exam findings may include peripheral edema, bibasilar rales, a third heart sound, jugular venous distension, and systolic heart murmur (as both functional mitral and tricuspid regurgitation can develop due to dilation of the ventricles). ECG findings are generally nonspecific but may show left ventricular hypertrophy, left atrial enlargement, and left bundle branch block, among others. The most valuable initial diagnostic test is the TTE and diagnostic criteria for DCM include an LV ejection fraction (LVEF) <45% and LV end-diastolic diameter >117% of the predicted value for age and gender. An analysis of serum creatinine kinase can be helpful, as some genetic forms of DCM manifest concomitant skeletal muscle involvement.

Genetic testing

As with HCM, most forms of DCM are also inherited in an autosomal dominant pattern [5]. From a clinical standpoint, the diagnostic criteria required to identify DCM as “familial” are two or more family members who also meet clinical criteria for diagnosis of DCM or a first-degree relative who died prior to age 35 years without any known explanation. In the group that meets the diagnostic criteria, only about 40% have an identifiable pathogenic variant [5], which suggests that many genes that cause DCM are yet to be discovered. The most common gene (about 20%–25% of familial DCM) in which pathogenic variants are found in DCM is *TTN*, which encodes titin and can be transmitted in an autosomal dominant (more common) or autosomal recessive manner. Other relatively common causes include pathogenic variants in *LMNA* coding for lamin (5%–8%, AD) and *SCN5A* coding for a voltage-gated sodium channel. Consequently, the genetics of DCM is considerably more complex and less well established when compared to HCM, with immense heterogeneity of over 50 identified DCM genes (Table 9.1) [6]. The genes implicated in DCM do not aggregate around a central molecular

Table 9.1 Genes associated with cardiomyopathy. Particularly well-known genetic causes of a particular cardiomyopathy are listed in red [3,5,7,8].

	HCM	DCM	ARVC	LVNC
% yield of genetic testing	50-60%	30-40%	50%	15-20%
Genes				
ACTC1	+	+		+
ACTN2	+	+		
ANKRD1	+			
BAG3		+		
CSRP3	+	+		
DES		+	+	
DSC2			+	
DSG2		+	+	
DSP		+	+	
GLA	+			
JPH2	+			
JUP			+	
LAMP2	+	+		
LMNA	+	+	+	
MYBPC3	+	+		+
MYH7	+	+		+
MYL2	+			
MYL3	+			
PKP2			+	
PLN	+	+	+	
PRKAG2	+			
RBM20		+		
RYR2			+	
SCN5A		+	+	
TAZ		+		+
TCAP	+	+		
TMEM43			+	
TMPM1	+			
TNNC1	+	+		
TNNI3	+	+		
TNNT2	+	+		+
TTN		+	+	
TTR	+			
ZASP/LBD3				+

biological pathway (such as the sarcomere unit in HCM or the desmosomes as seen in arrhythmogenic right ventricular cardiomyopathy [ARVC]). Pathogenic variants in numerous genes that are required for components of the sarcomere, Z-disk, cytoskeleton, nuclear skeleton, mitochondria, desmosomes, ion channels, and lysosomal membranes can cause DCM. Similar to HCM, genetic testing is recommended, and at-risk relatives should be evaluated by genetic testing (if available) and serial ECG and echocardiographic screening.

Management

The management of DCM is primarily related to the treatment of congestive heart failure and atrial/ventricular arrhythmias. Medications that are used to treat heart failure are

typically used and some patients may experience an initial substantial improvement (or even near recovery) that is less typical than of heart failure due to ischemic factors. Patients with LVEFs < 35% should be considered for ICD implantation as well as patients with highly arrhythmogenic gene mutations (e.g., *LMNA* and *FLNC*) due to the high risk of arrhythmias even with relatively preserved ejection fractions.

Arrhythmogenic right ventricular cardiomyopathy

Overview

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized primarily by dysfunction and dilation of the right ventricle (RV), as well as ventricular arrhythmias that originate from the RV. From a molecular biology and histopathology standpoint, ARVC is caused by pathogenic variants in genes coding for desmosomal proteins that lead to fibrofatty infiltration of the myocardium, primarily of the RV (although some variants can have more significant involvement of the LV) leading to scarring. The prevalence is estimated at 1 in 5,000 overall but as high as 1 in 2,000 in some European countries such as Italy and Germany [7].

Evaluation

Patients with ARVC typically present with arrhythmias often prior to heart failure and the first clinical manifestation can be syncope or even SCD. Classically, the ventricular arrhythmias associated with ARVC are triggered or worsened by adrenergic stimulation and thus can also be precipitated by physical or emotional exertion. Less severe presentations include palpitations or syncope. Physical exam findings can be minimal, and imaging and ECG studies are required for diagnosis. ECG findings that are associated with ARVC include epsilon waves and inverted T waves in the precordial leads [7]. Signal-averaged ECG analysis and arrhythmia monitoring are also helpful in assessment, and cMRI is considered standard in the evaluation. Biopsy findings can be diagnostic although they are not always performed and suffer from low sensitivity due to the patchy nature of the fibrosis and fibrofatty infiltration. Consensus diagnostic criteria have been proposed, and due to their complexity, the assessment of possible ARVC may be best performed by providers familiar with the condition and the criteria. Family history can be suggestive based on SCD and an autosomal dominant inheritance pattern; low penetrance confounds the recognition of ARVC in many families. Genetic testing has limited sensitivity, and current ARVC testing panels target genes encoding desmosomal proteins. The most common gene implicated is *PKP2*, which codes for plakophilin 2. Pathogenic variants in other genes commonly encountered include desmoplakin, desmoglein 2, and desmocollin 2. All known forms of ARVC are inherited in an AD fashion [7]. Roughly 50% of patients who meet clinical criteria for ARVC diagnosis will have an identifiable pathogenic variant.

Management

Although the data do not show conclusive mortality benefits, beta-blockers are generally prescribed to reduce arrhythmia risk and to reduce RV pathologic remodeling. Data are limited

for utility of adding anti-arrhythmic agents; however, amiodarone is sometimes used for treatment of symptomatic palpitations. Catheter ablation can be a palliative measure for persistent arrhythmias; however, it should not be thought of as a curative therapy as the progressive nature of ARVC tends to create new arrhythmogenic foci. As with any other indication, an ICD should be placed for secondary prevention in a patient with sustained or malignant ventricular arrhythmias. Primary prevention in individuals who harbor disease-causing variants in the absence of symptoms may be considered although consensus recommendations are largely lacking. Heart failure, when present, is approached using the standard treatment regimens.

Other cardiomyopathies

Restrictive cardiomyopathy

Other than the transthyretin (TTR) amyloidosis phenotype (discussed later in this chapter), restrictive cardiomyopathy (RCM) tends to be acquired or secondary resulting from infiltrative diseases, most commonly amyloidosis, sarcoidosis, hemochromatosis, diabetes, connective tissue disease, toxins, or radiation toxicity.

LV noncompaction

Some debate in the literature exists as to whether LV noncompaction (LVNC) is truly a unique cardiomyopathy or a phenotypic subtype of other cardiomyopathies. LVNC is characterized by a “spongy” appearance of the myocardium on imaging with prominent trabeculae and deep intertrabecular recesses and the presence of two distinct layers of compacted and noncompacted myocardial tissue. Originally the pathophysiology was thought to be driven by intrauterine defects in compaction of the myocardium; however, the tendency to progress and regress in adulthood makes it more likely to be an aspect of cardiac remodeling as seen in various other cardiomyopathies. The exact prevalence is unknown, but it is substantially less common than HCM and DCM. Frequently, LVNC is found in individuals who have family members afflicted with the more common HCM and DCM phenotypes, thus suggesting that there is some degree of overlap between these conditions. A genetic etiology can be identified in 15%–20% of patients with LVNC with the most commonly affected genes being those that are known to cause DCM (*TNNT2*, *ACTC1*, *TAZ*) and HCM (*MYH7*, *MYBPC3*) [3].

Peripartum cardiomyopathy

Peripartum cardiomyopathy that develops in women during late pregnancy or early postpartum period was originally considered to be a nongenetic disorder, which was distinct from DCM. The identification of pathogenic variants in genes that cause DCM in individuals with peripartum cardiomyopathy now supports a model whereby an underlying genetic risk is expressed during the stress of a pregnancy resulting in a DCM phenotype. The incidence estimates vary widely geographically with incidence in countries such as Nigeria and Haiti being between 1 in 100 and 1 in 300, to as low as 1 in 4000 in Europe and North America [9].

Inherited arrhythmogenic disorders

The group of diseases called inherited arrhythmogenic disorders (IADs) is a common cause of syncope and SCD in the young. Pathophysiology varies by condition, but they are typically caused by genetic defects that affect the function of ion channels that regulate sodium, potassium, or calcium handling, which are critical for normal cardiac contractility. Unfortunately, it is not uncommon for SCD, often in childhood, to be the first manifestation in an individual with IAD. The most common of these disorders is grouped into long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia (CPVT).

Long QT syndrome

Overview

LQTS is typically caused by either gain of function in sodium channels or loss of function in potassium channels, leading to delayed cardiac repolarization that manifests as an elongated corrected QT (QTc) interval on ECG. The prevalence has been estimated at 1 in 2500 [10]. The diagnosis is made with a QTc >500 ms (or 450–480 ms with other ECG findings and risk factors) in the absence of other factors that lengthen the QTc interval. Environmental factors can interact with the genetic defect as medications and electrolyte disorders can induce arrhythmias in mutation carriers.

Evaluation

Several different subtypes of LQTS were described clinically before causative genes were discovered; today it is more common to use genetic data to classify the subtypes. LQT1 is caused by pathogenic variants in *KCNQ1* that encodes for a potassium channel subunit. Mutations in this gene are responsible for 30%–35% of positive tests. Typically, arrhythmias are triggered by adrenergic stimulation (either physical or emotional) [10]. LQT2 is caused by pathogenic variants in *KCNH2*, which also encodes a potassium channel subunit. Pathogenic variants in this gene are responsible for 25%–40% of positive tests. LQT2 has the widest range of known triggers, including adrenergic stimulation, sleep, and the unique trigger of loud noises. LQT3 is caused by pathogenic variants in *SCN5A* (note that this gene is also implicated in DCM). This is responsible for 5%–10% of positive tests. Arrhythmias are most often triggered during sleep.

Genetic evaluation

To date, pathogenic variants in at least 15 genes have been known to cause this condition. Although the list is constantly growing, the vast majority of LQTS is associated with three genes, each of which leads to a somewhat unique phenotype designated as a subtype of LQTS as LQT1–3. Overall, about 80% of patients with the clinical diagnosis of LQTS will have an identifiable pathogenic variant with the currently used multigene panels [11].

Typically, LQTS is inherited in an autosomal dominant manner (one notable exception is Jervell and Lange–Nielsen syndrome) with incomplete penetrance, which can make pedigree analysis difficult. *De novo* variants are rare. Genetic testing is recommended for all patients with a clinical diagnosis of LQTS, for all individuals with a high pretest probability of the diagnosis wherein such testing would potentially confirm the diagnosis, and cascade testing in the first-degree relatives of affected individuals.

Vignette 9.2

RZ is an otherwise healthy 12-year-old male who was witnessed to have passed out during cross-country running practice. After a short period of unresponsiveness, he awakens and is alert and aware of his surroundings. Initial diagnostic evaluation in the emergency department is unremarkable. The patient does not have any history of similar events. The family denies any known history of “heart problems,” but careful evaluation does note a maternal cousin who died in his sleep in childhood and another cousin who drowned while swimming in open water at the age of 14 years.

Management

From a clinical management standpoint, one of the mainstays is to avoid competitive sports, rigorous exercise, QTc prolonging medications, and hypokalemia. Beta-blockers have been shown to have significant reduction in risk of SCD [11], but interestingly, this benefit is affected by genotype; individuals with LQT1 patients have the best response to beta-blockers and LQT3 patients have the least benefit [12]. Recent studies have shown that in patients with the LQT3 genotype who do not respond to beta-blockers, there is a reduction in the risk of SCD gained from using mexiletine (a class 1b sodium channel blocker) [12]. SCD prevention with ICD therapies are often considered in symptomatic patients, whereas recommendations for ICD in asymptomatic genotype-positive individuals is more complicated. Factors associated with higher SCD risks include QTc >500 ms despite medical therapy, female gender, recurrent syncope despite medical therapy, and pathogenic variants in the *SCN5A* gene. For patients with an ICD who are still experiencing frequent but appropriate shocks, despite optimizing medical therapy, can benefit from a left cardiac sympathetic denervation (LCSD) that decreases adrenergic input from the sympathetic network and limits the triggering of malignant arrhythmias.

A special case of LQTS is Jervell and Lange–Nielsen syndrome. This is caused by biallelic pathogenic variants in *KCNQ1* or *KCNE1* and has an autosomal recessive inheritance pattern. It is also associated with congenital sensorineural hearing loss and can present with severe arrhythmias at a very young age. Patients with this condition are considered to be at very high risk for arrhythmias and typically get an ICD placed early in the disease course.

Short QT syndrome

SQTS is characterized by an abnormally fast cardiac tissue repolarization and a short QTc interval. It is uncommon, and the prevalence has not been well estimated. There is significant overlap in the causative genes with LQTS; however, the mutation has an opposing effect to that observed in LQTS leading to a shortened QT interval pathophysiology. The most commonly implicated genes are *KCNH2*, *KCNQ1*, and *KCNJ2*. Clinical presentation is often with SCD but can include syncope and atrial fibrillation. The characteristic ECG findings are a QTc <320 ms, peaked T waves in the precordial leads, and evidence of early repolarization in the ST segment [11]. Given the rarity of this condition, there is a paucity of data on management strategies, recommendations for genetic screening, and ICD placement. Some hypothesize that using QTc prolonging medications (such as quinidine, sotalol, amiodarone, and propafenone) may be beneficial, but there are no large-scale data on the efficacy of this strategy.

Brugada syndrome

Brugada syndrome is the combination of a characteristic Brugada ECG pattern with clinical manifestation of a resulting arrhythmia. The prevalence is estimated at 1 in 5000 and 1 in 10,000 in the Western world and likely higher in South Asia [3]. To date, mutations in over 20 genes have been discovered to cause this disorder. Most exhibit autosomal dominant inheritance with variable expressivity and penetrance (exception to this is *KCNE5*, which is inherited in an X-linked manner). *De novo* variants are estimated at 1%. The earliest gene discovered to be related to Brugada syndrome was *SCN5A* (previously discussed as it relates to DCM and LQT3) and is still the most commonly mutated gene in the condition; however, it only accounts for about 20% of Brugada syndrome [11]. Other genes that have been associated with up to 5% of Brugada syndrome include *CACNA1c*, *CACNB2b*, *TRPM4*, *SCN10A*, and *ABCC9* [12]. The classic characteristic ECG findings (referred to as type 1 Brugada ECG) are a coved and down sloping ST elevation of >2 mm followed by T wave inversions in at least two of the right-sided precordial leads (V1–V3).

Similar ECGs are referred to as group 2 or 3 and can be diagnostic of Brugada pattern if they convert to type 1 with administration of a sodium channel blocker such as flecainide. In order to diagnose Brugada syndrome (rather than just Brugada pattern ECG), the patient should have a personal history of VT or VF, syncope, nocturnal agonal respiration or a family history of SCD in an individual <45 years of age or a type 1 ECG. Patients with Brugada syndrome should avoid sodium channel blocking medications such as class 1a (with the exception of quinidine that may be beneficial) and 1c antiarrhythmics, some antipsychotics and antidepressants (e.g., amitriptyline), and some anesthetic agents (e.g., bupivacaine). Also, fevers have been shown to trigger arrhythmias and must be treated promptly with antipyretics. Generally, most patients who meet criteria for Brugada syndrome are at high risk of arrhythmia and should receive ICD placement. In cases where the diagnosis is suggested solely by the ECG pattern and family history, ICD placement is more controversial;

electrophysiology studies are also used in challenging cases to guide IDC use. Genetic testing of patients with the diagnosis and targeted cascade screening of first-degree relatives is recommended if a pathogenic variant is found.

Catecholaminergic polymorphic ventricular tachycardia

Unlike the IADs previously discussed, catecholaminergic polymorphic ventricular tachycardia (CPVT) is distinct in that patients have normal resting ECGs and are prone to polymorphic ventricular arrhythmias with adrenergic stimulation. Pathogenic variants that affect the function of calcium channel rather than sodium or potassium channels seen in LQTS, SQTS, and Brugada cause CPVT. The prevalence of CPVT is estimated to be 1 in 10,000. The two most common genes in which variants are found include *RYR2*, which codes for a ryanodine receptor and has autosomal dominant inheritance, and *CASQ2*, which codes for a sarcoplasmic reticulum protein and has autosomal recessive inheritance patterns (Table 9.2). Due to the normal resting ECG, CPVT requires an exercise or pharmacologic stress test to elucidate the characteristic arrhythmias and diagnose the condition. The mainstay of management for CPVT is noncardiac selective beta-blockers, typically nadolol or propranolol, which blunt the adrenergic tone that triggers the arrhythmias. For patients who are resistant to treatment with beta-blockers during the diagnostic stress test, the addition of flecainide (a 1c Na channel blocker) has been shown to decrease the risk of SCD [12]. Competitive sports and exercise should be avoided. In addition to secondary SCD prevention, ICDs are indicated in patients who have recurrent syncope in spite of optimal medical therapy or in individuals who cannot tolerate medical therapy. LCSD can also be a consideration for these patients [11]. Genetic testing is recommended for all patients who meet clinical criteria for CPVT and if causative mutations are found, then cascade testing of first-degree relatives is indicated.

Familial hypercholesterolemia

Overview

Genetic contributions to lipid levels continue to be discovered and understood, driving the development of new strategies to better manage lipid disorders. Here, we discuss familial hypercholesterolemia (FH), the best-known example of a Mendelian lipid disorder. FH is a common, autosomal codominantly inherited genetic disease that leads to abnormally high levels of circulating low density lipoproteins (LDLs) cholesterol and early development of CAD among the other cardiovascular diseases. The common form is the heterozygous form where an individual has one defective copy of a gene involved in cholesterol metabolism and has a prevalence between 1 in 250 and 1 in 500 [13]. These patients tend to develop premature cardiovascular disease in their middle age. Homozygotes (1:160,000–125,000; history of familial hypercholesterolemia (HoFH)) are encountered more rarely, and due to having a pathogenic variant of both alleles, have a more severe phenotype [13]. They develop cardiovascular disease that is clinically apparent by the teenage years, sometimes earlier.

Table 9.2 Genes associated with different IADs. Particularly well-known causes are noted in *red* [3,11,12].

	LQTS	SQTS	Brugada	CPVT
% yield of genetic testing	80%	20%	35%	65%
Genes				
ABCC9			+	
AKAP9	+			
ANK2	+			
CACNA1c	+	+	+	
CACNA2D1		+		
CACNB2		+	+	
CALM	+			+
CASQ2				+
CAV3	+			
GPD1L			+	
HCN4			+	
HEY2			+	
KCNE1	+			
KCNE2	+			
KCNE3			+	
KCNH2	+	+		
KCNJ2	+	+		
KCNJ5	+			
KCNQ1	+	+		
PKP2			+	
RYR2				+
SCN10A			+	
SCN1B			+	
SCN3B			+	
SCN4B	+			
SCN5A	+		+	
SNTA1	+			
TRDN	+			+
TRPM4			+	

Evaluation

The diagnosis of HoFH should be strongly suspected with LDL levels of >500 mg/dl (untreated) and the appearance of cutaneous xanthomas prior to the age of 10 years. Suggestive physical exam findings of FH include corneal arcus (white lines of cholesterol and lipids surrounding the cornea) and xanthoma (yellowish plaques that tend to develop on the

eyelids, Achilles tendon, and elbows). Interdigital xanthomata are virtually pathognomonic for HoFH. There are multiple different published criteria used to confirm the diagnosis of FH, which rely on a combination of [13] LDL >190 mg/dL (untreated) in adults, >160 mg/dL in children, premature CAD or other cardiovascular disease, physical exam manifestations described above, family history of premature cardiovascular disease, and/or positive genetic testing.

Genetic testing

Pathogenic variants in three genes cause the vast majority of FH; the most common of which is *LDLR* that normally encodes the LDL receptor and is responsible for 60%–80% of FH (Table 9.3) [14]. Pathogenic variants in the *APOB* gene that leads to a defective apolipoprotein B and an inability for the LDL receptor to take up LDL-C are responsible for 1%–5% of FH. Finally, a gain-of-function variant in the *PCSK9* gene leads to the overexpression of the PCSK9 protein and overdegradation of the LDL receptor and is responsible for <3% of cases overall. Interestingly, different *PCSK9* variants cause low LDL levels and are protective against cardiovascular disease and drugs that block PCSK9 protein expression have been developed. Around 20%–40% of individuals with FH do not have an identifiable genetic variant and are likely caused by variants in genes that are not yet discovered.

When the diagnosis is confirmed in the proband, screening of family (both with LDL levels and targeted genetic screening when available) should be offered to at-risk relatives due to the severity of the condition and the ability to make meaningful interventions.

Vignette 9.3

KM, a 34-year-old man who hasn't seen a physician since childhood develops sudden onset, crushing substernal chest pain that radiates into his jaw. He drives himself to the emergency department where he is found to have an ST elevation myocardial infarction and is rushed to the cardiac catheterization lab where he is found to have severe CAD in all three of his major vessels. When asked about family history, he reveals "everyone" on his father's side of the family has a "bad heart" and that a paternal uncle also had a leg amputated. Physical exam reveals yellowish plaques on his eyelids that he states have been present since his twenties and a white ring around the outer aspect of his iris. A cholesterol panel reveals an LDL cholesterol level of 336, suggestive of FH.

Table 9.3 Genes associated with familial hypercholesterolemia (From GeneReviews) [14].

Familial hypocholesterolemia genes	Proportion of cases
<i>APOB</i>	1%–5%
<i>LDLR</i>	60%–80%
<i>PCSK9</i>	0%–3%
Unknown	20%–40%

Management

Management of this condition includes aggressive lipid lowering therapy, first with high intensity HMG-CoA reductase inhibitors (statins) such as atorvastatin or rosuvastatin with a goal of reducing LDL levels by 50% or <100 . If goal is not met in 3 months, typically the second line agent is ezetimibe; PCSK9 inhibitors are FDA approved in the United States and are increasingly being used as alternative therapy, particularly in those individuals with statin intolerance or those who are refractory to the standard therapies. For cases that are resistant to all pharmacologic methods (usually HoFH patients), LDL apheresis and even liver transplantation are extreme treatment options [13].

Tranthyretin amyloidosis

Overview

Amyloidosis is a pathophysiological process by which misfolded proteins form beta-pleated sheets and become deposited in various tissues. The heart, kidneys, liver, and nervous system are most often affected, but any organ system can be involved. A genetic form of amyloidosis occurs due to pathogenic variants in the *TTR* gene. *TTR* variants, of which there are >100 described, lead to abnormal TTR protein accumulation and cardiac amyloidosis [15]. Normal, “wild-type” TTR protein sometimes accumulates in healthy individuals without *TTR* variants. This is a natural, slow occurring process, which can lead to development of wild-type amyloidosis (also known as senile systemic amyloidosis) that is estimated to be present in 25%–36% of people over the age of 80 years [15] and is often found incidentally at autopsy. In the case of TTR amyloidosis, neuropathy and nephropathy are the most common clinical manifestations in addition to the cardiomyopathy.

Evaluation

Clinically, cardiac amyloidosis includes RCM leading to heart failure, angina, and even acute coronary syndrome despite normal epicardial coronary vessels, and electrical conduction system disease (includes atrioventricular block, sick sinus syndrome, atrial fibrillation, ventricular arrhythmias, and SCD). Interestingly, the prognosis for hereditary TTR cardiac amyloidosis is significantly better than for AL cardiac amyloidosis caused by immunoglobulin light chain deposition with a median survival time of 27 versus 5 months, respectively [15]. Classic ECG findings for cardiac amyloidosis include low voltage in all leads and a “pseudo-infarction” pattern that is characterized by pathologic Q waves, most often in the inferior and septal leads despite a lack of CAD [15]. The echocardiogram and cMRI findings for amyloidosis include increased LV wall thickness with diastolic dysfunction, evidence of infiltrative disease (often more global than other cardiomyopathies) and often a small pericardial effusion [15]. To formally make the diagnosis, a tissue biopsy is required; if a patient has all the signs of cardiac amyloidosis, the biopsy can be from an extracardiac site (most often abdominal fat pad, rectum, kidney). However, if the diagnosis is more uncertain, a cardiac

biopsy is preferred (the diagnostic sensitivity from biopsies of the RV septum is nearly 100% due to the diffuse nature of the disease) [15]. Genetic testing complements the biopsy workup and when pathogenic variants are detected, genetic counseling and testing of relatives are indicated.

Genetic testing

Genotype–phenotype relationships exist in this disease as certain variants cause different sets of symptoms, some of which tend to cause more cardiac involvement than others [15]. For example, the p.Val122Ile variant is well established as being common in the African-American population (estimated that 3.4% of this population carries the mutation) and is likely responsible for the significantly higher prevalence of cardiac amyloidosis in this population [15]. Other important cardiac *TTR* variants are the Thr60Ala (common in the Appalachian valley in families of Irish descent), p.Leu111Met (common in those with Danish background), and p.Ile68Leu (common in those with Italian background) mutations [16].

Management

An understanding of the molecular basis of disease has led to exciting novel therapies. Tafamidis is a small molecule stabilizer of the *TTR* tetramer and reduces amyloid formation, thereby slowing the disease progression. There are data to suggest tafamidis confers both a mortality benefit and a reduction in hospitalizations and an improvement in quality of life for patients with *TTR* cardiac amyloidosis [17]. For many phenotypes of *TTR* amyloidosis, particularly neuropathy, liver transplantation is offered as a way of removing the source of amyloidogenic protein production. Liver transplantation needs to be done early as some studies have shown that cardiomyopathy progression continues and that in some cases, consideration can be given to combined heart and liver transplant [15].

CAD risk and 9p21

A family history of CAD, particularly at a young age, is a well-established risk factor for its development. Several large genetic studies have tried to identify the genetic risks of CAD that presumably contribute to the family history risk. Single-gene models like FH apply to rare cases and have largely not been revealing for common forms of CAD. At this time, over 160 genetic markers have been identified to have some degree of predictive value on an individual's risk of CAD [18]. The most well-known and well-researched is the locus on 9p21. Multiple studies have shown a statistically significant relationship between allele variant in this locus and the risk of developing CAD in those without known disease with hazard ratios ranging from 1.19 to 1.32 [19,20]. While this association is interesting, the pathophysiologic mechanism of this relationship remains poorly understood, as the variants tend to be far from any protein-coding genes [19]. The clinical usefulness of this relationship has also been brought into question by the failure of adding this information to traditional risk factor based models to improve the model's ability to accurately predict risk of developing CAD [20].

A meta-analysis in 2014 demonstrated that variations in the 9p21 locus did not predict who would have subsequent CAD events in those already diagnosed with the disease [19]. This finding may suggest that 9p21 is somehow related to the development of atherosclerotic plaque over time but has limited, if any, role in acute plaque rupture or that treatment for CAD (whether it be statin or antiplatelet therapy) effectively suppresses any risk added by 9p21 variation.

Points to remember

- Several genetic variants can either directly cause cardiovascular disease or place a patient at the greater risk of developing disease.
- A detailed, three-generation family history is paramount to screening for potential genetic contribution to cardiovascular disease.
- In many cases, widely available genetic testing can impact the care of the patient as well as screen their family members for risk of disease.
- Pretest counseling is an important ethical consideration before any genetic testing.

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Hereditary connective tissue disorders

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Introduction

Individuals with connective tissue features such as extensive joint hypermobility, pectus deformity, scoliosis, pneumothoraces, clubfoot or contractures, cleft palate, or recurrent hernias warrant initial ophthalmology evaluation (assessing for ectopia lentis, astigmatism, myopia, glaucoma, and/or vitreous changes) and echocardiogram (assessing for valve disease such as mitral valve prolapse or bicuspid aortic valve (BAV) and thoracic aortic aneurysms). A thorough family history assessing for similar musculoskeletal features and history of sudden unexplained death and/or aortic/arterial aneurysm or dissection is recommended. Thoracic aortic aneurysm disease or abdominal aneurysm in an individual or family member of older age, with presence of smoking, hypertension, or cholesterol issues, may suggest an environmental rather than genetic etiology. Isolated, nonspecific musculoskeletal features may indicate a familial “body habitus,” but more significant disease in the presence of personal or family history of aneurysmal disease warrants genetic consultation for consideration of genetic testing. Isolated cases of aneurysm or significant connective tissue features in a family could also represent a genetic etiology, as many cases present with *de novo* variants. Typically, panel-based genetic testing (aortopathy or heritable disorders of connective tissue [HDCT]) is the most cost-effective and comprehensive testing strategy as phenotypes can vary widely between syndromic and nonsyndromic presentations even with variants in the same gene. A specific diagnosis can guide management including type and frequency of imaging surveillance, timing of surgical intervention, medication therapy, and guidance on other nonvascular manifestations. Early and correct diagnosis with appropriate surveillance strategies can decrease the risk of dissection or death.

Conditions with a primary vascular phenotype or aortopathy

Marfan syndrome

Overview

First described in the late 1800s, Marfan syndrome (MFS) is a classic disorder of connective tissue known for its effects on the cardiovascular, skeletal, and ocular systems. The main cause of morbidity and mortality in MFS is aortic disease, namely the significantly increased risk of aortic aneurysmal dilatation and dissection. MFS is inherited in an autosomal dominant pattern with an overall prevalence of 1:5000–1:10,000 [1].

The causative gene for MFS, *FBNI*, was identified in 1991 [2] and encodes the fibrillin-1 protein that is a major component of extracellular microfibrils present in connective tissues. Beyond their structural roles, these microfibrils are also thought to regulate transforming growth factor (TGF)- β signaling within the aortic wall. It is thought that abnormal TGF β signaling in the aorta due to pathogenic *FBNI* variants may contribute to aortic disease in MFS [3]. Notably, other genes in the TGF β pathway are associated with Loeys–Dietz syndrome (LDS), a condition with significant clinical overlap with MFS (see description below).

The cardinal manifestations of MFS involve the cardiovascular system and include progressive aortic root dilatation, a predisposition to dissection along the entire aorta, mitral, and tricuspid valve prolapse and pulmonary artery enlargement. Dislocation of lenses in the eyes (ectopia lentis) is also very common in MFS and occurs in about 60% of affected individuals [1]. Skeletal features of MFS include tall stature, disproportionately long extremities, pectus deformity, scoliosis, and other findings that contribute to the Marfan systemic score (<https://www.marfan.org/dx/score>).

Evaluation

The diagnosis of MFS is made using the revised Ghent criteria with emphasis placed on aortic root dilatation/dissection, ectopia lentis, and molecular testing of *FBNI* [4] (Table 10.1). Without a family history of MFS, the diagnosis is made by identifying a pathogenic *FBNI* variant associated with MFS and/or aortic disease along with a history of ectopia lentis and/or aortic root enlargement (Z -score ≥ 2.0). In the absence of *FBNI* testing, aortic root enlargement (Z -score ≥ 2.0) and ectopia lentis or a systemic score ≥ 7 is sufficient for the diagnosis.

Imaging modalities to assess the cardiovascular system in MFS include echocardiography and computed tomography angiography (CTA)/magnetic resonance angiography (MRA). Echocardiography is typically the first-line screening test for MFS and can show evidence of aortic root dilatation as well as valvular and/or overall cardiac dysfunction. CT/MR angiography can visualize aortic disease in the chest and abdomen/pelvis not seen on echo. Standardized Z -scores can be calculated (<https://www.marfan.org/dx/zscore>) to quantify the degree of aortic root dilatation. Ectopia lentis can be reliably diagnosed by slit-lamp examination.

Table 10.1 Revised Ghent criteria for diagnosis of Marfan syndrome [4].**Without FH**

1. Ao ($Z \geq 2$) AND EL = MFS
 2. Ao ($Z \geq 2$) AND FBN1 = MFS1
 3. Ao ($Z \geq 2$) AND systemic score (≥ 7 pts) = MFS^a
 4. EL AND FBN1 with known Ao = MFS
- Ao, aortic root dilatation or dissection.
 EL, ectopia lentis.
 FH, family history.

In the presence of FH

5. EL AND FH of MFS (as defined above) = MFS
6. Systemic score (≥ 7 pts) AND FH of MFS = MFS
7. Ao ($Z \geq 2$ in adults ≥ 3 in children) + FH of MFS = MFS

Systemic score

Feature	Value	Enter value if present
Wrist AND thumb sign	3	
Wrist OR thumb sign	1	
Pectus carinatum deformity	2	
Pectus excavatum or chest asymmetry	1	
Hindfoot deformity	2	
Plain flat foot (pes planus)	1	
Pneumothorax	2	
Dural ectasia	2	
Protrusio acetabulae	2	
Reduced upper segment/lower segment AND increased arm span/height ratios	1	
Scoliosis or thoracolumbar kyphosis	1	
Reduced elbow extension	1	
3/5 facial features	1	
Skin striae	1	
Myopia	1	
Mitral valve prolapse	1	
(positive ≥ 7)	Total	

^aWithout features of LDS, vEDS, or Shprintzen–Goldberg syndrome.*Genetic testing*

FBN1 is the only gene associated with MFS, and the gene testing is highly sensitive. A wide variety of pathogenic *FBN1* variants have been described, including missense, nonsense, frameshift, splice site, and small insertion/deletion changes [1]. About 75% of cases are inherited from an affected parent with the remaining 25% arising *de novo* [1]. Penetrance is high for individuals with MFS and a pathogenic variant in *FBN1* though intrafamilial clinical variability can occur. No true genotype–phenotype correlations exist for MFS with the exception of some variants between exons 24 and 32 of *FBN1* associated with a severe,

rapidly progressive form of MFS [5] (previously known as “neonatal MFS”). Given the overlap with other syndromes, we typically recommend sending a comprehensive aortopathy panel that includes *FBN1* as well as other genes associated with aortic aneurysm and dissection.

Vignette 10.1

*SM is a 33-year-old, previously healthy woman, who presents to the emergency room with sudden onset, sharp chest pain. Electrocardiogram (EKG) and cardiac enzymes are normal, but CXR shows a widened mediastinum. Stat CTA demonstrates an acute aortic dissection and aneurysmal enlargement of the ascending thoracic aorta. She is taken for surgery immediately and undergoes emergent composite aortic root replacement. An inpatient genetics consult is requested, and upon discussion with family, the patient’s mother underwent a similar aortic root repair at age 45 and her 25-year-old brother has a history of tall stature, lens dislocation, and incidentally noted mildly dilated aortic root on echo. On physical exam, the patient is noted to have several characteristics of MFS, including facial features, pectus deformity, hand and wrist sign, and increased arm span-to-height ratio. Given the high suspicion of a hereditary connective tissue disorder, a multigene aortopathy panel is sent that reveals a pathogenic missense variant in the *FBN1* gene, consistent with a diagnosis of MFS. The patient does well after surgery and is started on both a beta-blocker (BB) and angiotensin receptor blocker (ARB). She is discharged home with plans for close monitoring of her aortic diameter. At 6 months postop, her aortic measurements are stable without signs of aneurysm or dissection elsewhere in the aorta. Her mother and brother undergo genetic testing that reveals they share the same pathogenic *FBN1* variant.*

Management

Management of individuals with MFS typically involves a multidisciplinary team including cardiology, cardiothoracic surgery, ophthalmology, orthopedics, and clinical genetics with particular focus on the aorta.

Surveillance

- Aortic root: Echocardiography should be performed annually or more frequently if approaching surgical thresholds at the aortic root (~ 4.5 cm diameter), the rate of aortic dilation exceeds 0.5 cm/year, or there is significant valvular disease.
- Remaining aorta: Baseline CTA or MRA of the entire aorta (head to pelvis) should be done to assess for aortic aneurysms or dissections. Such imaging should be repeated at least annually in someone with a history of aortic root replacement, aneurysm, or dissection. Otherwise it should be repeated every 2 years with consideration of MRA to minimize radiation exposure.

Medical management

- Aggressively treat hypertension with BBs to reduce hemodynamic stress on the aortic wall. Consider adding ARBs that may attenuate abnormal TGF β signaling and slow aortic dilatation [6]. Calcium channel blockers should be avoided, if possible, as preliminary data suggest they may increase aortic complications [7].

- Annual ophthalmologic examination due to increased risk of ectopia lentis, cataract, glaucoma, and retinal detachment.
- Referral to orthopedist for severe or progressive scoliosis.
- Moderate aerobic activities (e.g., walking, cycling, swimming) are encouraged. Avoid contact sports, exercise to exhaustion, isometric activities (e.g., weight lifting, sit-ups, pull-ups, push-ups), scuba diving.
- Avoid medications such as decongestants that contain stimulants (e.g., ephedrine, pseudoephedrine), vasoconstricting agents (e.g., triptans), and fluoroquinolone antibiotics.

Surgical management

- Elective surgery: The threshold for elective surgical repair of the aortic root in MFS is an external diameter of 5.0 cm, a rate of increase of 1.0 cm/year, or severe aortic valvular disease [1], with the goal of preventing aortic dissection. Aortic root replacement may involve either a composite valve graft or a valve-sparing technique, the latter not requiring life-long anticoagulation. Elective repair in surgical centers with expertise in the surgery of the aorta is recommended.
- An acute aortic dissection is an indication for emergency surgery at any diameter when the ascending aorta is involved (type A). Dissections of the descending aorta (type B) are typically managed with aggressive BP control but may require surgical intervention. Endovascular stenting is generally not recommended in MFS patients due to tissue fragility.

Pregnancy

- Women with MFS should be followed by a high-risk obstetrician with echocardiography every 2–3 months during pregnancy and the immediate postpartum period. ARBs are contraindicated during pregnancy.

Genetic counseling

MFS is inherited in an autosomal dominant manner and close relatives of someone affected have up to a 50% chance of also being affected. All first-degree relatives (parents, siblings, children) of an individual with MFS should have baseline echocardiogram screening and should consider genetic testing if a pathogenic *FBN1* variant is known.

Loeys–Dietz syndrome

Overview

LDS is caused by inherited or *de novo* pathogenic variants in *TGFBR1*, *TGFBR2*, *SMAD2*, *SMAD3*, *TGFB2*, and *TGFB3*, all components of the TGF β -signaling pathway. It is an autosomal dominant condition with wide variability in physical and vascular characteristics. It classically presents and can be distinguished from MFS by the presence of hypertelorism, abnormal uvula (broad, bifid, raphe) or cleft palate and widespread aortic and arterial aneurysm. Neck artery tortuosity is common. Individuals may not have the tall stature of MFS

but may present with overlapping body habitus including flat feet, scoliosis, and pectus deformities. Overall, individuals with LDS have a more aggressive vascular course compared to those with MFS.

Evaluation

Additional features that may distinguish LDS from MFS include craniosynostosis, clubfoot, joint contractures, cervical spine instability, and allergic and inflammatory gastrointestinal disease. Food and/or environmental allergies are common, with increased prevalence of eosinophilic esophagitis and inflammatory bowel disease. Skin tends to be more translucent with wide, atrophic scarring. History may include surgeries for hernias and exotropia. Notably, ectopia lentis is not a feature of LDS [8].

As in MFS, the main cardiovascular feature seen on echocardiogram in LDS is aortic aneurysm at the level of the sinuses of Valsalva. Moreover, LDS is commonly associated with aneurysms and dissections of the thoracic and abdominal aorta and arterial branches of head, neck, lung, and lower extremities [9]. Congenital heart defects such as BAV, patent ductus arteriosus, and atrial septal defects can also be seen in LDS as well as mitral valve prolapse. Currently, no diagnostic criteria for LDS exist, and diagnosis is largely based on the positive genetic testing and presence of aortic aneurysm (personal or family history). Aortopathy gene panel testing should be pursued as part of the diagnostic workup.

Management

Surveillance

- Baseline head-to-pelvis MRA/CTA imaging to assess for arterial tortuosity and aortic/arterial aneurysms or dissections. Imaging should be performed every 2 years if no specific aneurysms are detected.
- Echocardiograms should be performed at least yearly and more frequently if approaching surgical thresholds for aneurysmal or valvular disease.
- Obtain flexion-extension X-rays of the neck due to risk of cervical spine instability.

Medical management

- Aggressively treat hypertension with BBs and/or ARBs. Mouse model evidence shows that ARBs at optimal dosage may provide enhanced benefit, outside treatment for hypertension
- Referrals to other specialists such as ophthalmology, gastroenterology, orthopedics and surgical specialties (cardiothoracic, vascular, neurovascular, general) as needed.
- Avoid contact sports, exercising to exhaustion, isometric activities (e.g., weight lifting, sit-ups, pull-ups, push-ups), scuba diving.
- Avoid medications such as decongestants that contain stimulants (e.g., ephedrine, pseudoephedrine), vasoconstricting agents (e.g., triptans) and fluoroquinolone antibiotics.

Table 10.2 Loeys–Dietz syndrome subtypes by gene.

LDS subtype	Gene	Inheritance	Proportion LDS cases	Notable findings
LDS type 1	<i>TGFBR1</i>	AD	20%–25%	Early aortic/arterial dissection
LDS type 2	<i>TGFBR2</i>	AD	55%–60%	Early aortic/arterial dissection
LDS type 3	<i>SMAD3</i>	AD	5%–10%	Osteoarthritis
LDS type 4	<i>TGFB2</i>	AD	5%–10%	Less severe aortic/arterial phenotype
LDS type 5	<i>TGFB3</i>	AD	Rare (< 5%)	Less severe aortic/arterial phenotype
LDS type 6	<i>SMAD2</i>	AD	Rare (< 5%)	Downslanting palpebral fissures

LDS, Loeys–Dietz syndrome.

Adapted from Bradley et al.[10], Meester et al. [9], and LDS Genereviews [11].

Surgical management

- Importantly, aneurysms of the aortic root tend to rupture or dissect at younger ages or smaller dimensions compared to MFS, and surgical decision-making can be influenced by size and rate of growth of aorta, gene involved or a specific genetic variant, and family history of early dissection or death. Thresholds for aortic surgery range from 4.0 to 4.5 cm depending on the LDS subtype (Table 10.2).

Pregnancy

- Women with LDS are at high risk of complications during pregnancy, delivery, and the postpartum period, including aortic dissection/rupture and/or uterine rupture. They should be followed by a high-risk obstetrician with frequent aortic imaging. ARBs are contraindicated during pregnancy.

Genetic testing

Similar to MFS, we recommend sending a comprehensive aortopathy panel that includes all known LDS genes due to the overlap with other aortopathies. About 75% of individuals with LDS result from a *de novo* pathogenic variant in one of the LDS genes while the remaining 25% are inherited from an affected parent [10].

Genetic counseling

LDS is inherited in an autosomal dominant manner and close relatives of someone affected have up to a 50% chance of also being affected. All first-degree relatives (parents, siblings, children) of an individual with LDS should have baseline echocardiogram screening and should consider genetic testing if a pathogenic variant in an LDS gene is known.

Vascular Ehlers–Danlos syndrome

Overview

Vascular Ehlers–Danlos syndrome (vEDS) is caused by inherited or *de novo* variants in *COL3A1* and is an autosomal dominant condition. Unlike MFS and LDS, vEDS typically does

not present with trackable thoracic aortic aneurysm disease but with family histories of variable spontaneous aortic and arterial dissections or hollow organ ruptures (most commonly bowel, less commonly uterus).

Evaluation

There may be mild skeletal features of connective tissue disorders, but key features are cutaneous in origin with easy and significant bruising and classic “cigarette-paper” scars. Pneumothoraces and clubfoot may also be present. Typically, patients with vEDS do NOT present with widespread and significant joint hypermobility, but finger joints can be flexible [11].

Echocardiogram screening often does not show any valvular or aneurysmal disease. When full vascular imaging is performed, observation of ectasia, aneurysmal disease or dissections in neck, abdominal branches or iliac arteries are not uncommon.

Genetic testing

Although clinical criteria have been suggested, the diagnosis is typically made by identifying a pathogenic variant in *COL3A1*, which can be identified through single gene or multigene testing. About 50% of cases are inherited from an affected parent, while the other 50% are *de novo*.

Management

Surveillance

- Echocardiograms every 1–2 years.
- Head-to-pelvis MRA/CTA imaging every 2 years, unless aneurysmal/dissections disease noted, then defer to neurosurgery or vascular surgery recommendations.

Medical management

- Aggressively treat hypertension; no strong data to suggest preference for ARB versus BB.
- Avoid constipation; treat with dietary fiber or Miralax. Avoid bowel stimulant laxatives.

Surgical management

- Avoid elective surgical procedures.
- Vascular surgical intervention, when performed prophylactically, should be performed by a surgeon experienced with vEDS.

Pregnancy

- Women with vEDS have an estimated 5.3% risk for death from peripartum arterial rupture or uterine rupture [12]. If pregnancy is desired, such women should be followed by a high-risk obstetrician.

Genetic counseling

vEDS is inherited in an autosomal dominant manner and close relatives of someone affected have up to a 50% chance of also being affected. Genetic testing for vEDS in childhood is a

complex issue. Complications in children are rare; however, introducing exercise restrictions early can be helpful. Thoughtful conversation of medical and psychological risks and benefits for testing children should occur.

Heritable thoracic aortic disease: smooth muscle disorders

Overview

Heritable thoracic aortic disease (HTAD) is characterized by familial thoracic aneurysm and/or dissection (FTAAD) in isolation, without significant skeletal, cutaneous, or pulmonary complications. Many genes identified are involved in the contractility of vascular smooth muscle. Multiple genes have been identified in FTAAD: *ACTA2*, *MYH11*, *MYLK*, *PRKG1*, *MAT2A*, *MFAP5*, and *LOX* [13]. Aneurysmal disease in smooth muscle disorders may be primarily in the aortic root or ascending aorta or could be a fusiform dilation of both.

Evaluation

HTAD is characterized by the absence of significant musculoskeletal disease, typically seen in MFS or LDS. Cutaneous features of livedo reticularis may be present. Iris flocculi can be noted on ophthalmologic exam. A subset of *ACTA2* pathogenic variants predispose to early-onset stroke or coronary artery disease or Moyamoya-like cerebrovascular disease. Patent ductus arteriosus, aortic coarctation, moyamoya-like cerebrovascular disease with stenosis and dilatation of cerebral vessels, retinal artery tortuosity, and brachial artery occlusion are present in patients with multisystemic smooth muscle dysfunction syndrome caused by the R179 variant in *ACTA2*. Pulmonary arterial hypertension, congenital mydriasis, hypoperistalsis, malrotation of the gut and hypotonic bladder are also common with this variant [14].

Management

Surveillance

- Yearly echocardiogram or more frequently if approaching surgical threshold.
- Baseline chest, abdomen, pelvis MRA/CTA. Repeat every few years.
- Consider baseline head and neck MRA/CTA to assess for cerebrovascular disease.

Medical management

- BP-lowering medications; no strong data to suggest preference for ARB versus BB.

Surgical management

- Guidelines suggest a 5.5 cm threshold for aortic surgery [15]. However, other studies showed that in up to 60% of patients with an acute type A dissection, the aortic diameter was less than 5.5 cm and that some had little to no aortic enlargement [16]. Suggest surgical consultation around aortic diameter of 5.0 and 4.5 cm in individuals with *ACTA2* variants.

Genetic testing

Approximately 30% families with HTAD who do not have a clinical diagnosis of MFS or another syndrome have a causative pathogenic variant in one of the known HTAD-related genes.

Genetic counseling

HTAD is primarily inherited in an autosomal dominant manner. Close relatives of someone affected with HTAD have up to a 50% chance of also being affected. All first-degree relatives (parents, siblings, children) of an individual with HTAD should have baseline echocardiogram screening and should consider genetic testing if a pathogenic variant in a HTAD gene is known.

Bicuspid aortic valve

Overview

Bicuspid aortic valve (BAV) is the most common congenital heart anomaly, affecting 1%–2% of all individuals with a slight male predominance. Some individuals show predisposition to thoracic aortic aneurysm, especially of the ascending aorta compared to the aortic root, as seen in MFS and LDS. In a family with BAV/aneurysm syndrome, there can be variations of (1) BAV without aneurysm, (2) aortic aneurysm with a normal tricuspid valve, and (3) BAV with aneurysm. Moreover, on the most severe end of the spectrum, there can be left-sided heart defects including coarctation of the aorta or hypoplastic left heart [17].

Only a small proportion of genetic etiology of BAV/aneurysm is known. Pathogenic variants in *NOTCH1* and *SMAD6* have been reported in some families. *NOTCH1* tends to present with calcific aortic stenosis as well. Importantly, BAV/aneurysm can show reduced penetrance and variable expressivity within its autosomal dominant pattern of inheritance [18].

Evaluation

Individuals with BAV/aneurysm tend not to show significant syndromic manifestations, unlike MFS or LDS. The pattern of aortic enlargement that is most typical for individuals with BAV is ascending aortic enlargement and an abnormal profile of the ascending aorta with a measurement above the sino-tubular junction, which is larger than the measurement of the aortic root. However, some individuals do present with larger aortic root measurements. Diagnosis of BAV is generally established by transthoracic echocardiography making sure to visualize the ascending aorta for signs of aneurysm formation.

Genetic testing

Genetic testing should include *SMAD6*, *NOTCH1*, *ACTA2*, and *GATA5* genes. However, it is important to note that only a small proportion of genetic etiology of BAV/aneurysm will be identified; thus familial echocardiogram screening is important. If echocardiogram is normal in first-degree family members, it should be repeated every 3–5 years, as aneurysmal disease can present over time.

*Management***Surveillance**

- Yearly echocardiogram is warranted to follow valve function and aneurysm progression.
- Screening for intracranial aneurysms in patients with BAV should be considered. [19]
- At this time, we do not have evidence to suggest a pattern of aneurysm formation outside of the aortic root/ascending aorta. However, because the natural history is unknown, every few years it may be advisable to get a CTA or MRA of the entire aorta.

Medical management

- There is no strong data to suggest preference for ARB versus BB.

Surgical management

- Guidelines suggest aortic diameter threshold of 5.5 cm for surgical intervention. However, surgical consultation around aortic diameter of 5.0 cm can also be considered.

Genetic counseling

Cascade testing is recommended for family members if a pathogenic variant is known in the family. Fetal echocardiograms for left ventricular outflow tract defects are warranted when a parent is affected, and there is a pregnancy.

Conditions with musculoskeletal and skin manifestations

Ehlers–Danlos syndromes

Overview

Ehlers-Danlos syndrome (EDS) refers to a heterogeneous group of connective tissue disorders characterized by different degrees of joint hypermobility, skin hyperextensibility, and tissue fragility. The International EDS Consortium currently recognizes 13 subtypes (Table 10.3), each with defined clinical criteria for diagnosis that were revised in 2017 [20]. Many types of EDS are due to abnormalities in different forms of collagen, which are important in the structure of many tissues and organs. We will focus on three EDS subtypes most likely to be seen in clinic: hypermobile EDS, classical EDS, and vascular EDS (described above).

Hypermobile Ehlers–Danlos syndrome

Overview

By far the most common of the EDS subtypes, hypermobile EDS (hEDS) occurs in as many as 1:5000 individuals and is considered the least severe form of EDS [21]. The genetic basis of hEDS remains unknown though it appears to be inherited in an autosomal dominant manner. The musculoskeletal system is affected most in hEDS, with joint dislocations and chronic joint pain being common manifestations, typically resulting from longstanding generalized joint hypermobility (GJH). Cardiovascular autonomic dysfunction (e.g., arrhythmias, postural orthostatic tachycardia syndrome [POTS]) can occur and may require tilt

Table 10.3 Clinical classification of the Ehlers–Danlos syndromes, inheritance pattern, genetic basis, and key features. Adapted from Malfait et al. (2017) [20].

EDS subtype	Abbreviation	Inheritance	Gene	Key features
Classical EDS	cEDS	AD	<i>COL5A1</i> , <i>COL5A2</i> , <i>COL1A1</i>	– Skin hyperextensibility and atrophic scarring – GJH
Classical-like EDS	clEDS	AR	<i>TNXB</i>	– Skin hyperextensibility with velvety skin texture and absence of atrophic scarring – GJH with or without recurrent dislocations – Easy bruising of skin/spontaneous ecchymoses
Cardiac-valvular	cvEDS	AR	<i>COL1A2</i> , <i>COL1A2</i>	– Severe progressive cardiac-valvular problems (aortic or mitral valve) – Skin hyperextensibility, atrophic scars, thin skin, easy bruising – Joint hypermobility (GJH or small joints)
Vascular EDS	vEDS	AD	<i>COL3A1</i>	– Family history of vEDS and <i>COL3A1</i> variant – Arterial rupture at a young age – Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology – Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineal tears – Carotid-cavernous sinus fistula formation in the absence of trauma
Hypermobile EDS	hEDS	AD	<i>Unknown</i>	<i>Note: There is a clinical spectrum ranging from asymptomatic joint hypermobility, through “nonsyndromic” hypermobility with secondary manifestations, to hEDS. See AJMG ref above for full criteria</i>
Arthrochalasia EDS	aEDS	AD	<i>COL1A1</i> , <i>COL1A2</i>	– Congenital bilateral hip dislocation – Severe GJH, with multiple dislocations/subluxations – Skin hyperextensibility
Dermato-sparaxis EDS	dEDS	AR	<i>ADAMTS2</i>	– Extreme skin fragility with congenital or postnatal skin tears – Characteristic craniofacial features – Redundant skin with excessive skin folds at the wrists and ankles – Increased palmar wrinkling – Severe bruisability with a risk of subcutaneous hematomas and hemorrhage – Umbilical hernia – Postnatal growth retardation – Short limbs, hand and feet – Perinatal complications due to connective tissue fragility

(Continued)

Table 10.3 (Continued)

EDS subtype	Abbreviation	Inheritance	Gene	Key features
Kyphoscoliosis EDS	kEDS	AR	<i>PLOD1</i> , <i>FKBP14</i>	– Congenital muscle hypotonia – Congenital or early-onset kypho-scoliosis (progressive or nonprogressive) – GJH with dislocations/subluxations (shoulders, hips, and knees in particular)
Brittle cornea syndrome	BCS	AR	<i>ZNF469</i> , <i>PRDM5</i>	– Early-onset progressive keratoconus or keratoglobus – Blue sclerae
Spondylo-dysplastic EDS	spEDS	AR	<i>B4GALT7</i> , <i>B3GALT6</i> , <i>SLC39A13</i>	– Short stature (progressive in childhood) – Muscle hypotonia (ranging from severe congenital to mild later onset) – Bowing of limbs
Musculo-contractural EDS	mcEDS	AR	<i>CHST14</i> , <i>DSE</i>	– Congenital multiple contractures – Characteristic craniofacial features – Skin hyperextensibility, easy bruisability, skin fragility with atrophic scars, increased palmar wrinkling
Myopathic EDS	mEDS	AD, AR	<i>COL12A1</i>	– Congenital muscle hypotonia, and/or muscle atrophy, which improves with age – Proximal joint contractures (knee, hip, and elbow)
Periodontal EDS	pEDS	AD	<i>C1R</i> , <i>C1S</i>	– Hypermobility of distal joints – Severe and intractable periodontitis of early onset (childhood or adolescence) – Lack of attached gingiva – Pretibial plaques – Family history of a first-degree relative who meets clinical criteria

GJH, Generalized joint hypermobility.

table testing, EKG, Holter, and so on for diagnosis. Easy bruising is quite common. Some hEDS patients may have symptoms of abnormal mast cell activation (e.g, itching, redness, hives) and report subjective improvement with antihistamines. Functional gastrointestinal disorders are also frequently identified in individuals with hEDS. Aortic root dilation, if present at all, is usually mild and the risk of aortic dissection is not increased in contrast to MFS, LDS, and vEDS [22].

Evaluation

Diagnosis of hEDS is based on the 2017 International EDS Consortium diagnostic criteria listed below. All three criteria must be present.

1. GJH (e.g., Beighton score ≥ 5 , see Table 10.4).
2. Two or more of Criterion A, B, or C.

Table 10.4 Beighton hypermobility scale. A score of at least five of the maximum of nine points is used to define hypermobility.

Criterion	Maximum points
Passive dorsiflexion of the fifth finger over 90 degrees	2 ^a
Passive apposition of the thumb to the volar surface of the forearm	2 ^a
Hyperextension of the elbow greater than 10 degrees	2 ^a
Hyperextension of the knee greater than 10 degrees	2 ^a
Ability to place the palms on the floor with knees fully extended	1
Total	9

^aOne point for each side.

- A. Five or more of the following: Soft velvety skin; mild skin hyperextensibility; striae (without weight gain or loss); bilateral piezogenic heel papules; abdominal hernias (multiple, not hiatal); atrophic scarring (not as severe as seen in cEDS or vEDS); prolapse of pelvic floor, rectum, or uterus; dental crowding and high palate; arachnodactyly (positive wrist and/or thumb sign); arm span-to-height ratio >1.05 ; mitral valve prolapse; or aortic root dilatation defined as a Z-score >2 standard deviations above the mean.
- B. Positive family history in first-degree relatives diagnosed with these criteria.
- C. Daily musculoskeletal pain in two or more extremities (for at least 3 months) or chronic pain for 3 months or more, recurrent joint dislocations, or atraumatic joint instability.
3. No evidence of unusual skin fragility and exclusion of alternative diagnoses including other connective tissue disorders and autoimmune rheumatologic conditions.

In individuals who do not meet the above criteria, a diagnosis of hypermobility spectrum disorder (HSD) can be made when there is symptomatic joint hypermobility and no other specific diagnosis can be established. Management of HSD is largely the same as hEDS and is aimed at symptomatic relief.

Genetic testing

Although no known causative gene for hEDS has been identified, an aortopathy or EDS-specific gene panel can be sent to exclude other syndromes with phenotypic overlap.

Management

Surveillance

- Baseline echocardiogram to assess the aortic root size and evidence of mitral valve prolapse.
- Daily calcium and vitamin D intake, annual vitamin D levels, periodic Dual-energy X-ray absorptiometry scans due to increased risk of low bone density.
- Periodic assessment with an ophthalmologist due to the increased risk of myopia.

Medical management

- Management of patients with hEDS is largely supportive and aimed at treating symptoms.
- Increasing salt and fluid intake for symptoms of POTS and orthostatic hypotension. For symptoms of autonomic dysfunction, consider Holter monitor and tilt table for diagnosis, cardiology referral for further management and/or treatment with midodrine, fludrocortisone, or BBs.
- Low impact exercising (e.g., swimming), joint braces, pain medication (Nonsteroidal anti-inflammatory drugs (NSAIDs), topicals), physical therapy for joint symptoms can be encouraged.
- Antihistamines can be taken for symptoms of abnormal mast cell activation.
- Emotional challenges (e.g., anxiety/depression), fatigue, and sleep disturbances are common in hEDS and should be addressed appropriately.
- Referral to other specialties as needed.

Pregnancy

There is no contradiction for pregnancy in patients with hEDS, but there is a risk of preterm labor and delayed healing with episiotomies and C-section scars. Labor and delivery may progress rapidly.

Genetic counseling

hEDS is inherited in an autosomal dominant manner with most affected individuals having an affected parent. Each child of an individual with hEDS has a 50% chance of inheriting the disorder.

Classical Ehlers–Danlos syndrome*Overview*

Classical Ehlers – Danlos syndrome is an autosomal dominant connective tissue disorder characterized by significant joint hypermobility, skin hyperextensibility, and atrophic scarring [23]. It is most commonly due to defects in type V collagen. Skin is described as “soft” and “doughy” and easily splits due to fragility. Wound healing is delayed and results in widened atrophic scars. Dislocations of any joint are common due to the hypermobility. Hernias, cervical insufficiency, and uterine prolapse have also been reported.

Evaluation

The diagnosis of cEDS should be suspected in individuals with the first major criterion (skin hyperextensibility and atrophic scarring) plus either the second major criterion (GJH) or at least three minor criteria. Major criteria include (1) skin hyperextensibility and atrophic scarring and (2) GJH. Minor criteria include soft doughy skin, easy bruising, fragile skin, molluscoid pseudotumors, subcutaneous spheroids, complications of joint hypermobility, epicanthal folds, hernias, and a positive family history. A final diagnosis requires confirmation by molecular testing demonstrating a pathogenic variant in *COL5A1*, *COL5A2*, or *COL1A1* (rarely).

Genetic testing

Diagnosis of cEDS can be confirmed by sequencing of *COL5A1*, *COL5A2*, and *COL1A1*, typically as part of a multigene EDS panel.

*Management***Surveillance**

- Baseline echocardiogram and yearly thereafter if aortic dilatation is present.

Medical management

- Similar to hEDS, low impact exercising (e.g., swimming), joint braces, pain medication (NSAIDs, topicals), and physical therapy can be recommended. Joint stabilization surgery does not typically provide long-lasting results.
- Skin wounds should be closed without tension, ideally in two layers.

Pregnancy

- There is increased risk of cervical insufficiency, preterm labor, uterine prolapse, postpartum hemorrhages, and skin lacerations. Patients should be followed by a high-risk obstetrician. A fetus with cEDS is at risk for hypotonia, breech presentation, and dislocation of hips and shoulders.

Genetic counseling

cEDS is inherited in an autosomal dominant manner with most affected individuals having an affected parent. Each child of an individual with cEDS has a 50% chance of inheriting the disorder.

Stickler syndrome*Overview*

Stickler syndrome is a HDCT that can include ocular findings of myopia, cataract, and retinal detachment; hearing loss that is both conductive and sensorineural; cleft palate (either alone or as part of the Robin sequence); and mild spondyloepiphyseal dysplasia and/or precocious arthritis [24].

Mitral valve prolapse has been reported in Stickler syndrome but may represent prevalence in the general population. There is typically no aortic dilation.

The majority of Stickler syndrome is inherited in an autosomal dominant manner (pathogenic variants in *COL2A1*, *COL11A1*, *COL11A2*), but rare autosomal recessive inheritance has also been reported (*COL9A1*, *COL9A2*, and *COL9A3*).

Evaluation

The diagnosis of Stickler syndrome should be suspected in an individual with a connective tissue disorder phenotype in association with eye abnormalities, hearing loss, joint issues, and facial dysmorphism including cleft palate. Presence of an aortic aneurysm with a cleft palate is more suspicious for LDS. The degree of hearing impairment is variable and may be progressive in Stickler syndrome. Early-onset arthritis is common and may be severe, leading

to the need for surgical joint replacement even as early as the third or fourth decade of life. More commonly, the arthropathy is mild, and individuals often do not complain of joint pain unless specifically asked. In individuals with intellectual disability and cleft palate/hearing loss, consider 22q11 deletion syndrome.

Genetic testing

When a clinical diagnosis of Stickler syndrome is suspected, diagnosis can be confirmed by testing specific Stickler genes or a multi-HDCT gene panel. There are some genotype–phenotype correlations (e.g., *COL2A1*: ocular disease including “membranous” congenital vitreous anomaly and milder hearing loss; *COL11A1*: typical ocular findings including “beaded” congenital vitreous anomaly and significant hearing loss; *COL11A2*: craniofacial and joint manifestations and hearing loss but without ocular findings). A pathogenic variant in *COL2A1* can be found in 80%–90% of individuals with Stickler syndrome. Pathogenic variants can be *de novo* or familial. Consider cascade familial variant testing.

Management

Surveillance

- Baseline ophthalmologic examination: follow-up as indicated. Counsel regarding retinal detachment risk and symptoms.
- Baseline audiogram: follow-up as indicated.

Medical management

- Treatment of symptomatic joint arthropathy.

Other conditions with phenotypic overlap

Homocystinuria

Homocystinuria is an autosomal recessive disorder caused by cystathionine β -synthase (CBS) deficiency. Individuals with homocystinuria may have a Marfan-like appearance (excessive height, long limbs, scoliosis, and pectus excavatum) and eye involvement, including ectopia lentis and/or severe myopia. In contrast to MFS, intellectual disability and thrombotic events are common in homocystinuria. The diagnosis can be made by measuring homocysteine levels, which are elevated in plasma, or identification of biallelic pathogenic variants in *CBS*, the gene encoding cystathionine β -synthase.

Beals syndrome

Beals syndrome, also known as congenital contractural arachnodactyly (CCA), is an autosomal dominant disorder characterized by a Marfan-like appearance (tall, slender, arachnodactyly, kyphosis/scoliosis) due to pathogenic variants in the *FBN2* gene. Unlike Marfan, most individuals with beals have contractures of major joints (knees and ankles) at birth and “crumpled” ears due to folded upper helices. Furthermore, the progressive aortic root dilation seen in Marfan is not characteristic of Beals.

Shprintzen–Goldberg syndrome

Shprintzen–Goldberg syndrome is an autosomal dominant connective tissue disorder with some overlap with LDS and MFS due to pathogenic variants in the *SKI* gene. It is characterized by craniosynostosis, craniofacial features (long/narrow head, widely spaced eyes), skeletal findings (arachnodactyly, long limbs, scoliosis), and cardiovascular anomalies. In contrast to LDS and MFS, however, individuals with SGS frequently have mild-to-moderate intellectual disability, hypotonia, and brain anomalies.

Points to remember

- Connective tissue disorders are multisystemic conditions with varied genetic basis.
- Consider sending comprehensive multigene panel to assist in diagnosis due to the phenotypic overlap between different connective tissue disorders.
- Baseline echocardiogram and additional aortic imaging may be indicated if a connective tissue disorder is suspected.
- Consider referral to cardiology, surgery, genetics, ophthalmology, and other specialties.

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Disorders that impact reproduction

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Introduction

The approach to an individual with infertility can be challenging as there are a wide range of genetic conditions associated with infertility. In some cases, the severity of the genetic variation results in a syndrome where infertility is not the most significant concern. In many others, infertility may be the first or only manifestation. In the latter scenario, identifying a pathogenic variant can be of significant burden to the affected individual as they not only have to grapple with their new diagnosis but also must decide whether they would like to proceed with having children to whom they may pass genetic variation—if they are able to have children at all.

Due to the complex interaction of genetics, hormonal, environmental, and social factors in infertility, it is important that reproductive potential be evaluated by trained professionals. Most individuals are referred from reproductive specialists for genetic counseling and testing after having been diagnosed with infertility. Some individuals who are referred may have a genetic disorder and may be interested in knowing their reproductive potential. While the role of a genetic counselor or a clinical geneticist is vital for the patient's understanding of how their condition may affect their lives and their potential offspring, it is important that these men and women be evaluated by male reproductive urologists and reproductive endocrinologists, respectively. As advances in assisted reproduction now allow men with rare spermatogenesis and women with diminished ovarian reserve to have biological children, the communication between specialists is key to allowing affected individuals to make informed decisions. Moreover, as noted below, there are some genetic conditions in which fertility may be present during early adult life but is subsequently lost. Individuals with these conditions should be referred early to discuss fertility preservation options.

Disorders that impact male fertility

47,XXY (Klinefelter syndrome)

Overview

Klinefelter syndrome (KS) occurs due to X chromosome aneuploidy and is the most common genetic abnormality associated with male infertility. It is estimated to occur in 0.15% of newborn males, 2% of men with infertility, and up to 9% of men with nonobstructive azoospermia (NOA) [1]. The X chromosome aneuploidy results in meiotic arrest and germ-cell death. Thus, few nonmosaic men will have sperm in the ejaculate and unassisted paternity is very rare.

Genetic testing

Chromosomal analysis is sufficient to diagnose KS. Approximately 90% of affected males carry two copies of the X chromosome (47,XXY); however, 7% have mosaicism and 3% have other sex chromosome aneuploidies. Although rare, large duplications of the X chromosome and translocation of sex-determining region Y (SRY) gene can result in similar presentations (discussed in the section on 46,XX DSD).

Vignette 11.1

AR is a 27-year-old man found to have mild hypogonadism and azoospermia during evaluation for infertility. His history is notable for learning difficulties and delayed puberty. He endorses low libido and difficulty maintaining erections. Physical exam is notable for tall stature, moderate gynecomastia, and sparse body hair. His testes are 4 cc and firm bilaterally. Laboratory evaluation demonstrates high levels of plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH), low testosterone, and azoospermia on repeat semen analysis with pellet.

The X chromosome contains genes whose expression levels are important for intellectual development, height, and spermatogenesis. Thus, aneuploidy of the X chromosome is commonly associated with above average height and deficits in executive functioning. KS results in testicular failure with characteristic hypergonadotropic hypogonadism and impaired/absent spermatogenesis. While estrogen levels may be normal or elevated, the testosterone-to-estrogen ratio is low resulting in gynecomastia and decreased virilization after puberty.

Management

Many boys and men with KS will be hypogonadal due to primary testicular failure and thus require testosterone supplementation. As exogenous testosterone suppresses spermatogenesis, it is critical that these men be referred for fertility evaluation prior to starting therapy. Men with sperm in the ejaculate should consider sperm banking. Foci of spermatogenesis are found in men with azoospermia approximately 44% of the time during testicular sperm extraction (TESE), regardless of age [2]. Thus, the benefit of prophylactic TESE in adolescent males is unclear unless there are plans to start testosterone therapy. In addition to

hypogonadism and infertility, men with KS are at increased risk for developing breast cancer, cardiovascular disease, and diabetes; thus, appropriate screening for these disorders should be initiated.

Genetic counseling

Unassisted paternity is very rare in men with nonmosaic KS as few will have any sperm in the ejaculate. Sperm aneuploidy rates in men with KS are similar to other groups of men with NOA. Interestingly, there have been no reported cases of sex chromosome aneuploidy in children of men with KS as of 2018 [3], suggesting that it is mainly the normal germ cells in men with mosaic KS that are producing sperm. Nonetheless, couples should be counseled that there is a risk of transmission of sex chromosome aneuploidy to offspring and preimplantation genetic screening (PGS) of embryos should be discussed.

47,XYY (XYY syndrome)

Overview

XYY syndrome occurs in about 1:850 newborn males. Despite similar incidence to KS, XYY syndrome is found 10–25 times less frequently among infertile men undergoing genetic testing, suggesting that most men with XYY syndrome are fertile. Thus, a 47,XYY finding may be incidental during the infertility evaluation and other possible etiologies should be investigated.

Cystic fibrosis/obstructive azoospermia

Overview

Pathogenic variants in *CFTR* are the most common genetic cause of obstructive azoospermia in men. Most men with classic cystic fibrosis (CF) have congenital bilateral absence of the vas deferens (CBAVD); however, pathogenic variants in *CFTR* are also found in 80% of men with CBAVD without pulmonary manifestations [4]. *CFTR* variants are also common in men with congenital unilateral absence of the vas deferens (CUAVD).

Genetic testing

All men with CBAVD and their partners should be tested for pathogenic variants in *CFTR*. While testing for the most common variants and the 5T allele are currently recommended, over 2000 variants have been identified in the *CFTR* gene, and the prevalence of specific variants varies considerably by ethnicity. Full-gene sequencing should be considered, especially in those with CBAVD without pulmonary manifestations and those of non-European descent. Given the prevalence of pathogenic *CFTR* variant carriers in the general population, it is recommended that the partner also undergo genetic testing even if *CFTR* sequencing is negative in the male. In men with negative sequencing, testing for variants in *ADGRG2* may be considered as these are found in approximately 20% of men with CBAVD without *CFTR* variants [5].

Vignette 11.2

RT is a healthy 32-year-old man found to have low volume azoospermia on evaluation for infertility. He endorses good libido and normal erectile function. Physical examination is notable for normal sized testes, thickened epididymal heads, and bilateral absence of the vasa deferentia. His semen sample has a volume of 0.5 mL, pH of 6.9, and no sperm in the pelleted fraction.

CBAVD is frequently associated with pathogenic variants in CFTR even in the absence of pulmonary manifestations. CBAVD is also usually accompanied with hypoplasia of the seminal vesicles. Testicular size is usually normal. As the seminal vesicle secretions are alkaline, contain fructose and contribute 70% of the fluid in the ejaculate, semen analysis in men with CBAVD is usually low volume, acidic, fructose negative, and uniformly devoid of sperm.

Management

Men with CBAVD should be evaluated for pulmonary and other complications associated with CF as mild disease manifestations may still be present. CUAVD is frequently associated with ipsilateral renal anomalies or agenesis, and these men should undergo renal ultrasound. Men with CUAVD usually have sperm in the ejaculate and may not have problems with fertility. Conversely, men with CBAVD never have sperm in the ejaculate; however, large numbers of sperm can be easily aspirated or extracted from the epididymis/testis. Success rates with intrauterine insemination using extracted sperm are low, so most men will ultimately proceed to in vitro fertilization or intracytoplasmic sperm injection (IVF/ICSI).

Genetic counseling

Men with biallelic variants in *CFTR* will pass one of those alleles on to their offspring. Their partner should also be tested for *CFTR* variants, as inheritance of an affected allele from the mother could result in CF in the offspring. The recent discovery of *ADGRG2* and other genetic variants that cause CBAVD challenge the dogma that men with CBAVD should be assumed to have *CFTR* variants even if sequencing is negative. Nonetheless, there may still be unknown genetic changes (e.g., within the introns or promoter), epimutations, or other potentially heritable factors that could affect *CFTR* gene expression or function and couples should be counseled of the risk of transmission to their offspring. Most men with CBAVD will require IVF/ICSI, and thus, PGS should be discussed. Furthermore, couples should be counseled that the rate of pregnancy loss is approximately two-fold when men have obstructive azoospermia due to *CFTR* variants compared to other causes (e.g., vasectomy).

Y Chromosome microdeletions

Overview

Y chromosome microdeletions (YCMDs) are the second most common genetic cause of male infertility. Meiotic recombination errors in highly repetitive sequences in the Y chromosome result in microdeletions. Three regions of the Y chromosome appear particularly susceptible to deletion and are termed AZFa, AZFb, and AZFc. Since most of the genes on the Y chromosome

are specific to spermatogenesis, men with YCMD are generally phenotypically normal. Testicular size may be variable depending on the degree of spermatogenic impairment.

Genetic testing

YCMD testing is typically performed by polymerase chain reaction amplification of several short segments within each AZF region, as microdeletions are too small to detect on karyotype. YCMD may be classified as complete or partial, depending on the number of primer pairs that fail to amplify. The karyotype should be checked in men with complete AZFa, AZFb, and AZFc deletions as a number of men may actually have $SRY + 46,XX$ DSD or have a ring chromosome Y. The majority of YCMD occur in the AZFc region.

Management

Complete deletion of the AZFa and/or AZFb regions are invariably associated with azoospermia [6]. Usable sperm is not found in the testes; thus, these individuals should not undergo testicular biopsy or TESE. Partial AZFa or AZFb deletions are occasionally encountered and may be associated with spermatogenesis. In men with AZFc deletions, sperm will occasionally be found in the ejaculate; however, viable sperm can be found during TESE 33%–50% of the time [6,7].

Genetic counseling

Men with complete AZFa or AZFb deletions should be encouraged to seek other methods of fatherhood (i.e., donor sperm or adoption). Men with AZFc deletions who have spermatogenesis should be informed that all of their male offspring will inherit the deletion. While none of the female offspring will inherit the deletion, there is weak evidence that YCMD may reflect genomic instability resulting in possible gene dosage changes in other chromosomes.

Disorders that impact female fertility

45,X (Turner syndrome)

Overview

Most cases of Turner syndrome (TS) result from loss of an X chromosome; however, large deletions or rearrangements of the X chromosome can result in TS-like syndromes. This condition occurs in approximately 1 in 2500 newborn girls, but it is estimated that up to 99% of fetuses with TS spontaneously abort [8]. The majority of females with TS will initially have normal ovarian function in utero but lose it prior to birth. Many will require hormonal therapy to enter puberty, although a small percentage may have normal ovarian function through early adulthood followed by early menopause.

Genetic testing

Chromosomal analysis is sufficient to diagnose TS and TS-like syndromes due to large deletions in the X chromosome. Mosaicism has also been reported in these individuals.

Vignette 11.3

An 18-year-old female presents for evaluation of primary amenorrhea. She has a history of coarctation of the aorta, for which she underwent repair as a child. On physical exam she is found to have short stature and webbing of the neck. She has no intellectual disability. She is Tanner stage 1 for breast development and Tanner Stage 3 for pubic/axillary hair. Hormonal profile demonstrates elevated LH and FSH levels.

Women with TS frequently present with normal female external genitalia, short stature, and a lack of secondary sexual characteristics, although there may be high variability in presentation. Loss of a copy of the SHOX gene on the X chromosome is associated with decreased height and skeletal abnormalities. Webbing of the neck and cardiac, renal, and endocrine abnormalities are common in women with TS. Approximately 30% of females will enter puberty without hormonal assistance; however, they may need additional hormonal supplementation later in adulthood due to subsequent ovarian failure.

Management

Girls with TS often have elevated LH and FSH levels before or around the time of puberty and may require estrogen supplementation. Short stature can be treated with growth hormone and low dose estrogen prior to puberty. Any virilization in a woman with TS should prompt investigation for mosaicism containing the Y chromosome, as the streak gonads in this setting are at high risk for germ-cell tumors and should be removed (see the section on Mixed gonadal dysgenesis). Streak gonads in a nonmosaic 45,X karyotype need not be removed. Females with TS are also at increased risk for cardiac abnormalities and other endocrine disorders including hypothyroidism and diabetes mellitus; thus, screening for these disorders should be instituted.

Genetic counseling

Most women with TS are infertile; however, up to 2%–5% may be able to conceive early in adulthood, although whether this is due to mosaicism is unknown [8]. It should be noted that there are high rates of chromosomal abnormalities in the embryos and high levels of spontaneous abortions (SAB), stillbirths, and congenital birth defects. Those with spontaneous menses may wish to consider oocyte preservation as diminished ovarian reserve and early menopause are common. Thus, women with TS who are having spontaneous menses should be cautioned of these risks. If they are undergoing IVF/ICSI, PGS should be offered. The few women who are able to get pregnant should be monitored closely during pregnancy for aortic dissections.

Disorders that impact both male and female fertility

Congenital adrenal hyperplasia

Overview

Congenital adrenal hyperplasia (CAH) results from enzyme deficiencies in the steroidogenesis pathway. Deficiencies of 21-hydroxylase, 11 β -hydroxylase, or 3 β -hydroxysteroid dehydrogenase result in the overproduction of pregnenolone, which is subsequently diverted toward testosterone production. Identification of CAH is critical as affected individuals may manifest with salt-wasting. CAH can occur in both males and females; however, in females it frequently results in ambiguous genitalia (masculinization without true sex reversal) that are detected at birth. While most cases of CAH are diagnosed early due to routine newborn screening, some milder, nonclassical presentations may potentially escape detection until puberty or later.

Genetic testing

Chromosomal analysis is important to detect the genetic sex of a child with ambiguous genitalia and rule out other conditions such as mixed gonadal dysgenesis. The clinical and biochemical phenotypes can give important clues to which of the genes coding for steroidogenic enzymes is deficient. The vast majority of cases are due to deficiency of 21-hydroxylase (95%), although deficiencies in 11 β -hydroxylase and 3 β -hydroxysteroid dehydrogenase can also cause masculinization of the female embryo. Traditionally single-gene sequencing and mutation testing have been performed; however, next-generation sequencing has facilitated rapid panel testing of multiple genes at a fraction of the cost of traditional sequencing.

Vignette 11.4

GG is a 19-year-old female who presents for evaluation of hirsutism, male pattern baldness, and irregular menstrual cycles. She reports no significant medical history. The age of menarche was 13 years. Physical exam is notable for coarse body and facial hair. Her external genitalia appear normal. Serum 17-hydroxyprogesterone and testosterone levels are elevated. Cortisol and aldosterone levels are low but detectable.

21-Hydroxylase deficiency is the most common cause of CAH. The most severe forms result in salt wasting in males and females and ambiguous genitalia in females (isolated or in combination with salt wasting). Less severe deficiency can result in a nonclassical presentation where external female genitalia are normal; however, overproduction of 17-hydroxyprogesterone results in symptoms of androgen excess in early adulthood and infertility. Given the severe phenotype and routine newborn screening, it is unlikely that the more severe forms will present in adulthood.

Management

Concern for CAH at the time of birth due to physical exam findings or newborn screening should prompt a rapid evaluation given the severity of the salt wasting forms. Management typically involves long-term replacement with glucocorticoids. Infertility affects most men and women with CAH [9,10]. Males may want to consider early sperm cryopreservation due to declining fertility with age. Adrenal rest nodules are common in the testes of males with CAH. Dexamethasone can be used to treat female fetuses in pregnant women with CAH to prevent masculinization, but the long-term effects are not known.

Genetic counseling

Genetic counseling regarding the risks of transmission of the pathogenic variant, if known, should be discussed. PGS to screen for affected embryos should be offered to those requiring IVF/ICSI who have known pathogenic variants. Some men and women with CAH may be fertile and able to conceive naturally.

Kallmann syndrome*Overview*

Kallmann syndrome is caused by gonadotropin-releasing hormone (GnRH) deficiency, most frequently due to impaired migration of the GnRH neurons from the hypothalamus to the anterior pituitary. Thus, it typically presents as delayed or absent puberty due to hypogonadotropic hypogonadism. There is often failure of migration of the olfactory neurons as well, resulting in impaired sense of smell. Males are typically born with micropenis and undescended testes, whereas females may appear normal. Both males and females generally do not undergo puberty spontaneously. As the defect is predominantly at the level of the pituitary, gonadotropin supplementation can allow for puberty and, potentially, fertility.

Genetic testing

Pathogenic variants in *ANOS1* (previously called *KAL1*) were the first to be associated with Kallmann syndrome; however, more than 20 other genes known to cause the phenotype have since been identified [11]. Some individuals may have more than one pathogenic variant. Nonetheless, the genetic etiology in 70% of cases of Kallmann syndrome remains unknown.

Vignette 11.5

ST is an 18-year-old male who presents for evaluation of delayed puberty. His medical history is notable for micropenis and bilateral cryptorchidism at birth. On physical examination, the sexual maturity is noted to be Tanner Stage 0. He is not able to identify any smells during testing. Serum testosterone levels are less than 50 mg/dL and LH and FSH levels are undetectable.

Kallmann syndrome results in pituitary failure and males and females with the condition generally do not undergo puberty spontaneously. Failure of the GnRH neurons and olfactory

bulb neurons result in the hypogonadotropic hypogonadism with anosmia phenotype commonly associated with the condition; however, there is variability in the presentation depending on the pathogenic variant.

Management

Management of Kallmann syndrome is largely centered around hormonal induction of puberty. While this can be accomplished with testosterone in males and estrogen in females, testosterone supplementation alone will usually result in sterility in males. Gonadotropin therapy can facilitate both induction of puberty and induce spermatogenesis in males. If semen analysis shows sperm, sperm banking should be offered prior to switching to testosterone supplementation. Females can be treated with estrogen/progesterone but may also require LH/FSH treatment to induce fertility.

Genetic counseling

Spermatogenesis and oogenesis can be induced in individuals with Kallmann syndrome. Nonetheless, the majority will not have a known genetic variant. Thus, risk of transmitting the condition to their offspring is often unclear since X-linked, autosomal dominant, autosomal recessive, and more complex inheritance patterns have been observed [11]. If the pathogenic variant is known, then PGS should be offered. There are other associated malformations that can occur with Kallmann syndrome and offspring may not necessarily have the same severity of phenotype as their parents.

Recurrent Spontaneous Abortions (SAB)

Overview

An estimated 10%–20% of known pregnancies result in SAB, also referred to as miscarriage; however, up to 50% of fertilized eggs may never reach full term, suggesting that most miscarriages occur prior to the woman learning that she was pregnant. The risk of miscarriage increases with age. While miscarriages are common and can be due to a multitude of factors, it is particularly frustrating when couples experience recurrent miscarriages. In some couples, a genetic cause may be identified; however, in many cases, the underlying etiology remains unknown.

Genetic testing

For couples who experience recurrent miscarriages, the initial genetic evaluation should include chromosomal analysis of both partners and the conceptus, if possible. Many different genetic abnormalities can result in nonviable fetuses. The human genome is very sensitive to aneuploidy, with only trisomy of chromosomes 13, 18, and 21; monosomy X, and polysomy of the X and Y chromosomes being compatible with life. Thus, all other aneuploidies will result in a miscarriage.

Translocations are found in approximately 4.5% of couples with recurrent miscarriages [12]. If the translocation remains balanced, the individual may be phenotypically normal;

however, the gametes of the individual will often be unbalanced [13]. Thus, depending on the location and extent of the translocation, the majority of the embryos may not be viable. More severe translocations may impair fertility due to meiotic nondisjunction during gametogenesis. In the event that the karyotypes are normal, another consideration is sperm aneuploidy testing, as high sperm aneuploidy can increase the likelihood of aneuploid embryos.

Management

Management of recurrent miscarriages can be challenging as the etiology may not be identifiable most of the time. While genetic causes are not modifiable, identification may identify potential health concerns for the affected individual.

When there are chromosomal abnormalities, PGS may be able to identify embryos that have inherited the normal copies of the chromosomes. In cases of high sperm aneuploidy, PGS can also be used. In either case, however, there is no mechanism currently available to identify which sperm or eggs have the normal chromosomes; thus, many or all of the embryos may be abnormal.

Genetic counseling

Individuals with karyotype abnormalities or high sperm aneuploidy should be advised that a majority of their gametes, and, thus, embryos may be affected. PGS should be offered if they are pursuing IVF/ICSI; however, they may want to consider donor sperm or eggs to facilitate successful pregnancy.

Disorders of sex development

A more detailed understanding of genital and gonadal development during embryogenesis has resulted in a change in the classification of the disorders of sex development (DSD). The classification focuses on chromosome analysis and the presence or absence and type(s) of gonads. Masculinization in individuals with 46,XX karyotype generally results from exogenous testosterone exposure or ectopic exposure due to CAH. In these cases, the gonad is generally an ovary, with the exception of those with *SRY* translocations, in which case *SRY* expression is sufficient to induce testicular development. Conversely, individuals with 46,XY generally will have testes (due to *SRY* expression), even though the external phenotype may be completely female. The one exception is 45,X/46,XY mixed gonadal dysgenesis (MGD), where both ovarian and testicular tissue may be present as an ovotestis. The degree of masculinization will be largely dependent on the percentage of cells with the 46,XY karyotype.

46,XX DSD

Overview

46,XX DSD is a rare condition that results in sex reversal (phenotypic male but genetic female). It occurs in approximately 1 in 20,000 male births. Most cases are due to *SRY*

translocation to the short arm of the X chromosome. *SRY* initiates testicular development and testosterone production resulting in a male phenotype. Hypospadias is commonly observed. As none of the other genes critical for spermatogenesis are present, all males with 46,XX karyotype are infertile.

Genetic testing

Chromosomal analysis is diagnostic. *SRY* translocations are found in 90% (i.e., 46,XX + *SRY*) [14]. Other genes have been implicated in the disease; however, testing for these other genes is not routinely performed. 46,XX DSD should be considered in any man with complete deletions of AZFa, AZFb, and AZFc, as this may reflect a 46,XX + *SRY* rather than a 46,XY karyotype. If the patient is found to have ovaries, CAH should be considered, as it is identified in 90% of cases of ambiguous genitalia in females.

Vignette 11.6

TM is a 42-year-old man found to be azoospermic during infertility evaluation. His medical history is notable for mild hypogonadism and delayed puberty. He was on testosterone therapy as a teenager. Physical exam reveals short stature (5'1"). He is normally virilized with normal penile length but has small testes. Semen analysis demonstrates normal volume with azoospermia on pellet analysis. His testosterone level is low with mildly elevated LH and FSH.

*Men with 46,XX + *SRY* are often fully virilized with normal-appearing external genitalia. The phenotype is more variable in men without *SRY* translocations and can vary from normal penile length and small testes to ambiguous genitalia. 46,XX + *SRY* men share many similarities to men with KS due to the presence of two X chromosomes; however, they often have short stature.*

Management

Men with 46,XX DSD are invariably sterile as they lack the Y chromosome genes necessary for spermatogenesis. Attempts to find sperm in the testes should not be undertaken, and they should be encouraged to pursue other methods of fatherhood (i.e., donor sperm or adoption). Oligozoospermic men found to have a 46,XX karyotype require further investigation as this indicates mosaicism or chimerism rather than a pure 46,XX genotype. As fertility is not an option in men with pure 46,XX DSD, testosterone supplementation may be used, and reduction mammoplasty may be indicated for those with gynecomastia. As with KS, there is an elevated risk for breast and testicular cancers, and these men should be screened accordingly.

Genetic counseling

Men with pure 46,XX DSD cannot make sperm and thus cannot father biological children. Nonetheless, men with mosaicism or chimerism may have sperm in the ejaculate. The risk of transmission to offspring in these men is theoretically low as all the spermatogonia would

require the presence of the Y chromosome; however, there are no data investigating this due to its rarity.

46,XY DSD

Overview

In contrast to 46,XX DSD where *SRY* expression is sufficient to cause sex reversal, 46,XY DSD can be caused by a number of genetic conditions. It is largely classified into three groups based on the primary site of the disorder: gonadal development, androgen synthesis, and androgen action (reviewed in [15]). Degree of masculinization can vary considerably from a phenotypically normal-appearing female with intra-abdominal testes (complete androgen insensitivity syndrome [CAIS]) to ambiguous genitalia and pseudohermaphroditism (e.g., partial androgen insensitivity syndrome [PAIS], testosterone biosynthesis problems) to phenotypically normal appearing males found to have a uterus and fallopian tubes (persistent Mullerian duct syndrome). Given this heterogeneity, individuals with 46,XY DSD may be raised and self-identify as a member of either gender. As cells have the Y chromosome, testes are usually present, and spermatogenesis may be observed in some individuals. Local production of androgen is required for Wolffian duct development, thus other structures of the male reproductive system may be absent. Incompletely differentiated gonads and ovaries have been reported.

Genetic testing

Chromosomal analysis is the primary test to identify individuals with 46,XY DSD, which is often performed shortly after birth due to ambiguous genitalia. Chromosomal analysis should be ordered in any phenotypically appearing female found to have testes or ovotestes as this suggests presence of a Y chromosome. Mutations in the androgen receptor are found in 80% of CAIS and 20%–30% of PAIS (> 800 pathogenic variants have been identified to date). Pathogenic variants in *SRY* are found in 10%–15% of individuals with gonadal dysgenesis. As many different genes and pathways can result in 46,XY DSD, causative variants are currently identified in less than 50% of affected individuals. Next-generation sequencing of gene panels for ambiguous genitalia/DSD allow rapid sequencing of over 100 genes simultaneously and may increase diagnostic yield moving forward.

Clinical Vignette 11.7

RS is a 22-year-old female presenting for evaluation of infertility. She reports normal childhood development and began puberty at age 12. She reports that she has never had any vaginal bleeding. She reports that sex is physically uncomfortable for both her and her husband. Physical exam reveals normally developed female genitalia; however, the vaginal vault is short and blind ending. No cervix is visualized. Her testosterone level was significantly elevated (750 ng/dL), with elevated levels of LH and FSH. Estradiol levels were normal. Ultrasound demonstrated absent uterus and fallopian tubes with solid ovoid masses near the internal

inguinal rings. Surgical pathology demonstrated testicular tissue with sparse germ cells and spermatogenic arrest.

This is a “classic” presentation of CAIS; however, there is high variability in the phenotype, and this is just one of several possible etiologies of 46,XY DSD. Most individuals are diagnosed at birth or during childhood due to ambiguous genitalia or abnormal pubertal development.

Management

Individuals with 46,XY DSD with incompletely developed gonads and/or undescended testes are at increased risk for developing gonadal tumors. Incompletely developed gonads are often removed prior to adulthood, and females can start estrogen/progesterone therapy. In women with CAIS, fully differentiated testes can be left in place until puberty is complete, as their testes can provide a source of estrogens and have a relatively low rate of malignancy [16]. Fertility preservation should be considered in any postpubertal individual prior to gonadectomy as some tissue may have viable spermatogenesis. Men with undescended testes should undergo bilateral orchidopexy to facilitate monitoring for testicular tumors and optimize spermatogenesis. Depending on the genetic variant, individuals often have significant other medical conditions that require specialized management and should be referred accordingly.

Counseling

46,XY DSD may have viable spermatogenesis that can be used to have children. Thus, offspring may inherit the pathogenic variant. Female offspring are less likely to be affected but may subsequently pass the variant on to their male offspring if the causative variant is not related to the Y chromosome. As many of these individuals will require IVF/ICSI for conception, PGS can be offered if the causative variant is known.

45,X/46,XY Mixed gonadal dysgenesis

Overview

45,X/46,XY MGD has a wide range of phenotypes, which vary based on the percentage of cells that carry the Y chromosome. There is high variability in the external and internal reproductive organs of individuals with MGD, including the presence of müllerian structures. While an intra-abdominal testis on one side and streak gonad on the other is the most common presentation, there may be one or two ovotestes or even an ovary.

Genetic testing

Chromosomal analysis is usually sufficient to identify 45,X/46,XY MGD.

Vignette 11.8

JK is a 32-year-old man with penoscrotal hypospadias and undescended testicles. During orchidopexy at age 17, he was found to have a tumor in an ovotestis on the right. He was also found to have a hemiuterus on that side, which was also removed. The left testis was brought

down into the scrotum. He now presents with a left testicular mass concerning for germ-cell tumor. He had delayed puberty but didn't require testosterone supplementation. Testosterone levels are low with high LH and FSH. Semen analysis reveals azoospermia. OncoTESE at the time of orchiectomy identified viable sperm that were able to be cryopreserved.

The presentation of 45,X/46,XY MGD is highly variable. In this case, incomplete masculinization with persistent müllerian structures and a unilateral ovotestis were found. Tumors develop in the gonadal tissue of up to 50% of individuals with MGD.

Management

Gender assignment is one of the biggest challenges in patients with DSD, especially MGD, as the degree to which the fetus may have been exposed to testosterone and how that may affect their future gender identity is unclear [17]. Gender confirming surgery can render an individual permanently infertile. Females with MGD may be able to bear children if the appropriate internal structures are present.

Ovotesticular tissue in individuals with MGD have a high rate of malignancy, especially if *WT1* variants are present; thus, these tissues are frequently removed at the time of diagnosis (malignancy risk and management in the different DSD classifications is reviewed in [18]). Undescended testes should also be considered for removal, but at a minimum should be brought down into the scrotum to permit routine evaluation/surveillance for malignancy. Fertility preservation should be discussed with any postpubertal individuals, and TESE/oncoTESE can be offered to look for sperm at the time of gonadectomy. Wilms tumor is more common in individuals with MGD, and thus appropriate screening should be instituted.

Genetic counseling

There are few reports of fertility in individuals with MGD; however, both spermatogenesis and paternity, as well as oogenesis and maternity, have been reported. There are limited reports regarding the quality of sperm and associated genetic risks. Couples should be counseled that there is a potentially increased risk of aneuploid embryos, and PGS should be offered although PGS cannot reliably detect mosaicism in embryos.

Other genes affecting gonadal differentiation

In addition to *SRY*, several other genes have been identified as having a role in gonadal development including, *WT1*, *NR5A1*, *SOX9*, *NR0B1 (DAX1)*, *WNT4*, *SPO1*, and *FOXL2*. Loss-of-function variants in *SOX9*, *WT1*, *NR5A1* result in 46,XY sex reversal (female phenotype), whereas *WNT4* and *RSPO1* loss-of-function variants result in 46,XX sex reversal (male phenotype). Non-loss-of-function variants can have variable phenotypes.

Points to remember

- KS is the most common genetic abnormality associated with male infertility followed by YCMDs.
- Pathogenic variants in the *CFTR* gene, associated with CF, are the most common genetic cause of obstructive azoospermia in men.
- Concern for CAH at the time of birth should prompt a rapid evaluation given the severity of the salt wasting forms.
- Kallmann syndrome presents with delayed/absent puberty due to hypogonadotropic hypogonadism and is associated with anosmia.
- Disorders of sexual development and differentiation are identified by chromosome analysis and the presence and type of gonads.

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Endocrine disorders

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Introduction

Hyperglycemia due to diabetes mellitus, hypercalcemia, hypocalcemia, abnormalities of serum phosphate, and tumors involving endocrine glands are very commonly encountered in the outpatient as well as in-hospital settings. Whereas the vast majority of such abnormalities are secondary to common disorders, for example, type 2 diabetes mellitus (DM) causing hyperglycemia and asymptomatic hypercalcemia in primary hyperparathyroidism, there is a significant overlap between the phenotypes of commonly occurring endocrine disorders and Mendelian endocrinopathies. This chapter provides an overview and approach to the diagnosis and treatment of monogenic endocrinopathies. Particularly, the approach to molecular diagnosis and its importance in instituting appropriate treatment and management strategies are discussed.

Monogenic causes of DM

DM comprises a spectrum of disorders with wide-ranging dysfunction of energy metabolism that is characterized by sustained hyperglycemia, which results in systemic complications. The pathophysiology of DM involves defects in insulin secretion, production, or action. The predominant, heterogeneous forms of DM are denoted as type 1 (with a predominant insulin secretory defect usually due to autoimmune destruction of the islets) and as type 2 (with both insulin resistance and inadequate insulin secretion). These two categories comprise more than 90% of all DM [1]. The genetic basis of types 1 and 2 DM is complex, variable, and polygenic, with each genetic variant contributing modestly to the phenotype [2,3]. Pathogenic variants in single genes also can cause DM; these variants often impact β -cell function, and insulin synthesis and secretion, rather than insulin resistance. In adults, the major categories are maturity-onset diabetes of the young (MODY) and mitochondrial causes of DM.

Maturity-onset diabetes of the young

Overview

MODY is a monogenic, nonketotic form of DM with an autosomal dominant (AD) inheritance pattern that typically presents before the age of 25 years. MODY accounts for 1%–4% of all cases of DM, depending on geography and ethnicity [4].

In 1996 Fajans and colleagues identified the first pathogenic variant responsible for MODY, in the gene encoding the transcription factor, hepatocyte nuclear factor 4 α (HNF-4 α) [5]. Since then, pathogenic variants in 14 different genes have been identified in families with MODY (Table 12.1). The majority of the genes encode factors responsible for the development or function of the insulin-producing β -cells of the islets of Langerhans [6].

Evaluation

MODY should be suspected in patients with the following clinical features: (1) onset of DM before 25 years of age, (2) strong family history of DM in three or more generations and AD pattern of inheritance, (3) absence of insulin requirement within 3–5 years after diagnosis of diabetes, and presence of measurable C-peptide level, and (4) lack of obesity or other features of insulin resistance (acanthosis). Although a young age at diagnosis and time interval between diagnosis and insulin use are important, these features may not always be true as MODY may also be present in patients older than 25 years and those already being treated with insulin [7]. In addition to these features, there also will be the absence of maternal inheritance with deafness (which raises suspicion for mitochondrial DM) and negative islet autoantibodies (GAD65Ab, IA1b, ZnT8Ab) that characterize autoimmune type 1 diabetes. Specific phenotypic features may increase the likelihood of certain MODY syndromes. For example, mild but nonprogressive fasting hyperglycemia suggests a diagnosis of MODY2. A low renal threshold causing glycosuria or a large increase in glucose on oral glucose tolerance test (OGTT) testing raises suspicion for MODY3. Renal dysfunction, renal cysts, or abnormal liver function may point the clinician to a diagnosis of MODY5.

Maturity-onset diabetes of the young 3

HNF-1 α is a transcription factor critical for the development of liver, kidney, intestine, and pancreatic islet cells. MODY3 is caused by pathogenic variants in *HNF-1 α* , which leads to decreased insulin secretion in response to glucose. More than 300 different pathogenic variants including gene deletion, frameshift, missense, nonsense, and splice-site variants have been described in more than 700 families [8]. Genotype has been shown to affect the age at diagnosis; additionally, environmental or other genetic factors also contribute to the clinical variability [9].

The penetrance is high and 95% of individuals with a pathogenic variant are diagnosed with DM by the age of 55 years [10]. Pathogenic variants in *HNF-1 α* cause DM due to haploinsufficiency or dominant-negative effect and the majority affect residues in the DNA-binding or transactivation domain of the HNF-1 α [11]. Mutational hotspots include an insertion in codon 291 (P291fsinsC, the most frequently reported to date), as well as

Table 12.1 Genetic classification of MODY syndromes.

MODY type	Gene	Chromosomal locus	Frequency	Gene product function	Effect of pathogenic variant in the gene	Other clinical features	Treatment
MODY 1	<i>HNF4A</i>	20q13	6%	Transcription factor	β -cell dysfunction (defect mainly in insulin secretion)	Low triglycerides, abnormal liver lipoproteins, higher birth weight/macrosomia, transient neonatal hypoglycemia	Sulfonylureas
MODY 2	<i>GCK</i>	7p13	25%	Glycolytic enzyme; β -cell glucose "sensor"	β -cell dysfunction (glucose-sensing defect leading to an increased threshold for insulin secretion)	Mildly elevated fasting glucose levels, small rise in glucose after 75 g-OGTT	Usually no treatment necessary; maybe insulin during pregnancy to prevent excess fetal growth
MODY 3	<i>HNF1A</i>	12q24	52%	Transcription factor	β -cell dysfunction (defect mainly in insulin secretion)	Elevated HDL, decreased hsCRP levels, glycosuria, large rise in glucose after 75 g-OGTT	Sulfonylureas, meglitinides, glucagon-like peptide 1 agonists, dipeptidyl peptidase 4 inhibitors, insulin
MODY 4	<i>PDX1/IPF-1</i>	13q12	1%	Transcription factor	β -cell dysfunction	Pancreatic agenesis (when homozygous), neonatal diabetes, pancreatic exocrine dysfunction	Diet, oral antidiabetic agents, and insulin
MODY 5	<i>HNF1B</i>	17q12	3%	Transcription factor	β -cell dysfunction, insulin resistance	Multi-organ involvement: renal cysts, dysplasia, renal tract malformations, hypoplastic glomerulocystic kidney disease, 40% with genital abnormalities, exocrine pancreatic dysfunction, rarely pancreatic aplasia, hypomagnesemia, hyperuricemia and gout, abnormal liver function tests, autism spectrum disorder	Insulin
MODY 6	<i>NEUROD1</i>	2q31	<1%	Transcription factor	β -cell dysfunction (reduced insulin production)	Neurological abnormalities, learning disabilities, overweight/obesity	Diet, oral antidiabetic agents, insulin

(Continued)

Table 12.1 (Continued)

MODY type	Gene	Chromosomal locus	Frequency	Gene product function	Effect of pathogenic variant in the gene	Other clinical features	Treatment
MODY 7	<i>KLF11</i>	2p25	<1%	Transcription factor	Decreased glucose sensitivity of β -cells	N/A	Oral antidiabetic agents, insulin
MODY 8	<i>CEL</i>	9q34	<1%	Bile salt-dependent lipase	Pancreatic endocrine and exocrine dysfunction	Exocrine pancreatic deficiency	Oral antidiabetic agents, insulin, pancreatic enzyme replacement
MODY 9	<i>PAX4</i>	7q32	<1%	Transcription factor	β -cell dysfunction	Associated with "ketosis prone diabetes"	Diet, oral antidiabetic agents, insulin
MODY 10	<i>INS</i>	11p15	<1%	Insulin precursor	β -cell dysfunction	Most present with neonatal diabetes but also may present in early childhood and adulthood	Diet, oral antidiabetic agents, insulin
MODY 11	<i>BLK</i>	8p23	<1%	Transcription factor	Insulin synthesis and secretion defect	Overweight/obesity in some	Diet, oral antidiabetic agents, insulin
MODY 12	<i>ABCC8</i>	11p15	<1%	K-ATP channel protein	ATP-sensitive potassium channel dysfunction	Associated with transient neonatal diabetes	Sulfonylurea
MODY 13	<i>KCNJ11</i>	11p15	<1%	K-ATP channel protein	ATP-sensitive potassium channel dysfunction	Associated with transient neonatal diabetes	Diet, sulfonylurea, insulin
MODY 14	<i>APPL1</i>	3p14	<1%	Adaptor protein	Insulin secretion defect	Overweight/obesity in some	Diet, oral antidiabetic agents, insulin

ABCC8, ATP-binding cassette subfamily C member 8; *APPL1*, adaptor protein phosphotyrosine interacting with pH domain and leucine zipper 1; *BLK*, B-lymphocyte kinase; *CEL*, carboxyl-ester lipase; *HDL*, high-density lipoprotein; *HNF*, hepatocyte nuclear factor; *hsCRP*, highly sensitive C-reactive Protein; *INS*, insulin; *IPF-1*, insulin promotor factor 1; *KCNJ11*, potassium voltage-gated channel subfamily J member 11; *KLF*, Kruppel-like factor; *MODY*, maturity-onset diabetes of the young; *NEUROD*, neurogenic differentiation; *OGTT*, oral glucose tolerance test; *PDX*, pancreatic duodenal homeobox; *PAX*, paired box gene.

insertion/deletion mutations involving codon 379. P291fsinsC results in a truncated protein with an intact dimerization domain that is unable to bind DNA but forms a heterodimer with the wild type protein, thereby inhibiting transcription factor activity in a dominant-negative manner [12]. Because of cell type-specific functions of HNF-1 α , MODY3 is characterized by a predominant β -cell phenotype. In addition, HNF-1 β has been found to compensate for loss of HNF-1 α in hepatocytes. In patients with MODY3, β -cell dysfunction is present before the onset of diabetes and exaggerated elevation of glucose levels in the plasma are found on OGTTs. HNF-1 α also has been shown to regulate the expression of the sodium-glucose cotransporter-2, and renal manifestations have been described in some MODY3 families, including carriers of the missense mutation p.Arg272His who manifest decreased reabsorption of glucose in renal tubules, leading to glycosuria [13].

Maturity-onset diabetes of the young 2

MODY2 is the second most common MODY syndrome and is caused by pathogenic variants in glucokinase (*GCK*) that encodes an isoform of hexokinase which is specifically expressed in β -cells and liver. *GCK* phosphorylates glucose, which is the rate-limiting step in glycolysis that is critical for the “retention” of glucose within the β -cell for subsequent metabolism and ATP generation that is required for insulin secretion. Thus, *GCK* serves as the “glucose sensor” of the β -cell and inactivating pathogenic variants elevate the threshold for glucose-stimulated insulin secretion in β -cells. In addition to having dysfunctional β -cells, patients with MODY2 have diminished accumulation of hepatic glycogen and a relatively high rate of gluconeogenesis after meals [14]. The V203A variant has been reported to also affect the function of *GCK* in liver cells [15]. Patients with MODY2 present with mild fasting hyperglycemia. In contrast to patients with MODY3, MODY2 manifests with only a slightly elevated level of glucose during an OGTT. They require no pharmacologic treatment, although sometimes insulin may be needed during pregnancy to prevent fetal macrosomia.

Other forms of MODY

MODY1 is caused by pathogenic variants in *HNF-4 α* and has a similar presentation to MODY3. MODY1 should be considered when genetic testing for *HNF-1 α* and *GCK* is unrevealing. Patients with MODY1 have higher birth weights, may have had transient neonatal hypoglycemia, and can be treated with sulfonylureas. MODY5 presents with renal and urogenital abnormalities. The other subtypes of MODY types are very rare (<1% of known MODY syndromes). Key features of the known MODY mutations are described in Table 12.1.

Genetic testing

Molecular genetic testing is sensitive and specific for diagnosing MODY. Next-generation sequencing (NGS) permits simultaneous analysis of multiple genes; alternatively, targeted gene sequencing by the Sanger method may be employed in certain patients, especially if there is suspicion of a specific MODY phenotype. Nearly one-third of individuals with MODY

5 have deletions involving *HNF-1 β* and thus tests that can detect copy number variation should be used routinely for diagnosis of this condition. It has been recommended that appropriate relatives are offered a biochemical test first (e.g., fasting blood glucose for MODY2 or OGTT for MODY3) before performing genetic testing.

Vignette 12.1

SK is a thin, 23-year-old man who was found to have a fasting blood glucose levels of 200 mg/dL on routine blood tests. Evaluation for islet antibodies for type I DM was negative. He has no previous history of DM and is not on any medications. He reports a family history of early-onset DM in his mother maternal grandfather, and multiple other relatives. There is no history of any muscle weakness, deafness, or stroke-like episodes in the family to suggest a mitochondrial disorder. These characteristics are highly suggestive of MODY. Genetic testing using a gene panel is required for diagnosis and further management.

Management

Patients with MODY3 can be treated with oral glycemic agents including sulfonylureas, meglitinides, glucagon-like peptide 1 agonists, dipeptidyl peptidase 4 inhibitors, or insulin. The therapeutic response to oral and noninsulin agents is an important reason for distinguishing MODY3 from type 1 DM. Systematic molecular phenotypic testing for the in vitro transcriptional activity of MODY3 mutants demonstrates a range in the level of dysfunction of the transcription factor.

Genetic counseling

It is important to note that *de novo* variants in the most common MODY genes (*GCK*, *HNF-1 α* , *HNF-4 α*) have been found in approximately 7% of families without a history of DM [16]. Once the proband has been identified, “cascade testing” is recommended as this approach maximizes chances of diagnosing monogenic DM in members of a family and is cost-effective for genetic testing. Through this process, relatives can be informed of their risk of monogenic DM and undergo further genetic workup. Genetic counseling is also important during pregnancy.

Genetic syndromes affecting multiple endocrine glands

Multiple endocrine neoplasia syndromes

Multiple endocrine neoplasia (MEN) syndromes are a rare group of disorders characterized by the hereditary predisposition to develop tumors in more than one endocrine gland. Currently, there are four recognized variants of the syndrome.

Multiple endocrine neoplasia type 1

Overview

Multiple endocrine neoplasia type 1 (MEN1) is the most common type of MEN and has a prevalence of approximately 1 in 30,000 individuals [17]. Although penetrance of this disorder is over 95% by age 40–50 years, clinical characteristics are variable. The clinical definition of MEN1 lies in the occurrence of two out of three tumors involving the parathyroid glands, pituitary gland, and entero-pancreatic neuroendocrine system. Familial MEN1 is defined as at least one individual with MEN1 and a first degree relative with one of the three most common tumors [18].

Evaluation

The prevalence of tumors in MEN1 is outlined in Table 12.2.

Parathyroid tumors

Primary hyperparathyroidism is the most common manifestation of MEN1 with a 95% penetrance by age 40. As MEN1 is rare in the general population, it accounts for only 1%–4%

Table 12.2 Prevalence of clinical manifestations of MEN1.

Parathyroid adenomas	90%
Neuroendocrine tumors	
Gastrinomas	40%
Insulinomas	10%
NF pancreatic polypeptide tumors	20%
Glucagonomas, vasoactive intestinal polypeptide-omas, somatostatinomas	2%
Thymic carcinoid tumors	2%
Bronchial carcinoid tumors	2%
Gastric enterochromaffin-like tumor	10%
Pheochromocytomas	<1%
Ependymomas	1%
Anterior pituitary tumors	
Prolactinomas	20%
GH, PRL, and NF adenomas	5%
ACTH-producing adenomas	2%
TSH-producing adenomas	rare
Other	
Adrenal cortical lesions (NF)	25%
Dermatological features	
Lipomas	30%
Facial angiofibromas	85%
Collagenomas	70%

ACTH, adrenocorticotrophic hormone; GH, growth hormone; NF, nonfunctional; PRL, prolactin; TSH, thyroid stimulating hormone.

Source: Brandi et al. Guidelines for the diagnosis and therapy of MEN type and type 2. J Clin Endocrinol Metab 2001;86:5658–71 [19].

of all individuals with hyperparathyroidism. The clinical presentation of hyperparathyroidism in MEN1 is unique because: (1) it generally presents at a younger age (median 25 years), (2) it affects both genders equally, and (3) it is caused by hyperplasia of more than one gland as opposed to one predominant adenoma as is seen in sporadic primary hyperparathyroidism [20].

Neuroendocrine tumors of the gastrointestinal system

Neoplasia of the pancreaticoduodenal neuroendocrine cells is the second most common manifestation of MEN1 and is observed in 40%–70% of patients [20]. Of these, gastrinoma is the most common and can present with diarrhea, esophageal reflux, peptic ulcers (i.e., Zollinger Ellison syndrome). Like parathyroid tumors, gastrinomas in MEN1 are characterized by an earlier age of presentation and multifocal tumors. Other tumors include insulinomas, glucagonomas, vasoactive intestinal polypeptide-omas (VIPomas; those secreting vasoactive intestinal peptide) and nonfunctional neuroendocrine tumors.

Pituitary tumors

Anterior pituitary tumors are the third most common manifestation and are observed in one-third of patients with MEN1. The distribution of hormones secreted is similar to those seen in sporadic pituitary adenomas, with prolactinomas being the most common (60% of cases), followed by growth hormone (GH)-secreting tumors (15%), and less commonly, non-secreting tumors and ACTH-producing tumors. Pituitary adenomas in MEN1 present similarly to sporadic adenomas, however there may be a tendency for larger tumors at presentation [20].

Other manifestations

Although there are no consistent physical exam findings of MEN1 (as they relate only to hormonal hypersecretion if present, such as GH over-production), there is an association of MEN1 with cutaneous findings. Lipomas, angiofibromas, and collagenomas are seen in 30%, 85%, and 70% of the affected individuals, respectively [20].

Genetic testing

The causative gene for MEN1, located on chromosome 11q13, was cloned in 1997 and the 610 amino acid protein encoded by the *MEN1* gene was named menin. Over 1200 germline and 200 somatic variants of *MEN1* have been reported with no obvious genotype-phenotype correlation. Nearly 75% of germline variants in *MEN1* are inactivating, consistent with the theory of its role as a tumor suppressor gene. Pathogenic variants at nine sites in *MEN1* account for 20% of germline variants. Approximately 5%–25% of patients with clinical MEN1 do not have a pathogenic variant in the *MEN1* gene. These patients may have pathogenic variants in genes not yet identified [21].

Management

Management of MEN1-related neoplasms should follow similar treatment principles as those used for sporadic endocrine neoplasia, with attention to the fact that the nature of MEN1

tumors can be more aggressive and resistant to treatment. Surgery is generally the first line for the management of hyperparathyroidism and pituitary adenomas related to MEN1. Treatment of entero-pancreatic neuroendocrine tumors can present a greater challenge, as location can complicate surgical resection and there is a high association with metastatic disease. Nonetheless, surgical management remains the primary source of therapy, along with chemotherapy and medical therapy for symptoms caused by excessive hormonal levels.

Surveillance for the development of additional neoplasia is a critical component of management. Close attention should be given to the development of symptoms and signs of MEN1-associated tumors, with additional yearly biochemical screening including serum levels of calcium, parathyroid hormone, prolactin, insulin-like growth factor, GH, chromogranin A and fasting gut hormones. Imaging including magnetic resonance imaging (MRI) of the pituitary and CT of the abdomen should be pursued at diagnosis and every 1–3 years as part of screening [20].

Genetic counseling

MEN1 is inherited in an autosomal dominant fashion. Germline heterozygous pathogenic variants of the *MEN1* gene are observed in 70%–90% of familial MEN1 cases.

MEN1-like phenotypes

Given the heterogeneity of presentation, MEN1 has an overlap with the phenotype observed in sporadic isolated endocrine tumors, or hereditary syndromes of single gland involvement, including familial isolated hyperparathyroidism (FIHP), familial hypocalciuric hypercalcemia (FHH), hyperparathyroidism-jaw tumor syndrome (HPT-JT), and MEN4.

Familial isolated pituitary tumor syndrome (FIPA) is caused by pathogenic variants in the aryl hydrocarbon receptor interacting protein gene (*AIP*) and is responsible for approximately 2% of pituitary adenomas. It has variable penetrance and is associated with the occurrence of large adenomas at a young age (often in adolescence or young adulthood). It most often leads to GH-secreting tumors (in 58% of cases) but can also cause prolactinomas, non-functional adenomas, and rarely Cushing's disease. FIPA is thought to be responsible for the historic familial gigantism described in Northern Ireland [22]. Pituitary tumors can also be seen in McCune–Albright syndrome and Carney.

Although not always possible to differentiate early MEN1 from the above-mentioned conditions, younger age of presentation, bilateral tumors, multifocality, and larger tumor size, and involvement of multiple endocrine glands should raise the suspicion for MEN1.

Multiple endocrine neoplasia 4

MEN4 is the most recently recognized MEN syndrome, with phenotypic similarities to MEN1. It was first described in 2002 in rats and termed “MENX,” and the causative gene was identified to be *CDKN1B* in 2006. The exact prevalence of MEN4 is unknown, and as of 2017 only 19 index cases have been reported in the literature [23].

MEN4 is associated with pituitary tumors, primary hyperparathyroidism, and neuroendocrine duodeno-pancreatic tumors in a similar fashion as MEN1. Primary hyperparathyroidism affects 80% of cases, presents at a later age than in MEN1 and has a female predominance. It is also thought to have a milder clinical course with no evidence of recurrence after surgery. Pituitary adenomas (both functional and nonfunctional) affect 37% of cases and are also thought to have a less aggressive clinical presentation than those of MEN1. Gigantism in pediatric patients has also been reported. Only seven cases of neuroendocrine tumors have been documented in MEN4, including nonfunctional and functional tumors secreting gastrin, insulin, ACTH, and VIP [23].

MEN4 is caused by pathogenic variants in *CDKN1B*, which is located on chromosome 12 and codes for p27 protein. *CDKN1B* is thought to be a tumor suppressor gene regulating cell cycle progression. Menin regulates the expression of p27, and the inactivation of menin leads to decrease in p27 expression. This suggests a common tumorigenesis pathway for MEN1 and MEN4 [23].

MEN2A, MEN2B (MEN3), and familial medullary thyroid cancer

Overview

The MEN2-associated syndromes are a group of disorders caused by pathogenic variants in the *RET* proto-oncogene. Patients with MEN2A have tumors arising in: (1) thyroid parafollicular cells, with 100% of patients developing medullary thyroid carcinoma (MTC), (2) catecholamine-secreting chromaffin cells with 50% of patients developing pheochromocytomas, and (3) parathyroid cells with 30% of patients having hyperparathyroidism. In addition, there is an association with the development of cutaneous lichen amyloidosis (CLA), and Hirschsprung's disease [which can also be seen in familial medullary thyroid cancer (FMTC)].

Evaluation

MEN2A accounts for approximately 70% of all MEN2-associated syndromes and manifests with MTC, pheochromocytoma, or parathyroid adenoma/hyperplasia. Patients with MEN2B also develop MTC and pheochromocytomas; however, this clinical syndrome is further characterized by facial characteristics such as thick vermilion of the lips, mucosal neuromas of the lips and tongue, marfanoid body habitus, ocular and skeletal abnormalities, and ganglioneuromatosis of intestines. FMTC accounts for 15% of cases of hereditary MTC. It is characterized by the presence of MTC, which tends to have a later age of onset and has less aggressive behavior than that of the MEN2-associated syndromes. There are varying criteria to define FMTC, with the minimum standards including four family members that are affected with MTC and no objective evidence of pheochromocytomas or parathyroid pathology [24].

Genetic testing

MEN2-associated syndromes are caused by activating pathogenic variants in the *RET* proto-oncogene. *RET* comprises 21 exons and is located on chromosome 10. Unlike MEN1, MEN2 is characterized by a strong genotype-phenotype correlation and the type of variants can

predict the likelihood of involvement of the different organs, as well as the aggressiveness of clinical course. The most common variants encountered in MEN2A are located in codons 609, 611, 618, and 620 on exon 10 and codon 634 on exon 11. The frequency of development of hyperparathyroidism and pheochromocytoma can vary depending on the *RET* codon involved. Similarly, the development of associated comorbidities (CLA and Hirschsprung's disease) is associated with certain pathogenic variants.

MEN2B is most commonly caused by pathogenic variants of exon 16 in *RET* (p.Met918Thr), followed by exon 15 (p.Ala883Phe). The p.Ala883Phe variant confers a less aggressive course for MTC compared to that of the p.Met918Thr [24]. Molecular diagnosis of MEN2-associated syndromes is of significant importance for the stratifying prognosis of an affected individual and in cascade testing of at-risk relatives and implementing surveillance measures.

Vignette 12.2

BL, a 25-year-old woman, presents for evaluation of abdominal distension, constipation, and diarrhea that have been present for many years. Her medical history is significant for “thyroid cancer” early during childhood for which she had to undergo thyroidectomy. She has a 2-year-old daughter who was diagnosed with MTC during infancy. Physical examination is remarkable for tall stature, long fingers and “bumpy lips.” The phenotype in Ms. BL is highly suggestive of MEN2. The gastrointestinal (GI) symptoms suggestive of intestinal ganglioneuromatosis and the early-onset MTC, marfanoid habitus, and facial features are key diagnostic clues. She should undergo genetic testing and appropriate surveillance measures should be instituted in her. Cascade testing of all individuals at risk is needed as aggressive MTC develops in almost all affected individuals and typically during infancy.

Management

Given the high penetrance and mortality associated with MTC in MEN2, management of patients identified to have MEN2-associated syndromes should include prophylactic total thyroidectomy. Furthermore, given the significant mortality risk with undiagnosed pheochromocytomas, all patients should undergo screening with plasma free or urine fractionated metanephrines prior to undergoing surgery. In the presence of a positive diagnosis, preoperative preparation with alpha and beta-adrenergic blockade should be undertaken. Management of pheochromocytoma includes surgical resection of the involved adrenal gland. Hyperparathyroidism surveillance and management should also be pursued with serum evaluation for calcium and parathyroid hormone and surgical resection as indicated.

Genetic counseling

The MEN2 syndromes and FMTC are inherited in an autosomal dominant pattern.

Cascade testing can be initiated when a pathogenic variant is known so that at-risk family members can begin surveillance and other appropriate management.

Genetic disorders of calcium metabolism

Familial hyperparathyroidism

Other than in MEN syndromes, hyperparathyroidism is also a manifestation of four other inherited disorders: HPT-JT, FIHP, FHH, and neonatal severe hyperparathyroidism. As with sporadic hyperparathyroidism, patients present with hypercalcemia and its sequelae, including hypercalciuria, nephrolithiasis, decreased bone mass, and osteoporosis (Table 12.3).

Hyperparathyroidism-jaw tumor syndrome

HPT-JT presents early in life, in the second or third decades, with primary hyperparathyroidism caused by a single adenoma or even parathyroid carcinoma in 10%–15% of affected individuals. The ossifying fibromas of the jaw manifest in 30%–40% of affected individuals. HPT-JT also is associated with renal cysts and hamartomas and even nephroblastomas (Wilm’s tumors). Benign uterine tumors also are associated with HPT-JT [25]. HPT-JT is inherited as an autosomal dominant disorder due to a pathogenic variant in *CDC73* which encodes the nuclear tumor suppressor parafibromin. Germline variants in *CDC73* also have been found in over a quarter of patients with parathyroid carcinoma [25].

Because the hyperparathyroidism of HPT-JT often is caused by a single adenoma, minimally invasive surgical removal can be appropriate, unless there are features of parathyroid carcinoma, which require a more aggressive local surgery, including en bloc resection. If surgery is not an option, the calcium mimetic, cinacalcet, can be used to attempt to gain pharmacologic control of hypercalcemia by allosteric activation of the calcium-sensing receptor (CASR). Jaw tumors require surgical resection to avoid local growth. For patients known to have *CDC73* pathogenic variants, biochemical surveillance of calcium and intact parathyroid hormone (PTH) should occur year from childhood onward along with radiographic examinations of the jaw every 5 years [25].

Disorders of CASR

The CASR is a G-protein coupled receptor (GPCR) that is most highly expressed in tissues that modulate circulating calcium levels, such as the parathyroid glands, kidney, and bone. PTH

Table 12.3 Inherited causes of hyperparathyroidism.

Multiple endocrine neoplasia 1	<i>MEN1</i>
Multiple endocrine neoplasia 2A	<i>RET</i>
Multiple endocrine neoplasia 4	<i>CDKN1B</i>
Hyperparathyroidism-jaw tumor syndrome	<i>CDC73</i>
Familial isolated hyperparathyroidism	<i>CASR</i>
	<i>CDC73</i>
	<i>GCM2</i>
Familial hypocalciuric hypercalcemia	<i>CASR</i>
Neonatal severe hyperparathyroidism	<i>CASR</i>

secretion is tonic unless CASR is stimulated by increased calcium levels, which activates $G_{i/o}$ to suppress adenyl cyclase and cyclic AMP (cAMP) production, decreasing PTH release. Pathogenic variants in *CASR* result in disorders that manifest with either inappropriately high or low calcium levels depending on whether the variants cause loss or gain of function, respectively. For example, gain-of-function variants in *CASR* can cause autosomal dominant hypocalcemia and hypocalcemia with Bartter syndrome and loss-of-function variants can cause neonatal hyperparathyroidism. CASR is also expressed in noncalcitropic tissues including the islets, central nervous system, gastrointestinal system, adipose tissue, the lungs, and vasculature where it has key roles in influencing development and cellular processes. Given its widespread expression, it is not unexpected that pathogenic variants in *CASR* also can have an impact on other disease states including asthma, Alzheimer's disease, and cardiovascular disease [26].

Familial hypocalciuric hypercalcemia

Individuals with FHH have inappropriately normal or mildly elevated parathyroid hormone in the presence of mild hypercalcemia and hypomagnesemia. The biochemical picture resembles primary hyperparathyroidism but can be differentiated in most cases because many patients with FHH have hypercalciuria and a calcium-creatinine clearance of $<1\%$, whereas many patients with primary hyperparathyroidism will have a measured value of $>2\%$. FHH is usually considered a benign disorder and most patients do not experience any morbidity from this disorder, but pancreatitis and chondrocalcinosis have been reported [27].

FHH is inherited in an autosomal dominant manner and is known to be caused by variants in at least three genes. FHH1 accounts for nearly two-thirds of cases and is caused by a loss-of-function variant of the *CASR* such that PTH secretion is not suppressed even when calcium levels are elevated. Pathogenic variants of the $G\alpha_{11}$ (*GNA11*) and $AP2\sigma$ (*AP2S1*) signaling genes downstream of the *CASR* give rise to FHH2 and FHH3, respectively [27].

Patients with FHH are not at risk for nephrolithiasis or nephrocalcinosis because of hypocalciuria. They also do not develop low bone density. For the most part, patients are asymptomatic and FHH is a benign disorder. However, there are a few case reports where neurologic symptoms (paresthesia, muscle cramps) and recurrent pancreatitis have been treated effectively with a calcium mimetic agent to decrease PTH secretion and calcium levels [28].

Familial isolated hyperparathyroidism

The definition of FIHP is an evolving one. After eliminating kindreds with pathogenic variants in *CASR*, *CDC73*, or *MEN1*, whole-genome sequencing of families identified a pathogenic variant in *GCM2* in 17.5% of probands with familial hyperparathyroidism. FIHP results from an activating variant in a regulatory C-terminal domain of *GCM2* resulting in autosomal dominant inheritance. Patients with FIHP may have multiple adenomas so that there often is postoperative persistence and/or recurrence of hyperparathyroidism. Parathyromatosis

(local dissemination of unencapsulated parathyroid tissue) or possible parathyroid carcinoma has been described in one patient [29].

Genetic disorders caused by Gs-alpha subunit

The Gs-alpha subunit (GNAS) is an essential protein for cellular signal transduction, communicating the activation of cell surface GPCRs to the cytoplasm for increased production of cAMP and other signaling cascades. It is not unexpected that pathogenic variants affecting GNAS functioning would manifest as endocrine disorders, given the number of peptide hormone receptors that fall in this category of GPCRs. There also is added complexity to GNAS-related diseases because it is an imprinted locus and the phenotypic manifestations can be dependent on paternal or maternal inheritance. Pseudohypothyroidism related disorders and McCune–Albright syndrome (MAS) are examples of disorders caused by inactivation and activation of GNAS, respectively [30].

Pseudohypoparathyroidism-related disorders

Overview

Disorders of GNAS inactivation include pseudohypoparathyroidism Ia, Ib, and Ic (PHP-Ia, -Ib, and -Ic), pseudopseudohypoparathyroidism (PPHP), progressive osseous heteroplasia (POH), and osteoma cutis.

Evaluation

Patients with PHP1a have hypocalcemia, hyperphosphatemia, and elevated PTH as manifestations of PTH resistance and may present any time after birth into early adulthood. Neuromuscular sequelae of hypocalcemia can be observed, including seizures. Additional manifestations of PHP1a are eponymously named Albright's hereditary steodystrophy (AHO) which includes subcutaneous calcium deposits, mineralization defects with dental hypoplasia and delayed tooth eruption, brachydactyly (shortened third and fifth metacarpals) and short stature due to early growth plate closure. The AHO phenotype also includes round facies, early-onset obesity, and cognitive impairment. Because of the role of GNAS in hormonal signaling, there can be the hormonal manifestations of early-onset hypothyroidism (TSH), hypogonadism and cryptorchidism (luteinizing hormone (LH) and follicle stimulating hormone (FSH)), and growth hormone releasing hormone (GHRH). Additional phenotypes related to PHP but lacking GNAS variants have also been described and are classified as PHP1b (PTH resistance without AHO), and PHP1c which manifests clinically similar to PHP1a. Patients with PHP2 have hypocalcemia and elevated PTH, but with a normal cAMP response to injection of PTH without phosphaturia indicating a defect downstream of cAMP production [30]. PPHP presents with AHO phenotype but without PTH resistance [30].

A related disorder, POH, is also caused by GNAS variants, and presents with dermal and deep connective tissue and muscle ossification, often along dermatomes, and without biochemical evidence of PTH resistance. Ectopic ossification in the dermis and fat also can be

seen in PHP1a and PPHP but is rarely seen in PHP1b; this manifestation is thought to be caused by differentiation of mesenchymal stem cells to extraskeletal osteoblasts [30].

Genetic testing

Suspicion of a *GNAS*-related disorder should prompt analysis for copy number variants, methylation, and DNA sequence analysis.

Management

For patients with a maternal *GNAS* pathogenic variant, regular (every 6–12 months) monitoring of serum calcium, phosphate, intact PTH and 25-hydroxy vitamin D3 (calcifediol) levels are suggested. Treatment of hypocalcemia includes vitamin D and calcium supplementation. Active vitamin D metabolites (e.g., calcitriol or 1,25 dihydroxy-vitamin D3) may be needed, especially when PTH levels rise to more than two times the upper limit of normal. The goal is to have serum calcium and phosphate levels within the normal range with avoidance of hypercalciuria. Imaging should be employed to detect nephrocalcinosis with persistent hypercalciuria. Parenteral calcium should be administered for acute, severe, symptomatic hypocalcemia. There is no role for recombinant PTH administration in these patients and phosphate binders are rarely required [31].

Thyroid function tests should be performed regularly (every 6 months in children and yearly in adults) and thyroid hormone replacement should be initiated as is indicated for hypothyroidism. There is a paucity of data regarding the outcomes in children and adults with GH deficiency and the initiation of replacement therapy should be decided on an individualized basis. Low bone density is a concern, but currently the role for monitoring bone density is not known. However, there may be a role for case detection in specific patients with multiple potential risk factors for decreased bone mass (hypogonadism and GH deficiency, in addition to PTH elevations). Osteopenia or osteoporosis, when discovered, should be treated as per guidelines, with the understanding that some therapies could exacerbate hypocalcemia.

Patients with the AHO phenotype develop early-onset obesity, possibly due to aberrant hypothalamic responses to appetite control signals (e.g., leptin, melanocortin) and body mass index should be monitored. Currently, there are no data regarding the utility of appetite suppressant medications in these disorders. Regular monitoring for metabolic sequelae of obesity also is important. Hypertension is common (>50%) and regular monitoring and standard of care therapy are suggested. Lipid abnormalities also have been described. Notably, there is evidence of a higher prevalence of type 2 diabetes and increased insulin resistance in adult patients compared with weight-matched controls. Patients should also be assessed for sleep apnea [31].

Genetic counseling

The *GNAS* locus is highly imprinted, so the phenotypic manifestations are influenced by whether *GNAS* expression in a particular tissue involves one or both parental alleles. PHP and related disorders have an AD pattern of inheritance in kindred studies [30].

PHP1a results when the *GNAS* variant is maternally inherited, and expression of the paternal allele is silenced by imprinting with methylation so that the mutated allele is predominantly responsible for *GNAS* expression in the kidney tubules, thyroid, gonads, and pituitary. In tissues with expression of both *GNAS* alleles, the AHO phenotype is displayed due to tissue-specific haploinsufficiency. When the *GNAS* variant is inherited from the father, the phenotype is of PPHP with expression of the nonmutated maternal allele in the kidney, hence the lack of PTH resistance, but with a manifestation of AHO due to the 50% reduction in *GNAS* expression in tissues with biallelic expression. POH also manifests from a paternal *GNAS* variant in most cases and it is unclear why some *GNAS* variants cause POH without the AHO phenotype. Initially, it was unclear if PHP1c is a distinct disorder from PHP1a, in spite of the similar phenotypic manifestations, because the function of erythrocyte derived *GNAS* from these patients was preserved. However, there is now evidence of *GNAS* pathogenic variants in some PHP1c kindreds, in particular in exon 13 which encodes an alpha helix in the C-terminus with a resultant decreased coupling with other cytoplasmic signaling proteins [30].

The other PHP-related disorders are not caused by *GNAS* variants and GPCR signaling is preserved. PHP1b results from aberrant imprinting of the maternal *GNAS* allele so that expression is abrogated in maternally expressed tissues, in some cases caused by a pathogenic variant in regulatory elements of maternal *STX16*. PHP2 appears to be caused by signaling protein variants downstream of *GNAS* and cAMP generation such as *de novo* pathogenic variants in *PRKARIA* or *PDE4D* [30].

McCune—Albright syndrome

Overview

The classical manifestations of MAS are café-au-lait spots and polyostotic fibrous dysplasia (FD) usually involving the skull base and femurs. Renal phosphate wasting also occurs, due to fibroblast growth factor 23 secretion from the bone. Sarcomas can develop in 1% of affected individuals and cardiomyopathy can be seen due to activation of the mitogen-activated protein kinase (MAPK) signaling pathway.

Evaluation

Due to the key role of *GNAS* in hormone receptor signaling, patients with MAS also manifest a variety of endocrine abnormalities due to autonomous activation of the hormone receptor signaling in the absence of a ligand. The most common endocrine presentation is precocious puberty, with autonomous ovarian estradiol production in women resulting in the early onset of vaginal bleeding and breast development. In men, elevated testosterone can be observed and there is a risk for development of Sertoli and Leydig tumors. Patients may develop hyperthyroidism manifested by suppressed TSH and elevated thyroid hormone (T3) levels. Elevated prolactin and GH levels are also seen in a subset of patients and somatotropinomas and prolactinomas can develop in these patients. Nodular adrenal hyperplasia can cause Cushing's syndrome in neonatal cases [32].

Genetic testing

Germline variants that cause *GNAS* activation are incompatible with life; MAS is caused by sporadic and somatic *GNAS* variants occurring early in postzygotic fetal development. The phenotypic presentations are thus variable and determined by the level of mosaicism. Because of mosaicism, genetic testing performed on a blood sample may be unrevealing; if required, testing may be performed on the tissue of interest when necessary to confirm an unclear clinical diagnosis [33].

Management

X-rays and other imaging including MRI or ^{99}Tc -MDP/SPET scanning could be of use to characterize the bone lesions. The FD can have a “ground-glass” appearance with cystic lesions and sclerosis. Laboratory parameters that evaluate bone and mineral metabolism including alkaline phosphatase, serum calcium and phosphate, 25-hydroxy vitamin D3, intact PTH and possibly bone turnover markers may be of help. Monitoring for hypophosphatemia and renal phosphate wasting is important as fracture risk is increased. Phosphate supplements are needed in some patients [34].

In cases of early sexual development, assessment for gonadal development with gonadotropin levels, estradiol or testosterone, and ovarian or testicular ultrasounds are recommended. For thyroid function, T3 levels should be measured routinely because hyperthyroidism could be missed using only a T4 assessment [34].

Genetic disorders affecting the adrenal glands

Congenital adrenal hyperplasia

Overview

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder caused by pathogenic variants in the cortisol biosynthesis pathway that lead to decreased cortisol production. Additional biochemical and clinical manifestations occur because of the accumulation of precursors upstream of the enzymatic block and diversion of these intermediates to other hormone synthesis pathways.

Evaluation

The majority of cases of CAH (90%) result from pathogenic variants in 21-hydroxylase (*CYP21A2*) that encodes a key enzyme required for adrenal steroids biosynthesis. “Salt-wasting” or classic CAH refers to a phenotype where both cortisol and aldosterone production are compromised, and patients present during neonatal period with hyponatremia and hyperkalemia. “Simple virilizing” CAH manifests as prenatal virilization of women.

Patients with nonclassic CAH (NCAH) can have a less severe presentation and can come to medical attention as adults [35]. Individuals with NCAH typically have a residual activity of 21-hydroxylase that still allows for some cortisol synthesis; the cortisol levels can increase

by increased stimulation by ACTH. However, this also leads to increased production of adrenal androgens. Dehydroepiandrosterone (DHEA) is not necessarily elevated but is converted to androstenedione, testosterone, and the potent androgen, dihydrotestosterone (DHT). The build-up of the precursor 17-hydroxyprogesterone (17OHP) also can drive its conversion to androsterone, androstenediol, and finally to DHT. In women, hyperandrogenism causes premature adrenarche, acne, hirsutism, menstrual irregularity or amenorrhea, infertility, and even signs of virilization (clitoromegaly, male pattern hair loss). Short stature may be observed. In a young adult female, phenotype can look similar to polycystic ovary syndrome. Elevated morning 17OHP (preferably in the menstrual follicular phase) or after ACTH stimulation can be used to confirm the diagnosis biochemically [35]. Men do not come to medical attention due to elevated androgen levels (unless there is premature adrenarche or pubarche) but may have gynecomastia.

Genetic testing

NCAH is an autosomal recessive disorder caused by homozygous or compound heterozygous pathogenic variants in *CYP21A2*. *CYP21A2* is adjacent to a pseudogene *CYP21Ap* and many *CYP21A* inactivating variants are caused by cross-over or gene conversion. The most common pathogenic variant found in NCAH patients is p.Val281Leu [35].

Management

Glucocorticoid treatment (usually prednisone, hydrocortisone, or dexamethasone) is clinically indicated for NCAH in symptomatic patients. For women, this could include disruptive hyperandrogenism, menstrual irregularities, and infertility or subfertility. Anti-androgen therapies, such as spironolactone, and oral contraceptive pills can decrease the manifestations of hyperandrogenism. Male patients also may have acne and decreased fertility and testicular adrenal rest tumors can form as nonmalignant, unencapsulated growths of adrenal-like tissue on histology. Patients with NCAH may also present with extreme fatigue during stressful illnesses or events, which could indicate that they require sick day treatment for adrenal insufficiency under those circumstances [35].

Genetic testing

CAH is an autosomal recessive disorder. Carriers can be identified by appropriate genetic testing to inform reproductive decisions.

Carney complex

Overview

Primary pigmented nodular adrenal cortical disease (PPNAD), also called micronodular hyperplasia, is present in a majority (60%–70%) of patients with Carney complex (CNC), manifesting as Cushing's syndrome with elevated cortisol and suppressed ACTH levels. CNC can also present with cardiac myxomas (30%–40%) and spotty skin pigmentation, lentigines,

and blue nevi. GH and prolactin producing pituitary tumors can develop. Additional endocrine manifestations include thyroid nodules, Sertoli and Leydig cell tumors, adrenal rest tumors, and ovarian cystadenomas [32].

Genetic testing

CNC is an autosomal dominant disorder and de novo pathogenic variants are seen in a significant proportion of patients. A pathogenic variant of protein kinase A regulatory type 1 subunit alpha on chromosome 17 causes CNC1, which is responsible for approximately 70%–80% of all CNC. CNC2 has a less severe phenotype and has been linked to chromosome 2p16 but the causative gene remains to be identified. Sporadic cases of bilateral micronodular adrenal hyperplasia, but without pigmentation, have been associated with variants in phosphodiesterase 11A (*PDE11A*) and *PDE8B* [32].

Management

Identification of a patient with findings consistent with CNC should prompt a cardiac evaluation, as cardiac myxomas can be a cause of mortality. Definitive therapy for Cushing's syndrome requires bilateral adrenalectomy. Detailed monitoring of tumor formation (physical exam and appropriate imaging) and of GH and prolactin excess is needed [32].

Bilateral macronodular adrenal hyperplasia

Overview

The nodules of bilateral macronodular adrenal hyperplasia (BMAH) are multiple and larger than those observed in CNC. BMAH was previously named ACTH-independent macronodular adrenal hyperplasia but the moniker was changed due to the finding that there is some paracrine involvement of ACTH stimulation locally in the adrenal. Patients present later in life, often in the fifth to sixth decade, with Cushing's syndrome manifested by mildly elevated cortisol, and low to suppressed ACTH and DHEA sulfate [36].

Genetic testing

Clinical characterization of early cases of macronodular hyperplasia identified aberrant expression of various hormonally activated GPCRs as the mediator for hypercortisolism and hyperplasia: these included receptors for glucose-dependent insulinotropic polypeptide, glucagon, vasopressin, adrenaline, luteinizing hormone, beta-human chorionic gonadotrophin, and thyrotropin hormone-releasing hormone. Since that time, approximately one-quarter of patients with familial BMAH and half of sporadic cases have been shown to have a pathogenic variant in armadillo repeat containing 5 locus which behaves as a tumor suppressor gene. Patients carry a germline variant, but a “second hit” or somatic variant is also found in the adrenal nodules [36].

Management

Cushing syndrome can be challenging to diagnose and the degree of hypercortisolism with BMAH can be subtle such that overt hypercortisolism (detected by urine free cortisol excess) can be absent and supplemental tests are required, such as dexamethasone suppression tests (lack of suppressibility) and midnight salivary cortisol (lack of circadian cortisol rhythm). ACTH should be below normal or suppressed. Although definitive treatment would require bilateral adrenalectomy, more conservative approaches such as medical therapy to block glucocorticoid action, or unilateral adrenalectomy of the most dominant (largest) side can be considered [37].

Points to remember

- MODY should be considered in younger patients who present with hyperglycemia, a strong family history of DM, and without autoimmunity or insulin resistance.
- The MEN syndromes are a heterogeneous group of disorders with the development of tumors in more than one endocrine gland.
- MEN1 is caused by pathogenic variants in the *MEN1* tumor suppressor gene and can lead to the development of pituitary adenomas, hyperparathyroidism, and pancreatic islet tumors as well as up to 20 different other endocrine and nonendocrine tumors.
- The MEN2 syndromes are caused by pathogenic variants in the *RET* proto-oncogene and have a strong genotype-phenotype relationship.
- MEN2A includes medullary thyroid carcinoma, pheochromocytomas, and hyperparathyroidism while 2B patients do not have hyperparathyroidism and have a marfanoid appearance with multiple ganglioneuromas.
- Maternal inactivating variants of the *GNAS* are responsible for the phenotypes of PHP and Albright's hereditary osteodystrophy. Activating *GNAS* variants cause MAS.

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Gastrointestinal disorders

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Introduction

In general, genetic and nongenetic gastrointestinal disorders present with similar signs and symptoms. For example, an individual with an inherited colorectal cancer (CRC) may present with iron-deficiency anemia, unexplained weight loss, or a bowel obstruction similar to the presentation of a sporadic CRC. Due to these similarities, it is important to maintain a high level of clinical suspicion for a genetic gastrointestinal disorder to ensure an accurate diagnosis. Red flags that should raise your suspicion for a genetic disorder include early onset of the disease (e.g., CRC <50 years old, precancerous colon polyps <50 years old, pancreatitis or liver failure in adolescents), multiple cancers or gastrointestinal polyps in one individual, and/or a family history of similar conditions. The accurate diagnosis of an inherited gastrointestinal disorder expedites appropriate treatment and surveillance for the affected individual and allows identification of asymptomatic family members to institute the necessary screening.

Diseases of the Colon

Hereditary CRC and polyposis syndromes

CRC is the second leading cause of cancer-related deaths for both men and women in the United States [1]. CRC develops from precancerous lesions such as colon polyps. The vast majority of CRCs are sporadic, while 25%–30% appear to have a familial predisposition caused by a combination of multiple genetic, environmental, and lifestyle factors shared within a family. Approximately 5% of CRCs are due to well-defined hereditary syndromes including Lynch syndrome, familial adenomatous polyposis (FAP), *MUTYH*-associated polyposis (MAP), and hamartomatous polyposis syndromes [2]. These syndromes are characterized by early-onset CRC or colon polyps, multiple types of tumors including extraintestinal tumors in an individual, multiple (>20) polyps over an individual's lifetime, and a

significant family history of multiple family members with the same or related diagnoses across generations.

Lynch syndrome

Overview

Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer) is the most common cause of hereditary CRC. It is an autosomal dominant condition due to pathogenic variants in the four DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Moreover, a germline deletion in *EPCAM* leads to inactivation of *MSH2* in a small number of individuals with Lynch syndrome [2]. The lifetime risk of CRC in Lynch syndrome can be up to 80% depending on the mutated gene. Lynch syndrome also confers an increased risk for several extracolonic cancers such as endometrial, ovarian, gastric, small intestine, hepatobiliary tract, urinary tract, central nervous system, pancreas, and skin (sebaceous neoplasms - Muir Torre syndrome). Recent data also have highlighted an association with prostate and possibly breast cancer.

Evaluation

Clinical suspicion for Lynch syndrome should be raised in all individuals with CRC <50 years old, right-sided colon cancers, CRC with high microsatellite instability (MSI-H), and a family history of multiple relatives with CRC or other Lynch syndrome-related cancers across more than one generation. An easy clinical tool to identify individuals who should be tested for Lynch syndrome is the Amsterdam II criteria [2]. The criteria can be remembered as the “3-2-1-0 Rule”: at least *three* relatives with Lynch syndrome-related cancers, across *two* generations, with at least *one* diagnosed under the age of 50 years and FAP has been excluded. Unfortunately, the Amsterdam II criteria have a low sensitivity and miss approximately 50% of individuals with Lynch syndrome. The Revised Bethesda guidelines were subsequently developed to assist in the diagnosis of Lynch syndrome by guiding tumor testing for MSI [2]. The Revised Bethesda guidelines state that tumors from individuals should be tested for MSI in the following situations:

- CRC diagnosed in a patient who is younger than 50 years
- Presence of synchronous, or metachronous, colorectal or other Lynch syndrome-related tumors regardless of age
- CRC with histology that is suspicious for MSI (e.g., mucinous or signet ring appearance, tumor infiltrating lymphocytes, Crohn’s-like lymphocytic reaction) in a patient <60 years of age
- CRC in a patient with one or more first-degree relatives with a Lynch syndrome-related cancer, with one of the cancers diagnosed under 50 years of age
- CRC in a patient with two or more first- or second-degree relatives with Lynch syndrome-related cancers regardless of age

Many centers are offering universal screening of all CRCs and uterine cancers for Lynch syndrome, regardless of age. Tumor screening consists of testing for MSI status and/or immunohistochemistry (IHC) staining (preferred) for the four DNA mismatch repair proteins (MLH1, MSH2, MSH6, and PMS2). Testing of rectal cancers should be done prior to neoadjuvant chemotherapy and radiotherapy. Furthermore, there are multiple online tools that are publicly available to identify patients who should be referred for consideration of genetic testing for Lynch syndrome: any individual with a $\geq 5\%$ risk calculated by the PREMM (1,2,6), MMRpro, and MMRpredict models or with a $\geq 2.5\%$ risk calculated by the PREMM5 model.

Genetic testing

Over 90% of CRCs in individuals with Lynch syndrome will be MSI-H and have loss of expression of at least one of the four DNA mismatch repair proteins. The IHC pattern can be used to guide germline genetic testing. Approximately 15% of sporadic CRC will be MSI-H and often show a loss of expression of MLH1 alone or in combination with PMS2 [2]. These patients will have normal germline genetic testing for the Lynch syndrome genes. Confirmatory testing can be done on the tumor for a *BRAF* (V600E) variant and/or hypermethylation of the *MLH1* gene promoter. All abnormal findings on tumor screening should be confirmed with germline genetic testing. The National Comprehensive Cancer Network (NCCN) Genetic/Familial High-Risk Assessment: Colorectal Guidelines provide a detailed description of additional testing strategies based on tumor testing results [3].

In cases with discordant results where tumor screening suggests Lynch syndrome and germline genetic testing are normal, paired tumor/germline testing can be performed. In this testing, somatic variants, copy number variants, and loss of heterozygosity are determined by comparing results between tumor and blood samples. Two variants of somatic origin or copy-neutral loss of heterozygosity and a somatic variant in the same Lynch syndrome gene that is concordant with the tumor IHC results suggest that the tumor is sporadic and indicates a significantly reduced likelihood of a Lynch syndrome diagnosis.

Vignette 13.1

A 40-year-old woman is referred for a diagnostic colonoscopy to evaluate iron-deficiency anemia. An ulcerated hemi-circumferential mass is identified in the ascending colon; biopsies confirm invasive adenocarcinoma, and she undergoes a right hemicolectomy. Histopathology of the surgical specimen shows a mucinous adenocarcinoma with a Crohn's-like lymphocytic reaction and loss of MLH1 and PMS2 on IHC staining. Upon further questioning, she reports having a hysterectomy 5 years ago due to abnormal vaginal bleeding and was told she had "dysplastic cells" in her uterus. She also has a family history of endometrial cancer in her mother at age 46 and colon cancer in her maternal grandfather in his 40s.

This patient should be referred for genetic testing for Lynch syndrome. She meets the Amsterdam II criteria, as well as the Revised Bethesda guidelines. Moreover, she had abnormal IHC staining on tumor screening that requires further workup. After meeting with a genetic counselor, she agrees to genetic testing and is found to have a pathogenic variant in

the *MLH1* gene. In this case, genetic testing can be done with a preset multigene panel from an established laboratory that includes *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* or with a more judicious approach based on the tumor screening by first testing for only *MLH1* and *PMS2* mutations. If this patient had been a 79-year-old woman with no prior personal history of cancer and no family history of cancer, clinical suspicion would be much higher for a sporadic colon cancer. In this case, before proceeding with germline genetic testing for Lynch syndrome, the tumor should be tested for the *BRAF* (V600E) mutation and/or hypermethylation of the *MLH1* gene promoter. If either of these tests are positive, the tumor is most likely sporadic.

Management

Due to the significantly increased risk of multiple cancers in Lynch syndrome, it is important to identify asymptomatic individuals so that appropriate screening, surveillance, and/or preventive interventions can be initiated [2]. Each mutated gene confers slightly different lifetime cancer risks; however, current recommendations for management are independent of the mutated gene. Table 13.1 summarizes lifetime cancer risks and management based on the NCCN recommendations [3].

Table 13.1 Lynch syndrome cancers: lifetime risk up to age 70 and management.

Cancer	General population risk	Lynch syndrome risk (gene dependent)	Management
Colorectal	4.5%	<i>MLH1/MSH2</i> : 52%–82% <i>MSH6</i> : 10%–22% <i>PMS2</i> : 15%–20%	Colonoscopies every 1–2 years starting at age 20–25
Endometrial	2.7%	<i>MLH1/MSH2</i> : 25%–60% <i>MSH6/PMS2</i> : 15%–26%	Consider risk-reducing hysterectomy after childbearing or screening with endometrial biopsies every 1–2 years
Ovarian	1.3%	<i>MLH1/MSH2</i> : up to 24% <i>MSH6/PMS2</i> : limited data	Consider risk-reducing bilateral salpingo-oophorectomy after childbearing
Gastric	< 1%	<i>MLH1/MSH2</i> : 6%–13% <i>MSH6/PMS2</i> : <3%	Consider upper endoscopy every 3–5 years starting at age 30–35
Small intestine	< 1%	3%–6%	Follow based on the family history
Hepatobiliary	< 1%	1%–4%	
Pancreas	< 1%	1%–6%	
Urinary tract	< 1%	1%–7%	
Brain/CNS	< 1%	1%–3%	Annual urinalysis starting at age 30–35 Annual physical and neurologic exam starting at age 25–30
Sebaceous neoplasms	< 1%	1%–9%	Annual skin exam
Prostate	11.6%	Up to 30%	Follow based on the family history

Genetic counseling

Lynch syndrome is an autosomal dominant condition, so all first-degree relatives of individuals with an identified pathogenic variant in the genes associated with Lynch syndrome should undergo testing. When a specific variant has been identified in a family, the current recommendation is for single-site variant testing for family members. In this situation, a negative test result is a true negative and that relative should be followed as general population risk for all Lynch syndrome-related cancers. Lynch syndrome screening typically does not occur prior to age 20, so genetic testing of individuals <18 years of age is generally not recommended.

Adenomatous polyposis syndromes

Overview

The adenomatous polyposis syndromes include both autosomal dominant (e.g., FAP/attenuated FAP) and autosomal recessive (e.g., MAP) transmission and are characterized by multiple adenomatous colon polyps, duodenal adenomas, and multiple fundic gland gastric polyps. These syndromes confer a significantly increased risk of CRC, as well as additional extracolonic malignancies (Table 13.2). The most common adenomatous polyposis syndromes (FAP/AFAP and MAP) are estimated to account for approximately 2% of all CRCs [2]. Many patients require surgical intervention to manage the polyp burden.

Evaluation

Individuals with a cumulative number of >10 adenomatous colon polyps should be considered for genetic testing for a hereditary polyposis syndrome. Evaluation should include a detailed family history specifically looking for relatives with multiple gastrointestinal polyps, as well as characteristic cancers (e.g., CRC, duodenal, thyroid). Physical exam and medical history should focus on characteristic findings such as osteomas, desmoid tumors, and congenital hypertrophy of the retinal pigment epithelium (Table 13.2). The number, size, and pathology of all polyps should be confirmed with pathology and procedure reports.

Genetic testing

In general, genetic testing for the adenomatous polyposis syndromes is recommended for individuals with: [2]

- > 10 cumulative adenomatous polyps on a single colonoscopy,
- ≥ 10 adenomas and a personal history of CRC, or
- a total of >20 adenomatous polyps in their lifetime.

Family history is important to help differentiate a possible autosomal dominant versus autosomal recessive syndrome. However, due to the high *de novo* mutation rate of the *APC* gene, this should be considered even if no family history is present. A number of genes with moderately increased lifetime risk for polyposis have recently been discovered and should be

Table 13.2 Adenomatous polyposis syndromes [2,3].

Syndrome	Gene(s) and inheritance	Diagnostic criteria/specific characteristics	Management
FAP	APC Autosomal dominant (~25–30% <i>de novo</i> mutations)	• 100–1000 adenomatous colon polyps beginning in teenage years • Nearly 100% lifetime risk of CRC • Extracolonic features: gastric polyps (fundic gland and adenomas), duodenal/ampullary polyps, desmoid tumors, CHRPE (multifocal/bilateral), osteomas, supernumerary teeth, epidermoid cysts • Extracolonic malignancies: papillary thyroid—cribriform-morular variant (1%–2%), duodenal/ampullary (4%–12%), stomach (<1%), pancreas (2%), brain-medulloblastoma (<1%), hepatoblastoma up to age 5 (1%–2%)	• Annual colonoscopy (preferred) or flexible sigmoidoscopy starting at age 10–15 • Prophylactic total proctocolectomy with ileo-anal pouch anastomosis is recommended by at least age 25 or sooner depending on polyp burden • Endoscopic surveillance of pouch every 1–3 years after surgery, depending on the polyp burden • Upper endoscopy with complete visualization of ampulla of Vater beginning at age 20–25 and continued every 1–4 years based on the polyp burden • Annual physical exam, including thyroid examination (consider thyroid ultrasound) • In at-risk children, consider serum AFP and liver ultrasound every 3–6 months
AFAP	APC Autosomal dominant (same <i>de novo</i> mutation rate as FAP) Mutations are generally in the 3' and 5' regions of the gene	• 10–100 adenomatous colon polyps beginning later in life than in FAP (typically after age 25) • ~70% lifetime risk of CRC • Extracolonic features: duodenal/ampullary polyps, fewer gastric polyps than FAP, CHRPE, and desmoid tumors are not typical for AFAP • Extracolonic malignancies: duodenal/ampullary; no hepatoblastoma; other cancer risks may be similar or less than with FAP	• Colonoscopy every 1–2 years starting in late teens/early 20s • Consider prophylactic colectomy with ileorectal anastomosis once polyp burden cannot be managed endoscopically • Endoscopic surveillance of rectum every 6–12 months after surgery, depending on the polyp burden • Upper endoscopic surveillance similar to FAP • Annual physical exam, including thyroid exam
MAP	MUTYH Autosomal recessive Two most common mutations are Y179C and G396D	• 10–100 adenomatous colon polyps in a lifetime (a higher number of serrated polyps can be seen than with FAP/AFAP) • ~80% lifetime risk of CRC • CRC risk for heterozygous carriers may be slightly increased compared to the general population	• Colonoscopy beginning at age 25–30 years and repeated every 2–3 years if normal or 1–2 years if polyps are found • Consider colectomy with ileorectal anastomosis if CRC or polyp burden cannot be managed endoscopically

SPS	Presumed familial inheritance <i>RNF43</i> mutations are a rare cause	• Extracolonic malignancies: duodenal cancer • Diagnosed if an individual meets one of the following criteria: 1. At least five serrated polyps proximal to the sigmoid colon with two or more >10 mm 2. Any number of serrated polyps proximal to the sigmoid colon in a patient with a first-degree relative with SPS 3. ≥ 20 serrated polyps of any size throughout the colon	• Upper endoscopy with complete view of ampulla of Vater beginning at age 30–35 years and continued every 1–4 years based on the polyp burden • Monoallelic mutation carriers can start colonoscopy at age 40 and repeat every 5 years • Colonoscopy every 1–3 years depending on the polyp burden • Consider colectomy if CRC, high-grade dysplasia, or polyp burden cannot be managed endoscopically • First-degree relatives should start colonoscopy at age 40, same age as youngest diagnosis of SPS, or 10 years earlier than a CRC diagnosis whichever comes first and repeat every 5 years

AFAP, Attenuated familial adenomatous polyposis; *CHRPE*, congenital hypertrophy of the retinal pigment epithelium; *CRC*, colorectal cancer; *FAP*, familial adenomatous polyposis; *MAP*, *MUTYH*-associated polyposis; *SPS*, serrated polyposis syndrome.

included in multigene panel testing: *POLE*, *POLD1*, and *GREM1*, which are autosomal dominant, and *NTHL1* and *MSH3*, which cause an autosomal recessive polyposis syndrome.

Vignette 13.2

A 50-year-old man was referred for genetic testing after a routine screening colonoscopy where a total of 15 polyps were removed. The majority of the polyps were tubular adenomas >6 mm, but two were tubulovillous adenomas >2 cm in size. Three-generation pedigree reveals a sister with colon polyps in her 40s, a father with multiple colon polyps and rectal cancer at age 60, and a paternal aunt with duodenal cancer in her 70s. Genetic testing was recommended for the *APC* gene. Testing was positive for a pathogenic *APC* variant.

The clinical phenotype of fewer than 100 colon polyps in the patient and his sister, as well as late-onset polyposis and rectal cancer in his father is consistent with AFAP. If the patient or a family member had hundreds of colon polyps, it would be suspicious for classic FAP. If there was no family history of polyposis or CRC, it would be suspicious for an autosomal recessive polyposis syndrome or a *de novo* variant in the *APC* gene, and the patient should have multigene panel testing that includes all of the above-mentioned genes.

Management

Management differs based on the polyposis syndrome ([Table 13.2](#)).

Genetic counseling

All first-degree relatives of individuals diagnosed with an autosomal dominant polyposis syndrome should be tested for the known familial variant. Whereas testing for children is generally not recommended for disorders that present in adulthood, testing is recommended when an individual will have direct, immediate benefit from the outcome of the test (e.g., testing for *APC* variants in children of families with classic FAP due to the risk of hepatoblastoma and need to start colonoscopy or sigmoidoscopy at an early age, but not for children of families with AFAP). In general, the timing of testing for *APC* gene variants should be based on the personal and family history. Genetic testing for siblings of individuals with an autosomal recessive polyposis syndrome is recommended. Genetic testing of their parents and offspring should also be considered if being a monoallelic carrier confers an increased cancer risk (e.g., *MUTYH* gene variants). Monoallelic carriers and their partners should be counseled on the risk for future offspring.

Hamartomatous polyposis syndromes

Overview

The hamartomatous polyposis syndromes are a heterogeneous group of rare, autosomal dominant conditions. They are identified based on the type of hamartomatous polyps (e.g., Peutz–Jeghers polyps, juvenile polyps) and carry an increased risk for both CRC and extra-colonic cancers ([Table 13.3](#)) [2]. These syndromes are caused by pathogenic variants in *STK11*, *BMPRIA*, *SMAD4*, and *PTEN*.

Table 13.3 Hamartomatous polyposis syndromes [2–4].

Syndrome	Gene(s) and inheritance	Diagnostic criteria/specific characteristics	Management
PJS	<i>STK11</i> Autosomal dominant (~30% <i>de novo</i> mutation rate)	- Clinical diagnosis can be made when an individual meets at least two of the following criteria: - Two or more Peutz–Jeghers polyps of the gastrointestinal tract (histopathology shows broad bands of muscularis mucosa smooth muscle) - Mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes, genitalia, or fingers - Family history of PJS ~40% lifetime risk of CRC - Extracolonic malignancies: breast (54%), pancreas (11–36%), stomach (29%), ovarian (21%), lung (15%), small intestine (13%), uterine/cervix (9%–10%), and testicle (<1%)	- Colonoscopy and upper endoscopy every 2–3 years starting in late teens - Computed tomography / magnetic resonance enterography or capsule endoscopy every 2–3 years starting at the age of 8–10 years - Pancreas cancer screening with endoscopic ultrasound or magnetic resonance cholangiopancreatography every 1–2 years starting at the age of 30–35 years - Annual mammogram and breast MRI with clinical breast exam every 6 months starting at age 25 - Annual testicular exam and ultrasound starting at age 10 - Annual pelvic exam and Pap smear with consideration of transvaginal ultrasound starting at age 18 - Smoking cessation
JPS	<i>BMPR1A</i> , <i>SMAD4</i> (only 50% of cases are found to have mutations in these genes) Autosomal dominant	- Clinical diagnosis can be made when an individual meets at least one of the following criteria: - At least three to five juvenile polyps of the colon (histopathology shows prominent cystically dilated glands and inflammatory stroma) - Multiple juvenile polyps throughout the gastrointestinal tract - Any number of juvenile polyps in an individual with a family history of JPS	- Colonoscopy and upper endoscopy every 2–3 years starting around age 15, repeat annually if polyps are found - Screening for vascular lesions (e.g., pulmonary and cerebral arteriovenous malformations) in patients with JPS-HHT due to a <i>SMAD4</i> mutation. This should occur within the first 6 months of life in infants with an identified <i>SMAD4</i> mutation

(Continued)

Table 13.3 (Continued)

Syndrome	Gene(s) and inheritance	Diagnostic criteria/specific characteristics	Management
<i>PTEN</i> hamartoma tumor syndromes (e.g., Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome)	<i>PTEN</i> Autosomal dominant (can be <i>de novo</i>)	• ~40% lifetime risk of CRC • Extracolonic features: gastric polyps (hyperplastic and juvenile polyps) are more common with <i>SMAD4</i> mutations, HHT syndrome can be seen with <i>SMAD4</i> mutations • Extracolonic malignancies: stomach, pancreas, and small intestine (21% for all combined) • Present with hamartomatous polyps, as well as intestinal ganglioneuromas and intramucosal lipomas • 9%–18% lifetime risk of CRC • Extracolonic features: macrocephaly, autism spectrum disorder, facial trichilemmomas, oral papillomas, cutaneous lipomas • Extracolonic malignancies: breast, endometrial, thyroid –follicular type, and renal cancers • Clinical diagnosis is made when patients meet a combination of major and minor criteria as detailed by the National Comprehensive Cancer Network guidelines [4]	• Colonoscopy starting at age 35 or 5–10 years before the earliest CRC diagnosis (whichever comes first) and repeat every 5 years • Annual thyroid ultrasound starting at the time of diagnosis • Clinical breast exam every 6–12 months starting at age 25 with transition to annual mammogram and breast MRI at age 30–35 or starting 5–10 years before the earliest breast cancer diagnosis (whichever comes first) • Renal ultrasound every 1–2 years starting at age 40 • Referral to gynecology for abnormal menstrual bleeding

CRC, Colorectal cancer; *HHT*, hereditary hemorrhagic telangiectasia; *JPS*, juvenile polyposis syndrome; *MRI*, magnetic resonance imaging; *PJS*, Peutz–Jeghers syndrome.

Evaluation

Individuals with hamartomatous polyps should undergo a complete history and physical exam, including a three-generation pedigree. Diagnostic workup may also include completion of endoscopic studies to evaluate for hamartomatous polyps in other areas of the gastrointestinal tract (e.g., upper endoscopy/enteroscopy if only a colonoscopy was previously performed). Further evaluation should focus on determining if a patient meets the diagnostic criteria and syndrome-specific characteristics outlined in [Table 13.3](#).

Genetic testing

Genetic testing should include a multigene panel with *STK11*, *BMPRIA*, *SMAD4*, and *PTEN*. Given the rarity of these syndromes, individuals who present with >5 hamartomatous polyps on colonoscopy, particularly those <40 years of age, should be referred for genetic testing.

Management

Management differs based on the polyposis syndrome (see [Table 13.3](#)).

Genetic counseling

All first-degree relatives of individuals with an identified hamartomatous polyposis syndrome, as well as relatives who meet the clinical criteria outlined in [Table 13.3](#), should have genetic testing. It is appropriate to offer genetic testing to children in families with an identified pathogenic variant since many of these syndromes present early in life and require surveillance. It is especially important for families with a *SMAD4* pathogenic variant due to the recommendation for hereditary hemorrhagic telangiectasia (HHT) screening within the first 6 months of life.

Diseases of the Stomach

Gastric cancer

Gastric cancer is the sixth most common cancer worldwide [\[5\]](#). Anatomically, one-third of all gastric cancers are located in the gastric cardia, while two-thirds are noncardia cancers. Histologically, gastric cancer is categorized as intestinal (cells are organized into glandular formations with intercellular junctions) versus diffuse (cell clusters that lack cell–cell cohesion), which includes signet ring cell adenocarcinoma. Approximately 80% of gastric cancers are sporadic. Nongenetic risk factors include *Helicobacter pylori* infection (more commonly associated with intestinal noncardia cancers), as well as obesity and gastroesophageal reflux disease (associated with gastric cardia cancers). The remainder of patients with gastric cancer have a familial clustering of the disease with 1%–3% attributed to known hereditary cancer syndromes such as Lynch syndrome, adenomatous, and hamartomatous polyposis syndromes, Li–Fraumeni syndrome, hereditary breast and ovarian cancer syndrome, and hereditary diffuse gastric cancer (HDGC) syndrome [\[6,7\]](#). Another rare type of tumor that can be found in the stomach, as well as the rest of the digestive tract, is gastrointestinal stromal tumor (GIST).

Hereditary diffuse gastric cancer

Overview

HDGC is characterized by early-onset diffuse-type gastric cancer with mean age of gastric cancer diagnosis of 37 years. HDGC is caused by pathogenic variants in *CDH1* in up to 50% of cases and is transmitted in an autosomal dominant manner. *CDH1* gene encodes E-cadherin, which is a transmembrane protein that plays a critical role in maintaining cell–cell cohesion and binds intracellular catenin. Lifetime risk of gastric cancer for individuals with a pathogenic *CDH1* variant by age 80 is 67% for men and 83% for women. Women with *CDH1* variants are also at significantly increased risk for lobular breast cancer [6,8].

Evaluation

Clinical presentation of individuals with gastric cancer due to HDGC is similar to gastric cancer from other etiologies and can include weight loss, abdominal pain, nausea, early satiety, anemia, and melena. Evaluation should include an upper endoscopy with biopsies of all abnormal lesions and examination for normal insufflation of the stomach. Advanced HDGC predominantly presents as *linitis plastica* with diffuse infiltration of the gastric wall; therefore poor distention on upper endoscopy should prompt further evaluation with computed tomography (CT) scan and endoscopic ultrasound (EUS) to better visualize the gastric wall layers.

Genetic testing

Genetic testing for *CDH1* gene variants is recommended for (1) individuals with diffuse-type gastric cancer before age 40, (2) families with two or more relatives with gastric cancer at any age with one confirmed diffuse-type cancer, and (3) families with both diffuse-type gastric cancer and lobular breast cancer (one of which diagnosed before the age of 50 years). *CDH1* testing should also be considered in individuals with bilateral or familial lobular breast cancer before age 50, diffuse-type gastric cancer and cleft lip/palate, and individuals with gastric biopsies showing in situ signet ring cells and/or pagetoid spread of signet ring cells adjacent to cancer [7]. A pathogenic variant in *CDH1* will not be identified in almost half of the individuals who meet the above criteria; they should still be considered to have a clinical diagnosis of HDGC.

Management

Individuals with a confirmed *CDH1* pathogenic variant should be managed by an experienced multidisciplinary team including genetic counselors/geneticists, gastroenterologists, surgeons, oncologists, pathologists, nutritionists, and psychosocial support. Due to the significantly increased lifetime risk of diffuse-type gastric cancer, individuals with a pathogenic *CDH1* variant are recommended to have a prophylactic gastrectomy. The timing of the procedure should be individualized with discussions beginning between 20 and 30 years of age. If surgery is refused or delayed, patients should undergo annual upper endoscopies with biopsies of all visible lesions including pale areas, as well as at least five random biopsies from the pre-pyloric area, antrum, transitional zone, body, fundus, and cardia (Cambridge protocol). A minimum of 30 biopsies is recommended [7]. Women with pathogenic *CDH1* variants should

begin annual mammograms at age 30 (or earlier based on the family history) with consideration for adding breast magnetic resonance imaging (MRI). Risk-reducing mastectomies should be considered based on the family history [4]. Individuals with a pathogenic *CDH1* variant and a family history of colon cancer should begin colonoscopies at age 40 or 10 years before the earliest diagnosis and continue every 5 years [7]. Patients who meet clinical criteria for HDGC but do not have an identifiable pathogenic variant or have a variant of uncertain significance should undergo intensive annual endoscopic surveillance as described above.

Genetic counseling

First-degree relatives of individuals with a confirmed *CDH1* variants should be offered genetic testing. Testing is not generally recommended prior to the age of consent (18 years). Testing younger unaffected family members should be individualized and based on the earliest age of gastric cancer in a family.

Gastrointestinal stromal tumor

Overview

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the digestive tract and are thought to arise from the interstitial cells of Cajal or precursors to these cells. Approximately 5000 new cases of GISTs are diagnosed in the United States each year [9]. The majority of GISTs are sporadic, found in the stomach and small intestine, and occur in individuals between the ages of 50–70 years old. In approximately 5% of individuals, GIST is part of an inherited (or familial) syndrome such as primary familial GIST syndrome, Carney–Stratakis syndrome, and neurofibromatosis type 1 [9].

Evaluation

Small, slow-growing GISTs are often asymptomatic and discovered incidentally on imaging studies or endoscopic exams. Larger GISTs can present with symptoms similar to other gastric cancers such as weight loss, abdominal pain, nausea, early satiety, hematemesis, melena, and anemia. Evaluation includes tests to diagnose/locate the tumor and tests to determine the likelihood of metastasis. Location of the tumor can be determined with upper endoscopy if the GIST is located in the stomach and by CT enterography/MR enterography or capsule endoscopy if the tumor is in the small intestine, and by colonoscopy for lower digestive tract tumors. Tissue sampling is necessary to confirm the diagnosis. In the case of gastric tumors, EUS with fine-needle aspiration (EUS-FNA) can often provide enough tissue for confirmation. If EUS-FNA is unable to obtain adequate tissue or the tumor cannot be reached by this method, surgical resection can be performed for further tissue analysis. Approximately 95% of GISTs will be positive for c-KIT protein (CD117) on IHC staining. The tumor mitotic rate (number of cells that are actively dividing out of 50 different microscopic fields) is also calculated. The higher the mitotic rate, the more aggressive the tumor and the higher likelihood of metastasis. Additional evaluation by a medical geneticist and/or dermatologist should be considered to identify other clinical manifestations of hereditary predisposition syndromes (Table 13.4).

Table 13.4 Features of syndromic/inherited GISTs [9].

Syndrome	Gene	Inheritance	Other clinical manifestations
Primary familial GIST syndrome	<i>KIT</i> (encodes the c-KIT receptor tyrosine kinase, a type III tyrosine kinase receptor)	AD, high penetrance	• Achalasia • Melanoma • Multiple lentigines • Perioral and perineal hyperpigmentation • Urticaria pigmentosa • GISTs can be anywhere in the GI tract
Primary familial GIST syndrome	<i>PDGFRA</i> (encodes platelet-derived growth factor receptor A, a type III tyrosine kinase receptor)	AD, high penetrance	• Lipomas • Fibrous tumors of the GI tract • Large hands • GISTs are primarily gastric
Neurofibromatosis Type I	<i>NF1</i> (encodes neurofibromin 1, a tumor suppressor protein)	AD, variable expression	• Café-au-lait spots • Neurofibromas • Pheochromocytomas • Other neuroendocrine tumors of the GI tract • Multifocal small bowel GISTs • Gastric-based multifocal GISTs
Carney–Stratakis syndrome	<i>SDHx</i> (encodes the succinate dehydrogenase complex composed of A, B, C, and D subunits)	AD, incomplete penetrance	• Paragangliomas • Renal cell carcinoma (rare)
Carney triad	<i>SDHC</i> promoter hypermethylation	Sporadic	• Carney triad = GIST, paraganglioma, pulmonary chondroma • May also be associated with adrenal cortical adenomas

GIST, gastrointestinal stromal tumor; AD, autosomal dominant.

Genetic testing

GISTs predominantly involve genetic changes in the *KIT* gene (75%), which encodes the c-KIT receptor tyrosine kinase and the *PDGFRA* gene (~10%) [9]. Pathogenic variants in these genes are seen in both sporadic and familial GISTs. In sporadic GISTs, the variants are somatic (i.e., acquired genetic changes that occur only in the tumor cells). Germline genetic testing for a syndromic/inherited GIST should be considered when:

- a person in the family is diagnosed with a GIST before age 50
- a person in the family has metachronous or synchronous GISTs
- two or more close relatives in a family are diagnosed with a GIST
- a person in the family with a GIST also has any of the syndrome-specific characteristics listed in Table 13.4

Management

There are no special management recommendations for familial GISTs compared to sporadic GISTs. Small, incidental GISTs can be monitored in carefully selected cases. Larger GISTs should be removed surgically if the patient is considered to be an overall good surgical

candidate. The first-line medical therapy for GISTs is imatinib; however, there is a high rate of resistance over time, and it is of limited utility in SDH-deficient tumors. Sunitinib and regorafenib are often effective for imatinib-resistant GISTs. There is no standard screening for individuals who have a germline *KIT*, *PDGFRA*, or *NF1* pathogenic variant and have not yet developed a GIST. However, individuals with SDH-deficient GISTs should consider having annual whole-body MRI and urine or plasma catecholamines to screen for paragangliomas and pheochromocytomas [9]. Other clinical manifestations of inherited GIST syndromes in affected individuals should be followed by appropriate specialists.

Genetic counseling

Genetic counseling should be offered to the first-degree relatives of individuals with a confirmed pathogenic *KIT*, *PDGFRA*, *NF1*, or *SDHx* gene variant. Genetic testing for familial GISTs is still evolving. There may be genes other than those previously mentioned that cause an inherited syndrome or that our current genetic testing technology cannot identify. Furthermore, not all GIST-associated hereditary predisposition syndromes are inherited in the traditional autosomal dominant manner. Complications of parent-of-origin effects, high *de novo* rates, and variable expressivity within families warrant consideration when testing family members. Testing of younger unaffected family members is dependent on the pathogenic variant in a given gene and the associated syndrome.

Diseases of the Pancreas

Pancreatic cancer

Overview

Pancreas cancer is the seventh leading cause of cancer death worldwide [5]. Histologically, pancreatic cancer can occur from exocrine or endocrine cells. Exocrine tumors (adenocarcinomas) account for 95% of pancreatic cancers, while endocrine tumors (pancreatic islet cell or pancreatic neuroendocrine tumors) account for less than 5%. The vast majority (~90%) of pancreatic cancers are sporadic. Nongenetic risk factors include pancreatitis, diabetes, obesity, and tobacco use. Approximately 10% of pancreatic cancers are due to a known hereditary cancer syndrome with germline *BRCA2* pathogenic variants explaining up to 17% of familial pancreatic cancers (FPCs). Pathogenic variants in *PALB2*, *CDKN2A*, *STK11*, and the Lynch syndrome genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*) are also associated with a significantly increased risk of pancreatic cancer [10]. Families who have at least two family members with pancreatic cancer who are first-degree relatives, but no identified germline variant, are considered to have FPC syndrome.

Evaluation

Clinical presentation of individuals with hereditary pancreatic cancer is similar to sporadic pancreatic cancer and can include unexplained weight loss, epigastric abdominal pain with radiation into the back, jaundice, and nausea. Recent onset of diabetes in individuals who lack classic risk factors for diabetes should also raise suspicion for pancreatic cancer. Evaluation

should include a full history and physical exam with a detailed family history. Initial bloodwork (complete blood count, basic metabolic panel, total bilirubin, alkaline phosphatase, AST, ALT, amylase, lipase, and CA 19-9/CEA tumor markers) and imaging with a CT scan or MRI should be performed. Additional imaging tests such as EUS and endoscopic retrograde cholangiopancreatography (ERCP) can be used to obtain tissue samples for diagnosis. There is not one specific blood test to diagnose a pancreatic neuroendocrine tumor; however, chromogranin A and pancreatic polypeptide levels are often elevated. Functional neuroendocrine tumors also produce specific hormones that can be measured in the blood (e.g., vasoactive intestinal peptide, gastrin, somatostatin, etc.).

Genetic testing

Genetic testing for hereditary pancreatic cancer syndromes is recommended for (1) individuals with pancreatic adenocarcinoma at any age by the NCCN, (2) families with a first- or second-degree relative with pancreatic adenocarcinoma, or (3) families with pancreatic adenocarcinoma that also meet criteria for the syndromes listed in [Table 13.5](#). Genetic evaluation may be considered for individuals and families with multiple pancreatic neuroendocrine tumors.

Table 13.5 Hereditary syndromes associated with an increased risk for pancreatic cancer.

Syndrome	Gene(s)	Lifetime pancreatic cancer risk	Syndrome characteristics
Pancreatic adenocarcinoma			
Hereditary breast and ovarian cancer syndrome	<i>BRCA1, BRCA2, PALB2</i>	Up to 7%	Female and male breast cancers, ovarian cancer
Lynch syndrome	<i>MLH1, MSH2, MSH6, PMS2, EPCAM</i>	Up to 6%	Colon, endometrial, ovarian, stomach, hepatobiliary, and skin (sebaceous) cancers
Familial atypical multiple mole melanoma	<i>CDKN2A, CDK4</i>	17%	Multiple young age melanomas
PJS	<i>STK11</i>	11%–36%	Small bowel, stomach, breast, ovarian, lung, uterine/cervical, and testicular cancers; PJS gastrointestinal polyps; mucocutaneous freckling
Familial adenomatous polyposis	<i>APC</i>	1%–2%	Multiple adenomatous colon polyps, ampullary adenomas, thyroid cancer, and gastric and duodenal polyps
Pancreatic neuroendocrine tumors			
von Hippel–Lindau syndrome	<i>VHL</i>	Up to 17%	Hemangioblastomas, renal cancer, and pheochromocytomas
MEN1	<i>MEN1</i>	40%–70%	Parathyroid, pituitary, and carcinoid tumors
Other familial risks			
Familial pancreatic cancer	N/A	4%–32%	Dependent on the number of immediate relatives with pancreatic cancer

MEN1, Multiple endocrine neoplasia type 1; *PJS*, Peutz–Jeghers syndrome.

Other genes that should be considered with pancreatic cancer include *TP53* (Li–Fraumeni syndrome), *BMPRIA* and *SMAD4* (juvenile polyposis syndrome), *PALB2*, *ATM* (heterozygous), *NF1* (neurofibromatosis type 1), *TSC1* and *TSC2* (tuberous sclerosis complex), and hereditary pancreatitis (HP) genes (*PRSS1*, *SPINK1*, *CFTR*).

Management

If a germline pathogenic variant is identified, individuals and their families should continue to be followed based on the management guidelines of the particular syndrome listed above in [Table 13.5](#). Alternative chemotherapy, clinical trials, and surgical options should be explored in pancreatic cancer patients with germline variants. Individuals should continue to be followed with an experienced multidisciplinary team including genetic counselors/geneticists, gastroenterologists, surgeons, oncologists, pathologists, nutritionists, and psychosocial support as needed. The International Cancer of the Pancreas Screening (CAPS) Consortium guidelines provide guidance for screening family members at risk for pancreatic cancer, which often includes ERCP or MRI/magnetic resonance cholangiopancreatography (MRCP) every 1–2 years beginning at age 50 but varies depending on the identified syndrome and number of family members with pancreatic cancer [\[10\]](#).

Genetic counseling

First-degree relatives of individuals with a confirmed pancreatic cancer predisposition pathogenic variant should be offered genetic testing. Appropriate age of testing is dependent on the syndrome identified and age of onset of symptoms in family members.

Recurrent pancreatitis

The pancreas is responsible for synthesizing enzymes for protein, fat, and carbohydrate digestion, as well as maintaining balance of blood sugar levels through production of insulin and glucagon. Pancreatitis is an episode of inflammation of the pancreas and usually presents with acute-onset epigastric abdominal pain, nausea, and vomiting. Causes of pancreatitis can be mechanical such as pancreatic duct outflow obstruction (e.g., gallstones, tumor, pancreas divisum), autoimmune, toxin- or drug-induced (e.g., alcohol, medications), metabolic (e.g., hypertriglyceridemia), infectious, or inherited. Approximately 30% of cases are idiopathic. Inherited causes should be considered in cases of acute recurrent pancreatitis starting in adolescence and early adulthood, no clear triggers for the pancreatitis, and individuals with a family history of recurrent pancreatitis.

Hereditary pancreatitis

Overview

Hereditary pancreatitis (HP) is a rare cause of acute recurrent and chronic pancreatitis. Clinical presentation is similar to pancreatitis caused by other etiologies. As mentioned above, HP should be considered in individuals with a personal history of pancreatitis at a young age including adolescence and early adulthood, multiple episodes of pancreatitis

without any clear triggers, and/or a family history of pancreatitis in two first-degree relatives or multiple second-degree relatives that span at least two generations [11]. Multiple recurrences of pancreatitis will eventually lead to chronic damage of the parenchyma with calcifications, fibrosis, ductal strictures, pancreatic duct stones, pancreatic exocrine insufficiency with steatorrhea, and malabsorption, as well as pancreatic endocrine insufficiency manifesting as diabetes mellitus. The median age at diagnosis of HP has been reported between 5 and 19 years with subsequent chronic pancreatitis by the second decade of life [12]. Multiple genes that affect the balance of naturally secreted pancreatic proteases and their inhibitors have been associated with HP and include *PRSS1*, *SPINK1*, *CFTR*, *CTRC*, and *CASR*.

Evaluation

Evaluation of individuals with acute pancreatitis should include a full history and physical exam with a detailed family history to elucidate potential etiologies. Initial bloodwork (complete blood count, basic metabolic panel, total bilirubin, alkaline phosphatase, AST, ALT, amylase, and lipase) and imaging (abdominal ultrasound, ERCP, or MRCP) should be aimed at identifying and treating modifiable causes of pancreatitis. If no clear etiology is identified and there are red flags to suggest an inherited reason (i.e., young age of onset, family history, multiple episodes with no trigger, chronic pancreatitis by the second decade of life), then genetic testing should be pursued.

Genetic testing

Genetic testing should include examination of the genes listed in [Table 13.6](#).

Eighty percent of cases of HP are due to pathogenic variants in the *PRSS1* gene and are inherited in an autosomal dominant manner. Autosomal recessive inheritance is much less common and is seen with some of the variants in *SPINK1* and *CFTR*. Finally, some variants are inherited in a complex pattern where the heterozygous genetic variant alone is unlikely to cause the clinical phenotype of HP but requires interactions with environmental factors or other genetic variants (e.g., co-inheritance of *CFTR* p.R75Q and *SPINK1* variants) [11]. Single-site variant testing can be done in families with a known pathogenic variant. However, due to the complex interplay between the genes associated with HP, multigene panel testing is appropriate.

Vignette 13.3

A 23-year-old woman presents to the ED with 24 hours of sudden-onset epigastric abdominal pain and intractable nausea and vomiting. She reports a history of similar symptoms twice as a child, but the episodes resolved quickly without medical treatment. She denies alcohol, tobacco, and IV drug use. Family history is significant for her mother passing away from pancreatic cancer at age 45.

Vital signs are stable. Physical exam is positive for tenderness to palpation in the epigastrium without rebound or guarding. Labs are significant for a mild hemoconcentration, slightly elevated creatinine, mildly elevated bilirubin with normal alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), and an

Table 13.6 Genes associated with hereditary pancreatitis.

Gene	Mechanism of action	Inheritance	Comments
<i>PRSS1</i> (serine protease 1 gene)	• Encodes cationic trypsinogen • Premature conversion of trypsinogen to trypsin leads to early activation of digestive enzymes prior to excretion from the pancreas	AD	• The three most common mutations include p. R122H (78% of mutations, 80% penetrance), p. N29I (12% of mutations, 93% penetrance), and p.A16V (third most common mutation, 43% penetrance) [11]
<i>SPINK1</i> (serine protease inhibitor Kazal type 1 gene)	• Encodes a trypsin inhibitor expressed in pancreatic acinar cells	AR ^a	• The most common mutations are p.N34S, p.P55S, and IVS3 + 2TC • Clinical pancreatitis may require interactions with other inherited mutations and/or environmental factors [11]
<i>CFTR</i> (cystic fibrosis transmembrane conductance regulator gene)	• Decreased insulin and digestive enzyme secretion from low pancreatic fluid volume • Increased acidity in the pancreatic duct lumen resulting in precipitation of proteins that cause pancreatic duct obstruction	AR and complex type	• Associated with cystic fibrosis • Pancreatitis can occur independently of the cystic fibrosis syndrome
<i>CTRC</i> (chymotrypsin C gene)	• Encodes a protease secreted by pancreatic acinar cells that degrades trypsin and trypsinogen	Complex type	• <i>CTRC</i> mutations alone may not result in HP but may act in conjunction with other genetic mutations and/or environmental factors
<i>CASR</i> (calcium-sensing receptor gene)	• Plays a role in maintaining calcium homeostasis and trypsinogen/trypsin stability	Complex type	• <i>CASR</i>-associated conditions include familial isolated hyperparathyroidism, hypocalcemia, and benign familial hypocalciuric hypercalcemia

AD, autosomal dominant; AR, autosomal recessive.

^aMost pathogenic variants are inherited in an autosomal recessive pattern, however, at least one *SPINK1* variant (c.27delC) may be autosomal dominant.

elevated amylase and lipase. Gallbladder ultrasound is normal. CT scan of the abdomen with pancreatic protocol reveals evidence of acute pancreatitis with a dilated main pancreatic duct and no peripancreatic fluid collections. After conservative management with normalization of amylase and lipase, she undergoes an ERCP that confirms a dilated pancreatic duct and shows multiple filling defects consistent with pancreatic duct stones. None of the stones are large enough to require lithotripsy and a pancreatic duct stent is placed.

Due to the history of likely multiple episodes of pancreatitis at an early age, evidence of chronic complications from recurrent pancreatitis, and no clear etiology for the episodes of pancreatitis, she is counseled on genetic testing for HP. Another suspicious factor is her mother's history of early-onset pancreatic cancer. Genetic testing reveals a pathogenic *PRSS1* variant.

Management

The basic management of an acute episode of pancreatitis due to HP is similar to the management of acute pancreatitis from other etiologies. Long-term management includes interventions to prevent future episodes of pancreatitis and management of chronic complications. Individuals with HP should be instructed to follow a low-fat diet and avoid alcohol and tobacco to help prevent recurrent pancreatitis. Long-term complications include pancreatic duct strictures and stones that may require ERCP with stent placement $\pm$ lithotripsy for duct stones, malabsorption, and steatorrhea from pancreatic exocrine insufficiency requiring pancreatic enzyme supplements, and diabetes mellitus from pancreatic endocrine insufficiency. As chronic pancreatitis can also result in chronic pain, patients may need invasive therapies such as a celiac plexus block or surgery for pain control. The most invasive and controversial surgery is a total pancreatectomy with islet autotransplantation. This should be reserved for younger patients with an identified genetic cause for chronic pancreatitis, severe pain leading to narcotic dependence, and residual islet cell function. Individuals with HP also have a significantly increased lifetime risk for pancreatic cancer, ranging from 19% to 40% [13]. Moreover, individuals with HP who smoke have a twofold greater risk for pancreatic cancer and are typically diagnosed with pancreatic cancer 20 years earlier than nonsmoking individuals with HP [13]. The Fourth International Symposium of Inherited Diseases of the Pancreas recommended screening for pancreas cancer in patients with HP every 1–3 years starting at 40–45 years of age or 10–15 years prior to the earliest pancreatic cancer diagnosis in the family [14]. Screening can be done with EUS or MRI/MRCP.

Genetic counseling

Testing asymptomatic family members should be coordinated with an experienced genetic counselor and only considered in individuals with a first-degree relative with a known pathogenic *PRSS1* variant. Positive testing in this setting predicts approximately an 80% risk of developing pancreatitis. In contrast, testing asymptomatic individuals for variants in the other genes associated with HP is of little value since the variants are common and most do not go on to develop pancreatitis. Testing is generally not recommended prior to 16 years of age.

Diseases of the Liver

The liver has many functions including aiding in digestion through secretion of bile, detoxifying chemicals, metabolizing drugs, and making proteins for clotting factors. Liver disease generally progresses from inflammation to fibrosis to cirrhosis and liver failure. Etiologies of liver disease, both acute and chronic, include viral (e.g., hepatitis A, hepatitis B, hepatitis C), toxins (e.g., alcohol), drugs (e.g., acetaminophen), metabolic syndrome leading to nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), autoimmune (e.g., primary sclerosing cholangitis), and inherited (e.g., Wilson disease, hereditary hemochromatosis). Worldwide there is a large geographical distribution of the major risk factors for liver disease [15], but in the United States, NAFLD is the most common cause. It should be noted that individuals may also have multiple etiologies for their liver disease (e.g., metabolic syndrome and alcohol). Diagnosis of an inherited liver disease requires a high index of clinical suspicion to order the correct labs and make the final diagnosis.

Wilson disease

Overview

Wilson disease is a disorder of defective copper excretion from the liver due to pathogenic variants in the *ATP7B* gene, which encodes a copper transporter expressed mainly in hepatocytes. A pathogenic variant in this gene leads to decreased hepatocellular copper excretion into bile and failure to incorporate copper into the soluble copper-carrying enzyme, ceruloplasmin [16]. Inability to excrete copper leads to build-up in multiple organs (e.g., liver, brain, cornea, kidneys), free radical production, and oxidative injury to organ systems with excess copper causing the clinical manifestations of Wilson disease.

Evaluation

Patients with Wilson disease typically present between the ages of 5 and 40 years with a wide spectrum of symptoms including hepatic (acute and chronic liver failure), hematologic (hemolytic anemia), neurologic (movement disorders), and psychiatric (depression, anxiety, compulsive behavior, psychosis) [16]. Hepatic presentation can range from biochemical abnormalities with no clinical symptoms to acute liver failure or cirrhosis. Acute liver failure due to Wilson disease often presents with elevated AST and ALT (typically <1000 U/L), normal or markedly low ALP, Coombs-negative hemolytic anemia, and elevated total and indirect bilirubin [17]. Patients should be evaluated for cirrhosis, which is present at the time of diagnosis in 35%–45% of patients [18]. Neurologic symptoms typically present later than liver disease. Most patients with a neurologic or psychiatric symptoms have Kayser–Fleischer rings on eye examination due to copper deposition in Descemet’s membrane of the cornea.

In patients suspected of having Wilson disease, or those with unexplained liver disease, workup should begin with serum ceruloplasmin, 24-hour urinary copper, and slit-lamp exam of the eyes. Approximately 85%–90% of patients will have a ceruloplasmin level <20 mg/dL [16].

If diagnosis is still unclear, liver biopsy can be obtained. A hepatic copper content >250 mcg/g dry weight is diagnostic of Wilson disease while a value <50 mcg/g dry weight excludes the diagnosis. Patients with hepatic copper content of 50–250 mcg/g dry weight should be referred for genetic testing [16,18]. Clinical presentation of Wilson disease can also overlap with autoimmune hepatitis and NAFLD. A high index of suspicion is essential to make the diagnosis.

Genetic testing

Wilson disease is an autosomal recessive condition due to pathogenic variants in the *ATP7B* gene. Whole-gene sequencing is available and should be performed in patients with a high clinical suspicion. Mutations are detected by sequencing all exons and their associated promoter regions with particular attention paid to exon/intron junctures, as well as excluding large deletions [19]. Clinical sensitivity to detect sequence variants in the *ATP7B* gene is 98%. Most patients with Wilson disease are compound heterozygotes with different variants on each of the *ATP7B* alleles. If Wilson disease has been confirmed, haplotype analysis or testing for known pathogenic variants can be performed in family members [15].

Vignette 13.4

A 22-year-old man is hospitalized for 2 days of confusion and 1 week of fatigue and jaundice. Family members report erratic movements over the past year and that he has gotten “clumsy.” The patient reports rare alcohol use and denies IV drug use. He is oriented to person, but not to place and time. He has icterus and abdominal exam reveals splenomegaly and ascites; the liver edge is not palpable. Neurologic exam shows asterixis and occasional spastic limb movements. Laboratory studies show anemia, decreased ALP, elevated International normalized ratio, AST, and ALT elevated between 5 and 10 times above normal, elevated indirect bilirubin, and LDH.

This patient’s neurologic symptoms (spasticity and declining motor coordination) are consistent with Wilson disease. The presence of splenomegaly and ascites suggest that he likely has cirrhosis. The laboratory abnormalities consistent with Wilson disease are AST:ALT ratio >2 , low Alk Phos, predominantly indirect hyperbilirubinemia, and elevated LDH/low haptoglobin, which suggest a hemolytic anemia. If the above patient were to have no neurologic findings and a strong family history of autoimmune disease, autoimmune hepatitis would also be high on the differential diagnosis. Workup should include ceruloplasmin, 24-hour urine copper and slit-lamp eye exam. If the initial workup is suggestive, but not conclusive for Wilson disease, a liver biopsy and referral for genetic testing would be prudent.

Management

Patients with Wilson disease require lifelong therapy. Discontinuation of therapy, even in patients with well-controlled disease, can lead to acute liver failure. Treatment most commonly involves a chelating agent (e.g., D-penicillamine, trientine) alone or in combination with zinc therapy. D-Penicillamine increases urinary excretion of copper, inhibits accumulation of copper in hepatocellular lysosomes, and solubilizes it for mobilization and

excretion [15]. Trientine is second-line therapy for patients intolerant of D-penicillamine. It increases urinary copper excretion and may interfere with intestinal absorption of copper. Treatment goal is a 24-hour urinary copper excretion of 200–500 mcg/day [16]. Zinc interferes with absorption of copper in the intestine and increases copper excretion in stool. Dietary modification alone is ineffective, but patients should avoid copper-rich foods including organ meats, mushrooms, chocolates, and nuts. Wilson disease patients with acute liver failure or decompensated liver disease unresponsive to therapy should be referred for liver transplantation.

Genetic counseling

If pathogenic *ATP7B* variants have been identified in the proband, all first-degree relatives should be tested for Wilson disease via direct mutation analysis or haplotype studies. If there is a clinical diagnosis of Wilson disease and no pathogenic variants have been confirmed, first-degree relatives can be screened with liver function testing, serum copper and ceruloplasmin, 24-hour urinary copper, and slit lamp eye exam [16]. Measurement of ceruloplasmin in Guthrie-dried blood spots or urine samples should be performed in newborns in whom there is concern for Wilson disease.

Hemochromatosis

Overview

Hereditary hemochromatosis is a disorder of disrupted iron homeostasis. It is commonly due to pathogenic variants in *HFE*, which increases intestinal iron absorption when the body already has sufficient iron stores. Other inherited forms of iron overload, classified as non-*HFE*-related hereditary hemochromatosis, are due to variants in genes for hepcidin (*HAMP*) and hemojuvelin (*HJV*), which cause juvenile hemochromatosis, transferrin receptor 2 (*TFR2*), or ferroportin (*SLC40A1*) [17]. Excess iron is deposited in the liver, heart, pancreas, and pituitary leading to the clinical manifestations of hemochromatosis.

Evaluation

The clinical manifestations of hereditary hemochromatosis are related to excessive iron deposition in tissues causing a range of symptoms including hepatomegaly, cirrhosis, diabetes mellitus, impotence in males, bronze or gray skin pigmentation, arthropathy (typically of the second and third metacarpophalangeal joints), cardiomyopathy, and conduction defects. Most patients with symptomatic hereditary hemochromatosis are diagnosed between the ages of 40 and 50 years although women usually develop symptoms after menopause [20]. Iron deposition in hepatocytes is not inflammatory, so hepatic fibrosis can occur even with normal serum aminotransferases. Elevated aminotransferases, if present, are usually mild and often discovered incidentally on routine blood chemistry panels. Diabetes mellitus results from iron deposition in the pancreas and is typically not seen in the absence of cirrhosis.

For individuals presenting with typical symptoms or physical exam findings and/or suspicious family history, workup should start with a serum ferritin, iron, total iron binding

capacity (TIBC), and transferrin saturation. A transferrin saturation >45% should prompt genetic testing for *HFE* gene variants [17]. Ferritin is frequently elevated to 300–3000 ng/mL [19]. However, ferritin may also be elevated in other liver diseases (chronic viral hepatitis, alcoholic liver disease, NASH), malignancies, and inflammatory conditions. Patients with non-*HFE*-related hereditary hemochromatosis typically have an elevated ferritin and normal transferrin saturation.

Genetic testing

HFE-related hereditary hemochromatosis is an autosomal recessive disorder due to pathogenic variants in *HFE*. Approximately 85%–90% of patients with hereditary hemochromatosis are homozygous for the C282Y variant. Two other well-documented *HFE* variants are H63D and S65C and typically present with the C282Y variant as compound heterozygotes [17]. Individuals who are homozygous for the C282Y variant have a high penetrance, whereas compound heterozygotes (e.g., C282Y/H63D) have approximately a 2% penetrance. There is a high-carrier frequency of the C282Y variant in individuals of European descent (~11%) and approximately one-third of northern European whites are carriers for either C282Y or H63D. Heterozygous carriers for the H63D variant are more common (northern Europeans: 25%; Hispanics: 18%; African Americans: 6%; and Asians: 8.5%).

Non-*HFE*-related hereditary hemochromatosis has been linked to autosomal recessive variant in *HAMP*-encoding hepcidin, *HJV*-encoding hemojuvelin, and *TFR2*-encoding transferrin receptor 2, as well as autosomal dominant variants in *SLC40A1*-encoding ferroportin. Pathogenic variants in these genes account for 10%–15% of patients with hereditary hemochromatosis [17]. Patients with a transferrin saturation >45% should be referred for *HFE* analysis. If no *HFE* gene variant is identified, other etiologies of liver disease (e.g., viral hepatitis, NAFLD, alcoholic liver disease) and secondary causes of iron overload (e.g., history of multiple blood transfusions, excess oral intake) should be ruled out before pursuing genetic testing for non-*HFE*-related hereditary hemochromatosis.

Management

Management of hereditary hemochromatosis involves staging the disease, treatment to reduce iron stores, management of complications of cirrhosis, and screening for hepatocellular carcinoma. *HFE* hemochromatosis can manifest in three distinct ways: (1) clinical diagnosis with signs and symptoms, (2) biochemical diagnosis with unexplained abnormal iron studies, and (3) nonexpressing homozygotes in whom there is a molecular diagnosis without presenting features. Management for each type is as follows:

- *Clinical HFE hemochromatosis*: Standard therapy is to reduce iron stores with weekly phlebotomy of approximately one unit of whole blood to achieve a serum ferritin concentration ≤ 50 ng/mL [19]. Once serum ferritin concentration is at goal, monitor serum ferritin every 3–4 months and perform maintenance phlebotomies as needed to maintain serum ferritin <300 ng/mL in men and <200 ng/mL in women. Standard screening for hepatocellular carcinoma should be done in individuals who have cirrhosis.

- *Biochemical HFE hemochromatosis and nonexpressing C282Y homozygotes:* Begin annual measurement of serum ferritin when the concentration exceeds 300 ng/mL in men and 200 ng/mL in women. In symptomatic individuals, begin phlebotomy when serum ferritin concentration is >300 ng/mL.

In patients with *HFE*-related hemochromatosis who are C282Y homozygotes or compound heterozygotes with elevated aminotransferases and ferritin >1000 mcg/L, a liver biopsy is recommended to stage the degree of fibrosis. Cirrhosis is present in approximately 20%–25% of patients with hereditary hemochromatosis at the time of diagnosis [17]. Treatment with proton pump inhibitors reduces intestinal absorption of iron and may decrease the need for phlebotomy. For patients unable to tolerate phlebotomy or who have cardiac manifestations of *HFE*-related hereditary hemochromatosis, the iron-chelating agent deferoxamine can be used [19]. Cirrhosis does not typically reverse with treatment, but many patients will have a decrease in fibrosis. Arthritis and hypogonadism do not improve with treatment, but diabetes mellitus may become easier to manage. Approximately 45% of patients with hereditary hemochromatosis will die from hepatocellular carcinoma [21]. Screening should include ultrasound or CT scan and serum AFP measurement every 6 months in cirrhotic patients [17]. When complications of end-stage liver disease develop, patients should be evaluated for liver transplantation.

Genetic counseling

All first-degree relatives of an individual with *HFE*-related hereditary hemochromatosis should be screened. Screening should include both genetic testing and serum iron studies (iron, ferritin, TIBC, and transferrin saturation). Relatives who are C282Y heterozygotes are not at risk for iron overload. For children of an affected individual, *HFE* testing of the other parent is recommended. Due to the high prevalence of *HFE* variants, there is a high likelihood that the other parent is heterozygous for an *HFE* variant. Parental genotyping is important to determine risks for siblings and/or future offspring. If both parents are heterozygous carriers for *HFE* gene variants, a child would have a 25% chance to be a homozygous affected offspring. However, if one parent is a heterozygous carrier and the other parent is homozygous, the risk to a child is increased to 50%. If one parent does not carry the C282Y, H63D, or S65C variant, the children are not at risk for iron overload and do not need to undergo *HFE* genotyping themselves [17].

Points to remember

- Lynch syndrome is the most common cause of hereditary CRC and should be considered in individuals with CRC <50 years old, right-sided colon cancers, CRC with high MSI, and a family history of multiple relatives with CRC or other Lynch syndrome-related cancers across more than one generation.
- Individuals with a personal history of >10 cumulative adenomatous colon polyps should be referred for genetic evaluation for a possible hereditary polyposis syndrome.

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- Hamartomatous polyposis syndromes are rare, autosomal dominant conditions characterized by the presence of hamartomatous polyps (e.g., Peutz–Jeghers polyps, juvenile polyps) and carry an increased risk for both CRC and multiple extracolonic malignancies.
- HDGC families will typically have family members with early-onset diffuse-type gastric cancer before age 40, as well as individuals with lobular breast cancer before age 50. Approximately 50% of families who meet these clinical criteria will have no *CDHI* pathogenic variant identified on genetic testing but should still be considered to have a clinical diagnosis of HDGC.
- The vast majority of GISTs (over 80%) are sporadic and 95% will be positive for c-KIT protein (CD117) on IHC.
- Germline pathogenic *BRCA2* variants explain up to 17% of FPCs.
- Inherited causes of recurrent pancreatitis should be considered in cases of acute recurrent pancreatitis starting in adolescence and early adulthood, no clear triggers for the pancreatitis, and individuals with a family history of recurrent pancreatitis.
- Individuals with HP (especially due to a *PRSS1* mutation) have up to a 40% lifetime risk for pancreatic cancer and should undergo regular pancreas cancer screening.
- Wilson disease can present with hepatic, neurologic, and psychiatric abnormalities and is characterized by acute liver failure, Coombs-negative hemolytic anemia, normal or markedly low-serum ALP, elevated total and indirect bilirubin, and serum ceruloplasmin <20 mg/dL.
- Hereditary hemochromatosis is a highly prevalent autosomal recessive condition most commonly due to pathogenic variants in the *HFE* gene and can present with hepatomegaly, cirrhosis, diabetes, skin pigmentation, arthropathy, and cardiac manifestations. Individuals with a transferrin saturation >45% should be referred for genetic testing for *HFE* gene variants.
- For children of an individual affected by hereditary hemochromatosis, *HFE* genetic testing of the other parent is recommended.

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Hematological disorders

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Introduction

Many heritable hematologic disorders are common enough to be frequently encountered during routine clinical practice. The clinical manifestations of these disorders are primarily dependent on whether the genetic variant affects the function of the red blood cells, white blood cells, platelets, or the coagulation cascade. As the clinical manifestations in these disorders can have a significant overlap with hematological disorders caused due to the more common causes such as nutritional deficiencies (e.g., iron deficiency) and immunologic mechanisms (e.g., autoimmune hemolytic anemia), detailed laboratory evaluation and expertise of hematologists is often required for their accurate diagnosis and management. In this chapter, we provide an overview of the well-known hereditary hematologic disorders.

Bleeding disorders

Overview

Von Willebrand disease (VWD) and clotting factor deficiencies are the most common heritable bleeding disorders. VWD caused by pathogenic variants in the Von Willebrand factor (VWF) gene is the most common bleeding disorder and has an estimated prevalence of 1:1000. Common disorders related to deficiencies of clotting factors include Hemophilia A (factor VIII deficiency) and Hemophilia B (factor IX deficiency). Hereditary deficiency of other coagulation factors (prothrombin, factors V, VII, X, XI, XIII, and fibrinogen) and qualitative disorders of platelets are rare, collectively accounting for approximately 5% of patients with bleeding disorders.

Von Willebrand disease

VWF is a multimeric protein that acts as a carrier for factor VIII and is responsible for platelet adhesion. Individuals with VWD commonly present with mucocutaneous bleeding such as epistaxis, easy bruising, menorrhagia, and gingival bleeding. VWD type 1 is the most common form of the disorder and is inherited in an autosomal dominant manner with variable expressivity and incomplete penetrance; thus, symptoms among affected family members may be different. VWD type 2 has several different subtypes: types 2A, 2B, and 2M are inherited in an autosomal dominant manner while type 2N is an autosomal recessive disorder [1]. VWD type 3 is the rarest form of the disorder and is inherited in an autosomal recessive manner.

Evaluation

Individuals with a hereditary bleeding disorder may present with spontaneous bleeding or prolonged bleeding in response to injury. Prolonged prothrombin time (PT) and partial thromboplastin time (PTT) may be the first diagnostic indicators of a bleeding disorder, at which point more targeted factor level assays and evaluation by a hematologist are warranted for diagnosis and management of specific disorders.

Clinical testing for VWD should include factor VIII activity, ristocetin cofactor, VWF antigen, and VWF multimer analysis [2]. In VWD type 1, laboratory evaluation typically reveals decreased VWF, FVIII activity, VWF antigen, and ristocetin cofactor activity, but with normal VWF multimers. A subtype of VWD type 1 has been described, called accelerated VWD clearance, or VWF type 1c, and is characterized by low VWF antigen, proportionately low ristocetin cofactor activity. VWD type 2A is identifiable by significantly low ristocetin cofactor in comparison to VWF antigen. Large and intermediate VWF multimers are absent. VWD 2B is characterized by decreased large VWF multimers and an increased affinity for platelet glycoprotein Ib. blood counts may demonstrate thrombocytopenia. VWD 2M is characterized by decreased or absent ristocetin cofactor, normal multimer structure, and decreased binding to collagen. Variants in the FVIII binding domain of the protein cause VWD type 2N. Thus, the laboratory findings in VWD type 2N phenotype resemble that of hemophilia A. In VWD type 3, laboratory evaluation typically reveals very low or undetectable levels of VWF.

Genetic testing

There is a strong genotype-phenotype correlation in VWD. VWD types 1 and 3 are caused by quantitative deficiency of VWF due to pathogenic variants in many exons of VWF. VWD type 2 occurs due to a qualitative abnormality and is caused mostly by variants in exon 28 of VWF. Despite these known associations, often, laboratory functional assays are sufficient for diagnosis [2]. Certain polymorphisms are more prevalent by ethnicity, and may affect VWF-ristocetin interaction, leading to abnormal laboratory evaluation, yet have no physiologic affect. Most variants causing type 2A are found in exon 28 of VWF, however, if type 2A is suspected, sequencing of the entire gene should be considered. All known variants

causing VWD type 2B are found in exon 28 of VWF and thus, targeted sequencing may be appropriate, when this subtype is considered.

Management

Patients with VWD type 1 are often successfully treated with desmopressin (DDAVP) which can release VWF from endothelial cell stores, thus increasing plasma levels of the protein. VWF types 2 and 3 are generally treated with factor replacement with VWF and FVIII concentrate. DDAVP treatment is usually not indicated in VWD type 2B but patients with type 2A may respond to DDAVP treatment.

Clotting factor deficiencies

Overview

Hemophilia A and B are caused by deficiencies of clotting factors VIII and IX, respectively and are the most common types of clotting factor deficiencies. Normal plasma factor levels range between 50% and 150% and the severity of hemophilia is defined by the degree of circulating clotting factor deficiency; mild hemophilia has factor levels between 6% and 49%, moderate hemophilia has factor levels between 1% and 5%, and severe hemophilia has levels less than 1%. Patients with moderate and severe hemophilia experience spontaneous bleeding episodes and excessive bleeding in response to injury. Without treatment, patients are at risk for bleeding into joints and internal organs, which could be life threatening. Patients with mild hemophilia generally only experience bleeding with a serious injury, trauma, or medical procedures; some may not experience any symptoms until adulthood. Coinheritance of variants in genes associated with hereditary thrombophilia, like factor V Leiden may decrease the risk of bleeding [3].

Evaluation

Factor levels and kinetics measurement may be sufficient to confirm a suspected diagnosis; however, molecular confirmation may be useful in distinguishing hemophilia A from VWD type 2N, and molecular confirmation in an affected male is useful in identifying at-risk female relatives.

Genetic testing

Hemophilia A is caused by pathogenic variants in the *F8* gene localized to Xq28. Hemophilia B is caused by pathogenic variants in *F9* on Xq27.1. Thus, these conditions are inherited in an X-linked manner. Approximately 30% of hemophilia occurs due to *de novo* variants. Approximately half of male patients with severe hemophilia A have inversion involving intron 22, which is almost always inherited from the patient's mother. If negative for intron 22 inversion, *F8* gene sequencing, deletion/duplication, and analysis to detect intron 1 inversion should be considered. Patients with large deletions and nonsense variants causing severe hemophilia A are at increased risk for the development of inhibitory antibodies to factor VIII [4]. Approximately 20% of patients with severe hemophilia A develop inhibitors.

Approximately one-third of patients with mild hemophilia A will have differing values between one-stage clotting and two-stage chromogenic assays and are characterized as having mild discrepant hemophilia A, which is associated with specific missense variants in *F8* [5].

Management

Patients may require prophylactic treatment with a plasma-derived or recombinant factor replacement product. Curative treatment strategies involving gene therapy have been investigated and are discussed in Chapter 30, Gene therapy for inherited diseases.

Women with bleeding disorders

Approximately half of the women who are carriers of hemophilia, have deficient factor levels and are at risk for bleeding complications, though symptoms and potential complications have historically been underappreciated. Factor level measurement is recommended for all women with a family history positive for a bleeding disorder, especially before any surgical procedure or before childbirth. A woman with normal factor levels may carry a single pathogenic variant, thus molecular testing for the known familial pathogenic variant is recommended to definitively determine a woman's carrier status.

Special care is required during labor and delivery and should be coordinated by an obstetrician familiar with bleeding disorders, and take place in a facility with appropriate hematologic, laboratory, pharmacy, and blood bank support. A birth plan should be established, following the routinely updated recommendations by the Medical and Scientific Advisory Council of the National Hemophilia Foundation. Postpartum management should be prolonged given the increased risk for postpartum hemorrhage. A network of federally-funded hemophilia treatment centers is an appropriate resource for patients with bleeding disorders, offering specialized nursing, physical therapy, social work, dental, and genetics services.

Platelet disorders

Overview

Platelet surface glycoproteins are essential for platelet adhesion (between platelets and vascular vessel wall) and aggregation (between platelets). Platelets also contain alpha storage granules which store clotting factors [6]. Patients with hereditary disorders of platelet function (Table 14.1) may present with mucocutaneous bleeding or excessive bleeding with trauma or surgery. Spontaneous bleeding is unusual and distinctive from the bleeding orders previously described.

Evaluation

Blood counts and peripheral blood smear should be the first analysis in the evaluation of an individual with a suspected platelet disorder. Patients with qualitative platelet disorders may

Table 14.1 Hereditary platelet disorders.

Platelet disorder	Genes (chromosomal locus)	Inheritance	Characteristics
Bernard–Soulier syndrome	<i>GP1BA</i> (17p13.2) <i>GP1BB</i> (22q11.21) <i>GP9</i> (3q21.3)	AR	Homozygotes - giant platelets on blood smear - decreased platelets which do not aggregate with ristocetin Heterozygotes - asymptomatic - thrombocytopenia and mild bleeding symptoms
Glanzmann thrombasthenia	<i>ITGA2B</i> <i>ITGB3</i>	AR	Homozygotes - failure of platelet aggregation - significant mucocutaneous bleeding, bruising, epistaxis, menorrhagia, and intracranial bleeding. - platelet counts are normal Heterozygotes may have - large platelets - thrombocytopenia - bleeding symptoms
MYH9-related disorders	<i>MYH9</i> (22q12.3)	AD	Homozygotes: not reported Heterozygotes - thrombocytopenia - giant platelets and Döhle-like inclusion bodies - nephritis - sensorineural hearing loss
Gray platelet syndrome	<i>NBEAL2</i> (3p21.31)	AR	Homozygotes - large platelets with the absence of the alpha storage granules - thrombocytopenia Heterozygotes: - asymptomatic - some decrease of alpha storage granules

AD, autosomal dominant; AR, autosomal recessive

also experience a quantitative deficiency of platelets. If the platelet count is low, bone marrow examination and platelet antibody analysis are warranted. If platelet count is normal, PT/PTT, platelet aggregation, and other platelet evaluations are warranted.

Hereditary thrombocytopenia with risk of malignancy: RUNX1 familial platelet disorder

Patients presenting with thrombocytopenia and a family history of acute myeloid leukemia should be tested for pathogenic variants in *RUNX1*. Thrombocytopenia is often the

presenting symptom but may be mild and undetected. Patients may have platelet abnormalities. Approximately one-third of patients develop leukemia, typically in the third decade of life. The disorder is transmitted in an autosomal dominant fashion. Patients presenting with a history of thrombocytopenia should also be evaluated for thrombocytopenia 2 (ANKRD26) and congenital amegakaryocytic thrombocytopenia, both of which also are associated with an increased risk for myeloid malignancies.

Genetic testing

There are many more hereditary disorders of platelet function than described here, and evaluation using platelet aggregation studies, as well as more in-depth analysis by a hematologist may be necessary to definitively determine a diagnosis. Once a hereditary platelet disorder is suspected, a test using a gene panel can be used for confirmation of diagnosis [7].

Management

Patients with platelet disorders may require platelet transfusion treatment under the care of a hematologist. If a familial pathogenic variant is identified, cascade testing can be performed on at-risk family members.

Bone marrow failure disorders

Fanconi anemia

Overview

Fanconi anemia (FA) is a rare disorder of aplastic anemia, that is typically inherited in an autosomal recessive manner. Patients with FA can present with growth deficiency, abnormal skin pigmentation, upper limb malformations, as well as renal and ocular manifestations. Absent or abnormal thumbs are among the most common abnormalities in FA. The phenotype is variable, and the condition may be first identified because of bone marrow failure or cancer diagnosis in adulthood. Pancytopenia is often the first hematologic indicator of FA. Malignancies including acute myeloid leukemia, solid tumors of the head, neck, genitourinary, and gastrointestinal tracts are well-described features of the disorder.

Evaluation

Screening test for the initial evaluation of FA is assessing increased chromosome breakage and chromosome fragmentation in response to diepoxybutane or mitomycin C exposure.

Genetic testing

There are twenty genes known to cause FA; biallelic pathogenic variants in *FANCA*, *FANCC*, and *FANCG* are the most common cause [8]. Carrier phenotypes are being described for several FA genes and should be shared with patients and families as new information and recommendations are developed.

Telomere disorders

Overview

Patients with a telomere disorder can present with abnormal skin pigmentation, dysplastic nails, progressive bone marrow failure, and increased risk for malignancy. Aplastic anemia generally occurs in childhood or early adulthood. However, anticipation can be observed in affected families, with individuals in subsequent generations exhibiting symptoms at earlier ages. Individuals may present in adulthood with pulmonary fibrosis, thus collaboration between pulmonology and hematology providers is crucial in recognizing the underlying etiology [9]. Other inherited bone marrow failure syndromes include Diamond Blackfan anemia and Shwachman–Diamond syndrome.

Evaluation

Telomere length analysis reveals very short telomeres in multiple cell types. Molecular characterization will definitively determine the affected gene. Clinical assays like erythrocyte adenosine deaminase levels, pancreatic function analysis, or telomere length help in narrowing the diagnosis.

Genetic testing

Given the genetic heterogeneity of telomere disorders, genetic testing for these groups of disorders typically involves the use of next-generation sequencing based gene panel tests. Genetic testing is required for accurate molecular diagnosis and testing of other at-risk family members.

Management

Bone marrow transplant may cure the hematologic symptoms of inherited bone marrow failure syndromes; however, patients should still be monitored for their additional cancer risks and counseled about recurrence risk to subsequent generations.

Thrombophilia

Overview

Normal coagulation is determined by a dynamic balance between procoagulant factors in the coagulation cascade and mechanisms that inhibit this cascade. “Inherited thrombophilia” is typically used to describe the increased familial risk for venous thromboembolic phenomena; however, in some instances, the risk for increased arterial thrombosis may also be increased. Hereditary thrombophilia is caused by heritable qualitative or quantitative defects in the proteins of the coagulation cascade which are often easily detected by specific assays. Despite the wide availability of testing for thrombophilia, the impact of such testing on clinical decision making is low and hence testing should be used in certain selective scenarios.

Table 14.2 Hereditary thrombophilia disorders.

Disorder	Genes (chromosomal locus)	Inheritance	Hazard risk of first VTE episode compared to the general population
Protein C deficiency	<i>PROC</i> (2q14.3)	AD	Heterozygotes: • 5-fold higher risk
Protein S deficiency	<i>PROS1</i> (3q11.1)	AD	Heterozygotes: • 7-fold higher risk
Antithrombin III deficiency	<i>SERPINC1</i> (1q21.1)	AD	Heterozygotes: • 16-fold higher risk
Thrombophilia due to factor V Leiden	<i>F5</i> (1q24.2)	AD	Homozygotes: • 80-fold higher risk Heterozygotes: • 4–5 fold higher risk
Prothrombin deficiency	<i>F2</i> (11p11.2)	AD	Heterozygotes: • 3–4 fold higher risk

AD, autosomal dominant

Evaluation

Presentation with a possible unprovoked venous thromboembolism necessitates obtaining a detailed history regarding recent surgeries, hospitalizations, injuries, travel, prolonged immobilization, use of venous catheters, pregnancy, medications like oral contraceptives and recent heparin use. Laboratory evaluation also includes testing for myeloproliferative neoplasms, malignancies, antiphospholipid antibody syndrome, and paroxysmal nocturnal hemoglobinuria. In the absence of such secondary causes, especially in a younger adult, the possibility of hereditary thrombophilia should be considered.

The most common causes of inherited thrombophilia are summarized in [Table 14.2](#). They can be categorized into the following broad categories [\[10\]](#).

1. Deficiency of anticoagulant protein, for example, protein C and S and antithrombin III deficiencies; all of which are negative regulators of the coagulation cascade
2. Increased procoagulant activity, for example, Factor V Leiden (the most prevalent of hereditary thrombophilia, as 5% of the general population is heterozygous) which makes Factor Va more resistant to proteolysis, prothrombin gene mutation which makes the mRNA of prothrombin more stable and increases its translation as well as increased Factor VIII level
3. Disorders of fibrinogen or fibrinolysis, for example, dysfibrinogenemia, heparin cofactor deficiency

Genetic testing

A common question is regarding when one should be tested for hereditary thrombophilia. Generally, in most individuals with a venous thromboembolism (VTE), this testing is not

required as it does not impact management. In our experience, we have found it to be most helpful in very young patients with unprovoked or unusual sites of thrombosis, especially those with a family history of VTE, or in women seeking to have successful pregnancies. Even in these cases, an individualized approach is recommended.

Vignette 14.1

CC is a 56-year-old Caucasian woman with no medical history, and no known family history of malignancy presented to the ER with acute dyspnea and is diagnosed with an unprovoked pulmonary embolism. Her pregnancy test is negative. She is placed on therapeutic anticoagulation with warfarin for a period of at least 6 months with a possibility of longer duration of therapy. Testing for antiphospholipid antibody syndrome is negative. She presents to you in the office to discuss the next steps and asks you the utility of hereditary thrombophilia testing.

Most experts agree that routine evaluation for hereditary thrombophilia is not indicated with the first episode of unprovoked VTE. However, patients with the following characteristics should be considered for genetic testing: (1) have one/more first-degree relative with documented unprovoked VTE before the age of 45 years, (2) recurrent thrombosis, (3) thrombosis in multiple venous sites or unusual vascular beds, and (4) history of warfarin-induced skin necrosis. CC does not meet any of these criteria and hence testing may be deferred.

Management

While thrombophilia testing may not impact clinical decision making regarding the type or duration of anticoagulation, the diagnosis of hereditary thrombophilia plays an important role in thrombo-prophylaxis during pregnancy to increase the chance of a viable pregnancy or during other high-risk conditions like the perioperative period. Currently, there are no consensus guidelines for testing and management of these disorders and anticoagulation treatment is undertaken on an individual basis.

Red cell defects

Hereditary defects of the red cell include the following: (1) deficiency of red blood cell enzymes, (2) red cell membrane defects that lead to increased fragility, and (3) hemoglobinopathies, characterized by abnormal hemoglobin and decreased ability to carry oxygen.

Red cell enzyme defects

Overview

The red cell possesses active machinery with enzymes to make help maintain its shape and motility as well as to maintain hemoglobin in its reduced form. Deficiencies in the activity of RBC enzymes lead to hemolytic anemias (Table 14.3). The two main pathways pertinent to the red cell enzyme defects that lead to hemolytic anemia are the glycolytic pathway and the hexose monophosphate shunt [11].

Table 14.3 Disorders of red cell enzyme defects.

Disorder	Genes, chromosomal locus	Inheritance	Characteristics
Glucose-6-phosphate dehydrogenase deficiency	<i>G6PD</i> , Xq28	X-linked	Hemizygous • Hemolytic anemia especially following oxidant stress and use of certain medications • Peripheral smear shows reticulocytosis, “bite cells” or blister cells. • More common in Africa, Europe, and Asia
Pyruvate kinase deficiency	<i>PKLR</i> , 1q22	AR	Homozygotes • Hemolytic anemia • Peripheral smear occasionally shows echinocytes • More common in individuals with of northern European and Chinese ancestry
Pyrimidine 5’ nucleotidase (cN-III) deficiency	<i>NT5C3A</i> , 7p14.3	AR	Homozygotes • Hemolytic anemia • Peripheral smear typically shows nonspecific basophilic stippling • More common in individuals of Navajo or Siberian ancestry

AR, autosomal recessive

Evaluation

The hemolysis is intravascular leading to reticulocytosis with a negative direct antiglobulin test (DAT or Coomb’s test) indicating a nonimmune etiology. The comprehensive metabolic panel may reveal indirect hyperbilirubinemia, elevated lactate dehydrogenase, and decreased haptoglobin. Glucose-6-phosphate dehydrogenase (G6PD) hemolytic anemia can vary widely in severity and in severe forms can present with acute hemolysis due to medications, acute illnesses, and foods that expose the red cell to oxidative stress. Within hours to a few days after exposure to the stressor, there can be an acute drop in the hemoglobin with reticulocytosis. G6PD level measured during acute hemolytic episodes may not be accurate.

Genetic testing

Genetic testing can be performed based on the disorder suspected. Single gene versus panel-based testing will help confirm a molecular diagnosis.

Vignette 14.2

FF is a 26-year-old African-American woman with no significant past medical history, who presented to the hospital ER with acute shortness of breath. Work up showed that she was anemic with a hemoglobin of 5 g/dL, with an elevated mean corpuscular volume (MCV) of 120. The reticulocyte count was 20%. Laboratory evaluation showed indirect hyperbilirubinemia,

elevated LDH, and decreased haptoglobin. The smear revealed several “bite” cells. About 3 days prior to this visit, she had been prescribed nitrofurantoin by her primary care physician for a urinary tract infection. Hemolytic anemia is suspected and a DAT is sent which is negative. This is a typical presentation of G6PD deficiency. Clinical diagnosis and G6PD enzyme assay are typically required for diagnosis and genetic testing is not routinely performed. Genetic testing may be helpful for cascade testing and identifying at-risk family members.

Management

A key principle of management is avoiding exposure to medications that trigger hemolysis in an individual with G6PD deficiency. Some of the common drugs to avoid include nitrofurantoin, primaquine, sulfamethaxazole, and dapsone. Hemolytic crises are managed symptomatically, and patients frequently require hospitalization.

Red cell membrane defects

Overview

The erythrocyte structure is maintained by cell surface proteins and cytoskeletal proteins. Pathogenic variants in the genes that encode components of the red cell membrane and its cytoskeleton lead to alteration in the shape and flexibility of the red cell and thereby, the survival of the red cells (Table 14.4).

Table 14.4 Disorders of red cell membrane defects [12].

Disorder	Genes (chromosomal locus)	Inheritance	Characteristics
Hereditary spherocytosis	<i>ANK1</i> (8p11.21) <i>SPTA1</i> (1q23.1) <i>SPTB</i> (14q23.3) <i>SLC4A1</i> (17q21.31) <i>EPB42</i> (15q15.2)	AD	Heterozygotes - Peripheral smear reveals extensive spherocytes - More common in Caucasians
Hereditary elliptocytosis	<i>SPTA1</i> (1q23.1) <i>SPTB</i> (14q23.3) <i>EPB42</i> (15q15.2)	AR	Homozygotes - Peripheral smear reveals extensive “elliptocytes” or cigar shaped red blood cells - More common in individuals of African and Mediterranean descent
Hereditary pyropoikilocytosis	<i>SPTA1</i> (1q23.1)	AR	Homozygotes Peripheral smear shows red blood cell morphology like that seen after a thermal burn
South Asian ovalocytosis	<i>SLC4A1</i> (17q21.31)	AD	Heterozygotes - Smear shows stomatocytes with longitudinal slits - High prevalence among individuals from southeast Asia—Malaysia, Papua New Guinea, and the Philippines

AD, autosomal dominant; AR, autosomal recessive

Evaluation

The typical presentation is that of extravascular hemolytic anemia where most of the hemolysis occurs in the spleen. This is in contrast to the enzyme defects where the hemolysis tends to be intravascular. The peripheral smear can often be abnormal and demonstrate abnormalities of red cell shape such as spherocytes in hereditary spherocytosis. There is reticulocytosis and an increase in the MCV and mean corpuscular hemoglobin concentration (MCHC). Indirect hyperbilirubinemia may be observed. DAT (or Coomb's test) is negative indicating a nonimmune etiology. A classic history is that of "pigment" gallstones, often in multiple members of the family. Another point to note is that the penetrance can be variable with generations demonstrating a variable clinical course.

Genetic testing

Clinical diagnosis is made by peripheral smear evaluation and evidence of hemolysis. Other confirmatory tests include osmotic fragility and eosin-5-maleimide binding for hereditary spherocytosis. Genetic testing is not routinely required for diagnosis.

Management

The overall goal of treatment is aimed at preventing complications of chronic hemolysis and anemia. General supportive measures include supplementation with folic acid and blood transfusions when required. In those with severe hemolysis, splenectomy may be considered. There are no specific treatments that address the underlying RBC membrane defect.

Hemoglobinopathies

Hemoglobin, the major oxygen-carrying protein in the red cell, is a hetero-tetramer. In adulthood, the most form is hemoglobin A which is composed of a heme moiety, 2 α globin chains, and 2 β globin chains. The word "hemoglobinopathy" refers to abnormalities in the structure of hemoglobin involving either of the globin chains (Table 14.5). The majority of hemoglobin variants does not have clinical significance and can be difficult to detect. However, many clinically significant hemoglobinopathies are relatively common and will be discussed in this section. The HGNC nomenclature is as follows: Beta-Globin-related hemoglobinopathies (HBB); subunit alpha 1 related hemoglobinopathies (HBA1:); subunit alpha 2 related hemoglobinopathies (HBA2:).

Sickle cell disease and related syndromes

Overview

Sickle hemoglobin, otherwise referred to as HbS occurs due to a missense variant in the β globin chain where glutamic acid is replaced by valine at the sixth amino acid. Sickle trait, that is, heterozygous state for HbS has a high prevalence (nearly 8%) in African Americans and is usually asymptomatic. Sickle cell disease is by far the most common inherited red cell disorder and includes the homozygous HbSS or a compound heterozygous state of sickle hemoglobin with another hemoglobinopathy (the most common one

Table 14.5 Hemoglobinopathies.

Disorder	Genes (chromosomal locus)	Inheritance	Characteristics
Sickle hemoglobin (HbS)	<i>HBB</i> (11p15.4) p.Glu6Val	AR	HbAS (heterozygous) trait • asymptomatic HbSS (homozygous) • anemia • moderate-to-severe vaso-occlusive crises
β thalassemia (β^+ and β^0)	<i>HBB</i> (11p15.4)	AR	HbA β (heterozygous) trait: • microcytosis without anemia Hb β/β (homozygous): dependent on type of mutation • moderate-to-severe anemia • ineffective and extramedullary hematopoiesis • iron overload
Alpha thalassemia (α thal)	<i>HBA1/HBA2</i> (16q13.3)	AR	HbA β (heterozygous) trait: • microcytosis without anemia Hb β/β (homozygous): dependent on the type of mutation • moderate-to-severe anemia • ineffective and extramedullary hematopoiesis • iron overload
Sickle-beta thalassemia	<i>HBB</i> (11p15.4) (Hb S β^+ /Hb S β^0)	AR	HbS β + • mild course of disease HbS β^0 : • moderate-to-severe anemia
Sickle- α thalassemia	<i>HBB</i> (11p15.4) <i>HBA1/HBA2</i> (16q13.3)	AR	Sickle alpha thalassemia has a milder clinical course than HbSS

AR, autosomal recessive

being HbS with beta thalassemia). The HbS, when deoxygenated, becomes insoluble leading to a rigid, “sickle” shaped cell which then causes micro and macrothrombi along with inflammation. The disorder thus clinically presents with a combination of hemolysis of the red cell and painful vaso-occlusive episodes. The severity of SCD is often not apparent in the first 6 months of infancy due to persistence of a form of hemoglobin called HbF or fetal hemoglobin.

Genetic testing

The US Preventive Services Task Force recommends screening all newborns for sickle cell disease [13]. Sickle cell anemia can be diagnosed with high-performance liquid chromatography and isoelectric focusing. There are more rapid methods that evaluate the solubility of hemoglobin under oxidative stress, but these methods are currently not as reliable as standard tests. In HbSS, HbA is absent; in compound heterozygous states, varying amounts of HbA can be detected along with the variant hemoglobin. Genetic testing is typically not

required for diagnosis. However, sequencing tests for identifying specific genetic variants that cause abnormal hemoglobins are available and could be of use for preimplantation diagnosis.

Vignette 14.3

AK is a 27-year-old AA man with sickle cell trait who presents to his primary care physician's clinic as he is getting married and wanted some information on how to obtain genetic counseling. His sister and maternal aunt have sickle cell disease and both of them receive transfusions. His CBC is normal with a hemoglobin of 14 g/dL and normal MCV, MCHC and reticulocyte count. His fiancée who is pregnant has sickle cell trait. Her Hb is 12 g/dL with an MCV of 73 and a normal reticulocyte count. She has no evidence of iron deficiency. He would like to understand the risk of their child having a sickle cell disease.

Sickle cell disease is an autosomal recessive disorder with a carrier frequency of 1 in 12 in individuals of African American ancestry. Given that AK and his fiancée have sickle cell trait, they have a 25% chance of having a child affected with sickle cell disease.

Management

Hemoglobin C disease: HbC is also due to a pathogenic variant in the β globin chain and leads to abnormal red cell membrane causing microcytosis and targets. It causes clinical manifestations in the homozygous or compound heterozygous states. HbC trait is generally asymptomatic.

Hemoglobin E disease: HbE is an example of an “unstable” hemoglobin and it is most commonly found in Southeast Asia. Most individuals who are homozygous for the variant are asymptomatic while those with compound heterozygous states with thalassemia can have mild to moderate anemia.

Sickle cell disorders and hemoglobinopathies are best managed by a hematologist with particular attention to infection prevention, nutrition and management of sickle cell crises often requiring hospitalization.

Thalassemia syndromes

Overview

Thalassemias are disorders characterized by defective synthesis of globin chains. The clinical presentation of these disorders is highly variable and dependent on the type of variant and the presence of a compound heterozygous state with other hemoglobinopathies. The defective biosynthesis of one of the globin chains leads to an imbalance in the globin chains and an excess of the “normal” or unaffected globin chain. Individuals with severe forms of the disease present with anemia and extramedullary hematopoiesis.

Evaluation

A diagnostic clue for thalassemia is the presence of low MCV. Microcytosis can be observed even in individuals with thalassemia trait. Hemoglobin electrophoresis reveals increased HbA2 in beta thalassemia and trait but can be normal in alpha thalassemia and trait.

Genetic testing

The diagnosis of thalassemia is best done by hemoglobin electrophoresis and analysis of peripheral smears. Genetic testing can be undertaken to identify at-risk family members and is also part of carrier screening available for preconception counseling, particularly in populations that have a high prevalence of the disorder.

Management

The overall goal is the management of anemia. Many individuals require frequent blood transfusions which may lead to iron overload. Splenectomy may be required in some individuals. Allogeneic hematopoietic stem cell transplant is potentially curative and may be considered in those with severe disease.

Hematological malignancies

Myeloid neoplasms

The 2016 WHO classification of myeloid neoplasms [14] includes a new section of myeloid neoplasms that are caused due to germline pathogenic variants (Table 14.6). Recognizing these as a specific entity raises the awareness to screen family members for inherited syndromes. Note that bone marrow failure syndromes (e.g., FA, Shwachman–Diamond syndrome, and dyskeratosis congenita) are discussed in the bone marrow failure section, though they are also associated with an increased risk to develop myeloid neoplasms.

Table 14.6 Disorders associated with susceptibility for myeloid neoplasms [14].

Disorder	Gene (chromosomal locus)	Inheritance	Characteristics
Myeloid neoplasms with germline predisposition without pre-existing disorder or organ dysfunction	<i>CEBPA</i> (19q13.11) <i>DDX41</i> (5q35.3)	AD AD	AML with germline <i>CEBPA</i> variants occur at an earlier age, usually during the third decade [15]
Myeloid neoplasms with germline predisposition and pre-existing platelet disorders	<i>RUNX1</i> (21q22.12) <i>ANKRD26</i> (10p12.1) <i>ETV6</i> (12p13.2)	AD AD AD	Lifelong thrombocytopenia and risk of developing myeloid malignancies [16–18]
Myeloid neoplasms with germline predisposition and other organ dysfunction	<i>GATA2</i> (3q21.3) <i>NF1</i> (17q11.2) <i>PTPN11</i> (12q24.13)	AD AD AD	Increased risk for juvenile chronic myelogenous leukemia in patients with neurofibromatosis, Noonan syndrome, and Noonan-like disorders
Juvenile Chronic myelomonocytic Leukemia (JMML)	<i>CBL</i> (11q23.3)	AD	

AD, autosomal dominant; *CEBPA*, CCAAT/enhancer-binding protein alpha

Lymphoid neoplasms

Acute lymphoblastic leukemia is an aggressive clonal neoplasm of a single hematopoietic B- or T-stem cell progenitor leading to the accumulation of blasts in the bone marrow or lymphatic tissues causing anemia, thrombocytopenia, lymphadenopathy, and splenomegaly (Table 14.7).

There is evidence to suggest that Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) as well as chronic lymphocytic leukemia also show a genetic predisposition.

Diffuse large B-cell lymphoma

Pathogenic variants in several different genes have been described in familial DLBCL. These include DNA repair and/or immune function, transcription, and chromatin remodeling and signal transduction genes. Other cancer syndromes and immunodeficiency syndromes have also been linked to familial DLBCL.

Non-Hodgkin lymphoma

First-degree relatives of individuals with NHL have a ~1.7-fold risk of developing NHL. Genome-wide association studies (GWAS) have found the association with a genomic locus on chromosome 3q27 located between encoding B-cell lymphoma protein 6 and encoding lipoma preferred partner.

Table 14.7 Syndromes associated with susceptibility to ALL.

Disorder	Gene (chromosomal locus)	Inheritance	Characteristics
Li–Fraumeni syndrome [19]	<i>TP53</i> (17p13.1)	AD	Heterozygotes Aggressive course with worse prognosis and risk of secondary malignancies
<i>PAX5</i> associated susceptibility [20]	<i>PAX5</i> (9p13.2)	AD	Heterozygotes Potential sensitivity to bcl2 inhibition
<i>SH2B3</i> associated susceptibility [21]	<i>SH2B3</i> (12q24.12)	AR	Homozygotes Also associated with developmental delay and autoimmunity
Thrombocytopenia type 5 [22]	<i>ETV6</i> (12p13.2)	AD	Heterozygotes Thrombocytopenia with bleeding tendency, red cell macrocytosis observed
ETV6 variations as the basis of a novel genetic syndrome associated with predisposition to childhood ALL [23]			

AD, autosomal dominant; AR, autosomal recessive

Hodgkin lymphoma

First-degree relatives of individuals with HL have an 8.5-fold elevated risk of developing HL. GWAS studies done in HL have provided further evidence for inherited susceptibility. They have demonstrated that risk loci influence disease through effects on B-cell regulatory networks, providing a mechanistic link between susceptibility and biology [24].

Points to remember

- *Hereditary bleeding disorders*: The most common disorder is VWD. There is a strong genotype-phenotype correlation in VWD. VWD types 1 and 3 occur due to a quantitative deficiency of VWF, while type 2 is a result of a qualitative abnormality.
- *Hereditary platelet disorders*: These are usually mild bleeding disorders but present with variable severity and may be associated with abnormal peripheral blood smear.
- *Hereditary bone marrow failure syndromes*: These have varying presentations and may be associated with specific cytopenias or pancytopenia. Bone marrow transplant may be the only definitive cure. Detection of familial syndromes is critical in choice of donor, conditioning regimens, and monitoring for additional cancer risks. Many of these syndromes as well as familial syndromes of myeloid neoplasms increase the risk of myelodysplastic syndromes and acute leukemia.
- *Inherited thrombophilia*: This is usually associated with an increased predisposition to venous thromboembolic events; however, testing is rarely applicable in most situations and should be approached with shared decision making with patients in the specific context where it will impact management.
- *Hereditary abnormalities of the red cell membrane*: This leads to increased fragility of the red cell due to structural defects, as in hereditary spherocytosis.
- *Hereditary disorders of hemoglobin*: These disorders like sickle cell disease lead to decreased ability of the protein to carry oxygen and are usually variants in the structure of the globin chains.

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Immunologic disorders

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Introduction

Patients with immunodeficiency present with infections that may be recurrent, severe, or caused by atypical pathogens. Most immune problems found in the adult population are secondary to existing systemic disorders, immunosuppressive therapy, or prolonged serious illnesses (Table 15.1). However, in some, immunodeficiency is a primary condition and results from an intrinsic genetic defect of the immune system (Table 15.2). These primary immunodeficiencies (PIDs) have overlapping clinical manifestations with acquired immunodeficiencies; therefore, evaluation to rule out the latter etiology is always the first goal of a clinical workup. PID may be observed as an isolated disorder or be part of a complex genetic syndrome. PID are clinically and genetically heterogeneous conditions and more than 300 entities described thus far. Most patients with PID manifest initial symptoms of immunodeficiency during infancy or early childhood. Only one-third of the patients have the onset of the disease during adulthood. This latter category not only includes adult-onset immunodeficiencies like common variable immunodeficiency (CVID) or selective IgA deficiency but also individuals with mild and nonclassical presentations of pediatric-onset PID in whom there has been a delay in diagnosis [1,2].

The clinical presentation of immunodeficiency can be quite variable but frequently involves infections that are: (1) recurrent or prolonged and caused by typical pathogens, (2) caused by atypical pathogens, (3) at unusual sites, or (4) severe, (5) resistant to standard therapies, and (6) associated with significant complications. Medical history should ascertain information about infections, anatomical anomalies that would increase risk of infections (e.g., renal anomalies in recurrent genitourinary tract infections), chronic medical problems with emphasis on any history of autoimmune disorders, lymphoproliferative disorders and cancer, medication use, allergies, adverse reactions to vaccination, and family history of similar problems. The physical examination should include evaluation of growth parameters, dysmorphic features, and skin and lymphoid tissue abnormalities. After potential causes of acquired immunodeficiency have been ruled out and if a diagnosis of PID is suspected, patients should have an initial laboratory workup that includes a differential blood count, absolute CD4 cell count, and CD4:CD8 ratio for patients with suspected T-cell deficiency

Table 15.1 Acquired causes of immunodeficiency.

Disease category	Examples
Infectious	Viral infections (HIV, EBV, VZV) Mycobacterial infections
Renal	Renal insufficiency Nephrotic syndrome
Rheumatologic	SLE
Nutritional	Alcoholism Undernutrition
Hematologic	Aplastic anemia Hematologic malignancy Sickle cell disease Splenectomy
Endocrine	Diabetes mellitus
GI	Hepatic insufficiency Hepatitis Protein-losing enteropathy
Iatrogenic	Immunosuppressants Chemotherapy Radiation therapy
Other	Cancer Chronic illness Chromosomal abnormality

Table 15.2 Classification of primary immunodeficiency disorders.

Immunodeficiency	Symptoms	Examples
Antibody deficiency	- Recurrent bacterial infections - Decreased serum antibody titers	- Selective IgA deficiency - Common variable immunodeficiency - X-linked agammaglobulinemia
Cellular deficiency and combined immunodeficiencies	- Recurrent infections by viruses, fungi, opportunistic pathogens - Decreased cellular immunity - Immunoglobulin deficiencies may be secondary to T-cell defect	22q11.2 deletion - Ataxia-Telangiectasia - Hyper IgE syndrome - Severe combined immunodeficiency
Phagocytic cell defects	- Cutaneous staphylococcal and Gram-negative pathogen infections - Deficient phagocytosis	- Chronic granulomatous disease - Chediak–Higashi syndrome - Leukocutye adhesion deficiency
Complement deficiencies	- Recurrent infections - Angioedema - Autoimmune disorders - Defective opsonization, phagocytosis and lysis of pathogens - Defective clearance of antigen complexes	- C1INH hereditary angioedema - C1 deficiency - C2 deficiency

and serum IgG, IgA, and IgM levels for individuals with suspected humoral deficiency. A comprehensive evaluation for PID is usually performed by allergy and immunology (A&I) specialists who orchestrate any additional necessary diagnostic evaluation. A clinical genetics service typically assists with the evaluation and management of patients with syndromic forms of PID, genetic counseling, and genetic testing [3].

Disorders with antibody deficiency

B-cell defects constitute the most prevalent category of immunodeficiency disorders as they account for more than 50% of all diagnosis of PID. Patients with antibody deficiencies are prone to infections with encapsulated pathogens including *H. influenzae* and *S. pneumoniae*. Recurrent bacterial pneumonia, sinusitis, meningitis, and sepsis are the most classical manifestations associated with B-cell defects. In addition, a high incidence of autoimmune disorders, particularly affecting the hematopoietic system, has also been reported. In most cases, antibody deficiencies manifest during childhood [e.g., Bruton agammaglobulinemia also known as X-linked agammaglobulinemia/hypogammaglobulinemia (XLA)]; however, some forms manifest later in life and are typically diagnosed during the teenage or young-adult years (e.g., CVID). B-cell deficiencies should be distinguished from acquired forms of antibody deficiency associated with chronic lymphocytic leukemia and protein-losing enteropathies. Currently, no targeted therapies are available to patients with PID. Antibiotic prophylaxis, intravenous immunoglobulin (IVIG) therapy and measure to prevent infections are standard treatment recommendations.

Here, B-cell deficiencies that are relevant to adults such as CVID and selective IgA deficiency are discussed. Additionally, XLA and hyper IgE, which represent model PID associated with B-cell deficiency and may have a nonclassical presentation with delayed onset of symptoms, are also discussed.

Common variable immunodeficiency

Overview

CVID is a PID that manifests with B-cell deficiency and impaired immunoglobulin production. The disease is clinically and etiologically heterogeneous. There is evidence that CVID could represent either a multifactorial or a monogenic disease, with the latter form being recognized in 15%–30% of individuals with the disorder. CVID is a diagnosis of exclusion and should be considered in patients who are at least 4 years old and have the following manifestations: (1) decreased serum concentration of IgG, (2) decreased serum concentration of IgA and/or IgM, (3) poor response to immunization, and (4) absence of features suggestive of other PID [4].

Evaluation

CVID is a common diagnosis among patients evaluated for PID. Most patients are diagnosed during adulthood, between the third and the fifth decades of life. Patients develop recurrent

infections, chronic lung disease, bronchiectasis, malabsorption, liver disease, and other gastrointestinal (GI) tract manifestations. Frequent upper and lower respiratory tract infections, GI tract infections, septic arthritis, and systemic infections are typically caused by encapsulated bacteria. Infections caused by viral and opportunistic fungal pathogens are observed less commonly. Dysregulation of the immune system leads to increased susceptibility for autoimmune disorders and malignancies. Hematologic autoimmune disorders (anemia, thrombocytopenia), rheumatoid arthritis, pernicious anemia, and autoimmune thyroiditis are especially frequent in this patient population. Increased risk of lymphoid malignancies, particularly non-Hodgkin lymphoma, has also been reported among patients with CVID.

On physical examination, nonspecific findings suggestive of chronic inflammatory diseases like weight loss, lymphadenopathy, splenomegaly, arthritis, or digital clubbing in individuals with chronic lung disease may be present. Diagnostic workup frequently demonstrates normal values of complete blood count and normal count of B and T lymphocytes. Immunoglobulin levels, including IgG and IgA, are significantly decreased. Concentration of IgM is usually low but can also be in the normal range. Patients have low or absent levels of IgG following vaccination (e.g., tetanus and diphtheria). The differential diagnosis includes both environmental and genetic causes. Decreased production of immunoglobulins may be associated with immunosuppressive therapies, systemic illnesses, malignancies, or conditions associated with increased loss of immunoglobulins. Other PIDs that mimic CVID include hyperimmunoglobulin M syndrome and combined immunodeficiencies, among others.

Genetic testing

The role of genetic testing in patients with CVID remains unclear and controversial. Monogenic defects are predominantly found in consanguineous families with affected individuals manifesting CVID-like phenotypes and in individuals with early-onset and severe humoral immunodeficiency. It has been estimated that 2%–10% of patients with a clinical diagnosis of CVID have monogenic causes for their immunodeficiency. Pathogenic variants in a number of genes including *TNFRSF13B*, *CD19*, *CD21*, and *BAFF-R* have been associated with CVID. Genetic testing of simplex cases of CVID usually does not reveal the molecular basis for the disorder [5].

Management

Targeted therapy for CVID is not available. Patients have been shown to benefit from immunoglobulin replacement therapy and prophylactic antimicrobial therapy. Acute infections should be treated with antibiotics as patients with CVID typically cannot fight infections without antibiotic treatment. Patients should not receive certain live-attenuated while inactivated vaccines are generally permitted. Patients are recommended to have a periodic evaluation for pulmonary, GI and autoimmune diseases. They should be monitored for hematologic malignancies, gastric cancer, and signs of other malignancies.

Genetic counseling

Genetic testing using array comparative genomic hybridization and multigene panel targeting PID genes or exome/genome sequencing should be considered for individuals with a family history of CVID and nonclassical presentation. Identification of familial pathogenic variants enables genetic counseling and cascade and prenatal testing for at-risk family members

Selective IgA deficiency

Overview

Selective IgA deficiency is the most common PID and has an estimated prevalence of 1:500 in the general population. This condition is clinically heterogeneous with most affected individuals demonstrating mild immunodeficiency or remaining asymptomatic throughout life. Patients with the condition report an increased frequency of bacterial infections, autoimmune disorders, and allergies. Laboratory studies demonstrate absent or very low concentration of IgA with normal or nearly normal concentrations of other immunoglobulin subclasses. The etiology of selective IgA deficiency is not fully understood. It has been found that ~20% of patients report family history of immune abnormalities.

Evaluation

Patients with selective IgA deficiency have frequent sinopulmonary, ear, and GI infections caused by *S. pneumoniae*, *H. influenzae*, and other typical bacterial pathogens. They are also prone to autoimmune disorders including rheumatoid arthritis, diabetes mellitus type 1, thyroiditis, immune thrombocytopenic purpura, and celiac disease among others. Patients have absent or significantly decreased concentrations of IgA (<0.07 g/L), normal concentrations of IgG and IgM, and demonstrate normal responses to vaccines. The differential diagnosis for the disorder includes CVID and secondary hypoglobulinemia which may present with symptoms and laboratory findings overlap for selective IgA deficiency. Secondary hypoglobulinemia may be caused by other disorders including nephrotic syndrome, protein-losing enteropathy, hematologic malignancies, and iatrogenic effects (e.g., complications of therapy with antiepileptic medications or angiotensin-converting enzyme inhibitors) [6].

Genetic testing

A positive family history of IgA deficiency has been recorded in 20% of patients with this diagnosis. Comprehensive genomic analysis of families with IgA deficiency has revealed pathogenic variants in *TNFRSF13B* in some patients with familial and sporadic forms of the IgA deficiency. Genetic testing for variants in *TNFRSF13B* may be considered in individuals with a positive family history of IgA deficiency and/or a nonclassical presentation.

Management

Targeted therapy for selective IgA deficiency is not available. Patients have been shown to benefit from prophylactic antibiotic therapy to decrease the risk of severe infectious

complications and aggressive treatment of infections. Patients with late-onset IgA deficiency should get chest radiographs to rule out thymoma.

Genetic counseling

A thorough family history should be documented in patients with IgA deficiency. Patients with a family history of frequent infections, autoimmune conditions, and known laboratory abnormalities concerning PID are at a higher risk of having a monogenic form of PID.

Bruton X-linked agammaglobulinemia (BXLA)

Overview

BXLA is a PID characterized by an absence of mature B cells, deficient antibody production, and increased susceptibility to bacterial infections. It is mainly diagnosed in children; however, the diagnosis can be delayed into early adulthood in individuals with a milder course of the disease and individuals with atypical presentations. BXLA is caused by pathogenic variants in Bruton tyrosine kinase (*BTK*) that encodes a tyrosine kinase involved in signal transduction in developing B-lymphocytes. The gene is localized to chromosome Xq22.1 and the condition follows an X-linked pattern of inheritance. The immunodeficiency predominantly affects men; only single case reports of affected women have been published in the literature. The latter cases have been attributed to skewed inactivation of the X-chromosome.

Evaluation

Patients with BXLA present with a history of frequent bacterial infections, particularly otitis media, chronic cough, rhinitis, postnasal drip, and failure to thrive in younger patients. Viral infections such as hepatitis and meningoencephalitis caused by enteroviruses have been also reported. A physical examination may reveal poorly developed tonsils and spleen; however, the lymph nodes are usually normal in size. In classical BXLA, patients start to develop frequent infections around the age of 6 months, after levels of maternal antibodies significantly decrease. It has been estimated that 50% of patients demonstrate symptoms by the age of 2 years. A diagnostic workup for patients with suspected antibody deficiencies and BXLA should include complete blood counts with differential counts, quantitative immunoglobulin levels, antibody titers, lymphocyte typing, and molecular analysis of *BTK*. Patients demonstrate decreased concentration of all classes of immunoglobulins, absent responses to vaccination, normal count of T cells, and absent/decreased count of B cells. The differential diagnosis varies and depends on the age of evaluation: transient hypoglobulinemia of infants, autosomal recessive hypogammaglobulinemia or severe combined immunodeficiency should be considered in younger patients, while older individuals should be evaluated for CVID [7,8].

Genetic testing

BTK is the only gene associated with BXLA. In 92% of patients, the cause is a single nucleotide variant. Copy number variants (CNVs), including whole gene deletions or partial

deletions, are detected in 8% of patients. It has been estimated that pathogenic variants in *BTK* are found in ~90% of patients demonstrating clinical symptoms associated with this condition. *De novo* variants are found in ~60% of patients. So far, over 500 variants in *BTK* have been reported. No clear genotype-phenotype correlations have been established; however, some mutations appear to result in milder phenotypes and later-onset of symptoms [9].

Management

No targeted therapy is currently available for patients with BXLA. Treatment options include avoidance of infections, antibiotic prophylaxis, IVIG, and hematopoietic stem cell transplantation [10].

Hyper IgE syndrome

Overview

Autosomal dominant hyper IgE syndrome (AD-HIES), also known as Job syndrome, is a multisystem disorder that manifests with a triad of skin abscesses, pulmonary infections, and elevated serum levels of IgE. Immunologic and infectious symptoms may present in infancy. Other features including facial dysmorphic features, skeletal abnormalities, and GI manifestations may become apparent later in life. Clinical overlap with other genetic and nongenetic disorders commonly results in delayed diagnosis of AD-HIES.

Evaluation

The first manifestations of Job syndrome may be present as early as at birth, or more frequently, during the first month of life. Affected children have an eczematoid or pustular rash that usually affects the face or scalp areas. Older children have skin boils that are considered a classical symptom of AD-HIES. Other early manifestations include bacterial pneumonias caused by *S. aureus*, *S. pneumoniae*, and *H. influenzae*. Frequent and prolonged infections result in the formation of pneumatoceles and bronchiectasis that increase the risk of secondary infections. Patients with AD-HIES develop additional systemic features during their adolescence or young-adult years. They are frequently found to have osteopenia, joint laxity, scoliosis and retained primary teeth. Facial dysmorphic features include facial asymmetry, high palate, increased nasal width, and deep-set eyes. Laboratory anomalies associated with AD-HIES include eosinophilia and marked elevation of serum IgE concentration. Initial laboratory evaluation should include a blood count with differential counts and serum immunoglobulin concentrations. Patients with an established diagnosis of AD-HIES should be evaluated for osteopenia, scoliosis and retained primary teeth. There is a significant clinical overlap between AD-HIES and atopic dermatitis. Both entities share some clinical characteristics including elevated serum levels of IgE and recurrent bacterial infections. Lack of systemic features associated with AD-HIES and a strong history of allergic reactions in atopic dermatitis set apart these two disorders. AD-HIES also shares several symptoms with other Mendelian disorders including autosomal recessive hyper IgE syndrome associated with *DOCK8* deficiency, *TYK2* deficiency, and Wiscott–Aldrich syndrome [11,12]

Genetic testing

AD-HIES is caused by pathogenic variants in *STAT3*. The molecular analysis of this gene allows for the identification of pathogenic variants in >95% of patients who meet the criteria for diagnosis of AD-HIES. Most cases are due to a *de novo* pathogenic variant in *STAT3*. The role of CNV in etiopathogenesis of AD-HIES is unknown. *STAT3* variants are considered to be fully penetrant. Genetic testing may be achieved through single-gene analysis of *STAT3*, an immunodeficiency panel or exome/genome sequencing. Molecular testing with multigene panels and exome/genome sequencing should be considered if the patient's presentation is not classical [12].

Management

Targeted therapy for AD-HIES is not available. Patients were shown to benefit from prophylactic antibiotic therapy to decrease the risk of severe infectious complications and aggressive treatment of infections. Patients are recommended to have periodic evaluations for osteopenia, scoliosis, and dental abnormalities. The diagnosis of AD-HIES is associated with an increased risk of developing lymphoma and arterial anomalies which, should be taken into consideration during periodic evaluations.

Vignette 15.1

JH is an 18-year-old man with a history of chronic atopic dermatitis and recurrent skin infections. He reported eczematous dermatitis changes in bilateral popliteal areas at age 8 years. The lesions were waxing and waning, but with time similar lesions also developed in the antecubital fossae and abdomen. His primary care physician prescribed topical steroids and moisturizers that did not alleviate his symptoms. JH also reported a history of respiratory problems including asthma and two episodes of pneumonias that required therapy with IV antibiotics. He did not report food allergies. The patient reported similar dermatologic problems in his younger brother and his 42-year-old mother. The physical examination revealed eczematous patches along the flexural areas of his upper and lower extremities, two boils on his back and scoliosis (10 degrees). Laboratory workup showed an elevated count of eosinophils and markedly elevated concentration of IgE (4500 IU/mL). His chest imaging studies demonstrated a normal appearance of the lungs. The patient was referred to the genetics clinic with a concern for Job syndrome.

*JH presents with symptoms that are concerning for Job syndrome, including eosinophilia, elevated concentration of IgE, two episodes of pneumonia, skin abscesses, eczema, and scoliosis. At the same time, similar symptoms may be observed in patients with atopic dermatitis with recurrent skin infections. To differentiate these two conditions, the National Institutes of Health (NIH) scoring system for kindreds with HIES should be applied. The patient scored 10 points for hyper IgE, 3 points for eosinophilia, 8 points for >4 skin abscesses, 4 points for two episodes of pneumonia, 2 points for scoliosis, and 4 points for severe eczema which gives a total of 34 points. According to NIH criteria, the diagnosis of HIES is indeterminate. The genetic testing with next generation sequencing (NGS) PID panel including *STAT3* and *DOCK8* genes was*

requested and did not reveal any pathogenic variants. This result does not exclude the diagnosis of HIES but makes it less likely. The patient was referred to an A&I clinic for further evaluation and management of his atopic dermatitis [11].

Disorders with T-cell deficiency

Cellular immunodeficiencies and combined cellular and antibody deficiencies are predominantly pediatric diseases with onset during infancy. Adult patients may have a nonclassical presentation of T-cell deficiency or a milder form of the disease leading to delays in diagnosis. Acquired cellular immunodeficiencies are common in adults and have heterogeneous etiologies including HIV infection, iatrogenic causes (immunosuppressive drugs), and secondary effects of some chronic medical conditions. All adult patients presenting with PID should be evaluated for acquired immunodeficiency first. Patients with both primary and secondary T-cell deficiencies report a history of chronic viral and fungal infections caused by herpesviridae, *Candida spp.* and infections caused by opportunistic and atypical pathogens like *Pneumocystis spp.* and atypical mycobacteria. They also have an increased risk of developing malignancies and lymphoproliferative disorders. Patients with cellular immunodeficiency may have isolated PID or multisystem disease that involves the immune system. Immunologic aspects of ataxia-telangiectasia (AT) and 22q11 deletion syndrome representing the latter category are discussed later.

Ataxia-telangiectasia

Overview

AT is a multisystem disorder that manifests with immunodeficiency in addition to other clinical symptoms including progressive cerebellar ataxia, choreoathetosis, and increased propensity for malignancy and sensitivity to ionizing radiation. AT is caused by pathogenic variants in the *ATM* gene and is inherited in an autosomal recessive fashion. In the United States, most patients are diagnosed in early childhood through a newborn screening program that identifies patients with AT and other abnormalities of T-cell development. Adult-onset AT, representing a nonclassic form of the disease, and adult patients with an established diagnosis of AT that require coordination of care and management recommendations may be referred to adult genetics services for further evaluation.

Evaluation

Patients with the classic form of AT demonstrate early symptoms of the disease between the ages of 1–4 years. They present with neurological manifestations including ataxia, oculomotor apraxia, dysarthria, and choreoathetosis. These symptoms are later accompanied by ocular telangiectasia, signs of premature aging, and endocrine anomalies. Immunodeficiency is present in the majority of patients and manifests with recurrent upper and lower respiratory tract infections, cutaneous viral infections—all caused by nonopportunistic pathogens. Patients are frequently found to have lymphopenia, immunoglobulin deficiencies (involving

classes IgG4, IgA, IgG2, IgE, and IgG) and abnormal antibody response to antigens. Other diagnostic findings that help in establishing diagnosis include cerebellar atrophy on brain imaging studies and elevated levels of serum alpha-feto protein [13–15].

Genetic testing

AT is an autosomal recessive disorder caused by pathogenic variants in *ATM*. Most patients (90%) with a clinical diagnosis have biallelic loss-of-function single nucleotide variants. Deletions and duplications of a part or the entire gene have been reported in 1%–2% patients. Sequence analysis combined with del/dup analysis of *ATM* allows for identification of the vast majority of molecular defects causing AT. Patients with a classical presentation of AT may be diagnosed with this condition through single-gene analysis of *ATM*. Adult patients with late-onset of the disease and/or nonclassical presentation may benefit from testing with an PID gene panel or exome and genome sequencing because of the significant genetic heterogeneity of disorders associated with T-cell deficiency.

Management

Most patients with classic AT live beyond the age of 25 years; pulmonary disease contributes considerably to the disease morbidity. Patients with AT have an increased risk to develop cancer particularly hematologic malignancies; other cancers associated with AT include breast, ovarian, and gastric cancer. It is most beneficial for patients to receive care from a multidisciplinary team familiar with the challenges of the disease. Management of AT includes IVIG to correct immunoglobulin deficiencies, pulmonary care, physical therapy, supportive care from neurology, and screening for early signs of cancer. The constellation of clinical symptoms associated with the classical spectrum of AT is quite distinct and enables clinical diagnosis. Atypical forms, manifesting with predominant symptoms of immunodeficiency, should raise a possibility of other rare disorders including Nijmegen breakage syndrome, RIDDLE syndrome, and other combined cellular and humoral deficiencies [13].

Genetic counseling

Parents and family members of individuals with AT should be tested for the familial variant in *ATM*. Testing of at-risk family members is important because heterozygous carriers are not only at risk to have a child with AT if their partner is also a carrier, but they are also at increased risk themselves to develop a malignancy, particularly breast cancer. Preconception and prenatal diagnosis are possible in families with determined pathogenic variants [16].

22q11.2 Deletion syndrome

Overview

22q11.2 deletion syndrome (22q11.2 DS), also called DiGeorge or velocardiofacial syndrome, is a multisystem genetic disorder resulting from a submicroscopic deletion of chromosome 22. The condition is inherited in an autosomal dominant manner.

The clinical spectrum of 22q11.2 DS is variable and ranges from severe multisystem disease to subclinical presentation.

Evaluation

The 22q11.2 DS is a multiorgan disorder. Most patients are born with congenital heart disease (conotruncal defects), palatal abnormalities (cleft palate, velopharyngeal insufficiency), and dysmorphic features (facial features, polydactyly). Other, less frequent anomalies affect the central nervous system, renal, endocrine, and ophthalmologic systems. Approximately 80% of patients with 22q11.2 DS have a diminished number of T lymphocytes secondary to thymic hypoplasia and abnormal production of mature T cells. Affected individuals have normal levels of immunoglobulins and appropriate proliferative responses. The immune defect leads to an increase in frequency of upper and lower respiratory tract infections. The incidence of autoimmune disorders, particularly hematologic autoimmune diseases and juvenile rheumatoid arthritis is also significantly increased. Autoimmune thyroiditis and selective IgA deficiency are also more commonly encountered in these patients [17].

Genetic testing

The 22q11.2 deletion can be identified by targeted analysis that employs fluorescence in situ hybridization or multiplex ligation-dependent probe amplification or through the comprehensive analysis of the human genome with chromosomal microarray (CMA). The latter approach allows for discrimination of the classic 3 Mb deletion found in 85% of patients from atypical smaller deletions. Increasingly individuals with 22q11 DS have been identified through exome or genome sequencing that allows for the detection of larger CNVs. In most patients (90%), the deletion results from a *de novo* event.

Management

Implementation of newborn screening for PID has enabled early identification of patients with 22q11 DS, as they present with reduced or absent T-cell receptor excision circles (TRECs). Patients with abnormal results of newborn screening are routinely tested for thymic aplasia with chest radiograph, and other immune abnormalities (leukopenia, abnormal immunoglobulin levels) by a pediatric immunologist. Abnormal immune workup prompts further investigations including screening for a 22q11 deletion and pathogenic variants in known PID genes. The presence of systemic features should instigate appropriate imaging and laboratory workup. Patients with an established diagnosis of 22q11 deletion are referred to immunology and other specialties depending on systemic involvement. Adult patients may benefit from input from immunology and rheumatology if they experience frequent infections and autoimmune problems ([18]).

Genetic counseling

Parental studies are recommended to rule out the presence of a deletion in one of the parents. Cases of gonadal mosaicism have been reported; therefore, genetic testing of siblings

born to a deletion-free parent of an affected individual may be considered, especially if symptoms concerning for 22q11 deletion are present in the sibling. Preconception and prenatal testing are available once parental origin of the deletion is confirmed.

Complement deficiencies

Complement deficiency disorders are a group of immune deficiency disorders following a Mendelian pattern of inheritance and demonstrate significant clinical heterogeneity. Patients with deficiencies of terminal components of the complement system demonstrate susceptibility to chronic or recurrent infections caused by *Neisseria spp.* and increased risk for autoimmune disorders. The infectious and autoimmune manifestations usually have an early-onset and associated complement deficiencies are typically diagnosed during childhood. In this chapter, deficiency of the C1 inhibitor (C1INH), which is generally diagnosed in adulthood and is characterized by recurrent episodes of edema, is discussed.

Hereditary angioedema

Overview

Hereditary angioedema (HAE) is an autosomal dominant condition that manifests with recurrent subcutaneous and submucosal attacks of severe swelling. Patients with HAE demonstrate a deficiency of C1INH resulting in impaired metabolism of bradykinin and increased vascular permeability. HAE due to C1INH deficiency (C1INH HAE) should be distinguished from rare acquired forms of C1INH deficiency (ACID) associated with B-cell lymphoproliferative disorders or angiotensinogen convertase enzyme inhibitor (ACEI)-associated angioedema, which affects ~0.7% of patients treated with ACEI [19].

Evaluation

Patients with HAE present with recurrent episodes of edema involving the face, tongue, uvula, lips, and limbs. Edema of the upper respiratory tract, especially with laryngeal involvement, may be life-threatening. GI tract involvement is frequent and manifests as abdominal pain, nausea, and diarrhea. Episodes of edema usually involve only one site, but in some cases, may be more widespread. The frequency of episodes ranges from a few episodes per year to weekly occurrences. The attacks usually last between 2 and 4 days and are generally self-limiting. Patients usually experience the first attack of angioedema during the adolescent years. Most patients are diagnosed with HAE in early adulthood or during the second and third decades of life. Mild trauma is the most common factor triggering episodes of angioedema. Some medications including ACEI, tamoxifen, and estrogens may increase the frequency and severity of HAE attacks. C1INH HAE should be suspected in individuals with: (1) recurrent episodes of swelling without urticaria, (2) recurrent episodes of colicky abdominal pain, (3) episodes of laryngeal swelling, (4) episodes of angioedema that last 2–4 days, are self-limiting and do not respond

to antihistamine therapy, (5) abnormal complement studies including low plasma concentration of C4 and decreased activity of C1INH, (6) family history of angioedema, and (7) negative history of therapy with ACEI.

Genetic testing

Patients with HAE demonstrate a functional deficiency of plasma C1INH that regulates the activity of plasma serine proteases including C1s and C1r proteases, Factor XII, and plasma kallikrein. There are two major types of HAE: HAE type I with decreased concentration of C1INH and type II with normal levels of nonfunctional C1INH. The former type is more prevalent and diagnosed in 85% of patients with HAE. Both types are clinically indistinguishable. Initial laboratory evaluation should involve serum levels of C4 and C1INH and activity of C1INH. A Decrease in C4 levels and decreased activity of C1INH confirm the diagnosis of C1INH HAE. *C1INH* gene SNV and CNV are detectable in 80% of patients meeting clinical criteria of HAE. Large deletions involving more than one exon have been found in 8% of patients [20].

Management

Acute treatment of angioedema involves the application of plasma-derived or recombinant C1INH, bradykinin B2 receptor antagonist (Icatibant), or kallikrein inhibitor (Ecallantide). In patients with laryngeal involvement, the above medications should be administered as quickly as possible. Long-term therapy includes regular injections of C1INH or monoclonal antibody to kallikrein (lanadelumab). Attenuated androgens and antifibrinolytics are still used; however, their application is associated with significant adverse effects. Patients should be educated about trigger avoidance and planning for acute treatment. They will require life-long surveillance in an A&I clinic.

Genetic counseling

HAE is an autosomal dominant condition with incomplete penetrance. Laboratory studies that include both biochemical and genetic testing for families with known C1INH pathogenic variants are recommended. Patients with clinical manifestations of HAE should first have biochemical evaluation of components of complement pathways (C4, C1INH). Biochemical abnormalities confirm the diagnosis of C1INH HAE. Genetic testing provides confirmation of the diagnosis on the molecular level and allows the testing of asymptomatic members of the family. Preconception and prenatal testing for families with a known C1INH pathogenic variant are possible.

Vignette 15.2

DS is a 22-year-old woman with a personal and family history of recurrent unexplained swellings. She reported having her first episode of lip swelling following wisdom tooth removal at the age of 18 years. The swelling was limited to her face (right cheek and upper lip) only. It subsided 3 days after its onset. The patient used benadryl and cold compresses with no

apparent effect. She was seen by her primary care physician, and this problem was attributed to the tooth extraction. Since the initial episode, the patient experienced four similar attacks that were limited to the face. They lasted 2–4 days and were not alleviated with antihistamine medications. She also had two unexplained episodes of colicky GI discomfort that was associated with diarrhea and lasted a few days. The patient denied a history of other medical problems, exposures or medication use. She reported a history of similar unexplained attacks in her 50-year-old mother. The diagnosis of C1INH angioedema was suspected. The initial workup included immune studies that demonstrated the decreased concentration of serum C4 and lower activity of C1INH. The patient was referred to a genetics clinic for genetic testing. Molecular analysis of the C1INH gene revealed a heterozygous p.Tyr199 pathogenic variant. The patient was provided with genetic counseling and referred to an A&I specialist to discuss further evaluation and management of her C1INH HAE.*

This case represents a classical presentation of C1INH associated angioedema. The onset of swelling attacks during adolescence years and delayed diagnosis until early adulthood are quite typical. The patient reported typical localized (face) episodes of swelling that were triggered by minor trauma (tooth extraction), were not associated with urticaria, did not responded to therapy with antihistamine drugs and lasted a few days. The patient did not report a history of systemic disorder or medication use (ACEI) that could trigger these episodes. The positive family history of similar attacks in her mother is a red flag that points to a possible genetic etiology. Initial workup demonstrated abnormal concentration of C4 component and decreased activity of C1INH. These laboratory findings confirm a presumptive diagnosis of C1INH HAE. Genetic testing involved molecular analysis of single gene: C1INH and enabled detection of pathogenic mutation. Detection of a C1INH pathogenic variant allows for informed genetic counseling in the family and targeted variant detection in family members. The patient was referred to an AI service to discuss prophylaxis and abortive therapy for angioedema attacks.

Autoinflammatory disorders

Autoinflammatory diseases (AIDs) are a category of innate immune system disorders that manifest with recurrent episodes of inflammation without a known cause. Some AID have monogenic etiology, others are multifactorial with a strong environmental component. Familial Mediterranean fever (FMF) is a model AID with the highest prevalence in this category of diseases.

Familial Mediterranean Fever

Overview

FMF is the most common monogenic AID. It is associated with pathogenic variants in the *MEFV* gene and is usually inherited in an autosomal recessive manner. The disease manifests with recurrent self-limiting inflammatory episodes with pain and fever. Amyloid

deposition in tissues and subsequent organ damage is the leading cause of mortality among patients with FMF.

Evaluation

Patients report recurrent febrile episodes accompanied by serositic attacks and cutaneous manifestations that last 2–3 days and are self-limiting. They have symptoms of sterile pleuritis, peritonitis, arthritis, and skin rashes. Patients frequently report a family history of FMF and Mediterranean ancestry. Laboratory findings include increased erythrocyte sedimentation rates (ESRs), leukocytosis, and elevated serum fibrinogen concentrations. Alleviation of patients' symptoms with colchicine therapy supports the diagnosis of FMF. Patients with untreated FMF are at increased risk of developing secondary amyloidosis that may affect the kidneys, spleen, liver, GI tract, and other organs. Renal amyloidosis may progress from asymptomatic proteinuria to progressive nephropathy with end-stage renal disease. The differential diagnosis of FMF is broad and includes other periodic fever syndromes, systemic rheumatic diseases, vasculitis, infections, and malignancies.

Genetic testing

FMF is caused by biallelic pathogenic variants in *MEFV*. Pathogenic variants are detected in 75%–90% of patients who meet the clinical criteria of FMF. No CNVs associated with FMF have been reported in the literature. It has been estimated that ~25% of patients meeting clinical criteria carry only one mutated *MEFV* allele. Genetic testing employs (1) targeted testing for frequent pathogenic variants in patients of Armenian, Turkish, Arab, and Jewish descent, (2) single-gene analysis of *MEFV*, (3) multigene panels targeting inflammatory diseases, or (4) comprehensive genomic testing with exome or genome sequencing.

Management

Acute treatment of FMF focuses on supportive care that includes intravenous hydration and the use of nonsteroidal anti-inflammatory analgesics. Identification of biallelic and monoallelic pathogenic variants in *MEFV* warrants preventive therapy with colchicine as it helps to prevent inflammatory attacks and amyloidosis. Patients who do not respond to therapy with colchicine should be considered for alternative anti-inflammatory agents. Patients with FMF should have regular (annual) evaluations that include laboratory studies for proteinuria, ESR, and serum fibrinogen concentrations.

Genetic counseling

FMF is usually inherited in an autosomal recessive manner, but there is growing evidence that some individuals with a heterozygous pathogenic variant in *MEFV* may manifest a mild-to-classical phenotype. Genetic testing provides confirmation of the diagnosis on a molecular level and allows testing of asymptomatic members of the family.

Points to remember

- Most immune problems found in the adult population are secondary to existing systemic disorders, immunosuppressive therapy, or prolonged serious illness.
- Typical PIDs have onset in childhood and manifest with recurrent bacterial and/or viral infections.
- PID may present as an isolated immune disorder or be accompanied by syndromic features.
- Many PID are associated with an increased risk of developing autoimmune disorders and cancer
- PID is clinically and genetically heterogeneous but can be effectively diagnosed with the application of genetic tests.
- Targeted therapy is not available for PID.

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Intellectual and developmental disabilities

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Introduction

Intellectual and developmental disability (ID) encompasses genetically and phenotypically heterogeneous disorders that have a negative impact on the physical, emotional, behavioral, and intellectual development of an individual. Individuals with ID typically have intelligence quotient (IQ) scores that are below the normal ranges and have significant limitations in adaptive behavior such as social and practical skills that are needed for daily living. Upon transition to adulthood, these individuals pose a unique challenge for the adult medicine general practitioner. The diagnosis and management of adults with ID have been revolutionized by the advent of modern clinical genomics including introduction of clinical microarrays and next-generation sequencing technologies. Over the past decade, healthcare providers have increasingly embraced this new era of genetic testing [1]. However, many adults with ID may not have had a genetic evaluation or may have had a limited evaluation.

ID can be classified based on severity: profound, severe, moderate, mild, and borderline intellectual functioning. It can also be categorized based on the cause, which could be due to both genetic and nongenetic factors. Table 16.1 illustrates some of the examples of the causes of ID. Genetic factors can lead to syndromic ID or nonsyndromic ID. As the name implies, syndromic ID refers to the presence of ID in association with clinical features suggestive of a particular syndrome while nonsyndromic ID is defined as the presence of ID by itself, without the presence of other clinical features suggestive of a syndrome. One common reason for a referral to medical genetics is for assessment of recurrence risk in family members. Genetic causes for ID include chromosomal aneuploidy, chromosomal rearrangements, deletions, duplications, or pathogenic variants in single genes. Establishing a molecular diagnosis and parental testing is helpful for assessing the recurrence risk in family members. If neither parent is a carrier then the family can be counseled that the variant is likely *de novo*, with the caveat of possible germline mosaicism [2]. As the etiology of disorders with ID is

Table 16.1 Causes of intellectual disability.

Nongenetic	Genetic syndromic ID	Genetic nonsyndromic
• Fetal alcohol syndrome • Maternal phenylketonuria • Congenital hypothyroidism • Lead poisoning • Neonatal encephalitis/meningitis • Neonatal hypoxic-ischemic encephalopathy • Neonatal hypoglycemia • Periventricular leukomalacia from prematurity • Others	• Trisomy 21 • Fragile X syndrome • Klinefelter syndrome • 22q11.2 Deletion syndrome • Williams syndrome • Prader–Willi syndrome • Angelman syndrome • Smith–Magenis syndrome • Potocki–Lupski syndrome • Phelan–McDermid syndrome • 1p36 deletion syndrome • Sotos syndrome • Pitt–Hopkins syndrome • Rubinstein–Taybi syndrome • Others	• Sequelae of some inborn errors of metabolism • Multiple single genes with no known association to a genetic syndrome

diverse and the number of disorders that can present with ID is large; in this chapter, we only provide a general overview for the evaluation of an adult with ID and discuss a few of the commonly encountered conditions.

Evaluation

The initial step in the diagnostic evaluation of adults with ID is to obtain a detailed medical history. The prenatal and perinatal history including exposure to teratogens, prematurity, or prolonged hospitalization during the neonatal period is important. Developmental history, particularly attainment of major motor, language, and social milestones and neuropsychological testing including IQ scores, when available, should be reviewed. Information regarding developmental regression, presence of seizures, and features suggestive of metabolic decompensation should be collected. Family history should focus on the presence of ID, neurodegenerative diseases, birth defects, and any known genetic disorders. Recurrent miscarriages in the family can be suggestive of a balanced translocation. Detailed physical examination including craniofacial dysmorphisms may help narrow the differential diagnosis. Certain facial features become less pronounced with age and thus, it may be helpful to review pictures from childhood. However, some distinctive features are evident even in adulthood, for example, long columella and broad thumb in Rubinstein–Taybi syndrome and almond-shaped eyes in Prader–Willi syndrome. In some conditions, the phenotype may evolve with age, such as progressive jaw protrusion in Smith–Magenis syndrome while maintaining the distinctive facial gestalt.

As the differential diagnosis for ID involves numerous disorders, unless history and physical examination are suggestive of a particular disorder or group of disorders, the testing

strategy generally involves a combination of tests including biochemical tests, enzymatic analysis, and genetic testing.

Genetic testing

In principle, all individuals with a diagnosis of ID for whom a cause has not been established should undergo a genetic evaluation [3]. A specific diagnosis allows for the implementation of appropriate management and helps to anticipate future health based on prognostic information specific to the condition. Additionally, a specific diagnosis may facilitate access to federal and state health insurance and disability benefits.

Chromosomal abnormalities were recognized as a common cause of ID in about 15% individuals with ID; Down syndrome (DS) was considered as the most common genetic cause of ID. With advances in molecular cytogenetic technologies, many microdeletions and microduplications were discovered as causes for ID and thus genetic tests such as chromosomal microarray analysis have become a commonly used test in the diagnostic evaluation [4]. Each syndrome has specific risks and medical complications, other than intellectual disability, that may result from the microdeletion or microduplications. Some deletions may include genes causative of inherited cancer syndromes, which would directly impact the future screening for the patient; [5,6] others may include genes causative of hematologic disorders, such as T-cell deficiency (e.g., 22q11 deletion) [7], or thrombocytopenia (11q23 deletion) [8]. As fragile X syndrome is a common cause for ID especially in males, triplet repeat expansion testing for fragile X is typically considered in the initial workup [9]. *FMRI* expansions are best detected by targeted testing, either by Southern blot or polymerase chain reaction (PCR). Additionally, based on the clinical features, biochemical tests including ammonia, plasma amino acids, urine organic acids, and others may be required.

If the initial workup is unrevealing, further testing using next-generation sequencing-based panel tests or whole-exome sequencing (WES) can be considered. The diagnostic yield of exome sequencing in individuals with neurological phenotypes has been estimated to be around 30% [10]. Whereas it would be reasonable to assume that a trio exome analysis would be better than a proband alone exome, in the practice of adult medicine, this approach may not always be possible as one or both of the parents may have passed away or be unavailable for testing. Using unaffected first-degree relatives to supplant the parent in the analysis can be an alternative. If exome sequencing is unrevealing, disorders that could be missed by WES, especially triplet repeat expansions, methylation, and imprinting disorders should be considered. Genome sequencing could help identify pathogenic variants outside of the coding sequence.

One challenge of genome-wide testing strategies is the detection of variants of uncertain significance; this is especially true of exome and genome sequencing [11]. Patients and caregivers should have pretest genetic counseling to educate them of the possibility of uncertain results. In some cases, it may be possible to reclassify the variant by establishing segregation with the disease or testing parents (if not done as part of an exome or a trio-based panel)

to establish if a variant is *de novo*. In some circumstances, research studies may be an additional option to establish a diagnosis.

Any research testing or procedures should be performed after informed consent is obtained according to the research protocol. In this scenario, the clinician must make it clear to the patient and caregivers that the workup is moving from standard clinical care to a research setting.

Genetic counseling

The traditional practice is to disclose the results of genetic testing in a face-to-face visit, although there is emerging evidence that telephonic disclosure may be acceptable [12]. If the results are negative, this affords an opportunity to discuss further evaluation. If a variant of uncertain significance is identified, the family can be informed of strategies to reclassify the variant.

When specific management guidelines are available (e.g., Trisomy 21, 22q11.2 microdeletion syndrome, Prader–Willi syndrome, fragile X, Klinefelter, and Williams syndrome), these should be shared with the family. Most guidelines pertain to the pediatric population and there are only a few that are specific to the care of adults [13]. If a microdeletion is identified, it is important to look at the genes involved in the genomic region to identify any genes that are associated with adult-onset disorders. For example, some deletions that cause Smith–Magenis syndrome include *FLCN*, which increases the risk for renal cell cancer risk and spontaneous pneumothoraces (Birt–Hogg–Dube syndrome) [14].

Syndromic intellectual disability

Fragile X syndrome

Overview

Fragile X syndrome is one of the most common inherited single-gene disorders that presents with ID [15]. The etiology is most often an expansion of a trinucleotide (CGG) repeat in the *FMR1* gene on chromosome X. Males with a full mutation (>200 repeats) typically have moderate-to-severe ID [16], whereas women with full mutations can be affected due to skewed X-chromosome inactivation and typically have mild ID [17]. This makes it imperative to consider fragile X syndrome in the differential diagnosis of intellectual disability in both genders.

Evaluation

Patients with fragile X syndrome often present with a history of hypotonia and a delayed acquisition of milestones, in particular, speech. Common medical problems include recurrent otitis media and gastroesophageal reflux. Some of the traits of fragile X become more evident in adulthood such as facial features, including long face with tall forehead,

prominent ears and jaw, joint hyperextensibility, pes planus, and strabismus. Macroorchidism appears after puberty. Some individuals can have mitral valve prolapse. IQ in males, most commonly in the moderate intellectual disability range, can decline over time in a substantial percentage of patients [18]. Other common cognitive defects include impairments in memory, mathematic, visuospatial abilities, and executive function. Behavioral issues are common and autism is seen in 15%–42% of affected individuals [19]. However, studies of adult males with fragile X syndrome suggest that there is a pattern of improvement in some symptoms with age such as restricted, repetitive behaviors, and impairment of verbal communication. Some degree of aggression has been reported by caregivers in a significant proportion of individuals with sensory concerns being predictive of both frequency and severity of episodes [20].

Genetic testing

Fragile X syndrome results from loss of function of the *FMR1* gene. This occurs due to the expansion of a CGG repeat in the 5' untranslated region of the gene with subsequent hypermethylation and silencing. Less commonly, there is mosaicism either of repeat sizes or methylation status, pathogenic variants of the *FMR1* gene, or partial or full deletions of the gene. *FMR1* expansions are best detected by targeted testing, either by Southern Blot or PCR [21]. If there is a high degree of clinical suspicion and the *FMR1* repeats are normal, the sequencing of the gene can be performed.

Vignette 16.1

JF is a 22-year-old man with a past medical history of hypotonia, joint laxity, recurrent otitis media requiring myringotomies at age 4, and obesity. He has moderate intellectual disability with significant emotional lability and behavioral issues that have escalated after puberty. His most noteworthy problems are perseverative behavior, social anxiety and gravitational insecurity with avoidance of escalators, elevators, and transitions across surfaces. Chromosome analysis performed during childhood was normal. Testing for FMR1 repeats reveals that he has one allele with 550 CGG repeats. This confirms a diagnosis of fragile X syndrome.

Management

Management consists of educational, psychological and behavioral intervention. An echocardiogram might be indicated if there is a clinical suspicion of mitral valve prolapse.

Genetic counseling

A new diagnosis of fragile X syndrome in a family has implications beyond the proband, as it often triggers cascade testing and requires genetic counseling for relatives. The presence of a full mutation in an affected patient almost invariably results from expansion at the time of maternal transmission of a premutation allele (55–200 CGG repeats). Mothers carrying a premutation allele are known to be at risk for fragile X-associated primary ovarian insufficiency and other menstrual dysfunction problems with consequences on bone and cardiovascular

health among others [22], as potentially are other female members of the family. Additionally, both men and women carrying premutations are at risk for fragile X-associated tremor/ataxia syndrome. These conditions are believed to be RNA-mediated toxicity rather than due to a reduction of product of *FMRI* [23]. Moreover, some authors have suggested premutation carriers can also have a higher rate of autistic and obsessive compulsive symptoms.

Down syndrome

Overview

DS is one of the leading causes of ID and is characterized by multiple congenital anomalies and comorbidities including congenital heart defects, Alzheimer's disease, leukemia, Hirschsprung disease, and cancer. The most common etiology of DS is the maternal nondisjunction of chromosome 21. Other less common causes include Robertsonian translocation of chromosome 21 with chromosome 14 and trisomy 21 mosaicism.

Evaluation

Individuals with DS are easily recognizable because of the characteristic features including upslanting palpebral fissures, epicanthal folds, brushfield spots, flat nasal bridge, small low-set ears, protruding tongue, excess nuchal skin, single transverse palmar crease, sandal gap, and short stature. The diagnosis is typically made prenatally or at birth. While making a diagnosis is not a major concern for DS in adults, it is important to know that individuals with DS are living longer and are known to experience accelerated aging. This means that they experience certain medical conditions in their 40s–50s, that are typically seen in the elderly in the general population, such as early cataracts, conductive hearing loss, hypothyroidism, obstructive sleep apnea, osteoarthritis, osteoporosis, and Alzheimer's disease.

Genetic testing

Trisomy 21 is one of the most common chromosomal abnormalities in liveborn children and has an estimated prevalence of one in 700 [24]. Advanced maternal age is a significant risk factor for having a child with DS (1 in 400 at age 35, 1 in 105 at age 40, and 1 in 12 at age 45); however, 80% of babies with DS are born to women younger than 35 years of age because of a higher fertility rate in that age group [25]. While the most common cause of DS is trisomy 21, other causes include Robertsonian translocation and isochromosome or ring chromosome. The diagnosis of DS can be made by routine cytogenetic analysis such as chromosomal analysis.

Prenatal screening for DS includes noninvasive and invasive diagnostic testing such as noninvasive prenatal screening using cell-free fetal DNA from maternal circulation, nuchal translucency, serum screening, ultrasound examination, and traditional cytogenetic analysis on fetal samples obtained through amniocentesis or chorionic villus sampling.

Management

The phenotype of DS is very variable. An interdisciplinary team of specialists should be involved in the care of a patient with DS to ensure timely screening and appropriate

management of comorbidities. These may include a clinical geneticist, developmental pediatrician, cardiologist, ophthalmologist, neurologist, orthopedic surgeon, psychiatrist, physical and occupational therapist, speech-language pathologist, and an audiologist. The American Academy of Pediatrics has issued a comprehensive guide to preventive screening and health maintenance in children with DS [26]. As individuals with DS grow older, there is an increased risk of common mental health disorders such as depression, anxiety, obsessive compulsive disorder and behavioral disturbances in adults. Because of the increased risk of Alzheimer's disease, individuals should be screened for memory impairment throughout mid-to-late adulthood. Transition from home care to assisted care may become necessary in some cases due to aging or death of parents of these individuals. Healthcare guidelines for adults with DS are listed in Table 16.2 [25].

Genetic counseling

Most cases of DS are not inherited and trisomy 21 occurs as a random event during gametogenesis. In about 2%–3% of cases, the parent is a carrier of a Robertsonian translocation that leads to trisomy 21 in the child and risk of recurrence in future pregnancies. At least half of all women with DS are fertile and can become pregnant. An estimated 35%–50% of children born to mothers with DS are likely to have trisomy 21 or other developmental disabilities. Information about fertility for men with DS is limited [25].

Table 16.2 Consideration of health screening in adults with Down syndrome.

Examination	Frequency	Additional comments
Audiology	Every 2 years	
Breast exam	Annual	
Cervical spine X rays	As needed for sports	Check for atlanto-axial dislocation
Echocardiogram	As indicated	Look for mitral/aortic valve problems
Education	As needed	Health, abuse prevention, sexuality, smoking, drug, and alcohol
Mammogram	Baseline at age 40, follow up every other year until age 50, then annual	
Monitor for obesity	Annual	Low calorie, high-fiber diet, and regular exercise
Neurological exam	As indicated if decline in function, memory loss, ataxia	
Ophthalmologic exam	Every 2 years	Look for keratoconus and cataracts
Pap smear and pelvic exam	Every 1–3 years after the first intercourse	If not sexually active, single finger bimanual exam or pelvic ultrasound every 2–3 years
Sleep apnea	As indicated	
Speech and language	As indicated	
Thyroid function test	Annual	

22q11.2 Deletion syndrome

Overview

22q11.2 deletion syndrome (22q11.2 DS), also known as velocardiofacial syndrome and DiGeorge syndrome, is the most common microdeletion syndrome and has an estimated prevalence of 1:3000–6000 children [27]. It is characterized by the involvement of multiple organ systems leading to congenital heart defects, immunodeficiency, hypoparathyroidism, palatal, gastrointestinal, skeletal and renal abnormalities, developmental and speech delay, and an increased risk for psychiatric illness. It is caused by a microdeletion on the long arm of chromosome 22 due to meiotic nonallelic homologous recombination mediated by a cluster of low-copy repeats on chromosome 22.

Evaluation

There is a large variability in phenotype but most of the individuals have a characteristic facies that include hypertelorism, prominent tubular nose with bulbous nasal tip, cleft palate, small open mouth, and retrognathia. While these facial dysmorphisms might be less reliable in adults, 22q11.2 DS should be considered in the setting of learning disabilities, velopharyngeal insufficiency and conotruncal defects such as tetralogy of Fallot, truncus arteriosus, interrupted aortic arch, and ventricular/atrial septal defects. Because of the increased prevalence of heart defects in 22q11.2 DS, the American College of Cardiology and the American Heart Association have issued guidelines for screening for genetic causes including 22q11.2 DS in all adults with conotruncal abnormalities [28]. The neurocognitive profile is also highly variable; however, the majority of patients have borderline IQ (70–84). There is an increased risk of developing several psychiatric disorders including attention deficit with hyperactivity disorder, autism spectrum disorder (ASD), anxiety and mood disorders, psychotic disorders, and schizophrenia.

Genetic testing

22q11.2 DS is caused by a three megabase pair (Mb) deletion on chromosome 22q that is flanked by low-copy number repeats. This deletion can be diagnosed by targeted techniques such as fluorescence in situ hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA) or genome-wide tests for deletion and duplications such as a chromosomal microarray (CMA).

Management

A multidisciplinary team approach is necessary to manage patients with 22q11.2 DS due to complex multisystemic involvement. Clinical practice guidelines have been published which includes anticipatory care for screening and management of associated conditions in patients with 22q11.2 DS [13,29].

Genetic counseling

Most of the 22q11.2 deletions are *de novo* events with unaffected parents. In about 10% of cases, the deletion is also present in a parent and hence parental testing is recommended

when a deletion is identified in the family, so that appropriate recurrence risk estimates can be provided. This is an autosomal dominant condition with a 50% chance of having an affected child in each pregnancy.

Nonsyndromic intellectual disability

Nonsyndromic intellectual disability (NS-ID) can often be associated with autism, epilepsy, and other neuromuscular deficits such as neuropathies and muscular dystrophies. While chromosomal aneuploidies or microdeletion/microduplications cause ID, there are many single genes that also cause ID, including NS-ID. Many of the genes associated with NS-ID are thought to be located on the X-chromosome which became the main focus of research in the 1990s. Pathogenic variants in more than 100 genes are now implicated in X-linked ID. However, some genes are known to cause NS-ID as well as syndromic ID depending upon the location of the pathogenic variant. For example, *ARX* is one of the most frequently associated genes with ID. Pathogenic variants in this gene are associated with several different overlapping ID phenotypes including NS-ID. NS-ID can be categorized as X-linked, autosomal dominant and autosomal recessive. Many recurrent copy number variants are *de novo* and considered to be the predominant cause of autosomal dominant ID [10]. On the other hand, very few recessively inherited genes have been identified that cause NS-ID. Overall, over 700 genes have been implicated in the molecular etiology of ID.

Autism spectrum disorder

The incidence of ASD in the general population is about 0.3%–0.6%. However, there is a significant overlap between ID and ASD. ID is found in two-thirds of individuals with ASD. Many studies have shown that the prevalence of ASD is about 8%–20% in patients with ID [30]. For example, many syndromic ID have a high incidence of concordant ASD such as fragile X syndrome (25%–47%); DS (5%–10%) and tuberous sclerosis (16%–48%) [31]. Thus, it is important to evaluate patients with ASD and ID for underlying genetic disorders that might explain their phenotype. With current advances in genetic testing, this can be done through panel-based testing or whole-exome/genome testing.

Conclusion

Technological advances have allowed us to improve our understanding of the causes of ID. However, while most of the research has focused largely on highly penetrant monogenic causes leading usually to severe ID, a large proportion of milder forms of ID are unexplained and may require more ongoing research into digenic and oligogenic forms of ID, variants in the noncoding regions, and somatic variants. It is envisioned that genome sequencing will likely become the first-tier diagnostic test for ID once it becomes affordable and widely available.

Points to remember

- Fragile X is one of the most common causes of ID in males.
- Current guidelines from American College of Medical Genetics and Genomics suggest that individuals with ID and ASD should get *FMRI* testing for fragile X syndrome and chromosomal microarray as first-line tests.
- DS is the most common chromosomal anomaly in liveborn infants and one of the most common causes of intellectual disability.
- 22q11.2 deletion syndrome is a multi-system disorder caused by a 3 Mb deletion on chromosome 22 leading to a varying neurobehavioral phenotype across the lifespan of an individual with an increased predisposition to psychiatric disorders in adulthood.
- Consider ID gene panels or whole-exome/genome sequencing in nonsyndromic ID.

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Metabolic disorders

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Introduction

Inborn errors of metabolism (IEM) may have mild, attenuated, and late-onset clinical manifestations. Typically, IEM in adults manifest with neurological or psychiatric symptoms, such as encephalopathy, stroke, psychosis, myopathy, or neuropathy. A high index of suspicion should be reserved for those patients presenting with recurrent unexplained symptoms, with signs and symptoms that are provoked by catabolic stress (such as exercise, infection, trauma, surgery, or postpartum period), or with features suggestive of systemic involvement (such as liver, heart, or eye involvement). This chapter outlines the more common presentations of commonly encountered IEM in adults and the differential diagnoses of these rare, but treatable genetic disorders.

Hyperammonemia

Overview

Ammonia is a neurotoxin and thus hyperammonemia causes encephalopathy. Whereas most commonly, hyperammonemia in adults is observed as a complication of advanced liver disease, in the absence of liver disease, it may be a clue for an underlying IEM. Urea cycle disorders (UCDs) are the most common genetic cause for hyperammonemia, yet other IEM such as organic acidemias and fatty acid oxidation disorders can present with this complication. Other nongenetic causes include portosystemic shunts, infection with urease-positive bacteria [1,2], and treatment with asparaginase [3] or valproic acid [4]. Importantly, false elevations of blood ammonia can occur with improper sample handling. The most accurate measurements require that samples be immediately placed on ice and analyzed within 15–30 minutes after collection.

The urea cycle consists of a series of six enzymes and at least two transporters that facilitate the conversion of waste nitrogen into urea, a product that is safely excreted in the urine (Fig. 17.1). In primary UCDs, one of these six enzymes is deficient, and consequently, waste nitrogen accumulates in the form of elevated ammonia levels in the blood (Table 17.1).

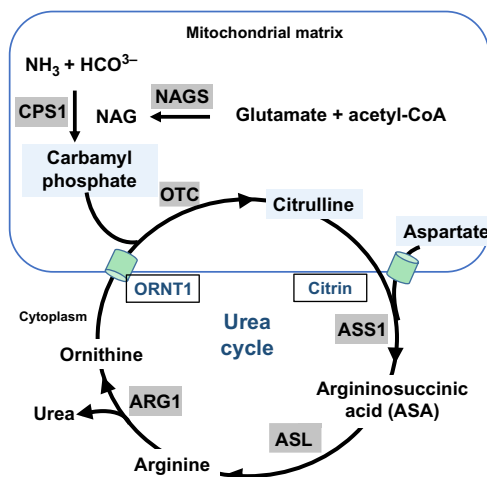


FIGURE 17.1 Urea cycle. The urea cycle consists of six enzymes (gray boxes) that convert waste nitrogen to urea, a safe waste product that is excreted in the urine. In addition, two transporters (white boxes) transport key urea cycle metabolites between the mitochondrial matrix and cytoplasm. When one of these enzymes or transporters is deficient, a urea cycle disorder results. NAGS, *N*-acetylglutamate synthase; CPS1, carbamoyl-phosphate synthase 1; OTC, ornithine transcarbamylase; ASS1, argininosuccinate synthase 1; ASL, argininosuccinate lyase; ARG1, arginase 1; ORNT1 is associated with HHH, hyperornithinemia-hyperammonemia-homocitrullinuria; citrin is associated with citrin deficiency.

Likewise, secondary UCDs result from defects in the transport of amino acids required for urea cycle function (Fig. 17.1), with hyperammonemia as a presenting feature. Although adult-onset presentations have been described in nearly all UCDs, the most common disorder diagnosed in adulthood is ornithine transcarbamylase (OTC) deficiency, an X-linked disorder with an estimated prevalence of approximately 1:15,000. All other UCDs are autosomal recessive disorders.

Adults with UCDs may present with altered mental status. Recognition of hyperammonemia as the cause for altered mental status in an individual with an undiagnosed UCD may be life-saving, as rising ammonia levels can be associated with coma and death if untreated. A first episode of hyperammonemia in such individuals may be triggered by an increased dietary protein load or other events associated with catabolic stress, such as surgery, menstruation, childbirth, fasting, or steroid administration. However, in some cases, no trigger is identified. Besides altered mental status, other presenting features include seizures, psychosis, and other neuropsychiatric manifestations [5,6]. Moreover, as acute liver failure can be a presenting feature of UCDs, especially OTC deficiency [7,8], evidence of liver disease does not always exclude the possibility of a UCD in the setting of hyperammonemia.

Evaluation

In an individual with hyperammonemia of unclear etiology, a careful history should include questions that may reveal clues to an underlying UCD. Individuals with UCD often avoid

Table 17.1 Urea cycle disorders.

UCD	Inheritance	Amino acids ^a	Urine orotic acid ^a	Confirmatory diagnostic tests
Primary UCDs				
NAGS deficiency	AR	Low plasma citrulline	Normal	Molecular genetic testing of <i>NAGS</i>
CPS1 deficiency	AR	Low plasma citrulline	Normal	Molecular genetic testing of <i>CPS1</i> , enzyme activity in liver biopsy specimen
OTC deficiency	X-linked	Low plasma citrulline	Elevated	Molecular genetic testing of <i>OTC</i> , enzyme activity in liver biopsy specimen
ASS1 deficiency or citrullinemia type I	AR	Elevated plasma citrulline Low plasma arginine	Elevated	Molecular genetic testing of <i>ASS1</i> , enzyme activity in fibroblasts
ASL deficiency or argininosuccinic aciduria	AR	Detection of argininosuccinic acid in urine or plasma Elevated plasma citrulline Low plasma arginine	Elevated	Molecular genetic testing of <i>ASL</i> , enzyme activity in RBCs and fibroblasts
ARG1 deficiency or hyperargininemia	AR	Elevated plasma arginine	Elevated	Molecular genetic testing of <i>ARG1</i> , enzyme activity in RBCs
Secondary UCDs				
Citrin deficiency	AR	Elevated plasma citrulline Elevated serine/threonine ratio	Typically normal	Molecular genetic testing of <i>SLC25A13</i>
HHH syndrome	AR	Elevated ornithine Elevated urine homocitrulline	Elevated	Molecular genetic testing of <i>SLC25A15</i>

^aThe laboratory features highlighted are the typical findings in each disorder. In some cases, the typical laboratory findings are not present, and a high level of clinical suspicion is required. In addition to the amino acids provided in the table, hyperalaninemia and hyperglutaminemia may be detected in individuals prior to diagnosis or in diagnosed individuals who have suboptimal control. *AR*, autosomal recessive; *ASS1*, argininosuccinate synthase 1; *ASL*, argininosuccinate lyase; *ARG1*, arginase 1; *CPS1*, carbamoyl-phosphate synthase 1; *HHH*, hyperornithinemia-hyperammonemia-homocitrullinuria; *NAGS*, *N*-acetylglutamate synthase; *OTC*, ornithine transcarbamylase; *UCD*, urea cycle disorder; *SLC25A13*, mitochondrial aspartate/glutamate carrier; *SLC25A15* mitochondrial ornithine transporter ORNT1.

protein-rich foods as high-protein loads may trigger unrecognized hyperammonemia. Likewise, individuals may endorse episodic vomiting or headaches, which may be signs of previously unrecognized hyperammonemia. Questions about inciting events for the current episode may also suggest a diagnosis of a UCD such as diet history (e.g., meal types and frequency), menstruation history, intercurrent illness, recent surgeries or childbirth, and medications.

Family history is also an important component of the evaluation of an individual with hyperammonemia. It is especially important in the setting of undiagnosed OTC deficiency because of the X-linked inheritance pattern. Typically, males with OTC deficiency have more severe disease than females, but males with mild disease or late-onset disease have been reported. Likewise, females with OTC deficiency can either have an early-onset, severe presentation or a much milder, late-onset presentation. Apparently asymptomatic females have also been reported. This variability in presentation in females with OTC deficiency is due to skewing of X-inactivation in the liver. Thus, a careful family history may reveal unexplained death in male infants or other individuals with features suggestive of unrecognized hyperammonemia. Physical examination is usually unremarkable. However, in the setting of hyperammonemia, altered mental status, lethargy, confusion, seizures, or even coma may be present. Spastic diplegia is a characteristic feature in arginase deficiency that is not typically noted in individuals with other UCDs.

Compared to the other UCDs, citrin deficiency is unique. Patients with citrin deficiency may present in infancy with transient intrahepatic cholestasis. As they grow older, they may demonstrate a preference for protein-rich foods with avoidance of carbohydrate-containing foods. As an adolescent or young adult, they may present with symptoms of hyperammonemia with or without prior symptoms of citrin deficiency. Interestingly, hyperammonemia may be triggered by medications, surgery, alcohol or sugar intake, but in some cases, the trigger is not identified. Citrin deficiency occurs due to pathogenic variants in *SLC25A13*, which encodes the aspartate-glutamate carrier. The unique dietary preferences have been attributed to altered cellular ratios of NADH/NAD⁺ in the setting of reduced cytoplasmic aspartate that impacts urea cycle function [9]. Carbohydrate intake is hypothesized to worsen the alteration in NADH/NAD⁺ ratios and thus, cytoplasmic aspartate deficiency, but with increased protein intake, cytoplasmic aspartate rises and restores urea cycle function [9].

In order to evaluate for UCDs in an individual with hyperammonemia, plasma amino acids and urine orotic acid should be ordered. The typical pattern of laboratory abnormalities for each disorder is provided in [Table 17.1](#). Other clues to the diagnosis may include low blood-urea nitrogen (BUN) and elevated plasma levels of aminotransferases.

Genetic testing

Single-gene testing or gene panel testing is useful for confirming a suspected diagnosis. The exact yield of genetic testing for each disorder has not been determined. However, genetic testing (sequencing and deletion/duplication studies) in individuals with OTC deficiency only detects pathogenic variants in approximately 85%–90% of individuals with the diagnosis, likely because the undetected variants are located in noncoding regions of the gene that are

not covered by routine clinical testing [10]. Thus, in 10%–15% of individuals, the diagnosis is based on clinical presentation and laboratory abnormalities or liver biopsy showing reduced OTC enzyme activity. Historically, allopurinol challenge was used to support a suspected diagnosis of OTC deficiency [11].

Vignette 17.1

SR is a 23-year-old female who presents to the ER with an altered mental status of unclear etiology. In the past 1 week, she started a high-protein, low-fat diet with the goal of losing weight. One day prior to admission, she developed a headache and two to three episodes of emesis. On the morning of presentation, she was confused and lethargic and was brought to the ER. Prior to starting the high-protein diet for weight loss, she avoided eating meat. She also had a history of episodic vomiting of unclear etiology. Her family history is remarkable for a full brother who died unexpectedly as an infant, but the cause for his death was never determined. Laboratory evaluation reveals a low BUN and normal liver function in the setting of an elevated ammonia level. Plasma amino acids reveal low citrulline and arginine with elevated glutamine. Urine orotic acid is also elevated. Treatment with intravenous fluids containing dextrose and intravenous forms of nitrogen-scavenging agents results in a normalization of blood ammonia levels. She is discharged from the hospital on a low-protein diet, citrulline supplementation, and an oral nitrogen-scavenging agent. She is also provided an emergency letter and anticipatory guidance relevant to her diagnosis. Several weeks later, DNA testing confirms the suspected diagnosis of OTC deficiency. Testing in her mother reveals that her mother also has the same pathogenic variant in OTC, and presumably, her brother died from the same diagnosis. Genetic counseling is provided to the family and testing for other at-risk relatives is offered.

The patient's history of protein avoidance, episodic vomiting, low BUN, and hyperammonemia after a protein load, in the absence of significant liver dysfunction, is suggestive of a diagnosis of a UCD. Laboratory evaluation including urine orotic acid and plasma amino acids is used to determine the specific UCD. Treatment is initiated once a UCD is suspected. DNA testing confirms the suspected diagnosis.

Management of UCDs

The acute management of hyperammonemia in the setting of a UCD should occur in a critical care unit, and consultation with an IEM specialist is recommended. To reduce the ammonia level, protein intake is temporarily discontinued, and anabolism is promoted with the use of intravenous fluids containing dextrose and insulin. Nitrogen-scavenging agents are used to divert nitrogen from the urea cycle, and supplementation with deficient amino acids (depending on the specific disorder) is beneficial. In patients with hyperammonemic coma, severe elevations in ammonia levels, or poor response to medical management, hemodialysis should be pursued given the risk for cerebral edema.

The chronic management of UCDs (except citrin deficiency) includes a low-protein diet and the use of nitrogen-scavenging agents. These include glycerol phenylbutyrate, sodium phenylbutyrate, and sodium benzoate, which divert nitrogen away from the urea cycle and

result in the production of safe nitrogen waste products that are excreted in the urine. The low-protein diet may be supplemented with essential amino acid mixtures that have reduced nitrogen load compared to natural protein. In addition, citrulline supplementation is used in OTC deficiency, *N*-acetylglutamate synthase (NAGS) deficiency, and carbamoyl-phosphate synthase 1 deficiency whereas arginine supplementation is provided in argininosuccinate lyase deficiency and argininosuccinate synthase 1 deficiency. Carbamylglutamate, a synthetic form of *N*-acetylglutamate, has been used in NAGS deficiency. When medical management does not result in good metabolic control, liver transplantation may be considered.

A key part of the management of UCDs is anticipatory guidance. Patients should avoid fasting and high-protein loads as well as skipping meals or medications, to reduce the risk of hyperammonemia. In addition, valproic acid and steroids should be avoided, when possible, given the potential increased risk for hyperammonemia. Events such as vomiting, dehydration, intercurrent illness, surgery, and childbirth require close management by a metabolic specialist. All individuals with UCDs should carry an emergency letter describing their diagnosis, initial management, and contact information for their primary metabolic specialist. Emergency bracelets are encouraged.

In contrast to the other UCDs, the treatment of citrin deficiency includes a high-protein, low-carbohydrate diet. Sodium pyruvate, arginine, and medium-chain triglyceride supplementation have also been used. Alcohol and high glucose infusions should be avoided in this disorder.

Genetic counseling

All UCDs except OTC deficiency are inherited in an autosomal recessive pattern. A patient's risk of having an affected child with the disorder will depend on their partner's carrier status. Thus, preconception genetic counseling is recommended.

OTC deficiency is unique among the UCDs because it is an X-linked disorder. Pathogenic variants in *OTC* can be *de novo* or inherited. Because of the possibility of skewed X-inactivation, the phenotype of OTC deficiency in females can vary from mild or even apparently asymptomatic to severe. Thus, evaluations of siblings and mothers of affected individuals are recommended. In addition, there are reports of males with OTC deficiency transmitting the disorder to their daughters, and therefore, the evaluation of fathers of affected females is also recommended. Appropriate genetic counseling of the affected individual with OTC deficiency should be provided. Although males with OTC deficiency do not transmit the disorder to their sons, all of their daughters will have the disorder. For females with OTC deficiency, each offspring, regardless of sex, will have a 50% chance of having the diagnosis. Therefore, preconception genetic counseling is recommended for both males and females with OTC deficiency. In addition, given the increased risks for hyperammonemia in an affected female during the peripartum period, consultation with a maternal-fetal medicine specialist and metabolic specialist is recommended before and during pregnancy so that appropriate plans can be made for evaluation and treatment of the mother and newborn [12].

Metabolic stroke

Overview

Stroke or stroke-like episodes in patients with IEM may manifest as focal neurologic deficits, migraine headaches, cortical blindness, altered mental status, or seizures. Metabolic-genetic etiology should be considered in young adults presenting with stroke, in patients with a family history of early-onset strokes, in cryptogenic stroke, and when brain imaging shows abnormalities in a distribution that does not follow a typical vascular territory. The differential diagnosis of metabolic disorders with such presentation include homocystinuria, cobalamin metabolism defect, organic acidemias (particularly in the setting of metabolic decompensation), Fabry disease, UCDs (specifically, in the setting of altered mental status and seizures), and mitochondrial disorders [such as mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)]. Several pathogenic mechanisms have been suggested, depending on the underlying metabolic disorder. For example, thromboembolic strokes in homocystinuria are associated with elevated homocysteine levels leading to hypercoagulability and atherosclerosis, whereas metabolic strokes in organic acidemias are suspected to be secondary to metabolic dysfunction in the basal ganglia and striatum that are vulnerable to energy deficiency [13]. In patients with mitochondrial disorders, stroke-like episodes are thought to be related to altered oxidative phosphorylation leading to energy failure and ischemic tissue injury [14].

In the mitochondria, oxidative phosphorylation occurs through a series of reactions executed by five enzyme complexes (Complex I–V), ultimately producing energy in the form of ATP. The complex subunits, along with regulatory factors, transfer RNA (tRNA) and ribosomal RNA molecules required to maintain the integrity and function of oxidative phosphorylation, are encoded by mitochondrial and nuclear genes. Pathogenic variants in these genes cause a subset of mitochondrial disorders. MELAS is a multisystemic disorder caused by pathogenic variants in the *MT-TL1* gene (most commonly m.3243A > G), or in other mitochondrial genes encoding mitochondrial tRNA or complex I, III, or IV subunits [15]. The major clinical symptoms in MELAS include stroke-like episodes, typically with onset prior to the age of 40 years, encephalopathy (seizures, cognitive decline), myopathy (muscle weakness, exercise intolerance) and lactic acidosis. Other clinical features may include cardiomyopathy, renal and GI dysfunction, diabetes mellitus, and sensorineural hearing loss [15].

Evaluation

In a patient with a stroke of undetermined cause, comprehensive history should be taken with special attention to recurrent symptoms of headaches, vomiting, and behavioral changes. Whereas stroke-like episodes can be seen with different IEM, the most commonly encountered diagnosis in adults is likely to be MELAS. Epilepsy is common in MELAS and may present as focal or generalized seizures. A history of exercise intolerance suggests myopathy. Gastrointestinal complaints may include recurrent vomiting, diarrhea or constipation. There may be a history of diabetes mellitus or glucose intolerance. Early development is

normal, but patients should be asked about learning disability, loss of skills, and cognitive decline. Family history should be obtained, specifically regarding similar signs and symptoms in maternal family members. Of note, MELAS is a maternally-inherited mitochondrial disorder, and the clinical presentation may vary between family members, depending on the distribution and burden of mutated mitochondrial DNA in different tissues (heteroplasmy). Physical examination may reveal short stature, and a thorough neurological examination is required to search for signs of neuropathy, muscle weakness, gait abnormality, and focal neurological deficits. Ophthalmology examination is recommended to rule out ocular complications, such as optic atrophy, retinopathy, and ophthalmoplegia. Cardiology assessment should be performed to rule out cardiomyopathy and conduction abnormalities. In addition to the lactic acidemia that is almost invariably present, laboratory workup may reveal anemia, renal dysfunction (renal Fanconi syndrome, proteinuria), or endocrine abnormalities. Muscle biopsy may be considered to evaluate for ragged-red fibers. Audiology examination is indicated to rule out hearing impairment, which may develop early in the course of disease. Psychiatric evaluation should be considered if there is any concern for personality change, mental disability, or dementia. Based on the history and clinical findings, other studies may be pertinent including brain magnetic resonance imaging (MRI)/MR spectroscopy (MRS), electroencephalogram (EEG), and nerve conduction study. Specifically, brain MRI during acute stroke-like episodes will not correspond to an ischemic stroke distribution but will show increased T2 signal in an asymmetric distribution, typically in the temporal, parietal and occipital lobes. MRS will show decreased cerebral *N*-acetylaspartate and increased lactate signals.

Genetic testing

In individuals suspected to have MELAS, single-gene testing for the most common mitochondrial pathogenic variant, m.3243A > G in *MT-TL1*, should be performed as the first step of genetic evaluation. To cover the broader differential diagnosis, a multi-gene panel (or exome) covering nuclear genes can be sent along with comprehensive mitochondrial DNA sequencing that includes *MT-TL1* as well as other mitochondrial genes. Due to heteroplasmy, if a mitochondrial disorder is suspected and if DNA sequencing from blood leukocytes does not reveal a pathogenic variant, testing can be done on other tissues, such as skin fibroblasts, urinary sediment or a muscle biopsy.

Vignette 17.2

A 22-year-old male is brought to the emergency room due to new onset of seizures. He was found at home confused and is complaining of severe headache. He has a history of complicated migraines. On physical examination, he is noted to have left arm weakness. Labs on arrival show acidosis with elevated lactate. Head computed tomography does not reveal specific evidence of ischemic stroke, intracranial bleeding or space-occupying lesion. Due to persistence of symptoms, brain MRI is ordered, which shows asymmetric T2 hyperintensities in the right parietal and left temporal lobes. On further questioning, the mother reports that she has hearing

impairment and migraine headaches and that his younger sibling has vision impairment, epilepsy, and learning problems. During the first day of his admission, the patient develops worsening left hemiparesis and altered mental status. With a clinical suspicion for MELAS, treatment with arginine infusion is administered. The patient is discharged from the hospital to rehabilitation but continues to have more episodes of seizures, headache, and confusion, ultimately leading to permanent disability.

A clinical diagnosis of MELAS was suspected in this patient who was meeting the following criteria [15,16]: (1) stroke-like episodes presenting earlier than 40 years of age, (2) encephalopathy characterized by seizures and/or dementia, (3) mitochondrial myopathy as evidenced by lactic acidosis. The history of recurrent headaches and vomiting, and the family history are supportive. The diagnosis was confirmed by mitochondrial DNA sequencing which revealed the m.3243A > G pathogenic variant in MT-TL1, with 60% heteroplasmy in blood.

Management

Outlining the treatment strategies for all IEM that present with stroke-like episodes is beyond the scope of this chapter and thus only the management strategy for MELAS will be outlined. Acute stroke-like episodes in MELAS are managed with arginine infusion (if a diagnosis of MELAS is established in the patient there is no need to wait for brain imaging which may delay initiation of treatment). The rationale for treatment with arginine, a nitric oxide donor, is based on evidence for nitric oxide deficiency contributing to MELAS complications [15,16], and studies suggest improved outcome with this treatment [17,18]. Prophylactic supplementation with oral arginine is recommended in patients that have a history of stroke-like episodes (the role of citrulline as an alternative nitric oxide donor is being studied [16]). Supplementation with coenzyme Q10, carnitine and creatine may be considered on a case-by-case basis. The treatment is otherwise supportive and based on symptoms. Annual ophthalmology, audiology, and cardiology evaluations are recommended, in addition to renal function and glucose studies to monitor for disease progression and long-term complications.

It is recommended to avoid treatment with valproic acid, aminoglycoside antibiotics, metformin, and topiramate due to toxic effects on the mitochondria, and/or risk of lactic acidosis [19].

Genetic counseling

MELAS is a maternally-inherited disorder caused by pathogenic variants in mitochondrial DNA. If the mother is carrying a pathogenic variant in mitochondrial DNA (the variant may not always be detected in blood and may require testing other tissues as noted earlier), all of her children will inherit the variant and her mother and siblings may be at risk. The symptoms in mother and other family members may vary—they may be symptomatic, oligo-symptomatic, or asymptomatic depending on mutation burden. A higher level of heteroplasmy in the mother's blood may increase her likelihood of having affected children [20]. The pathogenic variant may also arise *de novo* in the proband, in which case the mother and siblings will not be at risk. Rarely, patients may inherit the mitochondrial variant from their father [21,22].

Neuropsychiatric symptoms

Overview

Neuropsychiatric features can be the presenting symptom of a wide variety of IEM. These features can first become apparent in adolescence or adulthood and can include depression, anxiety, mania, and psychosis. Moreover, the phenotype may be difficult to distinguish from primary psychiatric disorders, and the psychiatric features may be present in the absence of any other underlying clues suggesting a metabolic disorder. As a result, the diagnosis may be delayed. Since treatments are available for many IEM, early diagnosis is essential for initiating appropriate therapy. Thus, a high level of clinical suspicion is necessary to recognize the possibility of an inborn error of metabolism in the setting of an adult with a neuropsychiatric presentation.

Disorders of cobalamin (also called vitamin B12) metabolism are one group of IEM that may present initially with a neuropsychiatric presentation in the adolescent or adult. Cobalamin, which is ingested through the diet is necessary for enzymatic reactions catalyzed by methionine synthase and methylmalonyl-CoA-mutase. Following ingestion, cobalamin undergoes a series of processing steps prior to utilization. Defects in the various steps of cobalamin transport and/or processing cause inborn errors of cobalamin metabolism. The most common inborn error of metabolism presenting in adulthood is cobalamin C disease.

Although disorders of cobalamin metabolism are the subject of this section, a host of other IEM can have a similar presentation, and some examples are provided in [Table 17.2](#).

Evaluation

A careful history and physical examination may reveal clues suggestive of a disorder of cobalamin metabolism. In the adult-onset form of cobalamin C disease, presenting features may include psychosis, early-onset dementia, or personality/behavioral change [23,24]. In addition to psychiatric features, individuals may have a history of thromboembolism, lethargy, fatigue, seizures, progressive neurologic decline, gait disturbance, vision abnormalities (e.g., bulls-eye maculopathy), myelopathy and/or urinary incontinence [9]. A hemolytic uremic syndrome is also a rare complication [24]. Family history may reveal siblings with similar symptoms. Physical examination may be remarkable for spasticity, myelopathy, and/or gait disturbance. The ophthalmologic examination may reveal reduced visual acuity, nystagmus, macular degeneration, optic nerve pallor, and/or pigmentary retinopathy [25]. Findings apparent on MRI of the brain may include brain atrophy and white matter abnormalities, and demyelination and vacuolation of the dorsal and lateral columns are also typical findings on spine MRI. Laboratory evaluation in cobalamin C disease will typically show normal vitamin B12 level, elevated plasma homocysteine level with normal to low methionine level, and elevated plasma methylmalonic acid level. Vitamin B12 deficiency can have a similar presentation but, in contrast to cobalamin C disease, the vitamin B12 level will be low. A subset of disorders of cobalamin metabolism will have only elevated plasma homocysteine or only elevated plasma methylmalonic acid. Moreover, 5,10-methylene-tetrahydrofolate reductase

Table 17.2 A selection of several inborn errors of metabolism in which a neuropsychiatric presentation is common.

Diagnosis	Inheritance	Reported neuropsychiatric presentation	Biochemical testing	Confirmatory diagnostic tests	References
Cerebrotendinous xanthomatosis	AR	Behavioral changes, personality changes, depression, cognitive decline, psychosis	Plasma cholestanol, tissue cholestanol, urine and plasma bile alcohols	DNA testing for <i>CYP27A1</i>	[1]
Disorders of cobalamin metabolism	AR ^a	Personality change, behavior alteration, dementia, psychosis	Plasma homocysteine, plasma methylmalonic acid, plasma vitamin B12 level	Gene panel or single-gene testing	[2,3]
GM2 gangliosidosis	AR	Psychosis, mania, depression, cognitive decline	Hexosaminidase A activity (serum or leukocytes)	DNA testing for <i>HEXA</i>	[4]
Metachromatic leukodystrophy	AR	Cognitive decline, emotional instability, memory issues, psychosis, mania, personality change	Arylsulfatase A enzyme activity (leukocytes), urine sulfatides	DNA testing for <i>ARSA</i>	[5,6]
Niemann–Pick type C	AR	Cognitive decline, memory impairment, psychosis, behavior change, mood disorder	Filipin staining (fibroblasts)	DNA testing for <i>NPC1</i> and <i>NPC2</i>	[7]
Wilson disease	AR	Personality change, depression, cognitive decline, anxiety, psychosis, catatonia	Serum copper, serum ceruloplasmin, urine copper	DNA testing for <i>ATP7B</i>	[8]
X-linked adrenoleukodystrophy	XL	Personality change, behavioral change, mania, psychosis, cognitive decline	Very long chain fatty acids, serum ACTH, baseline cortisol	DNA testing for <i>ABCD1</i>	[9]

^aMost disorders of cobalamin metabolism are inherited in an autosomal recessive inheritance pattern. However, cobalamin X is a rare X-linked disorder that typically presents in childhood. *ACTH*, adrenocorticotrophic hormone; *AR*, autosomal recessive.

deficiency has also been associated with neuropsychiatric symptoms including seizures, spastic paraplegia, psychosis, and cognitive decline in adulthood in the setting of elevated plasma homocysteine [26]. In addition, anemia and other blood cytopenias may be present.

The initial presentation of Wilson disease, an autosomal recessive disorder of copper transport associated with biallelic variants in *ATP7B*, may be a mood disorder, behavioral disturbances, or personality changes [27]. Cognitive decline and psychosis have also been reported [27]. Other phenotypic features that may be present include tremor or movement disorder and/or liver disease. The characteristic greenish-brown Kayser-Fleischer ring may be identified on ophthalmologic examination but is not always present at the time of diagnosis. Low serum ceruloplasmin, low serum copper, and elevated urinary copper are characteristic laboratory findings in Wilson disease. Treatment includes copper-chelating agents, such as penicillamine, and zinc supplementation.

Likewise, cognitive decline, psychosis, behavioral disturbances, and mood disorders are a common presenting feature of the late-onset form of Neimann–Pick Type C [28]. Physical examination may reveal vertical supranuclear gaze palsy and/or splenomegaly, but these examination findings are not invariably present. Likewise, dystonia, ataxia, and movement disorders may be present. A biochemical diagnosis can be confirmed by demonstrating impairment of cholesterol esterification and positive filipin staining in cultured fibroblasts. The identification of biallelic variants in either *NPC1* or *NPC2* provides molecular confirmation of this autosomal recessive disorder. Other IEM that can have psychiatric features as the initial presentation include X-linked adrenoleukodystrophy, which can present with personality change, behavioral disturbance and mania [29], and cerebrotendinous xanthomatosis which can present with personality/behavior change, depression, psychosis, or even catatonia [30]. Late-onset forms of metachromatic leukodystrophy and GM2 gangliosidosis can also present with similar presentations [31–33]. Lastly, episodic hyperammonemia in an undiagnosed UCD (see previous section) can mimic psychiatric disorders leading to a delay in diagnosis [34,35].

Genetic testing

When a particular disorder of metabolism is suspected, single-gene testing or a multi-gene panel can be ordered to confirm the diagnosis molecularly. When the phenotype is not clearly distinguishable from other inherited disorders, exome sequencing may be considered.

Vignette 17.3

HZ is a 27-year-old man who presents with a two-month history of reduced appetite, depression, and loss of ability to do activities of daily living. Over the last week, he has lost the ability to ambulate and developed urinary incontinence and catatonia. On examination, the patient does not respond to questions. Other remarkable examination findings include decreased reflexes in lower extremities with apparent spasticity of the lower extremities. However, the examination is difficult given apparent lack of cooperation. His phenotype is initially attributed to a new diagnosis of schizophrenia, and antipsychotic medications are initiated. In the

meantime, additional laboratory and imaging evaluations are ordered. The MRI of the brain reveals brain atrophy and white matter abnormalities, and MRI of the spine reveals demyelination of the dorsal and lateral columns. Laboratory evaluation reveals anemia, elevated plasma homocysteine, elevated plasma methylmalonic acid, and normal vitamin B12 levels. An ophthalmologic evaluation reveals mild optic atrophy. The history and presentation are suspicious for a disorder of cobalamin metabolism, and a metabolic specialist is consulted. Daily subcutaneous injections of hydroxocobalamin (high dose) are started and result in normalization of plasma homocysteine and methylmalonic acid levels. One month later, he is no longer catatonic, his mood and appetite have improved, and he no longer has urinary incontinence. His spasticity has not resolved, but physical therapy has resulted in improvements in movements of the lower extremities. DNA testing reveals biallelic variants in *MMACHC*, the gene associated with cobalamin C disease. Interestingly, evaluation of his four asymptomatic siblings reveals elevated homocysteine and methylmalonic acid levels in one brother. Thus, treatment is initiated in his brother and DNA testing later confirms his diagnosis. Both brothers will continue hydroxocobalamin injections with close monitoring of homocysteine and methylmalonic acid levels and with regular ophthalmology follow-up evaluations as they grow older.

This patient's abnormalities on physical examination (including ophthalmologic examination) and brain imaging are suggestive of a potential metabolic diagnosis, which was confirmed with laboratory evaluations. Moreover, identification of the correct diagnosis led to improvements in the phenotype. Lastly, patients with cobalamin C disease may be apparently asymptomatic prior to their initial metabolic decompensation or neuropsychiatric presentation. Thus, testing of at-risk relatives is an important component of the management for families.

Management of cobalamin metabolism disorders

Treatment for cobalamin C disease includes regular subcutaneous or intramuscular hydroxocobalamin injections. If normalization of plasma homocysteine does not occur, betaine is also used. If there is secondary carnitine deficiency, levocarnitine supplementation may be used. Regular monitoring of plasma homocysteine and methylmalonic acid levels is recommended to assess the adequacy of therapy. Individuals with cobalamin C disease are also advised to avoid prolonged fasting and nitrous oxide, as nitrous oxide inhibits the methionine synthase enzyme [36]. Regular ophthalmology visits are also an important component of the management to monitor for potential ophthalmologic complications associated with the disorder [25].

Genetic counseling

Most inborn errors of cobalamin metabolism, including cobalamin C disease, are associated with an autosomal recessive inheritance pattern. Since full siblings have a 25% or $\frac{1}{4}$ risk of having the same diagnosis, and given the possibility of late-onset disease, an evaluation is warranted for all full siblings. Recurrence risks for offspring of an affected individual will depend on their partner's carrier status. Thus, preconception genetic counseling is recommended for affected individuals.

Rhabdomyolysis

Overview

Rhabdomyolysis is characterized by muscle pain and elevation of serum creatine kinase (CK), with or without myoglobinuria. It may lead to acute renal failure when CK levels increase up to several thousands of units and can be complicated by life-threatening electrolyte imbalances. Metabolic-genetic etiology should be sought in patients with nontraumatic rhabdomyolysis, particularly when rhabdomyolysis is triggered by stress, physical activity, heat, febrile illness, prolonged fasting or general anesthesia. Other features that may suggest a metabolic diagnosis include recurrent episodes of rhabdomyolysis and myoglobinuria, persistently elevated CK (between episodes), history of fatigue, myalgia, muscle weakness or exercise intolerance, and family history of rhabdomyolysis.

Metabolic myopathies may be caused by impaired carbohydrate metabolism (glycolysis or glycogenolysis), fatty acid oxidation, or oxidative phosphorylation leading to energy deficiency in muscle cells. Accordingly, the metabolic differential diagnosis of recurrent rhabdomyolysis includes glycogen storage diseases (muscle glycogenoses such as glycogen storage disease types III, IV, V, VII, VIII, IX, and X), fatty acid oxidation metabolism defects (in particular, disorders of long-chain fatty acid oxidation), and mitochondrial disorders. *LPIN1* deficiency typically presents in childhood, but rare cases of adult-onset have been reported [37]. Pompe disease (glycogen storage disease type II) may be suspected in an adult patient with myalgia, muscle weakness and exercise intolerance, although the myopathy would be chronic and progressive, with no history of acute rhabdomyolysis. Nonmetabolic genetic disorders that may be associated with rhabdomyolysis, such as Limb-Girdle Muscle Dystrophy, *RYR1*-related disorders, and dystrophinopathies are beyond the scope of this chapter but should be considered in the differential diagnosis. It is important to evaluate and identify genetic causes of rhabdomyolysis, as this will impact the management and allow for proper genetic counseling for the patient and at-risk family members.

Evaluation

When evaluating a patient with rhabdomyolysis, specific history should be sought regarding the timing of symptoms in relation to exercise. The onset of symptoms immediately after starting exercise suggests muscle glycogenosis, whereas onset with prolonged exercise may be more consistent with a diagnosis of a fatty acid oxidation disorder. Other than physical activity, rhabdomyolysis may be provoked by increased dietary fat intake (in carnitine palmitoyltransferase II deficiency), fasting (fatty acid oxidation disorders, *LPIN1* deficiency), infections, exposure to cold or medications (such as valproic acid or anesthetic agents) [38]. Patients should be asked about exercise intolerance, myoglobinuria, fatigue and episodic muscle cramps, which may suggest muscle breakdown in response to minor or nontraumatic physical stress (it is important to differentiate between routine activity level and strenuous exercise, as the latter may predispose to exertional rhabdomyolysis even in the absence of an underlying metabolic perturbation). A “second wind” phenomenon, meaning spontaneous improvement in muscle pain and fatigue after short rest, is typical of glycogen storage

disease type V (McArdle disease). Multisystemic disease, including ophthalmologic, neurological, and cardiac manifestations, as well as lactic acidosis may suggest a diagnosis of mitochondrial myopathy. A history of long-standing, progressive muscle weakness and myalgia may be more compatible with a diagnosis of Pompe disease, or muscular dystrophy rather than acute rhabdomyolysis. The physical examination may reveal sensory deficits suggesting peripheral neuropathy (in mitochondrial trifunctional protein deficiency), or muscle weakness (for example in Pompe disease, or as a late manifestation in glycogen storage disease type III). Laboratory workup is most informative during acute rhabdomyolysis episodes, although abnormalities (such as CK elevation) may persist even when the patient is asymptomatic. Distinctive patterns of plasma acylcarnitines (including free and total carnitine levels) can differentiate between fatty acid oxidation disorders. Urine organic acids may detect dicarboxylic aciduria in fatty acid oxidation disorders or mitochondrial metabolites. Lactate may be elevated in mitochondrial myopathies and in glycogen storage disorders. Hemolytic anemia may be seen in aldolase A deficiency (glycogen storage disease type XII) or phosphoglycerate kinase deficiency. Muscle biopsy may be considered as part of the diagnostic workup and may reveal glycogen deposition in glycogen storage disorders, mitochondrial abnormalities in mitochondrial myopathies, or abnormal muscle fibers in muscular dystrophy. An echocardiogram should be considered to rule out cardiomyopathy, which may develop in patients with oxidative phosphorylation disorders, *LPN1* deficiency, and a subset of glycogen storage diseases. It is important to note that normal physical examination, laboratories or muscle biopsy between episodes do not rule out a metabolic diagnosis as the cause of rhabdomyolysis. Therefore, if there is a strong clinical suspicion, molecular testing should be pursued.

Genetic testing

In patients suspected to have a specific metabolic etiology based on initial history and lab findings, targeted genetic testing may be done to confirm a diagnosis. However, because of the overlap in features and nonspecific signs and symptoms, in most cases, the preferred approach would be to send more comprehensive genetic testing such as a gene panel or exome sequencing to cover the broad differential diagnoses.

Vignette 17.4

RS is a 32-year-old male who is admitted to the internal medicine unit with muscle weakness, myalgia, and dark-colored urine. One day prior to admission he was helping a friend move out of his apartment. Urinalysis at primary physician's office was notable for blood, and he was sent to the emergency room, where he was found to have myoglobinuria and significantly elevated CK at 80,000 U/L. On further questioning, he recalls a similar episode when he was younger and had a febrile illness. Another episode happened last year when he had to fast for dental surgery under general anesthesia. Both of these episodes resolved without special evaluation or treatment. He denies chronic muscle weakness.

Upon admission, treatment was started with intravenous fluids, and renal function was determined to be within the normal range. The weakness and myalgia improved within

24 hours, and the CK level trended down. Metabolic screening labs showed moderate elevation of C16 and C18:1 on acylcarnitine profile, and dicarboxylic acids were detected on urine organic acids analysis. Rhabdomyolysis gene panel was sent which detected two heterozygous pathogenic variants in CPT2, consistent with a diagnosis of carnitine palmitoyltransferase II deficiency (a fatty acid oxidation disorder). At his follow-up visit, a screening echocardiogram was done and showed normal ventricular function with no concern for cardiomyopathy. Upon diagnosis, treatment was initiated with fat-restricted diet, medium-chain triglyceride supplementation, and avoidance of triggers including strenuous physical activity.

Management

Long-chain fatty acid oxidation disorders such as *CPT2* deficiency are managed by restricting long-chain dietary fat while maintaining the intake of essential fatty acids and medium-chain triglycerides. Medium-chain triglycerides are important because they can enter the mitochondria and be metabolized independently of *CPT2* and the enzymes involved in metabolism of long-chain fatty acids. This special metabolic diet is a life-long treatment that requires follow-up by a metabolic-genetics specialist and a metabolic nutritionist. In addition, triggers that may precipitate rhabdomyolysis, including fasting and prolonged exercise should be avoided. During acute illness high caloric intake and good hydration should be provided. During acute episodes of rhabdomyolysis intravenous fluids are administered along with close monitoring of electrolytes and kidney function, and in severe cases dialysis may be required.

Low-dose carnitine supplementation may be considered only when free carnitine is markedly reduced. The use of triheptanoin, a triglyceride with potential anaplerotic effect, is currently under investigation.

Consensus guidelines for the treatment of long-chain fatty acids oxidation disorders have been published [39].

Genetic counseling

Long-chain fatty acid oxidation disorders including *CPT2* deficiency are associated with an autosomal recessive inheritance. Once a genetic diagnosis is confirmed, testing can be offered to at-risk family members and prenatal diagnosis is possible by testing chorionic villi or amniotic fluid samples.

Peripheral neuropathy

Overview

Peripheral neuropathy can be caused by a variety of infectious agents, toxins, chronic diseases, and inherited disorders. A variety of genetic disorders has been associated with peripheral neuropathy. In particular, peripheral neuropathy can be a presenting feature of numerous IEM (Table 17.3).

Table 17.3 IEM with peripheral neuropathy in adulthood.

Diagnosis	Inheritance	Peripheral neuropathy phenotype	Biochemical testing	Confirmatory diagnostic tests	References
Adult polyglucosan body disease	AR	Chronic sensory-motor axonal polyneuropathy and other features such as the neurogenic bladder, spastic paraplegia with gait disturbance	Polyglucosan deposition in nerve biopsy, enzyme activity in fibroblasts or muscle tissue	<i>GBE1</i> DNA testing	[10]
Fabry disease	X-linked	Small fiber peripheral neuropathy which can be very painful, and other features including angiokeratomas, sweating abnormalities, corneal and lenticular opacities, stroke, cardiac disease, and renal disease	Enzyme activity in plasma or leukocytes (males only as females can have normal enzyme activity)	<i>GLA</i> DNA testing	[11,12]
Krabbe disease	AR	Chronic sensory-motor demyelinating (or hypomyelination) polyneuropathy and other features such as weakness and progressive neurologic decline	Enzyme activity (fibroblasts or leukocytes)	<i>GALC</i> DNA testing	[13]
Metachromatic leukodystrophy	AR	Chronic sensory-motor demyelinating polyneuropathy and other features including seizures, neurologic symptoms and behavior change	Enzyme activity (leukocytes); urine sulfatides	DNA testing for <i>ARSA</i>	[14,15]
Mitochondrial diseases	Mitochondrial or AR	Sensory axonal neuropathy, sensory-motor axonal neuropathy, sensory-motor axonal demyelinating neuropathy, motor axonal neuropathy, and various other features	Lactic acid may be elevated in some disorders	mtDNA sequencing and deletion studies, AR mitochondrial gene sequencing	[16]
Refsum disease	AR	Chronic sensory-motor polyneuropathy, may be asymmetric, and other features such as retinitis pigmentosa, anosmia, deafness, ataxia, ichthyosis	Elevated serum or plasma phytanic acid or fibroblast enzyme activity	<i>PHYH</i> and <i>PEX7</i> DNA testing	[17]
Tangier disease	AR	Sensory-motor demyelinating polyneuropathy or mononeuropathy, pseudosyringomyelic neuropathy, and other features including yellow tonsils, hepatosplenomegaly, enlarged lymph nodes, xanthoma, early coronary artery disease, corneal dystrophy	Reduced HDL	<i>ABCA1</i> DNA testing	[18]
X-linked adrenoleukodystrophy and related disorders	X-linked	Sensory-motor axonal neuropathy, sensory-motor demyelinating and other features including behavior/personality change and adrenocortical impairment	Elevated very long chain fatty acid in males and some females	<i>ABCD1</i> DNA testing	[19]

AR, autosomal recessive; HDL, high-density lipoproteins.

One of the most notable examples of an IEM that often presents with peripheral neuropathy is Fabry disease. The characteristic painful, small fiber neuropathy characteristic of Fabry disease may be the only presenting feature of the disorder for many years. Later, other phenotypic features that may develop include hypohydrosis, abdominal pain and gastrointestinal symptoms, hearing loss, cardiac disease, renal disease, and stroke. Lack of recognition of this peripheral neuropathy can lead to a delay in diagnosis. In fact, the average delay in diagnosis from the initial presentation is nearly 20 years in individuals with Fabry disease [40]. Recognition of this diagnosis in the early stages of disease is important so that enzyme replacement therapy can be offered.

Other IEM have been associated with both sensory and motor neuropathies. For instance, chronic sensory-motor demyelinating neuropathy can be a feature of several lysosomal storage disorders such as Krabbe disease and metachromatic leukodystrophy. Chronic sensory-motor axonal polyneuropathy can be a feature of adult polyglucosan body disease, a glycogen storage disorder, and adult-onset forms of X-linked adrenoleukodystrophy. Mitochondrial disorders can be associated with a wide variety of different forms of peripheral neuropathy. In many cases, other phenotypic features may provide a clue to the underlying diagnosis (Table 17.3).

Evaluation

A careful history and physical examination may reveal clues suggestive of a diagnosis of Fabry Disease. Periodic episodes of burning pain in the extremities is a characteristic presenting feature of Fabry disease. These pain episodes may be triggered by exercise, temperature change, or stress. Patients may also report periodic abdominal pain and gastrointestinal symptoms and hypohidrosis. Given the X-linked inheritance pattern, males typically have more severe and earlier onset symptoms as compared to females with the diagnosis. Thus, when taking the family history, it is important to inquire about males with unexpected renal disease, cardiac disease, or early-onset stroke. The physical examination should include a careful skin examination (including genital area) to search for angiokeratoma, the classic skin lesions associated with the disorder. Moreover, an ophthalmologic examination can be helpful to identify the characteristic corneal and lenticular opacities observed in Fabry disease. Proteinuria may be an early sign of renal disease and should be evaluated in individuals with a suspected diagnosis. In addition, individuals with suspected or confirmed Fabry disease should have audiology, neurologic, cardiac, and renal evaluations. Tangier disease, a disorder of cholesterol metabolism, can be associated with a sensory-motor demyelinating polyneuropathy or mononeuropathy or a characteristic pseudosyringomyelic neuropathy. Other phenotypic features of Tangier disease include the yellowish color of the tonsils, hepatosplenomegaly, enlarged lymph nodes, xanthoma, early coronary artery disease, and corneal dystrophy.

Vignette 17.5

A 26-year-old female presents with an episodic burning sensation in her hands and feet. The pain lasts for days and is usually triggered by stress or cold sensation. She also has episodic abdominal pain of unclear etiology. She reports a family history of a brother who was recently diagnosed with kidney disease. Her brother also has a similar burning sensation of his hands and feet. On examination, she has angiokeratoma near her umbilicus. You suspect a diagnosis of Fabry disease and refer to ophthalmology for evaluation. The ophthalmologist identifies corneal and lenticular opacities consistent with the diagnosis. Because α -galactosidase A activity in leukocytes is not reliable for the diagnosis of Fabry disease in females, you send DNA testing for GLA which identifies a heterozygous known pathogenic variant in GLA. She shares this information with her brother, and DNA testing reveals the same pathogenic variant in GLA in his sample. Following the diagnosis, both your patient and her brother elect to start enzyme replacement therapy for Fabry disease. Moreover, the identification of the pathogenic variant in these two siblings facilitates testing of other at-risk relatives. In addition to starting enzyme replacement therapy, you refer your patient for cardiology, audiology, and neurologic evaluations, and you evaluate her kidney function, which is currently normal.

This patient presented with the classic extremity pain characteristic of Fabry disease. Moreover, the angiokeratoma, which may not always be present, and the corneal and lenticular opacities provided clues to the diagnosis. Although enzyme activity can be used to diagnose Fabry disease in male patients, the enzyme studies are not reliable in females.

Management

Enzyme replacement therapy for Fabry disease is U.S. Food and Drug Administration (FDA) approved, and recommendations for managing adult patients with Fabry disease using this therapy have been published [41]. Enzyme replacement therapy should be complemented with supportive therapies for various complications, such as renal, cardiovascular, neurologic, and gastrointestinal, as needed [41].

Genetic counseling

Because Fabry disease is an X-linked disorder, males with Fabry Disease tend to have more severe disease with earlier age of onset for various complications as compared to females with the diagnosis. Testing for at-risk relatives (e.g., siblings, parents) is recommended. In addition, genetic counseling regarding recurrence risk in offspring should be provided. Although males with Fabry disease do not transmit the disorder to their sons, all of their daughters will have the disorder, but the severity of the disorder in females cannot be predicted due to skewing of X-inactivation. For females with Fabry disease, each offspring, regardless of sex, will have a 50% chance of having the diagnosis.

Points to remember

- A blood ammonia level should be assessed in all individuals with altered mental status and neuropsychiatric symptoms of unclear etiology.
- Altered mental status secondary to hyperammonemia may be the presenting feature of a urea cycle disorder.
- A metabolic-genetic diagnosis should be suspected in patients presenting with stroke at an early age, with a family history of stroke at an early age, or with atypical findings on brain imaging.
- An ophthalmologic examination may provide important clues to the diagnosis of some inborn errors of metabolism.
- The metabolic differential diagnosis of recurrent rhabdomyolysis is broad and may include glycogen storage disorders, fatty acid oxidation disorders and disorders of oxidative phosphorylation.
- Delays in diagnosing Fabry disease can result in delays in treatment which can impact the long-term outcome for individuals with this disorder.

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Mitochondrial disorders

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Introduction

Mitochondria and the respiratory chain

Mitochondria are subcellular organelles, and their numbers vary in different cell types ranging from zero in mature erythrocytes to a few thousand per cell in hepatocytes and muscle cells. The important structural components of the mitochondria include the outer mitochondrial membrane, the inner mitochondrial membrane organized into folds called cristae, the intermembrane space, and the mitochondrial matrix. Several enzymatic reactions in key metabolic pathways including pyruvate oxidation, Krebs cycle, urea cycle, metabolism of amino acids and fatty acids, and synthesis of various lipids and hormones occur within the mitochondrial matrix. The components of the respiratory chain (RC) are anchored to the inner mitochondrial membrane. The mitochondrial RC generates adenosine triphosphate (ATP) via oxidative phosphorylation and provides the primary source of energy for metabolically active tissues including the brain, muscle, heart, liver, kidney, and pancreatic islet cells.

The RC consists of five multimeric complexes (I–V) localized to the inner mitochondrial membrane. Reduced nicotinamide adenine dinucleotide (NADH) is oxidized by complex I, and succinate is oxidized by complex II. Subsequent reactions involve the transfer of these electrons to the electron carrier coenzyme Q (ubiquinone) yielding ubiquinol and then to complex III (ubiquinol:cytochrome *c* oxidoreductase) and to cytochrome *c*. Reduced cytochrome *c* generated in the process is oxidized by complex IV (cytochrome *c* oxidase), which transfers the electrons to molecular oxygen. As the reaction proceeds, protons are simultaneously pumped to the intermembrane space of the mitochondria creating an electromotive force. The restoration of the electrochemical gradient is achieved at the level of complex V (ATP synthase) where the energy from the inward flow of electrons via complex V is captured as a high energy bond between ADP and Pi resulting in ATP. This coupling of oxidation and phosphorylation (OXPHOS) to yield ATP is a key function of mitochondria. Other important roles of mitochondria include calcium homeostasis, generation and regulation of

the production of reactive oxygen species (ROS), regulation of cell death/apoptosis, retrograde signaling, and gene regulation [1].

Unique features of mtDNA

mtDNA is a circular double-stranded molecule that is 16,569-bp long. It encodes two rRNAs, 22 tRNAs and 13 of the 45 polypeptides of the RC, of which 7 are components of complex I (NADH dehydrogenase), one (cytochrome *b*) subunit of complex III, three components of complex IV (cytochrome *c* oxidase), and two subunits of complex V (ATP synthase). The rest of the polypeptides of the RC complexes, including all the subunits of complex II (succinate dehydrogenase), as well as approximately 1500 other proteins that function in mitochondria and the enzymes involved in mtDNA maintenance and expression, is encoded by nuclear genes, synthesized in the cytoplasm by the cell's translational machinery and subsequently imported into mitochondria. These nuclear encoded genes are diploid and follow Mendelian inheritance [2].

Each mitochondrion, on average, has about five genomes [3]. Mitochondria are dynamic organelles that exhibit motility, fusion and replication. Mitochondrial replication proceeds independently of cell division and occurs even in postmitotic tissues. The compact mtDNA is prone to mutations and has a much higher mutation rate than nuclear DNA (nDNA) [4]. When a new mtDNA mutation occurs, a mosaic pattern of normal and mutant mtDNA is created within the cell, and this effect is referred to as heteroplasmy. Heteroplasmy may also exist within the tissue level, where cells with varying populations of normal and mutant mitochondria are seen. When the level of heteroplasmy or mutant load exceeds a certain threshold, mitochondrial function is impaired and clinical features become apparent [5]. The threshold for disease may be tissue specific and may be lower in metabolically active tissues with high oxidative demands such as the brain and retina, heart, skeletal muscle, liver, endocrine glands, and kidney tubules making them more vulnerable to the effects of mitochondrial dysfunction. Consequently, mitochondrial disease manifests often in these tissues and involvement of more than one organ system is common [6].

During normal cell division, there can be a random distribution of mitochondria between the two daughter cells and consequently the level of heteroplasmy may vary between the daughter cells. Changes in the distribution of mutant mtDNA and progressive accumulation of mtDNA mutations underlie the progressive nature of mtDNA diseases. The opposite effect may also be observed, and mutant mitochondria may be selected against and lost especially in rapidly dividing cells including white blood cells. This has important implications in the diagnosis of these disorders as pathogenic mitochondrial variants may not be detected in peripheral blood but may be present in muscle tissue in a patient affected with mitochondrial myopathy [7].

Mitochondrial deoxyguanine kinase (DGUOK) and mitochondrial thymidine kinase (TK2) mediate the first steps in purine and pyrimidine nucleotide salvage and are encoded by nuclear genes and imported into the mitochondria as are other proteins. The mitochondrial replication machinery contains a battery of enzymes including the mtDNA polymerase (polymerase γ [POLG]) and 5'-3' DNA helicase (Twinkle). Mitochondrial nucleotide transporters, notably the adenine nucleotide translocator (ANT), facilitate transfer of nucleotides across the mitochondrial membrane [8]. Other proteins such as the mitofusins (MFN1 and MFN2) and

dynamin related GTPase (encoded by OPA1) facilitate mitochondrial fusion allowing for exchange of mitochondrial content and accurate stoichiometry of the mitochondrial enzymes. Pathogenic variants in these nuclear genes are inherited in an autosomal recessive or less commonly autosomal dominant manner and result in depletion of the mitochondrial DNA pool leading to the clinical phenotype of mitochondrial DNA depletion syndromes. In addition, pathogenic variants in these genes result in multiple mtDNA deletions of varying sizes indicating a defect in mitochondrial DNA maintenance [9].

Evaluation of mitochondrial disorders

Primary mitochondrial diseases (PMDs) refer to those disorders affecting the structure and function of mitochondria including the RC thereby impacting OXPHOS. PMD is clinically and genetically heterogeneous and has an estimated prevalence of 1:4300 [10].

These disorders manifest with a wide range of clinical symptoms and can involve any organ or tissue. Multisystem disease with involvement of the most metabolically active tissues is a common finding. The clinical course is typically progressive, and patients may report worsening of the disease during periods of intercurrent illness or other stress. Patients presenting during childhood often have severe and progressive diseases and recessively inherited disorders affecting the nuclear genes are common in this age group while mtDNA pathogenic variants predominate in adult-onset diseases [11]. However, there are notable exceptions to this rule. Leigh syndrome due to mtDNA mutations in *MT-ATP6* and *MT-TL1* and Pearson syndrome (PS) due to single large deletions of mtDNA manifest during childhood. Mutations in genes involved in the mitochondrial replication including *POLG* and *RRM2B* that are typically associated with recessively inherited severe childhood phenotypes may cause late-onset disease with milder phenotype manifesting in adults [12–14].

Common presentations of mitochondrial disease in childhood include Leigh syndrome that usually begins in early infancy or childhood following a variable period of normal growth and development. Symmetric spongiform degeneration of the basal ganglia, brainstem, and demyelination is seen. Although a disease of childhood, late-onset manifestations in adolescents and adults have also been reported. Leigh syndrome is genetically heterogeneous, and over 75 genes encoded on nDNA and mtDNA have been implicated [15].

Leigh syndrome and other mitochondrial disorders often present with progressive muscle weakness including involvement of extraocular muscles manifesting as ptosis and ophthalmoplegia. Fatigue, exercise intolerance, and exhaustion are common. These symptoms are often accompanied by multiorgan dysfunction and “mitochondrial red flag” symptoms include dementia, encephalomyopathy, stroke-like episodes, seizures, ataxia, optic atrophy, pigmentary retinopathy, sensorineural hearing loss, cardiomyopathy, cardiac conduction defects, endocrine abnormalities including diabetes, thyroid or adrenal dysfunction, gastrointestinal dysmotility, hepatopathy, nephropathy, and peripheral neuropathy. Extreme weight loss, psychiatric disturbances including anxiety, and depression are also seen. Any combination of these symptoms may occur, and when there are other affected family members,

variability in age of onset and presentation is not uncommon. The first clinical presentation with stroke or cardiac conduction defects may be fatal in these patients.

Clinical features of some of the well-known mitochondrial disorders in adults are summarized in [Table 18.1](#).

Dysfunction in a subset of nuclear genes involved with mtDNA maintenance is now recognized as an important cause of mitochondrial disease in adults. These include *POLG* [16], *OPA1* [17], *RRM2B* [18], *TWINK* [19], and *RNASEH1* [20]. A common feature of this cohort of diseases is progressive external ophthalmoplegia (PEO) and the secondary accumulation of multiple deletions and/or depletion of muscle mtDNA. The clinical spectrum of this group of disorders is broad and can include psychomotor retardation, ptosis, ataxia, cardiomyopathy and conduction defects, dysphagia, constipation or pseudo-obstruction, muscle weakness and fatigue, and depressive mood [21,22]. Recessive mutations in *MPV17* have been identified in axonal type Charcot-Marie-Tooth disease and are associated with neuropathy, leukoencephalopathy, and multiple mitochondrial DNA deletions in the muscle [23].

Laboratory testing

The laboratory investigation of mitochondrial disease is complex, and a multipronged approach including clinical and functional studies as well as genetic testing is employed. When a mitochondrial disorder is suspected, biochemical studies in blood, urine, and cerebrospinal fluid (CSF) are performed as initial screening studies as these are minimally invasive and may help to direct more invasive or expensive testing.

The initial evaluation in blood for mitochondrial disease should include complete blood count, creatine phosphokinase, liver and kidney function studies, lactate and pyruvate, plasma amino acids, acylcarnitine profile, and quantitative urinary organic acid analysis. A complete blood count may reveal megaloblastic or sideroblastic anemia, neutropenia, and thrombocytopenia or pancytopenia if the bone marrow is affected. Elevations of plasma aspartate and alanine aminotransferases, hypoalbuminemia, and coagulopathy may indicate hepatic involvement. Metabolic acidosis and lactic acidosis may be seen. Persistent elevation of blood lactate (> 3 mM/L) suggests the presence of mitochondrial dysfunction and indicates impaired utilization of pyruvate generated during glycolysis. However, lactate is a non-specific biomarker, and elevations are often spurious and associated with difficult phlebotomy or poor sample processing or may be due to secondary mitochondrial dysfunction that can be seen in poor perfusion states or other inborn errors of metabolism. More importantly, many patients with primary mitochondrial disorders do not have elevations in serum lactate. Pyruvate elevation, specifically in the presence of lactate elevation, points to defects of pyruvate metabolism [24,25].

If CSF is obtained, a specimen should be sent for amino acids, lactate, pyruvate and 5-methyltetrahydrofolate measurements. 5-Methyltetrahydrofolate levels are reported to be low in mtDNA deletion syndromes [26]. Elevated CSF lactate is not specific to mitochondrial disease and may be seen in seizure disorders, stroke, central nervous system (CNS) infection, or malignancy

Table 18.1 Common disorders due to mtDNA or nuclear gene mutations.

Disease	Clinical features	Genetic defect	Mutant load	Inheritance	Diagnosis	Management	Agents/ circumstances to avoid
Point mutations in mtDNA							
Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELASs)	Multisystem disorder with protean manifestations. Vast majority become symptomatic before age 40 years. Symptoms: stroke-like episodes, seizures, dementia, muscle weakness, exercise intolerance, headaches, recurrent vomiting, hearing impairment, short stature. Early psychomotor development may be normal or there may be learning delay	Pathogenic variants in any one of the following genes: <i>MT-TL1</i> (> 80% of cases. This occurs in a mutation hotspot mt3243A > G) <i>MT-ND5 MT-TC MT-TF MT-TH MT-TK MT-TL2 MT-TQ MT-TV MT-TW MT-TS1 MT-TS2 MT-ND1 MT-ND6 MT-CO2 MT-CO3 MT-CYB</i> Varying degrees of heteroplasmy in affected and unaffected tissues. Most reliably detected in skeletal muscle and clinically affected tissues		Maternal/ mitochondrial	Lactic acidemia. Elevated CSF protein and lactate may be seen. Increased T2 weighted signal not corresponding to vascular distribution. Strokes are asymmetric, typically involve temporal, parietal, and occipital lobes. Slow spreading of stroke-like lesions in weeks following first symptoms Increased apparent diffusion coefficient (ADC) on diffusion-weighted imaging (unlike decreased ADC seen in ischemic strokes) Muscle biopsy: ragged red fibers, mitochondrial proliferation. RC enzyme analysis: Multiple partial defects involving Complex I and/or Complex IV Muscle biopsy is not required if diagnosis is established on molecular genetic testing	Treatment of manifestations and supportive therapy Arginine 10 g/m ² body surface area IV bolus followed by a similar dose as IV infusion over 24 h for the next 3–5 days. Maintenance therapy: prophylactic arginine daily Mitochondrial supplement cocktail	Mitochondrial toxins: Valproic acid Metformin Aminoglycoside antibiotics Linezolid Propofol Statins Nucleoside reverse transcriptase inhibitors

(Continued)

Table 18.1 (Continued)

Disease	Clinical features	Genetic defect	Mutant load	Inheritance	Diagnosis	Management	Agents/ circumstances to avoid
Maternally inherited diabetes and deafness (MIDD)	Type I or Type 2 diabetes and sensorineural hearing loss. Associated cardiomyopathy, myopathy, and other organ involvement may be seen	Pathogenic variant m3243 A > G in <i>MT-TL1</i>	Varying levels of heteroplasmy	Maternal			
Myoclonic epilepsy with ragged red fibers (MERRFs)	Multisystem disorder Myoclonus may be first symptom Generalized epilepsy, ataxia, peripheral neuropathy, dementia, optic atrophy, hearing loss, WPW syndrome and cardiomyopathy Subcutaneous lipomas are a recognized feature in some patients	Pathogenic variants in <i>MT-TK</i> account for >80% of all cases. This occurs in a mutation hotspot m8344 A > G. Other genes implicated: <i>MT-TF</i> <i>MT-TL1</i> <i>MT-TI</i> <i>MT-TP</i>	Most reliably detected in skeletal muscle and clinically affected tissues Varying degree of heteroplasmy in tissues may occur	Maternal	Lactic acidosis in blood and CSF Brain MRI: brain atrophy and basal ganglia calcification common Muscle biopsy: ragged red fibers, COX-negative fibers, hyperactive SDH fibers RC enzyme analysis: decreased activity of subunits I, III, IV, V, especially IV RC enzyme analysis may be normal	Treatment of manifestations and supportive therapy Mitochondrial supplement cocktail	Mitochondrial toxins: Valproic acid Metformin Aminoglycoside antibiotics Linezolid Propofol Statins Nucleoside reverse transcriptase inhibitors
Leber hereditary optic neuropathy (LHON)	Bilateral subacute painless visual loss in young adult males. Enlarging dense central scotoma is followed by optic atrophy. Some improvement in vision may be seen but	One of the three pathogenic variants in: m.3460 G > A in <i>MT-ND1</i> m.11778G > A in <i>MT-ND4</i>	largely homoplasmic	Maternal	Brain MRI: usually normal. White matter abnormalities, signal abnormalities in optic nerves may be seen RC enzyme activities: usually	Treatment of manifestations and supportive therapy Mitochondrial supplement cocktail Idebenone has been approved in the EU	mitochondrial toxins

	significant improvement is rare Other symptoms: tremors, peripheral neuropathy, myopathy and cardiac arrhythmia. Males are four to five times more likely to be affected. A multiple sclerosis like illness has been reported in females	m.14484T > C in <i>MT-ND6</i>				normal but reductions in Complex I enzyme activity especially with the m.3460 G > A variant in <i>MT-ND1</i>	
Neuropathy, ataxia, retinitis pigmentosa (NARP)	Late-childhood or adult-onset peripheral neuropathy, ataxia, pigmentary retinopathy Seizures, hearing loss, and cardiac conduction defects may also occur. Children who have a high mutant load usually in excess of 90% heteroplasmy may develop features of mitochondrially inherited Leigh syndrome (MILS) with high morbidity and mortality in childhood	Two Pathogenic variants in <i>MT-ATP6 gene</i> : m8993T > G m8993T > C	Heteroplasmic. Higher mutant loads over 90% may occur within younger patients with MILS while adults with lower mutant loads <35% may be asymptomatic	Maternal	Brain MRI: basal ganglia lucencies Abnormal electroretinogram Sensorimotor neuropathy	Treatment of manifestations and supportive therapy. Mitochondrial supplement cocktail	

Single large-scale deletion in mtDNA

Kearns S ayre syndrome (KSS)	Onset before age 20 years Classic triad: pigmentary retinopathy, progressive external ophthalmoplegia including ptosis,	Single large-scale deletion ranging from 1.1 to 10 kb of the mtDNA by quantitative PCR or next-	Heteroplasmic with a high mutant load present in all tissues and can be detected in skeletal muscle, peripheral blood leukocytes, urinary sediment.	Generally sporadic due to deletions in mtDNA occurring de novo	CSF protein exceeding 100 mg/dl Low CSF Methyltetrahydrofolate Lactic acidosis in blood and CSF Brain MRI: leukoencephaopathy	Treatment of manifestations and supportive therapy. Mitochondrial supplement cocktail Folinic acid	Mitochondrial toxins: Valproic acid Metformin Aminoglycoside antibiotics Linezolid Propofol

(Continued)

Table 18.1 (Continued)

Disease	Clinical features	Genetic defect	Mutant load	Inheritance	Diagnosis	Management	Agents/ circumstances to avoid
	cardiac conduction defects Additional features: endocrinopathies, progressive myopathy, dysphagia, cerebellar ataxia, sensorineural hearing loss, cognitive decline, short stature, renal impairment	generation sequencing			cerebral or cerebellar atrophy. EMG/NCV: Myopathy + neuropathy Muscle biopsy: Ragged red fibers, COX-negative fibers, hyperactive SDH fibers. RC enzyme analysis: decreased activity of subunits I, III, IV. RC enzyme analysis may be normal		Statins Nucleoside reverse transcriptase inhibitors
Pearson syndrome	Failure to thrive, short stature, sideroblastic anemia, exocrine pancreatic insufficiency that is often fatal in infancy or early childhood Survivors may develop features of KSS, endocrinopathy, conduction defects and myopathy	Single large-scale deletion ranging from 1.1 to 10 kb of the mtDNA	Heteroplasmic. Cells of the hematopoietic lineage have a higher mutant load. Progressive accumulation of mutant mitochondrial in other tissues with age influences the phenotype	Generally sporadic due to deletions in mtDNA occurring de novo			
Progressive external ophthalmoplegia (PEO)	Ptosis, impaired eye movements due to paralysis of extraocular muscles, myopathy with weakness, and exercise intolerance and occasional conduction defect	Single large-scale deletion ranging from 1.1 to 10 kb of the mtDNA	Heteroplasmic and largely confined to skeletal muscle	Generally sporadic due to deletions in mtDNA occurring de novo			

Mitochondrial disorders due to pathogenic variants in nuclear encoded genes

Mitochondrial neurogastrointestinal encephalopathy (MNGIE)	Severe GI dysmotility, cachexia, ptosis, external ophthalmoplegia, sensorimotor neuropathy	Autosomal recessive disorder due to biallelic mutations in <i>TYMP</i> that encodes thymidine phosphorylase	Brain MRI: diffuse WM changes with increased flair or T2 weighted signal NCV: Mixed axonal and demyelinating neuropathy Increased plasma thymidine and deoxyuridine Reduced thymidine phosphorylase activity

POLG-related disorders

Epilepsy syndromes

Myoclonic epilepsy, myopathy, sensory ataxia (MEMSA)	Myopathy, epilepsy ataxia without ophthalmoplegia. (Previously knowns as spinocerebellar ataxia with epilepsy SCAE) Recurrent seizures accompanied by interictal encephalopathy	Autosomal recessive disorder due to biallelic mutations in <i>POLG</i> gene	EEG: rhythmic high amplitude delta with superimposed spikes (RHADS) with occipital predilection, focal or multifocal changes Brain MRI: stroke-like lesions with occipital predilection. May be normal CSF oligoclonal bands may be seen Blood lactate elevated or normal

Ataxia neuropathy spectrum (ANS)

Mitochondrial- recessive ataxia syndrome (MIRAS)		Subtotal loss of large myelinated in sural nerve, axonal neuropathy, atrophy of dentate nuclei, posterior spinocerebellar tracts, posterior spinal column

(Continued)

Table 18.1 (Continued)

Disease	Clinical features	Genetic defect	Mutant load	Inheritance	Diagnosis	Management	Agents/ circumstances to avoid
Sensory ataxia, neuropathy, dysarthria, ophthalmoplegia (SANDO)							
Progressive external ophthalmoplegia							
arPEO Autosomal recessive progressive ophthalmoplegia	Progressive weakness of extraocular muscles with symmetrical ptosis Associated features: myopathy, ataxia, SNHL, parkinsonism, depression, cataracts, and premature ovarian failure	Autosomal recessive due to biallelic pathogenic variants in <i>POLG</i> gene					
adPEO Autosomal dominant progressive external ophthalmoplegia		Autosomal dominant due to single pathogenic variant in <i>POLG</i> gene					
Movement disorder syndromes							
Progressive ataxia, palatal tremor (PAPT) syndrome	Earlier onset Parkinsonism around age 30–40 years, initially asymmetric features, restless legs, palatal tremor, ataxia	Both autosomal dominant and -recessive mutations in <i>POLG</i> gene			Dopamine transporter imaging: Bilateral nigrostriatal dopaminergic defect Hypertrophic degeneration of inferior olives in PAPT		

Alterations to the cellular redox status due to OXPHOS dysfunction result in biochemical abnormalities including abnormal plasma amino acid profile with elevated alanine or proline, glycine or threonine. Elevated urinary amino acids indicate renal tubular impairment. Abnormal acylcarnitine profile reflective of altered fatty acid and ketone metabolism and abnormal urine organic acid profile with elevations of dicarboxylic acids, 3 methylglutaconic acid, intermediaries of the Krebs cycle are often seen [27]. Evidence of rhabdomyolysis with elevated serum creatinine kinase levels has been seen in some mitochondrial myopathies [28]

Newer technologies such as whole transcriptome sequencing (RNAseq) using RNA from affected tissues will be helpful in unsolved cases and will facilitate the interpretation of variants identified by whole-exome sequencing (WES) and whole-genome sequencing (WGS). Transcriptomic analysis might also reveal biomarkers of mitochondrial disease. Fibroblast growth factor 21 (FGF21) and growth and differentiation factor (GDF15) have been shown to be useful biomarkers in mitochondrial disorders [29,30].

Imaging studies

Neuroimaging with magnetic resonance imaging or computed tomography of the brain is routinely employed in the initial diagnosis and follow up of these patients. Stroke-like lesions in a nonvascular distribution, bilateral, sometimes symmetrical involvement of the deep gray matter, diffuse white matter involvement, and increased brain metabolites, specifically lactate, on brain MR spectroscopy are classic findings that raise the possibility of mitochondrial disease [31].

Tissue diagnosis

Histopathology, biochemistry, and electron microscopy of tissues, specifically muscle, have traditionally been considered the gold standard for diagnosing mitochondrial disease [32]. However, with newer genetic testing that is now available, invasive tissue biopsies are no longer the first line of diagnosis in most instances of suspected mitochondrial disease. Selective tissue testing remains a very informative procedure, especially when genetic testing is not informative or if additional functional studies are required to validate the results of genetic studies [25]. A needle biopsy may be performed in some centers; however, an open biopsy may be needed to provide sufficient tissue for detailed analysis and for biochemical testing. Tissue diagnosis requires extensive coordination between specimen collection and the diagnostic laboratory. The studies are typically done on tissue that is snap frozen in liquid nitrogen immediately after collection and shipped to diagnostic centers. It is important to preserve additional tissue in glutaraldehyde for electron microscopy. Histology typically includes hematoxylin and eosin (H&E) staining, modified Gomori trichrome staining to detect subsarcolemmal proliferation of mitochondria and myofibril degeneration that is seen as ragged red fibers, succinate dehydrogenase (SDH), cytochrome c oxidase (COX), NADH tetrazolium reductase (NADH-TR) [33,34]. Fresh muscle allowing for testing intact mitochondria increases the diagnostic yield, but this may not be routinely available outside of select centers. Skin biopsies with subsequent testing of cultured fibroblasts have benefits in select

settings [35]. Buccal swabs have been used for functional studies, but the results have not been conclusively proven and validated.

Genetic testing

DNA testing has now emerged as the new gold standard and first line testing of patients with suspected mitochondrial disease. Histopathological, biochemical, and additional genetic analysis of tissue including muscle remain important tools to further delineate the phenotype and ascertain the relevance of any genetic variants identified in blood but should no longer be considered first or second-line tests when suspicion of a primary mitochondrial disease (PMD) is high and appropriate genetic testing is available.

MtDNA mutations can affect mitochondrial encoded subunits of the RC or the mitochondrial tRNA or rRNA genes. Point mutations and large-scale single deletions of the mtDNA are the common causes of mitochondrial disease due to mtDNA defects. Point mutations may arise throughout the mitochondrial genome. However, there are a few mutation hot spots that are associated with canonical mitochondrial syndromes such as mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes (MELASs), myoclonic epilepsy with ragged red fibers (MERRFs), or Leber hereditary optic neuropathy (LHON). Point mutations are commonly maternally inherited. Single large deletions of the mtDNA varying in size from 2 to 10 kb that involve several tRNA genes are common and result in well-recognized clinical phenotypes such as Kearns Sayre syndrome (KSS), PS, and PEO [6]. Deletions of the mtDNA are typically sporadic though exceptions are known to occur. When a mother harbors a mtDNA deletion, the risk of transmitting it to her offspring is <5% [36].

Some genotype phenotype correlation exists for disorders caused by mtDNA mutations. For example, mutations in *MT-TK* are the most common cause of MERRF syndrome, while LHON typically results from one of the three common pathogenic variants in mitochondrial genes encoding complex I such as *MT-ND1*, *MT-ND4*, and *MT-ND6*. However, it is important to note that in many instances, there is no firm correlation between any given mutation and the clinical phenotype. For example, mutations in the *MT-TL1* gene are found in >80% of all patients with MELAS. However, mutations in other mtDNA genes including mitochondrially encoded subunits of complex I: *MT-ND1*, *MT-ND5*, and other *mitochondrial tRNA genes*: *Mt-TH* and *MT-TV* also result in clinical features of MELAS. Conversely, *MT-TL1* mutations may result in cardiomyopathy or mitochondrially inherited diabetes and deafness (MIDD). These clinical features may occur in isolation or combination, and the degree of heteroplasmy and mutant load in various tissues results in this wide individual variability even in affected members of the same family [37].

The majority of genes involved in mitochondrial structure and function are coded by the nuclear genome. These nuclear genes are translated by the cytosolic translation machinery and targeted to the mitochondria where they perform key functions. Pathogenic variants in more than 250 nuclear genes have now been described [38], and the number continues to increase as both new genes and new roles implying mitochondrial function for known genes

are being described [39]. Pathogenic variants in nDNA follow autosomal dominant, autosomal recessive, and X-linked inheritance or may occur as *de novo* events [38,39]. These nuclear gene defects can be broadly classified as (1) disorders that affect the structural subunits of the respiratory complexes and their assembly, (2) disorders affecting mtDNA replication and maintenance affecting mtDNA, RNA, and protein synthesis, (3) disorders affecting cofactor metabolism including coenzyme Q₁₀ biosynthesis, (4) disorders affecting the mitochondrial milieu, transmembrane transport, mitochondrial motility, fusion and fission, and homeostasis, (5) disorders upstream of the OXPHOS system affecting substrates including Krebs cycle defects, defects of pyruvate, fatty acid and ketone body metabolism, and (6) disorders that result in accumulation of toxic intermediaries that exert an inhibitory effect on mitochondrial function [38].

In patients with nDNA defects, genetic testing is typically done using extracted DNA from peripheral blood. DNA from other sources including skin fibroblasts, muscle, chorionic villi, or amniocentesis may also be used. Next generation sequencing (NGS) is very effective in identifying mtDNA defects including point mutations, low-level heteroplasmy, and mtDNA deletions, and again peripheral blood may be the most accessible specimen. However, it may be necessary to test other tissues, including muscle, urinary sediment, or affected tissues to identify mtDNA mutations and heteroplasmy as peripheral blood may not be revealing in all cases [40].

If mitochondrial DNA depletion syndromes are suspected, a separate assay using quantitative real-time polymerase chain reaction (PCR) will be required for quantification of mtDNA content in affected tissues [34]. These technologies, however, are still new, and the clinicians caring for patients with suspected mitochondrial disease often encounter genetic variants of uncertain significance in known genes or in genes that have not been associated with disease phenotypes and whose function is not fully known. Networking platforms and data resources such as Genematcher, Leigh Map, and MSeqDR can enhance the data interpretation [41,42].

These genetic studies are typically performed on peripheral blood, which is easily obtainable, and many diagnostic centers are adopting an exome-first approach along with NGS of mtDNA as the initial diagnostic test when a mitochondrial disease is suspected. A muscle biopsy or skin biopsy is performed later only if additional information or functional data are needed to prove pathogenicity of identified mutations. However, if a patient is unstable, collection and preservation of affected tissues, especially liver, muscle, and skin, and simultaneous tissue diagnosis may be necessary.

Despite the advances in genetic testing with WES/WGS, the genetic cause is confirmed in 25%–50% of patients but remains elusive in the rest. The reasons for this are complex and in many instances are due to prohibitive costs and nonavailability of testing but also include challenges in identifying *de novo* mutations, cryptic splice site defects, copy number variants, and mutations in deep intronic or regulatory regions [43].

Management

Treatment of mitochondrial disease remains a challenge given heterogeneity and rarity of these disorders and lack of effective ways to monitor therapeutic response. For the vast

majority of patients, therapy remains supportive and is targeted toward management of specific symptoms. Aerobic conditioning and endurance exercise training induce mitochondrial biogenesis and increase mitochondrial enzyme activity, and studies have shown improvement in clinical symptoms, muscle strength, and quality-of-life scores in patients with mitochondrial myopathies [44,45].

A variety of dietary supplements including vitamins and cofactors are used as empiric treatments in an effort to enhance ATP production by increasing availability of substrates, increasing RC activity, enhancing electron transfer, and reducing oxidative stress and accumulation of toxic metabolites. There has been limited research and a paucity of prospective randomized controlled trials to systematically assess the effects of these supplements in improving mitochondrial function, and most available data are subjective and retrospective in nature. The majority of patients diagnosed with primary mitochondrial disorder are on a cocktail of several supplements, the most commonly used being coenzyme Q₁₀, levocarnitine, and riboflavin [46]. However, these supplements may be therapeutic in only specific cohorts of patients and may rescue some phenotypic changes.

CoQ₁₀ (ubiquinone) is the electron carrier shuttling electrons from complexes I and II to complex III. It is an important compound in the cellular oxidoreductase systems and has other roles in fatty acid oxidation, prevention of lipid peroxidation, and maintenance of mitochondrial membrane integrity [47]. Primary CoQ₁₀ deficiency results from defects in nuclear encoded genes involved with the biosynthesis of CoQ₁₀ and is responsive to CoQ₁₀ supplementation [48]. CoQ₁₀ is commonly used in most forms of mitochondrial dysfunction with reported benefits, but these have not been formally validated. Reduced CoQ₁₀ or ubiquinol is the most bioavailable form and is preferred and should be administered with food to enhance absorption. Idebenone, an analog of CoQ₁₀, was studied in patients with LHON, and while a statistically significant outcome in restoring visual acuity was not shown, it was found to have a protective effect in preventing further vision loss [49].

Riboflavin is an integral component of flavoproteins that are important to the function of several dehydrogenases and the electron transport chain. Patients with defects in these nuclear encoded genes and those with deficiency of riboflavin transporters respond well to riboflavin therapy [39,48], but benefits across the spectrum of mitochondrial diseases are unproven. Patients with mitochondrial deletion syndromes have been shown to have low CSF 5-methyltetrahydrofolate [50], and folinic acid supplementation is commonly used. Other B complex vitamins are also routinely used, but objective data from clinical trials are lacking.

Stroke-like episodes are a common presentation of mitochondrial syndromes such as MELAS and are thought to be secondary to reduced nitric oxide (NO) production and decreased cerebral blood flow secondary to impaired vasodilatation. A therapeutic role for NO precursor L-arginine has been demonstrated in patients with MELAS [51], and intravenous (IV) L-arginine therapy during the acute phase of stroke ameliorates the symptoms while maintenance therapy with oral arginine has been shown to decrease the frequency of the stroke-like episodes [34]. Recent studies have shown that citrulline that can be converted to arginine and NO may also be effective and perhaps more efficacious than arginine in increasing NO production and improving perfusion in MELAS patients [52]. L-Arginine has also found to be beneficial in other mitochondrial syndromes presenting with stroke [53].

Management during critical illness

Patients with mitochondrial disease are prone to intermittent worsening of symptoms especially during periods of stress, prolonged fasting, intercurrent illness, anesthesia, or surgery when there are increased energy demands placed on an already compromised system. In some cases, no acute precipitating events are apparent. Evaluation of critically ill mitochondrial patients should include a comprehensive assessment of all body systems with careful evaluation for any new organ system involvement. Following initial triage referral to a tertiary center equipped to provide comprehensive care should be considered [54].

Cardiopulmonary failure and acute cerebral events such as seizures and stroke have been described as the leading cause of mortality in adult mitochondrial patients [55]. In critically ill patients, comorbidities such as myopathy, impaired mobility, feeding difficulties with swallowing dysfunction, chronic intestinal pseudo-obstruction (CIPO), cardiomyopathy, cardiac conduction defects including Wolf–Parkinson–White syndrome, endocrine dysfunction with diabetes, adrenal insufficiency, and renal impairment with tubulopathy are common, increasing their risk of morbidity and mortality. These patients may be chronically malnourished with micro and macronutrient deficiencies that add to their disease burden. Management goals should include addressing the presenting symptoms and careful scrutiny for comorbidities with blood and urine chemistries, ventilatory assessment, electrocardiogram (EKG), echocardiogram, and CNS imaging as needed. IV fluids with dextrose and saline can be used to maintain IV hydration. A solution containing 5% dextrose is preferred over solutions containing higher concentrations of dextrose to avoid increase flux from lactate to pyruvate and consequent worsening of lactic acidosis. Lactated Ringer's solution is conventionally avoided. Dextrose concentrations may have to be lowered or avoided in patients with diabetes or those who are established on a ketogenic diet. A trained dietician should be involved in the management plan [54].

Vitamin supplements and cofactor therapy should be continued when possible. Electrolytes and acid base status should be closely monitored and promptly corrected with further evaluation of renal or adrenal function as needed. Persistent unexplained hypotension should raise the possibility of adrenal insufficiency and may require stress dose steroids. Elevations of lactate above baseline may occur during the acute phase of an illness; however, normalizing the lactate level should not be the primary management goal as lactate will usually return to baseline when the patient begins to recover and is hemodynamically stable. Any change in baseline neurological exam warrants neuroimaging and comparison to previous findings. Liver dysfunction and synthetic failure with hypoalbuminemia, hypoglycemia, and coagulopathy may occur and should be managed appropriately.

Certain medications interfere with mitochondrial function and mitochondrial replication and may potentiate toxicity. Valproic acid and its derivatives are contraindicated in *POLG*-related disorders and may be poorly tolerated in patients with other forms of mitochondrial dysfunction [56]. Use of metformin for diabetes and topiramate for seizures may worsen lactic acidosis [54,57]. Exposure to aminoglycosides may precipitate hearing loss in patients with susceptible mtDNA mutations [58]. Retroviral therapy for HIV infections and statins

may worsen myopathy, peripheral neuropathy, and weakness [59,60]. Vigabatrin may worsen mitochondrial depletion in patients with defects in the salvage pathway [61]. Patients are at increased risk for anesthesia-related complications. They may be hypersensitive to volatile anesthetics and muscle relaxants. Propofol is a known mitochondrial toxin and may unmask features of a mitochondrial dysfunction. Prolonged use of propofol is not recommended as propofol infusion syndrome is a known risk [62]. Adequate preoperative assessment, avoidance of prolonged fasting, maintenance of hydration, and nutrition in the perioperative phase and close surveillance before discharge is essential. There is no clear evidence of malignant hyperthermia during anesthesia in these patients [54].

Patients should be provided a summary document by their mitochondrial disease care center of clinical symptoms, organ-specific symptoms, baseline cardiorespiratory function, special nutritional needs, medications, allergies, and contraindicated therapies. A medic alert bracelet may be useful.

Emerging therapies

The phospholipid, cardiolipin, is unique to mitochondria and is expressed on the inner mitochondrial membrane where it plays an important structural role in maintaining membrane contours, organization, and anchoring of the OXPHOS complexes and ancillary proteins on the inner mitochondrial membrane, thus facilitating the function of the RC. Mitochondrial dysfunction and consequent increase in ROS are known to cause oxidative damage to cardiolipin that in turn triggers disarray in the organization of OXPHOS complexes, altered calcium homeostasis with increased permeability, and leaking of cytochrome *c* and pro apoptotic proteins eventually resulting in cell death by apoptosis. Elamipretide, a synthetic tetrapeptide, binds to cardiolipin and prevents its oxidation and consequent downstream apoptotic events [63]. The drug is currently under clinical trials as a first-in-class cardiolipin protective compound for mitochondrial myopathy, Barth syndrome, and LHON.

Metabolically active tissues increase their mitochondrial population in response to increased energy demands by increasing the activation of peroxisome proliferator-activated receptor- α coactivator (PGC)1 α transcriptional co-activator that regulates mitochondrial biogenesis. Drugs used for other clinical indications including bezafibrate (used in patients with dyslipidemia), 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) (used in hyperinsulinemia), epicatechin, and proprietary compounds such as RTA 408 (a synthetic isoprenoid) have been shown to increase mitochondrial biogenesis mediated by increased PGC1 α and are under evaluation as potential therapy for mitochondrial disorders [64].

Targeted therapy for specific mitochondrial disorders is also under evaluation. TK2 is the first enzyme in the deoxypyrimidine salvage pathway in mitochondria and provides reduced levels of nucleotides (deoxythymidine [dThd] monophosphate and deoxycytidine [dCyt] monophosphate) for mitochondrial DNA synthesis. This results in mitochondrial DNA depletion syndrome (i.e., less number of functional mitochondrial DNA). Autosomal recessive mutations in *TK2* gene cause nucleotide imbalance resulting in mtDNA depletion and or multiple mitochondrial deletions. Three main phenotypes of TK2 deficiency are recognized

depending on the age of onset and severity. Those phenotypes are: 1) infantile-onset form with myopathy and early death from respiratory failure, 2) a childhood-onset presentation with generalized proximal limb weakness, respiratory insufficiency, and median survival till the early teenage years, and 3) a late-onset form with bulbar and limb myopathy, PEO, respiratory muscle weakness, and mtDNA deletions [65]. Clinical trials where patients with TK2 deficiency will be treated with open-label dThd and dCyt, which are nucleotide precursors to promote mitochondrial DNA synthesis, are underway.

Bone marrow or solid organ transplantation has been attempted in patients with mitochondrial disease with mixed results. Allogenic bone marrow transplantation for mitochondrial neurogastrointestinal encephalopathy (MNGIE) disease results in biochemical correction of the thymidine and deoxyuridine imbalances in blood and some clinical improvement, but morbidity and mortality are very high [66]. Liver, kidney, and cardiac transplants have been attempted in patients in whom the disease burden was highest in these organs at presentation, but results have not been uniformly satisfactory, and progression of co-existing neurological disease has a negative effect on the long-term outcomes [67–70].

Allotropic gene therapy for patients with LHON due to *MT-ND4* pathogenic variants using intra-vitreal injections of adeno-associated viral (AAV) vectors expressing MT-ND4 has been studied, and initial results have documented safety and increased visual acuity in some patients [71]. Additional studies are in progress.

Mitochondrial replacement therapy involves the transfer of the nuclear genes from an oocyte or zygote at the metaphase II spindle, polar body, or pronuclear stage into an enucleated donor oocyte or zygote using in vitro fertilization. By minimizing transmission of maternal mtDNA, the risks of passing on a mitochondrial inherited defect from a mother to offspring are significantly reduced. Mitochondrial donation was debated and approved by both houses of parliament in the United Kingdom, but this is not yet available in the United States [11].

Genetic counseling

The majority of mtDNA mutations are maternally inherited though the mother herself may be asymptomatic or oligo symptomatic in many instances. A clinically unaffected mother may harbor mutant mitochondria in her oocytes. However, a genetic bottleneck is thought to exist between the ovum and the blastocyst of the embryo, and therefore, only a small number of the maternal mitochondria are transmitted to the fetus. A smaller (25%) but significant number are thought to be de novo [72]. The paternally inherited mitochondria are ubiquitinated and destined for destruction although extremely rare instances of paternally inherited mutations in myopathies have been described [73].

Despite improved efficiency in diagnosis of primary mitochondrial diseases due to mtDNA mutations, it is often difficult to predict the recurrence risk in the offspring of a female who is known to harbor pathogenic mtDNA variant. Unlike disorders due to nuclear encoded gene mutations, antenatal testing of fetal tissue for mtDNA pathogenic variants may not accurately predict onset or severity of diseases in the fetus because of heteroplasmy.

Points to remember

- Mutations in nuclear or mitochondrial DNA can lead to mitochondrial disorders.
- Mitochondrial disorders tend to involve multiple organ systems and almost always involve metabolically active tissues.
- Investigating mitochondrial diseases is a complex process requiring a multipronged approach and is often initiated with biochemical studies in blood, urine and CSF.
- Invasive muscle biopsies were traditionally the gold standard for diagnosing mitochondrial disorders but have now been largely replaced with genetic testing using next generation sequencing methodologies.
- Supportive therapy is key in managing patients with mitochondrial disorders.

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Neurologic disorders

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Introduction

The evaluation should begin with a detailed history that includes the age at onset, the patient's description of symptoms, and the temporal course of the disease (static, episodic, relapsing/remitting, or chronic progressive). In the case of disorders such as epilepsy, dementia, or autism that affect consciousness, memory, or language, observations by family members or caretakers are critical. History regarding systemic symptoms, prior medical and surgical history, medication use, and reported alcohol and drug use may provide vital clues. The history helps to determine the nature of the problem and to focus the neurological exam and the diagnostic evaluation. Clues to a genetic etiology include history of similarly affected family members, onset of neurodegenerative disease before the age of 65 years, as well as disease-specific clinical features such as café-au-lait spots in neurofibromatosis type 1 or vertical ophthalmoplegia in Niemann – Pick type C, and characteristic findings on neuroimaging. Whenever a genetic etiology is considered, a complete three- to four-generation pedigree should be recorded, with special attention paid to noting the presence or absence of consanguinity, which is relevant for rare autosomal recessive conditions, ethnic background because of founder effects, noting details of neurological conditions and confirming critical information by obtaining medical records and any prior genetic test results for the affected relatives.

The neurologic exam should be performed to confirm or rule out the affected anatomic region(s) of the nervous system and to identify clinical features suggestive of specific disorders. A thorough and informative neurologic exam is a skill that can only be mastered by examining numerous patients with and without neurologic disease to acquire confidence in one's findings, and familiarity with the range of normal findings at different ages and under different circumstances.

Based on the impression from the history and physical examination, the diagnostic workup most relevant for neurogenetic diseases includes electroencephalography for

epilepsy, electromyography/nerve conduction velocity for neuropathies, motor neuron disease and muscular dystrophies, and neuroimaging (computed tomography scan, positron emission tomography scan, and magnetic resonance imaging, which are the most sensitive and versatile modalities for most genetic disorders affecting the central nervous system). Nerve, muscle, and brain biopsy have been largely, but not entirely, superseded by genetic testing. Genetic testing will be discussed in more detail in the context of specific neurogenetic conditions. Pretest and posttest genetic counseling is an essential part of the evaluation to ensure understanding of the risks, benefits, limits, and implications for family members and is covered in more detail in Chapter 4, Genetic counseling for adults.

Neurogenetic disorders

While it would not be possible to outline the clinical features, diagnostic strategy, and management for all of the well-known neurogenetic disorders, in this chapter, we provide an overview of some of the commonly encountered disorders.

Movement disorders

Movement disorders are classified as hyperkinetic (excessive activity) or bradykinetic (reduced activity). Hyperkinetic movement disorders can be focal or generalized, rhythmic (e.g., tremor), jerky (e.g., tic), sustained (e.g., dystonia), or brief and random (e.g., myoclonus and chorea), elicited only with stress or certain tasks and can remit or be progressive. Bradykinetic movement disorders are often accompanied by rigidity, postural instability, or tremor. A movement disorder diagnosis is a clinical diagnosis based on informed observation and examination. Movement disorders frequently affect gait or balance and can be associated with dementia and psychiatric manifestations. This section will focus on three movement disorders which are likely to be referred for neurogenetic evaluation: ataxia, Parkinson disease (PD), and Huntington disease (HD).

Ataxia

Evaluation

Ataxia results from disease of the cerebellum or its afferent and efferent pathways and results in impaired gait, speech, coordination, and eye movements. Peripheral sensory loss also results in an ataxic gait, but not in speech or eye movement abnormalities. Acute and subacute onset of symptoms, over hours to weeks, is more likely from alcohol, medications, infections, or illicit drugs. Unilateral symptoms are more likely to result from vascular, demyelinating, infectious, or neoplastic causes. Most of the inherited ataxias are characterized by symmetric, chronic, progressive disease, and cerebellar atrophy on magnetic resonance imaging (MRI) (Fig. 19.1A). The exception to this rule is the inherited episodic ataxias.

Inherited ataxias are characterized by extreme clinical and genetic heterogeneity. The history and physical exam should attempt to narrow down the diagnostic possibilities by

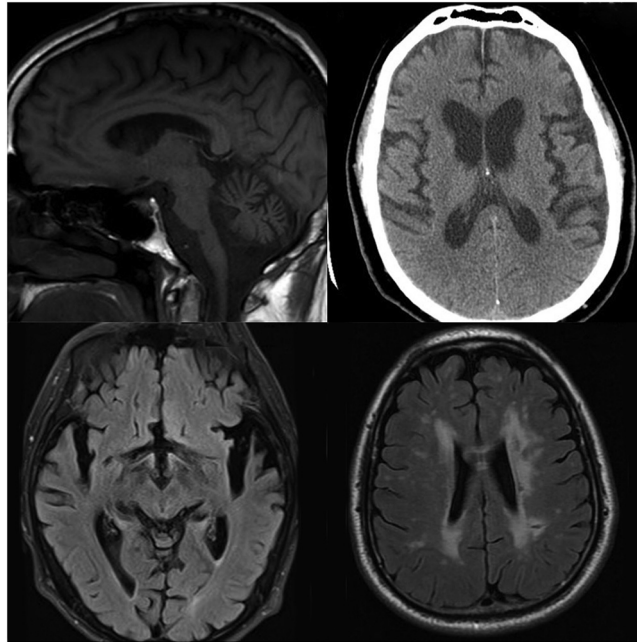


FIGURE 19.1 A (upper left panel): Sagittal T1 MRI of a patient with spinocerebellar ataxia type 2 reveals cerebellar atrophy. B (upper right panel): CT of a patient with Huntington disease shows an atrophy of the cortex as well as a moderate caudate atrophy resulting in a “butterfly” appearance to the lateral ventricles. C (lower left panel): Axial T2 FLAIR MRI of a patient shows a clinical diagnosis of frontotemporal dementia. MRI shows marked Left > Right temporal atrophy. D (lower right panel): Axial T2 Flair MRI of a patient with CADASIL shows extensive white matter abnormalities.

assessing age at onset, rate of progression of disease, symmetry, and whether the ataxia is nonsyndromic, or syndromic with additional features such as intellectual disability, dementia, ophthalmoplegia, oculomotor apraxia, pigmentary retinal degeneration, spasticity, peripheral neuropathy, and cardiomyopathy, among other features. Ataxia can be inherited as an autosomal dominant, autosomal recessive, X-linked, or mitochondrial condition. Thus, a detailed family history is essential. For example, a clear family history of persons in three successive generations with ataxia strongly supports a genetic etiology, and the pattern of autosomal dominant inheritance limits the possible genetic causes.

Genetic testing

There are over 40 genetically distinct autosomal dominant cerebellar ataxias, also known as spinocerebellar ataxias (SCAs); however, the majority of them are extremely rare, with some forms identified in only one or a few families worldwide [1]. SCAs present in adulthood, with SCA3 being the most common dominantly inherited ataxia, due to a founder variant in individuals with Portuguese ancestry, followed by SCA1, 2, 6, and 7. Because of significant phenotypic overlap among the SCAs, testing with a multigene panel is preferred over a single

gene or serial genetic testing. Many of the SCAs are caused by repeat expansions in coding or noncoding regions of the gene; importantly, repeat expansions are *not* detectable by conventional sequencing methods or exome sequencing.

Autosomal recessive ataxias are caused by biallelic pathogenic variants in a wide variety of genes, including numerous individually rare diseases that range from disorders of peroxisome biogenesis to congenital disorders of glycosylation, and vitamin E deficiency. The single most common recessive ataxia is Friedreich ataxia (FA).

The most common X-linked ataxia, fragile X-associated tremor and ataxia syndrome (FXTAS), occurs most commonly in men, after the age of 50 years and is caused by a premutation in *FMR1*. Because of the dynamic nature of the trinucleotide CGG repeat, usually there is no family history of ataxia; however, there may be a history of a female relative with premature menopause or a grandson, or other male relative with fragile X syndrome. In addition, symmetric, increased signal intensity in the middle cerebellar peduncles is a specific sign on brain MRI.

Vignette 19.1

BC is an 18-year-old boy who presented with a 2-year history of progressive incoordination and staggering gait. On exam, he was found to have impaired saccadic eye movements, dysarthria, intention tremor, truncal titubation, loss of vibratory and position sense, absent ankle reflexes, pes cavus, extensor plantar response, ataxia as well as scoliosis and a cardiac murmur. Brain MRI demonstrated cerebellar and spinal cord atrophy. There were no other affected family members.

The sensory loss, absent ankle reflexes, and pes cavus in addition to ataxia in a young adult is suggestive of FA. The cardiac murmur could indicate cardiomyopathy, which occurs in a majority of patients. The negative family history is compatible with autosomal recessive inheritance, and unlikely to be an autosomal dominant cerebellar ataxia. Because FA is the most common recessive ataxia, trinucleotide repeat testing of the FXN gene should be ordered. If the testing shows homozygosity for expanded GAA repeats in the first intron of FXN this is diagnostic of FA. More than 95% of patients have expanded GAA repeats; rarely single-nucleotide pathogenic variants in the coding sequence have also been known to cause the disease. FXN encodes frataxin, and loss of expression leads to accumulation of oxidized mitochondrial iron. Because cardiac hypertrophy and conduction defects are common in FA, a baseline cardiac evaluation as well as regular screening for diabetes are recommended.

Management

Ataxia has numerous hereditary and acquired causes, and the management should be based on the underlying diagnosis. There is disease-specific treatment available for many acquired ataxias, and for a limited number of hereditary ataxias, including ataxia with vitamin E deficiency, ataxia due to mitochondrial, or peroxisomal disorders (Refsum disease). Supportive treatment for symptoms includes assistive devices for gait ataxia and other activities of daily

living such as eating or dressing. Computers can also be used to assist with writing and to improve communication when there is dysarthric speech.

Genetic counseling

Identification of a specific syndrome in the proband such as FXTAS (X-linked) or SCA1 (autosomal dominant) enables accurate risk assessment and predictive testing for family members. Because ataxia syndromes encompass all modes of inheritance, the recurrence risk for children ranges from 0% (son of an individual with FXTAS), to <1% (children of an individual with autosomal recessive ataxia), to 50% in any autosomal dominantly inherited ataxia. Parents of a child diagnosed with ataxia-telangiectasia should be counseled regarding the risk for heterozygous carriers of a pathogenic variant in *ATM* to develop breast and pancreatic cancer. Genetic counseling for a patient with ataxia, with apparently negative family history, in whom genetic evaluation is negative has several possible explanations: an acquired (nongenetic) cause, the testing did not include the causative gene, a class of pathogenic variant undetectable by the testing performed (noncoding, chromosomal translocation, trinucleotide repeat expansion), or a novel genetic cause.

Parkinson disease

Evaluation

PD is a progressive degenerative neurologic condition with the onset typically in the fourth to sixth decades of life. The physical characteristics are resting tremor, bradykinesia, and rigidity. Patients typically have a flat masked face, stooped posture, and take small rapid steps. Mild-to-moderate cognitive impairment can occur later in the disease course. The major pathology is neuronal loss in the substantia nigra with the accumulation of Lewy bodies containing aggregates of α -synuclein. DaTscan measures dopamine transporter activity, which is reduced in PD. Effective symptomatic treatment includes L-dopa and deep-brain stimulation of the sub-thalamic nucleus. The disease duration can be 10–20 or more years.

Genetic testing

PD is usually a sporadic disorder but pathogenic variants have been identified in 15 or more genes. These genetic forms of the disease are transmitted in an autosomal dominant, autosomal recessive, or X-linked manner [2]. Missense mutations in the *LRRK2* gene are the most common cause of autosomal dominant PD. Mutations in *LRRK2* are especially common in North Africa, the Basque population in Spain, and individuals with Ashkenazi Jewish ancestry. Pathogenic variants in the α -synuclein gene are rare, but appropriately match the known pathology in the substantia nigra. Carriers of pathogenic variants in the *GBA* gene associated with Gaucher disease are at increased risk for PD and this occurs most commonly in those with Ashkenazi Jewish ancestry. The onset of PD before the age of 30 years may be associated with autosomal recessive pathogenic variants in the *PARK2* gene. The other less common autosomal recessive form of PD occurs due to pathogenic variants in *PINK1*. A rare X-linked form of PD is associated with pathogenic variants in *RAB39B*.

Genetic testing for PD is best accomplished by use of multigene panels specifically designed for PD or neurodegenerative diseases.

Vignette 19.2

JC is a 55-year-old man with a 5-year history of slowly progressive resting tremor and bradykinesia who was given a diagnosis of PD by a neurologist. The patient's mother had died at the age of 75 years with an onset of PD at 54 years and there was a similarly affected maternal uncle. JC has two younger siblings and three young adult children. Following genetic counseling, testing was undertaken using a multigene panel that included 15 genes associated with PD. The testing revealed a p.Gly2019Ser pathogenic variant in LRRK2. This is the most common pathogenic variant in LRRK2. Because this is an autosomal dominant form of PD the siblings and children should receive genetic counseling. JC was being successfully treated with L-dopa.

Management

PD is usually managed by a neurologist. Medications (L-dopa/Cabidopa) and sometimes deep-brain stimulation are therapeutic interventions. Physical and occupational therapists can help with gait and activities of daily living.

Genetic counseling

LRRK2-related PD is transmitted in an autosomal dominant manner. Penetrance is age-related and nearly complete by age 80 years. Gaucher disease, caused by pathogenic variants in *GBA*, is inherited in an autosomal recessive manner, with some genotype-phenotype correlation. Both individuals with biallelic pathogenic variants in *GBA* with Gaucher disease and heterozygote carriers are at increased risk to develop PD. The exact risks are unclear but are on the order of 15%–30% by age 80 years. Pathogenic variants in *PARK2* and *PINK1* are also autosomal recessive and only persons with biallelic pathogenic variants are affected with PD.

Huntington disease

Evaluation

HD is a progressive neurodegenerative disorder with especially severe degeneration of the caudate and putamen in the striatum evident on computed tomography (CT), MRI, and at autopsy (Fig. 19.1B) [3]. The most common manifestations are chorea, behavioral changes (ranging from apathy to agitation and sometimes including delusions or antisocial activity), and cognitive decline in the later stages of the disease. Individuals with HD are also at risk for depression, anxiety, and suicide ideation. Onset of symptoms is typically in the fifth and sixth decades with the disease duration historically quoted as 15–20 years. Although usually a disease of midlife, the age at onset can range from early childhood (juvenile) to the elderly years.

Genetic testing

HD is an autosomal dominant disorder associated with a CAG triplet repeat expansion in the *HTT* gene located on chromosome 4p. HD testing is currently accomplished with repeat expansion analysis of the *HTT* gene; however, advances in long-read sequencing may allow for detection of CAG triple repeats via sequencing in the future.

Vignette 19.3

*PW is a 40-year-old school teacher who reports decreased ability to manage her classroom curriculum, deterioration in her handwriting and new inability to control her temper. Her father was estranged from the family and she had not seen him since childhood. She had heard a rumor that her paternal grandfather had been committed to a state psychiatric hospital. Physical examination revealed repetitive twitching of her face and hands. The history is suspicious for HD and after genetic counseling, genetic testing revealed 45 CAG repeats in the *HTT* gene. This result confirms the clinical suspicion of HD. PW's care may be managed by a multi-specialty team, such as that which can be found at an HDSA Center of Excellence. Her siblings, children, and other at-risk family members would require genetic counseling.*

Management

Management of HD is primarily directed by a neurologist. Chorea can sometimes be improved with medications (tetrabenazine, fluphenazine, and haloperidol); antidepressants and antipsychotics are often required. Psychiatrists, clinical psychologists, and social workers are frequently involved in the care. Palliative care and hospice are often needed in the end stage of the diseases.

Genetic counseling

HD is autosomal dominant, so each child of an affected person is at 50% risk of inheriting the CAG expansion mutation. Expansion of 40 CAG repeats or greater are associated with full penetrance during a normal life span but an exact age of onset cannot be predicted. Expansions of 34 – 39 repeats are abnormal but in the reduced penetrance range, often associated with the onset after age 60. Expansions of 60 or greater are often associated with juvenile onset (before age 20). Guidelines are available from the Huntington Disease Society of America regarding genetic counseling and testing of at-risk individuals.

Motor neuron and neuromuscular diseases

Weakness caused by neurological disease can arise from pathology at any site in the neuraxis. A neurological history and exam can determine the anatomic localization of the pathology—upper motor neuron (UMN), lower motor neuron (LMN), peripheral nerve, neuromuscular junction, or skeletal muscle. The UMN begins in the motor cortex of the brain and descends through the pyramidal tract to synapse with the LMN. The LMNs originate in the brain stem and spinal cord and innervate the fibers of the muscle spindle.

Peripheral nerves have motor, sensory, and autonomic functions; both acquired and inherited peripheral neuropathies can affect one, two, or all three functions. Anatomically, peripheral neuropathies may affect the cell body, the axon, or the myelin sheath, and can affect large fiber or small fiber peripheral nerves. The exam is helpful in differentiating the type and extent of neuropathy.

Most muscle diseases cause symmetric weakness with intact sensation and reflexes. The distribution of muscle weakness in history and exam is critical. Ptosis, extraocular muscle or facial weakness, scapular winging, proximal or distal muscle weakness, and the presence of myotonia, cramping, and myoglobinuria can help in narrowing the diagnostic possibilities. Neuroimaging is generally not helpful for diagnosis and is ordered to exclude structural diseases. In contrast, electromyogram (EMG)/nerve conduction velocity (NCV) is very useful as an adjunct to the clinical examination of the peripheral nervous system.

Amyotrophic lateral sclerosis

Evaluation

Amyotrophic lateral sclerosis (ALS) is a progressive, degenerative disease whose hallmark is the involvement of both UMNs and LMNs. It begins with cramping and limb muscle weakness, or facial weakness affecting swallowing, and talking, followed by muscle atrophy, and spontaneous muscle fiber twitching (fasciculations). UMN signs include hyperactive deep tendon reflexes, and spasticity. Purely UMN or LMN signs should suggest alternative possible diagnoses such as hereditary spastic paraplegia or spinal muscular atrophy, respectively. When considering a diagnosis of ALS, treatable causes that mimic ALS should be excluded including cervical myelopathy and multifocal motor nerve conduction block.

ALS occurs most commonly as a sporadic disease in 90%–95% of cases, tends to occur at older ages, and has a male preponderance. ALS is associated with frontotemporal dementia (FTD) that can manifest with either or both conditions in an individual with *C9orf72*-related disease or the much rarer *CHCHD10*-related ALS/FTD. Genetic causes of ALS in adults are inherited as autosomal dominant traits, so family history is important. Juvenile forms of ALS can be inherited as autosomal recessive or dominant traits.

Genetic testing

The most common genetic causes of familial ALS are *SOD1*-related (~20% of heritable ALS), and *c9orf72*-related (25%–30% of heritable ALS), followed by *TARDBP* and *FUS*, accounting for less than 4% each [4]. *SOD1* and *c9orf72* mutations have also been reported in simplex cases so the family history may be negative. There are a number of additional genes causing familial ALS, which can be assayed using a multigene panel test when there is no previously identified cause in the family.

Management

ALS has no treatment to reverse or stop the underlying cause, so the disease is fatal, usually within five years of diagnosis; with some exceptions, most notably the renowned physicist,

Stephen Hawking. The mainstay of therapy consists of physical therapy and devices to assist mobility, speech, and respiration. In 1995, the US Food and Drug Administration (FDA) approved riluzole for treatment of ALS. In 2017 a second drug, edaravone, which reduces oxidative stress, was approved. Because of the paucity of effective treatment for ALS, eligible patients should be offered the opportunity to participate in clinical trials.

Genetic counseling

The most common genetic forms of adult-onset ALS are inherited as autosomal dominant conditions. Simplex cases with a *SOD1* pathogenic variant may have an unaffected parent because of incomplete penetrance. Predictive testing of an unaffected individual at risk raises similar challenges as for any untreatable, adult-onset disease such as Huntington or Alzheimer disease. Referral to a genetics specialist for predictive testing is strongly recommended. This is especially beneficial when interpreting negative or uncertain genetic test results, particularly when the affected relative dies without having genetic testing.

Peripheral neuropathies

Overview

Peripheral neuropathies can be broadly divided into acquired forms and genetic forms. Acquired forms can be caused by diabetes mellitus, inflammatory conditions, medications (e.g., certain cancer chemotherapy agents) and toxins (e.g., alcohol and lead). Genetic forms of peripheral neuropathy can be transmitted in autosomal dominant, autosomal recessive, X-linked, or mitochondrial modes of transmission. The most common genetic forms are often also referred to as Charcot – Marie – Tooth (CMT) disease [5].

Evaluation

An investigation into the causes of peripheral neuropathy usually requires consultation with a neurologist. The evaluation includes careful history regarding employment or exposure to drugs and toxins, testing for metabolic factors such as diabetes, and EMG and NCV studies. Genetic forms are often divided into demyelinating (CMT1 with slow NCV) and axonal (CMT2 with normal or near normal NCV) forms. A positive family history is an important clue to possible genetic causes. Persons with typical CMT often have onset in childhood or adolescence of a slowly progressive peripheral neuropathy that includes sensory loss in the feet, distal muscle weakness, and atrophy (which first affect the feet and then the hands), loss of deep tendon reflexes, and high arches with pes cavus foot deformity.

Genetic testing

There are more than 100 genetic causes of hereditary peripheral neuropathy, but most are quite rare. The most common autosomal dominant forms are caused by a duplication of the *PMP22* gene (CMT1A) and missense mutations in the *MPZ* gene (CMT1B). Missense mutations in the *GJB1* (Connexin-32) gene are the most common X-linked cause (CMTX1) and missense mutations in *GDAP1* and *SH3TC2* are the most common autosomal recessive

causes. Missense mutations in the *MFN2* gene are the most common cause of axonal CMT2. Most commercial laboratories have large hereditary neuropathy multigene panels which test for 30–50 or more genes. Testing for the duplication of *PMP22* is often not included in these panels and should be done first because it is the most common cause of CMT. 80% of autosomal dominant demyelinating CMT1 is caused by *PMP22* dup.

Vignette 19.4

TM is a 15-year-old boy who presented with a five-year history of progressive difficulty running and climbing stairs. Examination revealed depressed tendon reflexes, bilateral foot drop, and high arches. NCV showed mildly slow nerve conduction velocities. His mother had a loss of vibratory sensation in her toes and a maternal uncle had a history of peripheral neuropathy. Genetic testing for PMP22 duplication was negative but a hereditary neuropathy panel revealed a missense mutation in the GJB1 gene, the most common cause of X-linked CMT. The family should receive counseling for an X-linked disease in which females are sometimes mildly to moderately affected.

Management

Management of hereditary peripheral neuropathy is usually accomplished by a specialist in rehabilitative medicine (physiatrist) and physical therapists. Affected persons sometimes require ankle-foot orthoses (AFOs), canes, forearm crutches, or walkers. Rarely individuals may also need a wheelchair.

Genetic counseling

In the autosomal dominant forms of CMT, each child of an affected person is at 50% risk to inherit the condition. Penetrance is usually complete by the third decade, but occasional specific pathogenic variants lead to presentations after the age of 30 or 40 years. In the autosomal recessive forms, heterozygous carriers are asymptomatic and the onset in homozygotes is frequently during early or mid-childhood. The common *CMTX1* (*GJB1* mutations) is essentially X-linked dominant with milder expression in female carriers. Affected males usually have a full CMT phenotype whereas carrier females may be asymptomatic or have a mild-to-moderate phenotype and even rarely full clinical manifestations.

Muscular dystrophies

Overview

There are a very large number of muscular dystrophies, but this section will focus on the few that are most common, namely, X-linked Duchenne muscular dystrophy (DMD), autosomal dominant myotonic, and fascioscapulohumeral (FSHD) dystrophies, and limb-girdle muscular dystrophies (LGMDs), which can be autosomal dominant or autosomal recessive.

Evaluation

The most common form of muscular dystrophy is X-linked DMD which typically has its onset in early childhood in boys, with slowly progressive lower limb weakness associated with pseudo-hypertrophy of the calves and very elevated serum creatine kinase. Myotonic dystrophy can occur at any age and its two salient characteristics are slowly progressive muscle weakness and associated myotonia (slow relaxation of a muscle contraction). It can be associated with a large number of additional characteristics including frontal balding, cataract, testicular atrophy, cardiomyopathy with or without arrhythmia, and diabetes mellitus. FSHD typically has an onset in childhood or adolescence and is associated with progressive weakness and atrophy of the face, muscles of the upper arm and prominent winging of the scapula. Both FSHD and myotonic dystrophy can be associated with distal weakness of the lower limb producing foot drop. The limb-girdle dystrophies have onset in childhood or young adulthood of slowly progressive proximal weakness of the upper and lower limbs which may or may not be associated with a cardiomyopathy (depending on the subtype) and is usually not associated with the additional characteristics seen in some of the other dystrophies such as pseudo-hypertrophy, cataracts and winging of the scapula.

Genetic testing

DMD is associated with a variety of mutations in the dystrophin gene including small deletions and duplications. Myotonic dystrophy type 1 (DM1) is associated with a CTG repeat expansion in the noncoding region of a muscle kinase gene *DMPK*. A separate, but clinically overlapping form called DM2 is associated with a very large expansion in an entirely different gene and was previously called paroxysmal myotonic myopathy (PROMM). There are at least eight forms of autosomal dominant LGMD, most frequently occurring due to pathogenic variants in *CAV3* or *DES*. There are more than 20 forms of autosomal recessive LGMD, the most common being caused by pathogenic variants in *CAPN3*, *DYSF*, and *FKRP*. FSHD1 is caused by a contraction of a DNA repeat sequence called D4Z4 on one copy of chromosome 4q35. A less common variety (FSHD2) with very similar clinical manifestations is caused by pathogenic variants in the *SMCHD1* gene. Genetic testing for DMD, myotonic, and FSHD dystrophies must be done on an individual gene basis. Genetic testing panels are available for the large number of LGMDs.

Vignette 19.5

A 38-year-old man presented with several years of slowly progressive proximal and distal weakness in both legs that produced bilateral foot drop. He had no family history of muscle or nerve disease. He was initially thought to have either CMT or LGMD, but genetic testing with multigene panels for these two disease categories was negative. Further evaluation with an EMG revealed electrical evidence of myotonia and it was noted that he had mild difficulty releasing the grip of his hands. In addition, his mother had had cataract surgery in her early 50s and he had a maternal first cousin with a cognitive developmental disability and a diagnosis of cerebral palsy. Directed genetic testing for myotonic dystrophy was positive with 250

CTG repeats in the DMPK gene. This established the diagnosis of classic DM1. Genetic testing of his mother revealed 80 CTG repeats and his cousin had 1500 CTG repeats. Thus, his mother had latent DM1 and his cousin had anticipation with congenital DM1. The index patient's aunt (mother of his cousin) must also have a DM1 expansion. All children of his mother and aunt are at 50% risk for DM1 and each of the index person's children is at 50% risk.

Management

The muscular dystrophies require careful management with a medical team including physical therapists, occupational therapists, physiatrists, social workers and neurologists. Exon skipping therapy for DMD has been approved by the FDA and is discussed in Chapter 28, Precision medicine in the genomic era. Otherwise, there are no specific treatments for these dystrophies although attempts at genetic approaches to DMD and myotonic dystrophy are underway.

Genetic counseling

DMD is an X-linked-recessive disorder with full manifestations in boys who are usually in wheelchairs by early adolescence but may live into adulthood with intensely focused physical and respiratory treatment. A less severe type termed Becker MD (BMD) does not require a wheelchair and is more slowly progressive. BMD is associated with in-frame deletions of the *DMD* gene. Myotonic muscular dystrophy is an autosomal dominant disorder with highly variable expressivity and clinical severity varies from mild to moderate to severe [6]. CTG expansions greater than 50 in *DMPK* are in the abnormal range. The disease demonstrates genetic anticipation such that there is an early childhood and more severe form associated with large expansions of the CTG repeat usually in excess of 1000. FSH dystrophy is also an autosomal dominant disorder with wide variability in severity that is not closely associated with the size of the D4Z4 contraction [7]. LGMDs can be autosomal dominant or autosomal recessive depending on the specific causative gene mutations [8,9].

Dementias

Dementia is characterized by a decline in cognition from a previously attained level of function, affecting at least one domain such as language, learning, and memory, executive function, or social cognition, and interferes with an individual's ability to function independently. Alzheimer disease is the most prevalent form of dementia, followed by vascular dementia. Additional dementia syndromes include dementia with Lewy bodies, PD-related dementia, and frontotemporal dementia. Dementia risk increases strongly with age. The onset of dementia in patients before the age of 65 years is considered young-onset dementia, and it is this subset of patients most likely to be referred for genetic evaluation.

Alzheimer disease

Overview

Alzheimer disease (AD) is the most common form of dementia with increasing incidence over each decade of adult life. It is rare in the 40s and uncommon in the 50s, but probably affects 30%–40% of the population over age 85 years. It is a clinicopathological diagnosis with progressive memory loss and behavioral changes associated with microscopic amyloid plaques and neurofibrillary tangles (containing tau protein) in the brain.

Neuropathological findings are often complicated by signs of vascular disease and Lewy body (α -synuclein) aggregation. Disease duration is typically 8–12 years. Autosomal dominant genetic causes are rare and account for 1%–2% of disease [10]. The much more common sporadic form of the disease is known to be etiologically complex with numerous genetic risk factors each of low risk, except for the ApoE4 allele which is associated with substantial risk.

Evaluation

Most patients with AD present with slowly progressive memory loss followed by confusion, disorientation, and sometimes a change in personality. Brain imaging reveals diffuse cerebro-cortical atrophy which is often marked in the hippocampus. The amyloid Positron Emission Tomography (PET) scan shows excessive accumulation of β -amyloid. Evaluation of cerebrospinal fluid (CSF) by lumbar puncture reveals decreased β -amyloid and increased *tau* proteins. Early-onset disease (prior to age 50 or 60 years) with a positive family history suggests a direct genetic cause.

Genetic testing

Pathogenic variants in one of the three genes, *APP*, *PSEN1*, and *PSEN2* cause early-onset familial AD with onset typically in the 40s or 50s. The clinical syndromes associated with these three genes extensively overlap such that genetic testing should include all three genes. Many commercial laboratories have genetic panels for dementia or neurodegenerative disorders that include these three genes. The clinical manifestations often overlap with PD and FTD such that genes for all these diseases are often included in the panels. *ApoE4* is the most common genetic risk factor for sporadic AD, but its presence is neither necessary nor sufficient for the disease. The age versus risk tables are available for individuals who have had ApoE testing. Several other genes have been associated with increased risk for AD, but the associated risks are very low and testing of these genes is presently only used in research studies.

Management

There is no cure, prevention, or highly effective treatment for AD. Patients in the early- to mid-stage of the disease, who experience memory loss may sometimes benefit from anticholinesterase medications (e.g., donepezil). Physical therapy, art, music, and exercise can be

part of a management program. Affected persons often require residence in a memory care unit of a retirement home or placement in a nursing home or adult care facility.

Genetic counseling

Families with pathogenic variants in *APP*, *PSEN1*, or *PSEN2* have an autosomal dominant disease. Each child of an affected person is at 50% risk for early-onset familial AD. Onset is typically in the 40s and 50s with nearly complete penetrance by age 75 years. Adult children at 50% risk occasionally request genetic testing which should only be done with careful genetic counseling including an explanation of risks and benefits. Heterozygote carriers of ApoE4 have an increased risk of developing AD on the order of 15%–20% in the 70s. Homozygotes for ApoE4 have an even greater risk on the order of 30%–40% in the 70s.

Frontotemporal dementia

Overview

FTD is probably the most common cause of progressive dementia in midlife (40s–60s). It is distinguished by focal atrophy in the frontal and temporal regions of the brain. The disease most commonly causes a change in personality with behaviors such as apathy, agitation, or inappropriate antisocial activities [11]. Problems with language can also occur including progressive aphasia. In addition to the focal brain atrophy, the disease is often distinguished from Alzheimer dementia because memory loss is absent or relatively mild in the early stages of the disease. Neuropathology may include aggregates of tau or TDP-43. Approximately 40%–50% of the affected persons have a genetic cause.

Evaluation

Affected persons usually present with a progressive behavioral change which is often initially misdiagnosed as a personality disorder or unexplained psychosis. Brain imaging will show atrophy in the frontotemporal areas (Fig. 19.1C) and fluorodeoxyglucose (FDG) PET scanning shows decreased metabolism in these same regions. The clinical presentation needs to be differentiated from Alzheimer disease, vascular or Lewy body dementia, drugs, toxins, and metabolic encephalopathy.

Genetic testing

Pathogenic variants in at least 10 different genes have been associated with FTD. The most common are *MAPT* (tau), *GRN*, and *C9orf72* [12]. Many commercially available genetic testing panels for dementia or neurodegenerative diseases include *MAPT* and *GRN*. However, the mutation in *C9orf72* is a hexanucleotide repeat expansion, which must be tested separately.

Management

There are no highly effective treatments for FTD. The management is usually by a neurologist and/or psychiatrist and may include sedatives and antipsychotics. Behavior therapy may

be helpful. Placement in a memory disorders unit or nursing home may be necessary. The disease duration is usually 5–10 years.

Genetic counseling

Pathogenic variants in the three disease genes (*MAPT*, *GRN*, *C9orf72*) cause an autosomal dominant disorder with 50% risk to each child of an affected person. Penetrance is very high by the seventh decade, but examples of unaffected elderly gene carriers are known. The repeat expansion in *C9orf72* may also be associated with ALS. Some families with mutations in this gene express FTD or ALS or both.

CADASIL

Overview

CADASIL stands for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. This disorder is said to have a prevalence of approximately two to five cases per 100,000, but it is probably underrecognized because of its multitude of nonspecific clinical manifestations. CADASIL is often misdiagnosed as multiple sclerosis.

Evaluation

The three most common clinical characteristics of CADASIL are migraine headache, transient ischemic attacks (TIA) and stroke, and dementia all of which tend to occur episodically over a period of decades. Migraine with aura often begins during the fourth decade and TIA with strokes often occurs during the fifth and sixth decades of life followed by slowly worsening dementia. Dementia affects both memory and executive functions and may be associated with depression, bipolar symptoms or psychotic features. MRI shows diffuse bilateral white matter hyper-intensities that usually begin in the anterior temporal regions ([Fig. 19.1D](#)). Neuropathology reveals diffuse white matter degeneration associated with characteristic granular osmophilic material in the vessel wall of arterioles.

Genetic testing

CADASIL is caused by missense mutations in the *NOTCH3* gene that are usually associated with the addition or deletion of a cysteine residue [\[13\]](#). Hundreds of mutations have been reported throughout the gene with many clustering in exons 3 and 4. With a high index of suspicion, genetic testing can be ordered just for the *NOTCH3* gene. However, many commercial laboratories include the *NOTCH3* gene in panels for dementia or leukodystrophies.

Management

Management is symptomatic and should be conducted by an internist or neurologist. Because patients with CADASIL are at an increased risk for cerebral micro-hemorrhages, there is a concern that antiplatelet agents (such as aspirin) and anticoagulants (such as warfarin and related drugs) may be contraindicated. However, these factors have not been systematically studied and such treatment is controversial.

Genetic counseling

CADASIL is an autosomal dominant disorder with 50% risk to each child of an affected person. Penetrance of symptoms is very high by the sixth decade of life, but the severity is unpredictable and varies from mild to moderate to severe. Some persons with migraine receive a routine MRI which surprisingly shows unexpected diffuse white matter hyperintensity and are discovered to have a pathogenic variant in *NOTCH3*.

Paroxysmal disorders

Headaches, seizures, periodic paralysis, and vertigo are examples of neurologic symptoms that occur episodically. Sometimes they are triggered by external factors such as sleep deprivation, skipping medication doses, stress, or infection. Neurologic exam between episodes is often entirely normal, and neuroimaging may also be normal. Seizures, movement disorders, and cardiac events such as syncope can all appear very similar to an observer. Therefore, a careful history (including information from witnesses) or family member videotaping brief events can be useful for diagnosis. In some disorders, there are lab abnormalities only during episodes, such as hyperkalemia in *SCN4A*-associated periodic paralysis, or electrographic seizures during electroencephalogram (EEG). In selected cases, inpatient video EEG monitoring to record events while withdrawing medication under medical supervision is required. In other disorders, there are persistent abnormalities interictally on EMG, EEG, or MRI.

Epilepsy

Evaluation

Epilepsy refers to a group of diseases with recurrent, unprovoked seizures due to abnormal, synchronized neuronal activity. Epilepsy is common, affecting 1 in 26 people. Diagnosing the types of seizures and the epilepsy syndrome are vital to treatment and prognosis. The International League Against Epilepsy has recently published updated guidelines on classification [14]. Seizures may be focal, meaning they originate in a localized brain region, or generalized, meaning they arise in both brain hemispheres. Genetic forms of epilepsy are most prevalent in childhood, and particularly those with onset prior to age 5 years [15]. In adults, epilepsy is most prevalent in older adults, often acquired, and the consequence of injury from stroke, head trauma, alcohol withdrawal, brain tumor, taking medications or drugs that lower the seizure threshold, or in the setting of degenerative disease. Therefore, the age at diagnosis, the types of seizures, epilepsy diagnosis, presence of comorbidities such as autism or intellectual disability, EEG and MRI findings are most useful in genetic evaluation of a patient with epilepsy.

Inheritance of genetic epilepsies can be autosomal recessive, autosomal dominant, mitochondrial, or X-linked. Severe, early childhood epilepsies commonly arise as *de novo* dominant conditions.

Genetic testing

Early infantile epileptic encephalopathies comprise a large, genetically heterogeneous group of disorders of infants and early childhood characterized by multiple types of seizures, developmental delay or regression, and frequent EEG abnormalities. Examples include Dravet syndrome caused by *SCN1A* pathogenic variants and West syndrome caused by *ARX* or *CDKL5* pathogenic variants. With improvements in medical treatment, young adults with lifelong epilepsy may present for updated genetic evaluation. The yield of genetic testing is highest in individuals with epilepsy onset prior to 2 years of age [16]. Gene discovery in epilepsy has identified novel mechanisms of disease affecting: ion channels, neurotransmitter receptors, chromatin remodeling, mechanistic target of rapamycin kinase (mTOR) pathway, intracellular signaling, transcriptional regulation or synaptic vesicle trafficking. Developmental brain disorders affecting neuronal migration comprise another large group of disorders that manifest with a combination of epilepsy, intellectual disability, and developmental delay. The age of onset ranges from infancy to adulthood, and the degree of disability ranges from severe to none. The brain MRI is abnormal but can be subtle, requiring careful review by an expert. Examples include filamin A and doublecortin (*DCX*) related disease.

Epilepsy genetic testing can be done by a panel of relevant genes, or by clinical exome or genome sequencing. Testing should include deletion/duplication testing since copy number variation has been shown to cause epilepsy.

Vignette 19.6

KL is a 42-year-old man with onset of epilepsy at age 9.5 years. His seizures began with exposure to light filtering through trees while riding in a car, followed by a generalized tonic-clonic seizure. His epilepsy diagnosis is progressive myoclonic epilepsy (PME). EEG showed slowed background, frequent epileptiform discharges, and myoclonic jerks. Photoc stimulation caused activation of epileptiform activity. On exam, his speech and thought processes were slowed; he was confined to a wheelchair because of frequent myoclonic jerks elicited by movement (action myoclonus) noted on the neurologic exam. KL is of northern European ancestry; there is no family history of epilepsy.

*KL has a form of PME [17]. This narrows the differential to a subset of epilepsy that includes: myoclonic epilepsy with ragged red fibers (MERRFs), Unverricht – Lundborg disease (ULD), Lafora body disease, neuronal ceroid lipofuscinosis, PRICKLE1-related PME with ataxia, amongst others. Because of the age at onset, long survival, and negative family history compatible with autosomal recessive inheritance, ULD was suspected to be the most likely cause. Unfortunately, the available epilepsy panels, even those specific for PME, only offer sequencing of the *CSTB* gene; none includes testing of the 5' untranslated region dodecamer expansion in the *CSTB* gene that causes more than 90% of ULD. Therefore, one should consider recommending targeted testing for the expansion, and if negative, a PME panel for further work-up. This case illustrates the importance of the ordering clinician being knowledgeable about the genes on the panel, the types of variants resulting in disease, and the limitations of testing.*

Management

There are a growing number of scenarios where the results of genetic testing affect the management of epilepsy. These include identification of a pathogenic *SCN1A* variant in Dravet syndrome, for which sodium-blocking anti-epileptic drugs such as phenytoin or lamotrigine can worsen the epilepsy, *SLC2A1*-related epilepsy causing a deficiency of glucose transport into the brain, for which a ketogenic diet is indicated, and rare reports of individuals with *KCNT1* gain-of-function variants, who have benefitted from quinidine, or patients with *GRIN2A* or *GRIN2D* variants who have benefitted from “re-purposed” drugs developed for other diseases. Clinical trials are needed to prove the utility of some of these novel treatments.

Genetic counseling

Identification of a *de novo* cause of epilepsy in a young child greatly reduces the recurrence risk for subsequent children for a couple. Because of germline mosaicism, however, the risk is not lowered to the population risk. When the same diagnosis is made in a young adult with intellectual disability and refractory epilepsy, the risk for his or her unaffected siblings' children is reduced to the population risk. The risk to inherit the genetic cause should clearly be differentiated from the ability to predict the risk of epilepsy or intellectual disability. In tuberous sclerosis complex (TSC), the children of an affected patient are at 50% risk to inherit TSC, but accurate predictions regarding risk of epilepsy or intellectual disability are not possible.

Headache

Overview

Headache is one of the most common clinical problems in the general population and is more common in females than males. The two most common subtypes are muscle tension headache and migraine. Migraine is usually unilateral and throbbing. The headache may be preceded by an aura which is often a visual disturbance or sensory phenomena on one side of the body. When the patient experiences unilateral weakness along with the headache, this is termed hemiplegic migraine.

Evaluation

Familial hemiplegic migraine can be genetic and is most often caused by pathogenic variants in the *CACNA1A* gene. Familial headache is common, but genetic forms of migraine are often associated with other findings such as transient unilateral weakness (hemiplegia), triggered by mild head trauma, brain stem features (dysarthria, diplopia, hearing loss), confusion or evidence of brain edema on MRI. Familial hemiplegic migraine can also be associated with ataxia or seizures (during or in between headaches). Individuals with these additional characteristics are candidates for genetic testing [18].

Genetic testing

Pathogenic variants in three genes have been associated with familial hemiplegic migraine, the most common being *CACNA1A* and the others being *ATP1A2*, *SCN1A*, or *PRRT2*. All are associated with autosomal dominant inheritance with incomplete penetrance and highly variable clinical manifestations. Pathogenic variants in *CACNA1A* can also be associated with cerebellar ataxia. In fact, an adult-onset cerebellar ataxia, known as SCA6, is associated with a small CAG repeat expansion in this gene.

Management

Patients with genetic forms of migraine often respond to calcium channel inhibitors or a 5-hydroxytryptophan (5-HTP) agonist.

Genetic counseling

Pathogenic variants in the *CACNA1A*, *ATP1A2*, *SCN1A*, or *PRRT2* genes are associated with autosomal dominant inheritance. However, the penetrance is variable and the clinical manifestations can be mild, moderate, or severe. Some persons with a pathogenic variant in *CACNA1A* eventually develop permanent ataxia associated with cerebellar atrophy on MRI.

Points to remember

- Most common adult neurological problems have a subset of patients with a genetic etiology. It is not appropriate to offer genetic testing to all adult patients with epilepsy, stroke, or dementia since 90% or more will not be hereditary, but familiarity with the genetic disorders and recognition of patients with them is the first step in a neurogenetic evaluation.
- A careful medical and family history, and a complete neurological exam followed by appropriate nongenetic testing (such as imaging, EEG, or EMG/NCV) is essential prior to ordering any genetic testing, and results should always be interpreted in the context of the phenotype and family history.
- Genetic diagnosis of a neurological disorder informs prognosis, recurrence risk, can affect treatment choices for some conditions, and is required for many clinical trials.
- Genetic diagnosis for muscular dystrophies, inherited neuropathies, and neurodegenerative diseases has eliminated the need for muscle, nerve, or brain biopsy in many cases.
- Neurogenetic disorders are frequently genetically heterogenous (ataxia, LGMD, PD), so a negative gene panel or exome does not necessarily exclude a genetic etiology.

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Pulmonary disorders

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Introduction

Heritable forms of pulmonary disorders are rare. However, some of these disorders can present with clinical features that have a significant phenotypic overlap with the more commonly occurring disorders of the lung. For example, the symptoms and signs of pulmonary disease in individuals with alpha-1 antitrypsin deficiency (AATD) can be indistinguishable from that of symptoms observed in chronic obstructive pulmonary disease (COPD). Many heritable forms of pulmonary disorders are multisystem disorders that present early during childhood. A knowledge of the nature and the extent of the involvement of the other organ systems is critical for diagnosis and instituting appropriate management. In this chapter, we discuss selected heritable disorders that present with obstructive lung disease, restrictive lung disease, pleural disease (pneumothorax), and pulmonary vascular pathology.

Obstructive lung disease

Alpha-1 antitrypsin deficiency

Overview

Alpha-1 anti trypsin (AAT) is a protein belonging to the family of serine protease inhibitors (PIs) that is encoded by the gene *SERPINA1*. AATD is an autosomal recessive disorder characterized by increased risk for COPD and liver disease. The pulmonary disease occurs due to a “toxic loss-of-function” mechanism wherein loss of AAT leads to reduced inhibition of neutrophil elastase and inflammation-mediated destruction of alveolae. In contrast, the liver disease occurs due to a “toxic gain-of-function” where accumulation of specific variant forms of AAT in the hepatocytes leads to hepatic injury. The prevalence of the disorder in the United States in individuals of northern European heritage is approximately 1 in 5000. The pulmonary manifestations of AATD resemble that of COPD. Liver disease can manifest as chronic hepatitis, cirrhosis, and hepatocellular carcinoma.

Evaluation

Pulmonary symptoms in AATD include dyspnea, cough, sputum production, and wheezing [1]. Smoking is a risk factor that determines the age of onset and progression of the pulmonary disease. In smokers, the onset is typically in the fourth decade of life. Nonsmokers typically develop disease in the sixth decade. Some individuals with AATD never develop pulmonary disease. In adults, hepatomegaly, fibrosis, and cirrhosis can occur even in the absence of a history of neonatal- or childhood-onset liver disease. Liver disease is typically seen with AAT variants that accumulate in the liver (e.g., PI*ZZ genotype). The risk for hepatocellular carcinoma is increased compared with the general population. In addition to these manifestations, AATD can also present with panniculitis and vasculitis.

A diagnosis of AATD should be considered in an individual with: (1) emphysema occurring at age <45 years, (2) emphysema occurring in the absence of smoking or occupational risk, especially if characterized by basilar changes, (3) unexplained bronchiectasis, and (4) adult-onset asthma. Supportive family history and history of liver disease should be actively sought.

Genetic testing

The diagnosis is established by measuring the serum levels of AAT, protease inhibitor (PI) typing, and gene sequencing. Serum levels of AAT below 20 $\mu\text{mol/L}$ are typically diagnostic of AAT. PI typing using isoelectric focusing is the gold standard blood test for identifying AAT variants. Normal alleles are associated with normal levels and function of AAT (PI*MM). Alleles that are associated with plasma AAT levels less than 35% of the average normal level are termed deficient alleles. The most common deficient alleles associated with emphysema is PI*ZZ. Null alleles lead to absence of detectable AAT protein in the plasma. Sequencing of *SERPINA1* is indicated when the ATT levels and the PI typing are discordant or when the phenotype cannot be explained by the reported genotype.

Management

Supportive care for the treatment of pulmonary disease includes cessation of smoking, avoidance of inhalational irritants, administration of influenza and pneumococcal vaccinations, use of inhaled bronchodilator and anti-inflammatory therapy, and treatment of respiratory infections. Intravenous augmentation by infusion of pooled human AAT is approved for treatment of AATD. The selection of patients, dosing, and monitoring is best managed by expert centers with experience in such therapy. Individuals with severe disease may be candidates for lung and/or liver transplantation.

Genetic counseling

AATD is an autosomal recessive disease. When both parents of an affected individual are heterozygotes, each sib of an affected individual has a 25% chance of being affected with the disorder. Cascade testing may identify other family members at risk for AATD. It is estimated that approximately 3% of North Americans are heterozygotes for PI*MZ. Generally, there is no increased risk of pulmonary disease in heterozygotes; however, some studies have

reported that these individuals may be slightly more susceptible to the development of air-flow obstruction [2].

Cystic fibrosis

Overview

Cystic fibrosis (CF) is a multisystem disorder that affects airways, lungs, intestines, liver, exocrine pancreas, sweat glands, and the reproductive tract. CF is caused due to pathogenic variants in *CFTR*, which encodes a chloride channel belonging to the ATP-binding cassette transporter superfamily on the epithelial cell surfaces. The incidence of CF in the United States is estimated to be 1 in 3200 live births in individuals of Northern European background; it is the most common life-limiting autosomal recessive disorder in that population [3]. As CF can be diagnosed by newborn screening, the diagnosis in a majority of individuals in the United States is now made very early during life. However, prior to the newborn screening programs, one study estimated that 7% of individuals with CF, especially the milder forms, were diagnosed during adulthood [4]. Whereas the manifestations of CF can be highly variable, most individuals eventually develop chronic respiratory tract infections and significant pulmonary disease.

Evaluation

Sinopulmonary symptoms are common in the majority of patients with classic CF. Nasal congestion, chronic cough, postnatal drip, headaches, nasal polyps, and chronic sinusitis are the commonly reported sinus-related symptoms. Thick secretions in the airways lead to decreased clearance of and colonization by bacteria, recurrent airway infection, damage to airway walls, bronchiectasis, and progressive lung disease. Exocrine pancreatic insufficiency develops in nearly 85% of individuals; recurrent bouts of pancreatitis can be a disabling feature. Hepatomegaly and elevated plasma aminotransferase and alkaline phosphatase activity can be observed; however, progressive liver disease is not common. Most men with CF are infertile because of congenital bilateral absence of vas deferens (CBAVD). The diagnosis of CF is made based on clinical features, biochemical testing, and genetic testing. The primary biochemical test used for confirmation of diagnosis is the sweat chloride test. Sweat chloride level > 60 mmol/L is abnormal and supports a diagnosis of CF.

Genetic testing

Genetic testing has significant implications for the diagnosis and management of CF. In individuals with milder phenotypes and in those with intermediate sweat chloride values, testing can help confirm a diagnosis. In individuals with classic CF, in whom the diagnosis is not in question, genetic testing can be of help in choosing appropriate genotype-specific therapy. For diagnostic testing, typically, full gene sequencing and assays to detect deletions or duplications are preferable. For carrier screening, in addition to full gene sequencing, panel-based tests that assess for the most commonly occurring pathogenic variants are available. Whereas the number of pathogenic variants that are included in the panel-based tests varies

depending on the diagnostic laboratory, the American College of Medical Genetics recommends a panel that contains at least 23 of the most commonly occurring pathogenic variants. Sequencing of *CFTR* is also helpful in evaluation of individuals without classic CF but other *CFTR*-related manifestations such as recurrent pancreatitis and male infertility. It is important to note that parental testing may be required to establish the phase of two variants detected in an individual, particularly when the clinical phenotype is atypical.

Management

Management of CF requires a multidisciplinary approach and treatment of pulmonary disease is best coordinated at centers with experience in treating individuals with CF. Clearance of airways using hypertonic saline and inhalational DNase, prevention of infections with influenza and pneumococcal vaccinations, use of inhalational bronchodilators, and anti-inflammatory therapy are standard measures to treat pulmonary disease. Individuals with pancreatic insufficiency require pancreatic enzyme replacement and appropriate supplemental nutritional therapies. Disease-specific therapy with *CFTR* modulators, which improve the conductance through *CFTR*, is an exciting new development in the treatment of CF. Some modulators such as elexacaftor and tezacaftor facilitate the processing and trafficking of F508del-*CFTR* and increase the amount delivered to the cell surface. Ivacaftor potentiates the *CFTR* channel opening at the cell surface in individuals with specific genotypes (e.g., G551D, G1244E, and G1349D, among others). Recent studies have shown that in individuals who are homozygous for two F508del variants, a combinatorial therapy with elexacaftor-tezacaftor-ivacaftor leads to improvement in respiratory symptoms and decrease in episodes of acute exacerbation [5]. Gastrointestinal and liver disease are best managed by specialists. Men with CF are infertile but may father children using assisted reproductive technologies.

Genetic counseling

Classic and nonclassic forms of CF and CBAVD are inherited in an autosomal recessive manner. *CFTR*-related recurrent pancreatitis in the absence of other features of CF may occur due to heterozygous variants in *CFTR*, especially when co-occurring with variants in genes that increase the risk for pancreatitis such as *SPINK1* and *CTRC*. The carrier frequency for a pathogenic variant in *CFTR* is estimated to be 1 in 25 in non-Hispanic Whites, 1 in 24 in individuals with Ashkenazi Jewish heritage, 1 in 58 in Hispanics, and 1 in 60 in African Americans. Several professional societies recommend that carrier screening should be offered to all couples planning pregnancy and to all pregnant women. The standard carrier screening panel includes 23 common pathogenic variants. For individuals of non-white, non-Ashkenazi Jewish ethnicities, a panel that interrogates a higher number of pathogenic variants than just the 23 common pathogenic variants could theoretically provide a higher carrier detection rate; however, it is hard to generalize this as the variants included in the expanded panels vary. Many carrier tests offer full gene sequencing. Reflex testing for intronic variants in *CFTR* is required in specific circumstances. If one of the variants identified is the Arg117His, additional testing to assess a poly-T tract in the noncoding region should be performed. If the Arg117His variant is found *in cis* (i.e., on the same chromosome) with a 5T

intronic variant, and there is a second known pathogenic variant *in trans*, the phenotype generally tends to be of classic CF. However, if instead of 5T, the poly-T tract had 7T or 9T variants, then the phenotype is less severe and can range from no symptoms to mild pulmonary disease. If the Arg117His variant is found in *trans* (i.e., on the opposite chromosome) with a 5T intronic variant, the likelihood of CBAVD is significantly increased in men.

Primary ciliary dyskinesia

Overview

Primary ciliary dyskinesia (PCD) comprises a heterogeneous group of disorders that are characterized by either congenital absence of cilia or abnormalities in ciliary structure and motility. PCD is caused by a deficiency of proteins required for the normal structure or function of central core (axoneme), ciliary membrane and matrix, or proteins required for ciliary assembly. To date, more than 30 different types of PCD that are caused by pathogenic variants in over 40 genes have been described. As ciliary motility is critical in the functioning of respiratory epithelia, the most common features of PCD are recurrent infections of the upper and lower respiratory tract. In some forms of PCD, heterotaxy (discordance of right and left patterns of ordinarily asymmetric structures) and situs inversus can be observed due to embryonic and nodal ciliary abnormalities [6]. Most men with PCD are infertile due to immotile spermatozoa.

Evaluation

There is significant heterogeneity in the clinical presentation of PCD; however, most patients manifest symptoms during childhood. Chronic and perennial rhinosinusitis is a classic symptom of PCD. Chronic otitis media and recurrent episodes of acute otitis media are common during childhood and can lead to conductive hearing loss. Cough with expectoration and wheezing are features that suggest lower airway infection and inflammation. Recurrent inflammation of airways leads to bronchiectasis. Individuals with heterotaxy commonly have congenital cardiovascular malformations including septal defects, transposition of the great vessels, double outlet right ventricle, and anomalies of the vena cavae. The diagnostic evaluation should include appropriate imaging of chest and lungs, pulmonary function tests, as well as evaluation for congenital heart disease.

Genetic testing

Diagnostic testing for PCD include measurement of nasal nitric oxide and tests for ciliary ultrastructure and function, such as high speed videomicroscopy analysis and transmission electron microscopy. However, the applicability and utility of these tests are limited by their availability and the expertise needed for their interpretation. Thus, genetic testing has become a first line test for diagnosis. Panel-based tests for PCD are available. If such testing does not identify a molecular cause, exome and genome sequencing may be considered.

Management

To date, there are no disease-specific therapies to improve ciliary function. Management should include aggressive measures to enhance clearance of mucus, prevent respiratory infections, and treat bacterial infections.

Genetic counseling

Most forms of PCD are inherited in autosomal recessive manner; however, X-linked (*PIH1D3* and *OFD1*) and autosomal dominant (*FOXJ1*) modes of transmission are also known. Identifying molecular basis of PCD is important for cascade testing and prenatal testing.

Restrictive lung disease

Idiopathic familial pulmonary fibrosis

Overview

Interstitial lung diseases (ILDs) are characterized by alveolar abnormalities. There are different subtypes of ILD based on specific clinical features; fibrosing ILDs is one subtype characterized by both interstitial abnormalities and irreversible pulmonary fibrosis. When a secondary etiology is not identifiable for a fibrosing ILD, the condition is categorized as idiopathic pulmonary fibrosis (IPF). IPF is relatively rare and has an estimated annual incidence of 10 cases per 100,000 person years [7]. When IPF is observed in two or more members of a family, it is known as idiopathic familial pulmonary fibrosis or familial interstitial pneumonia (FIP). About 20% of pulmonary fibrosis is familial in nature.

Evaluation

Individuals with IPF complain of dry cough and shortness of breath with exertion. Some develop fever, weight loss, fatigue, myalgias, and arthralgias. The clinical manifestations are usually observed between the fifth and seventh decades of life. The physical examination typically reveals inspiratory crackles on auscultation. Pulmonary function tests reveal a restrictive pattern of lung disease. The American Thoracic Society has established diagnostic criteria for IPF [8]. A diagnosis of IPF is made in the presence of at least three of the following four criteria:

- Absence of exposure to environmental agents
- Abnormal pulmonary function tests
- Computed tomography (CT) scan reveals bibasilar reticular opacities with or without ground glass
- Exclusion of alternative diagnosis by surgical lung biopsy or transbronchial or bronchioalveolar lavage

Familial pulmonary fibrosis is diagnosed in an individual with IPF who has at least one other first-degree relative with IPF and/or a pathogenic variant in one of the four genes known to cause IPF. The differential diagnosis for IPF includes other genetic disorders with

parenchymal lung disease such as Hermansky–Pudlak syndrome, neurofibromatosis type 1, Niemann–Pick disease type B, Gaucher disease, and familial hypocalciuric hypercalcemia type 1.

Genetic testing

Common and rare genetic variants are associated with the development of pulmonary fibrosis. Approximately one-third of IPF is caused by common variants. These are variants with a minor allele frequency (MAF) of $>5\%$. These common variants can be found in both sporadic and familial pulmonary fibrosis and are characterized by incomplete penetrance. The most widely known common variant linked to pulmonary fibrosis is the rs35705950 located in the promoter region of the *MUC5B* gene [9]. Single-nucleotide polymorphisms in other genes such as *TERT*, *TOLLIP*, and *SPPL2C* have also been associated with PF [10].

Rare variants with lower population allele frequencies ($\text{MAF} < 0.1\%$) and larger effect sizes have also been identified as causes for IPF. This includes two categories of genes: (1) those involved in telomere maintenance and (2) those involved in surfactant metabolism. Abnormalities in pathways affecting telomere maintenance lead to shortening of telomeres, activation of the DNA damage response, and cellular senescence. Genes that encode proteins required for telomerase enzyme (*TERT* and *TERC*), telomere biogenesis (*DKC1*, *PARN*, *NAF1*), and telomere protection (*RTEL1*, *TINF2*) have been implicated in the etiology of pulmonary fibrosis. The most commonly associated gene is *TERT*. Pathogenic variants in *TERT* are found in $\sim 15\%$ of families with IPF and in $\sim 3\%$ of individuals with sporadic IPF. Genes encoding surfactant proteins A and C (*SFTPA1*, *SFTPA2*, and *SFTPC*) have been associated with adult-onset pulmonary fibrosis. Pathogenic variants in these genes lead to gain of toxic function from protein misfolding and increased endoplasmic reticulum stress. Pathogenic variants in these genes are found in 8%–15% of cases of FIP and in 1%–3% of sporadic IPF cases.

Management

The management strategy depends on the type of IPF and is largely symptomatic. Nonpharmacologic treatments include oxygen therapy and pulmonary rehabilitation. Medical therapy with corticosteroids, immunosuppressive and cytotoxic medications, and antifibrotic agents has been used to slow the decline of lung function. Lung transplantation is considered in individuals who are unresponsive to medical therapy. Periodic pulmonary function tests and high-resolution CT scans of the chest are recommended for surveillance.

Genetic counseling

The inheritance of pulmonary fibrosis is somewhat unclear; autosomal dominant, with reduced penetrance seems likely, particularly with pathogenic variants in genes involved in maintenance of telomeres.

Spontaneous pneumothorax

Overview

Pneumothorax, a condition characterized by the collection of air in the pleural cavity, can be a result of trauma, underlying airway disease, pulmonary infections, ILD, congenital lung malformations, and connective tissue disorders. Pneumothorax that occurs without a precipitating external event is termed primary spontaneous pneumothorax (PSP). A majority of PSP are sporadic and often occur in tall, thin individuals between the ages of 18 and 40 years. A positive family history is present in 10% of individuals with PSP. Some of the common etiologies for the familial forms of PSP are listed in [Table 20.1](#).

Evaluation

It is important to identify familial PSP as many genetic disorders that present with pneumothorax have extrapulmonary manifestations that can have serious complications such as aortic dissection and cancer. While the diagnosis of pneumothorax can be easily made by history and imaging, elucidating the underlying genetic etiology may be more challenging and requires a high level of clinical suspicion. Common clinical presentation of pneumothorax includes dyspnea and acute onset of sharp and steady pleuritic chest pain. Taking a detailed family history as well as looking for extrapulmonary features may help narrow down the differential diagnosis.

Genetic testing

Genetic testing for a panel of genes known to cause genetic disorders that present with PSP would be valuable in evaluation of familial forms of PSP. This is particularly desirable when the phenotype is not classical for any specific genetic disorder or if the patient is young and may not have developed other extrapulmonary manifestations.

Table 20.1 Common etiologies of primary spontaneous pneumothorax.

Pathophysiology	Disorders	Comments
1. Pulmonary disease	Alpha-1 antitrypsin deficiency Cystic fibrosis	This chapter
2. Connective tissue disorders	Cutis laxa Homocystinuria Loeys–Dietz syndrome Marfan syndrome Vascular EDS	Chapter 10
3. Defects of tumor suppressor genes	Birt–Hogg–Dube syndrome Tuberous sclerosis	Chapters 8 and 24

Management

The acute management of pneumothorax involves assessing the clinical status of the individual and the size of the pneumothorax. Individuals with small pneumothoraces (size <3 cm at the lung apices and <2 cm at the hilum), and normal respiratory rate, heart rate, blood pressure, and oxygen saturation can be managed conservatively with supplemental oxygen. Individuals with large pneumothoraces and cardiopulmonary compromise will need emergent chest tube thoracostomy. Individuals with multiple blebs or cysts, recurrent episodes of pneumothorax, or individuals with prolonged air leak after tube thoracostomy may need a definitive procedure like pleurodesis to prevent recurrence.

Vascular disorders

Pulmonary hypertension

Overview

Pulmonary arterial hypertension (PAH) is characterized by mean arterial pressure >20 mm Hg, normal left atrial pressure, and pulmonary vascular resistance >3 wood units. PAH has been subdivided into five groups:

Group 1: Idiopathic or familial PAH with or without germline variants in genes known to cause PAH.

Group 2: PAH due to left heart disease

Group 3: PAH due to chronic lung diseases and hypoxemia

Group 4: PAH due to pulmonary artery obstruction or chronic thromboembolic conditions

Group 5: PAH due to unidentified mechanisms

The heritable form of PAH can be subclassified into familial (two or more family members with PAH) and simplex (single occurrence in the family) forms. Familial segregation is seen in 6%–10% of patients with PAH.

Evaluation

The clinical presentations of PAH include symptoms such as dyspnea during exertion, syncope, dizziness, edema of ankles or legs, chest pain, and palpitations. The most frequent symptom is persistent dyspnea on exertion. PAH usually presents in the third or fourth decades of life with the mean age of diagnosis being 36 years. The average survival period following diagnosis is 2.8 years. PAH affects women more than men with the affected female-to-male ratio being approximately 3:1. The diagnostic evaluation typically begins with EKG, echocardiogram, and chest radiographs. Other investigations such as pulmonary function tests, ventilation-perfusion scans, high-resolution CT scan of the chest, arterial blood gases, and pulmonary angiography may be useful for making the diagnosis. Finally, right heart catheterization helps to make the definitive diagnosis.

Genetic testing

To date, about 16 genetic risk factors have been identified. Most heritable PAH is caused by a pathogenic variant in *BMPR2* (53%–86% of patients with family history of PAH) [11]. Pathogenic variants in *BMPR2* are also found in 14%–35% of patients with idiopathic/sporadic PAH. Other genes such as *CAV1* and *KCNK3* are also implicated in PAH. Hereditary hemorrhagic telangiectasia (HHT) and PAH can co-occur in the same family if the disorder is caused due to pathogenic variants in *ACVRL1*. PAH is generally inherited in an autosomal dominant manner with reduced penetrance. Autosomal recessive forms of PAH have also been identified with overt features of venous or capillary involvement and is associated with pathogenic variants in genes such as *EIF2AK4*. Besides monogenic causes, various genetic modifiers that determine the clinical phenotype of PAH have been identified.

Management

The supportive management of PAH includes cessation of smoking, initiation of exercise program, pulmonary rehabilitation, influenza and pneumococcal vaccinations, supplemental oxygen therapy, anticoagulation, and diuretics. Patients are advised to avoid: appetite suppressants (e.g., fenfluramine/phentermine), agents that cause vasoconstriction, high altitude, and oral contraceptives and other estrogen-containing medications. Therapies that can reduce the pulmonary arterial pressure include prostacyclin analogs, endothelin receptor antagonists, phosphodiesterase inhibitors, guanylate cyclase stimulators, and oral calcium channel blockers. Potential future therapies include medications such as macitentan and a tissue-targeting novel ET-1 receptor antagonist. Other therapies such as sildenafil, tyrosine kinase inhibitors, selexipag, and riociguat are being investigated in clinical trials [12].

Genetic counseling

The majority of types of PAH are inherited in an autosomal dominant manner with incomplete penetrance. Genetic counseling and testing for at-risk family members can be undertaken if the pathogenic variant is known in the family.

Hereditary hemorrhagic telangiectasia

Overview

HHT is a disorder characterized by the presence of multiple vascular malformations. Individuals with the disorder typically have telangiectasias (small dilated blood vessels) on the face, lips, oral mucosa, tongue, and nailbeds. Telangiectasia in the nasal cavity lead to recurrent epistaxis, which can sometimes be difficult to control. Bleeding from telangiectasias or arteriovenous malformations (AVMs) in the pulmonary, hepatic, gastrointestinal, cerebral, and spinal vasculature can result in serious complications. Pulmonary, liver, and cerebral AVM are found in nearly half, one-third, and one-tenth of individuals with HHT, respectively [13,14]. HHT is caused by pathogenic variants in genes that encode proteins involved in the transforming growth factor-beta signaling pathway. Nearly 90% of HHT is caused by pathogenic variants in three genes: *ENG*, *ACVRL1*, and *SMAD4* [14].

Evaluation

The age of onset and severity of symptoms in HHT is highly variable. More than two-thirds develop some symptoms during the second decade of life and approximately 90% manifest signs of disease by the age of 40 years. Spontaneous recurrent epistaxis is the most common clinical manifestation. Mucocutaneous telangiectasia may not be evident in children or young adults and may develop later during life. Telangiectasia can occur throughout the gastrointestinal tract but are common in the stomach or duodenum. Acute gastrointestinal bleeding can be life threatening and slow, but recurrent gastrointestinal bleeding can present as iron-deficiency anemia. Whereas most individuals with pulmonary AVM are unaware of their presence, large AVM can result in a right-to-left shunt and present with hypoxia, cyanosis, and polycythemia. Bypassing of the pulmonary capillary filters can result in paradoxical embolism leading to transient ischemic attacks (TIAs), embolic stroke, and cerebral abscess. Cerebral and spinal AVM can cause symptoms by pressure effects; bleeding from these vascular malformations can have devastating neurological consequences. Individuals with HHT due to pathogenic variants in *SMAD4* can have hamartomatous polyps in the gastrointestinal tract. This disorder is termed juvenile polyposis/HHT syndrome [15].

The diagnosis of HHT is made based on the Curaçao diagnostic criteria that include (1) spontaneous and recurrent epistaxis, (2) mucocutaneous telangiectasia at the typical sites, (3) visceral involvement (e.g., AVM in pulmonary, cerebral, or hepatic circulations and gastrointestinal telangiectasia), and (4) first-degree relative with HHT [16]. From these criteria, a definitive diagnosis of HHT is made when three or more criteria are met. The diagnosis of HHT is “suspected” when two criteria are met and is considered “unlikely” when one or none of the criteria are met.

Complete blood count is required in all to assess for anemia. Contrast echocardiography and/or noncontrast thin cut CT of the chest to detect pulmonary AVM are recommended in all individuals. Routine screening for cerebral AVM in asymptomatic individuals is controversial, especially in children; however, if screening is desired, magnetic resonance imaging with contrast is the preferred diagnostic modality.

Genetic testing

The diagnosis may be confirmed by identification of a pathogenic variant in genes that are known to cause HHT. Whereas the number of genes included in a panel vary, *ENG*, *ACVRL1*, and *SMAD4* are almost always included. It is to be noted that in 10%–15% of individuals in whom HHT is suspected, genetic testing may not reveal a molecular cause for the disorder. Genetic testing can be of considerable utility in cascade screening of individuals who are at risk for the disorder in a family.

Vignette 20.1

BC, a 26-year-old woman who has a history of fatigue, effort intolerance, recurrent epistaxis that occurs two times per week, and black tarry stools presents to the clinic for evaluation. The family history is significant for recurrent epistaxis in her father, her brother, and two of her

paternal uncles. Her father also had a history of many colon polyps. On examination, you note that she has small dilated pinpoint blood vessels on her lip.

This clinical presentation is consistent with juvenile polyposis/HHT syndrome. The management should focus on confirming molecular diagnosis and evaluation for anemia, pulmonary AVM, liver functions, and gastrointestinal bleeding. Given that the risk for gastrointestinal cancer is increased in this condition, upper gastrointestinal endoscopy and colonoscopy should also be instituted.

Management

For epistaxis, local measures such as saline spray, nasal lubrication are recommended in all. For individuals who have recurrent epistaxis, evaluation by an otorhinolaryngologist is recommended. Laser therapy is generally preferred over cauterization for epistaxis. Systemic therapies with tamoxifen, tranexamic acid, or bevacizumab may be required in a subset of patients. Individuals with pulmonary AVM should get prophylactic antibiotics prior to dental and nonsterile procedures. Definitive treatment of AVM is required in individuals with pulmonary symptoms such as hypoxia and dyspnea. Such treatment is also important for the prevention of paradoxical embolization. Outlining the specific strategies for management of pulmonary AVM is beyond the scope of this chapter, and readers are encouraged to refer to guidelines from professional societies [17]. Generally, large AVM with feeding artery size greater than 2 or 3 mm requires consideration for occlusion via embolotherapy or devices. The management of cerebral lesions is very complex and should be performed by centers with experience in such procedures.

Genetic counseling

HHT is inherited in an autosomal dominant manner. Most affected individuals have an affected parent; however, the clinical manifestations demonstrate considerable heterogeneity and it is possible that mildly affected members may not have reported symptoms. *De novo* variants in *ENG* and *ACVRL1* are rare. There are some genotype-phenotype correlations; pulmonary and cerebral AVMs tend to be more common with pathogenic variants in *ENG1* while hepatic disease is more common with *ACVRL1*. Individuals with juvenile polyposis/HHT will need to undergo cancer surveillance for upper gastrointestinal and colonic cancers.

Lung cancer

Lung cancer is the second most commonly diagnosed cancer, worldwide. The leading cause of lung cancer is smoking. However, about 10%–15% of lung cancer occurs in individuals who have never smoked [18]. Risk factors in this population include environmental exposures such as radon, asbestos, air pollution; genetic alterations; and lung diseases such as tuberculosis, emphysema, and chronic bronchitis. There are two distinct types of lung cancer—small cell lung cancer (SCLC), which accounts for 15%, and nonsmall cell lung cancer (NSCLC), which accounts for 85% of all lung cancers. NSCLC can be further divided into three subtypes based on the histology—large cell, squamous cell, and adenocarcinoma.

More than two-thirds of lung cancer in never smokers is adenocarcinoma. It is widely known that lung cancer is predominantly associated with environmental carcinogens included in tobacco smoke and air pollution, but familial clustering of lung cancer is also observed [19]. Whether this familial aggregation is due to shared environmental factors or shared genetic factors is poorly understood and difficult to tease out. Many studies have demonstrated that individuals with a family history of lung cancer (particularly in their siblings) are two to three times more likely to develop lung cancer [20]. Several somatic variants in genes such as *EGFR*, *TP53*, and *KRAS* have been identified in lung cancers and are known to impact therapy. However, to date only a few germline variants that confer a significant risk for lung cancer have been identified. Genome-wide association studies have shown an increased risk of lung cancer in individuals with certain high-risk alleles located on chromosomes 15q and 6p and variants in candidate genes including *HER2* and *EGFR*. More molecular and whole-genome analyses are necessary to identify genes causing familial lung cancer. Genetic testing for hereditary lung cancer is not done routinely but testing for somatic genetic alterations including *EGFR*, *ALK* rearrangements, c-MET amplifications, *ROS1* rearrangements, and *KRAS* mutations has now become standard of care, as they impact choice of therapy for lung cancer such as erlotinib, crizotinib, and EGFR tyrosine kinase inhibitors.

Points to remember

- AATD presents with signs and symptoms of COPD.
- CF is the most common life-limiting autosomal recessive disorder in individuals of Northern European background
- Genotype-specific targeted therapies are available for the treatment of CF.
- In individuals with chronic sinopulmonary symptoms and heterotaxy or situs inversus, PCD should be considered in the differential diagnosis.
- Genetic testing in individuals with recurrent PSP can help diagnose conditions in which other organ system involvement can cause serious morbidity.
- PAH and HHT can present with significant morbidity due to involvement of pulmonary vasculature.
- Few Mendelian forms of lung cancer have been described, and thus germline testing is not likely to have a high diagnostic yield.

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Psychiatric disorders

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Introduction

An evaluation of mental health disorders/mental illnesses must include both a physical examination and a psychological assessment. The purpose of the physical examination and possible laboratory tests is to rule out underlying physical concerns such as thyroid dysfunction. There is no laboratory test that can determine if someone has mental illness. Instead, laboratory tests can be used to rule out other conditions such as thyroid dysfunction. Mental illnesses are typically diagnosed based on specific behaviors and emotions exhibited by the patient. In addition, features can be quite dynamic even throughout one individual's lifetime. An individual may be diagnosed with depression at one stage in their life and later present with bipolar disorder. While there are several well-described genetic syndromes that are associated with an increased likelihood for mental illnesses, many of these syndromes have other characteristic features such as autism, developmental delays, or medical complications.

The psychological assessment will include asking questions about a patient's symptoms, behaviors, and feelings. Multiple options such as the Patient Health Questionnaire can be helpful as an initial approach to an assessment. These questionnaires often include questions about behaviors such as suicidal thoughts, feelings of depression, sleep patterns, energy levels, eating habits, and substance abuse, among others. It is important to determine the severity, timing/onset, and duration of symptoms. In addition to current symptoms, it is also important to assess the patient's social history; If there has been abuse, trauma, a particularly stressful or unstable living environment or family situation, etc. All of these factors can play a role in someone's overall mental health and become an important part of the evaluation. Assessing the family history for any mental illnesses can be informative for assessing risk and the patient's perception of what having a mental illness means.

Multiple individuals in the family with a psychiatric disorder (of any type), individuals with birth defects, developmental delays, or seizures, among other findings can be suggestive of a hereditary increased risk for a genetic predisposition to mental illnesses or a genetic syndrome with mental illness as a feature.

Mental health conditions

Mental illnesses affect one in four individuals worldwide. Most people with a mental illness are never diagnosed with a specific genetic syndrome. Therefore, this group of conditions is different from many other genetic conditions. Mental illnesses are the result of a combination of complex influences including inherited factors, environmental exposures, and personal experiences.

Major mental illnesses commonly seen are a major depressive disorder, generalized anxiety disorder, schizophrenia, and bipolar disorders, which are identified based on the *Diagnostic and Statistical Manual of Mental Disorders* version 5 (DSM-5) criteria.

Major depressive disorder

At least five of the following symptoms are observed during the same 2-week period most of the day or nearly every day. At least one is either a depressed mood or loss of interest/pleasure and this must be different than previous functioning/mood.

- Depressed mood
- Markedly diminished interest/pleasure in all or almost all activities
- Change in appetite
- Insomnia or hypersomnia
- Psychomotor agitation or retardation (observable by others)
- Fatigue or loss of energy
- Feelings of worthlessness or excessive/inappropriate guilt
- Diminished ability to think, concentrate, or indecisiveness
- Recurrent thoughts of death, suicidal ideation with or without a specific plan

Generalized anxiety disorder

Excessive anxiety and worry about multiple events or activities that are difficult to control more days than not for at least 6 months. These feelings impair social, occupational, or other areas of functioning and are not attributed to physiological effects of a substance, medical condition, or other mental disorder. At least three of the following symptoms are present for adults, but only one of the following is found in children.

- Restlessness or feelings of being on edge
- Easily fatigued
- Difficulty in concentrating
- Irritability
- Muscle tension
- Sleep disturbances

Schizophrenia

The level of functioning in one or more major areas such as self-care, work or interpersonal relations must be markedly decreased and at least two of the following must be present for a significant portion of time during a 1-month period (if untreated). At least one must be the first, second, or third listed. Continued signs of disturbance persist for at least 6 months and are not attributed to the physiological effects of a substance, medical condition, or other mental disorder.

- Delusions
- Hallucinations
- Disorganized speech
- Grossly disorganized or catatonic behavior
- Negative symptoms such as diminished emotional expression

Bipolar disorders

Bipolar I is the presence of a manic episode that is either preceded or followed by a hypomanic or major depressive episode. While bipolar II is a current or past hypomanic episode and a current or past major depressive episode.

A manic episode is a distinct period of abnormally and persistently elevated, expansive, or irritable mood, goal-directed activities/energy that is lasting at least 1 week, present most of the day, nearly every day and results in marked impairment in social or occupational functioning and may require hospitalization to prevent harm. A hypomanic episode lasts for four consecutive days and is present most of the day, nearly every day but does not cause marked impairment in social or occupational functioning or require hospitalization. These mood changes cannot be attributed to the physiological effects of a substance, medical condition, or other mental disorder.

Manic/hypomanic episode—at least three of the following are present and considered a noticeable change from usual behavior

- Inflated self-esteem or grandiosity
- Decreased need for sleep
- More talkative or sense of pressure to keep talking
- Flight of ideas or racing thoughts
- Distractibility
- Increase in goal-directed activities or psychomotor agitation
- Excessive involvement in activities that have a high potential for negative consequences

The incidence of these disorders can vary with anxiety (of any type) being the most common, affecting approximately one in three individuals and schizophrenia or bipolar disorder being less common affecting approximately 1 in 250 to 1 in 100 individuals [1].

A combination of genetic “nature” and environmental “nurture” factors interact and may lead to the development of a psychiatric disorder [2]. An individual’s environment includes

nutrition, personal experiences, upbringing, stressors, socioeconomic status, lifestyle choices, and other external factors that may result in changes in how one's genes function or are expressed. Such modification of gene expression is known as an epigenetic effect [3]. In addition, certain genetic variants may influence the environments in which someone chooses to participate. For example, preliminary data suggests that genetic changes in the dopamine transporters or serotonin receptor genes may predispose someone to be more impulsive and/or to seek out risky behaviors [4]. Finally, it is important to know that even though a large combination of inherited genetic factors are likely to play a role in an individual's overall mental health, each gene may have only a very small impact on the outcome and not all of these genetic factors or predispositions are inherited.

Genetic conditions associated with an increased risk for mental illness

There are several well-described genetic syndromes that are associated with an increased likelihood for mental illnesses such as schizophrenia, depression, anxiety, bipolar disorder, and/or autism spectrum disorder, in addition to other accompanying physical features or characteristics. The following is a brief description of several of these syndromes and is not an exhaustive list of possible syndromes or their associated features [5].

Chromosome microdeletion/duplication syndromes

Overview

Chromosome microdeletion/duplication syndromes are the result of deletions or duplications of segments of chromosomes that may include one or more genes. Different syndromes have now been identified since the availability of comparative genomic hybridization technologies. Many of these conditions may include mental illnesses as part of their phenotype.

Evaluation

The following are some examples of microdeletion or microduplication syndromes that include a psychiatric component. For each condition, the physical as well as cognitive or psychiatric features are summarized in brief.

- *15q13.3 deletion*
 - Physical/medical: epilepsy but no specific constellation of physical features
 - Cognitive/psychiatric: developmental delays/intellectual disabilities, schizophrenia, attention problems, mood disorders, and/or autism spectrum disorder
 - Prevalence: approximately 1/5000 individuals
 - Symptom onset: childhood

- *16p11.2 deletion syndrome*
 - Physical/medical: macrocephaly, vertebral anomalies, and/or epilepsy
 - Cognitive/psychiatric: developmental delays/intellectual disabilities, autism spectrum disorder, and/or attention-deficit hyperactivity disorder (ADHD)
 - Prevalence: approximately 1/3000–1/4000 individuals
 - Symptom onset: childhood though some individuals have such mild symptoms they are not identified until adulthood
- *17p11.2 deletion syndrome* (also known as Smith – Magenis syndrome)
 - Physical/medical: hypotonia, hyporeflexia, short stature, ophthalmological abnormalities, otolaryngologic abnormalities, dental abnormalities, neuropathy, brachycephaly, synophrys, deep-set eyes, midfacial retrusion, and/or coarsening of face with age
 - Cognitive/psychiatric: developmental delay/intellectual disabilities, sensory integration problems, self-injurious behaviors, sleep disturbances, hyperactivity, and/or aggression
 - Prevalence: 1/15,000–1/25,000 individuals
 - Symptom onset: childhood
- *22q11.2 deletion syndrome* [also known as DiGeorge syndrome or Velo-Cardio facial syndrome (VCFS)]
 - Physical/medical: congenital heart defects, palatal abnormalities, immune deficiencies, hypocalcemia, characteristic facial features including hypertelorism, cleft lip/palate, craniosynostosis, prominent nasal root, and/or bulbous nasal tip
 - Cognitive/psychiatric: developmental delays/intellectual disabilities, autism spectrum, anxiety disorder, and/or psychiatric illness (specifically schizophrenia)
 - Prevalence: approximately 1/4000 individuals
 - Symptom onset: childhood though some individuals have such mild symptoms they are not identified until adulthood

Genetic testing

Testing typically consists of comparative genomic hybridization (array CGH also known as a chromosomal microarray) or possibly fluorescence in situ hybridization. Tests evaluate for missing or extra segments of DNA.

Genetic counseling

When an individual has one of these conditions, there is typically a 50% chance that each of their children may inherit the condition. In some cases, these disorders are inherited from a parent (especially in the cases of those that can be quite mild), while in others, the genetic change is *de novo*. Individuals should be counseled regarding the availability of genetic testing and the variable expressivity of these conditions.

Single-gene Mendelian disorders

Overview

Single-gene Mendelian disorders are typically the result of pathogenic single-nucleotide variants within a specific gene. These conditions may be *de novo* or inherited in an autosomal dominant, autosomal recessive, X-linked, or mitochondrial manner.

Evaluation

When considering the medical history of a patient, it is important to remember that the onset of mental illnesses can be incredibly variable from childhood to adulthood. Symptoms can begin prior to a formal diagnosis and a significant overlap is common. The diagnosis of a mental illness is based on psychological evaluation using *DSM-5*; not a physical examination, biochemical testing or genetic testing; however, these evaluations may be warranted when it is suspected that the patient has an underlying condition, in addition to or associated with, the psychiatric disorder [6]. It is possible for an individual to receive an initial diagnosis of anxiety or depression and later be diagnosed with bipolar disorder. This does not mean that the initial diagnosis was incorrect, but demonstrates a patient's current state since mental health is dynamic. The following are some examples of the Mendelian disorders that may include a psychiatric component. For each condition, the physical as well as cognitive or psychiatric features are summarized in brief.

- *Bardet – Biedl syndrome* (autosomal recessive)
 - Physical/medical: rod-cone dystrophy, polydactyly, and/or genitourinary abnormalities
 - Cognitive/psychiatric: developmental delay/intellectual disabilities, anxiety, mood disorders, depression, bipolar, obsessive compulsive disorder, and/or autism spectrum disorder
 - Genetic testing: at least 19 genes have been identified, a multigene molecular sequencing panel is often necessary
 - Prevalence: approximately 1/100,000 individuals
 - Symptom onset: childhood
- *CADASIL* (autosomal dominant)
 - Physical: Ischemic stroke and/or diffuse white matter lesions on brain
 - Cognitive/psychiatric: Progressive cognitive decline as an adult, migraines, mood disturbance, manic episodes, depression, and/or dementia
 - Testing: genetic molecular sequencing of *NOTCH3* gene
 - Prevalence: approximately 1/33,000 individuals
 - Symptom onset: variable (childhood to adulthood)
- *Fabry disease* (X-linked—males are more severely affected than females)
 - Physical/medical: pain in extremities, anhidrosis or hypohidrosis, corneal opacity, proteinuria with deterioration of renal functions, cardiomyopathy, vascular lesions, strokes, and/or fatigue
 - Cognitive/psychiatric: depression and/or anxiety

- Genetic testing: genetic molecular sequencing of *GLA* gene or α Gal A enzyme levels
- Prevalence: variable—approximately 1/3000 individuals (Italy) to 1/117,000 individuals (other ethnic groups)
- Symptom onset: childhood or adolescence (males) adulthood (females)
- *Hexosaminidase A deficiency* (autosomal recessive). This is a variable group of disorders with severity from infantile (also known as Tay Sachs) to chronic and adult onset
 - Physical/medical:
 - Infantile-progressive weakness, seizures, and death typically by 5 years of age;
 - Chronic/adult-muscle wasting, weakness, fasciculations, dysarthria, and/or cerebellar atrophy
 - Cognitive/psychiatric:
 - Infantile-severe developmental delays and lack of developmental progress;
 - Chronic/adult-psychotic depression, bipolar, schizophrenia, and/or paranoia
 - Genetic testing: genetic molecular sequencing of the *HEXA* gene. Hexosaminidase A enzyme levels can also be performed.
 - Prevalence: variable—approximately 1/3600 individuals (Ashkenazi Jewish population) to 1/250,000 individuals (other ethnic groups)
 - Symptom onset: variable (increased severity correlates with younger age at onset)
- *Huntington disease* (autosomal dominant)
 - Physical/medical: progressive chorea, bradykinesia, overall motor decline typically, and/or weight loss
 - Cognitive/psychiatric: cognitive decline, personality changes, depression, disinhibitions, hallucinations, depression, and/or anxiety among others
 - Prevalence: approximately 1/6000 individuals
 - Genetic testing: trinucleotide repeats in the *HTT* gene (> 40 trinucleotide repeats)
 - Symptom onset: adulthood
- *MELAS* (mitochondrial inheritance)
 - Physical/medical: stroke-like episodes, lactic acidosis, encephalopathy with seizures, muscle weakness, exercise intolerance, headaches, vomiting, hearing loss, neuropathy, short stature, and/or cardiomyopathy
 - Cognitive/psychiatric: developmental delays/intellectual disabilities, bipolar disorder, anxiety, psychosis, possible dementia
 - Genetic testing: molecular mitochondrial DNA sequencing
 - Prevalence: variable—approximately 1/2400 individuals (Australia) to 1/500,000 individuals (Japan)
 - Symptom onset: childhood to adulthood
- *Phenylketonuria* (autosomal recessive). Note: If treated with dietary restrictions, symptoms are significantly reduced or eliminated.
 - Physical/medical: if untreated —epilepsy, musty body odor, and decrease in pigmentation
 - Cognitive/psychiatric: if untreated—developmental delays/intellectual disabilities. In addition some individuals can have anxiety, depression, phobias, and/or panic attacks (more common if treated but still not well controlled)

- Genetic testing: genetic molecular sequencing of the *PAH* gene or plasma phenylalanine concentrations
- Prevalence: approximately 1/5000–1/10,000 individuals
- Symptom onset: shortly after birth
- Note: an unaffected baby of a mother with phenylketonuria that is not well controlled by diet can have microcephaly, brain malformations, congenital heart defects, limb malformations, tracheoesophageal fistula, and intellectual disabilities. In addition, if an adult begins to deviate from the recommend diet, symptoms such as the cognitive and psychiatric effects can develop as an adult.
- *POLG-related disorders* (most autosomal recessive)
 - Physical/medical: encephalopathy, intractable epilepsy, neuropathy, hepatic complications, lactic acidosis, myopathy, hearing loss, pancreatitis, ataxia, ophthalmoparesis, and/or ataxia
 - Cognitive/psychiatric: developmental delays, depression, mood disorder, and/or psychosis
 - Genetic testing: genetic molecular sequencing of the *POLG* gene
 - Prevalence: approximately 1/51,000 individuals. Some carrier parents can have milder symptoms as well
 - Symptom onset: variable (increased severity correlates with younger onset)
- *Tuberous sclerosis complex* (autosomal dominant)
 - Physical: hyperpigmentation of skin, facial angiofibromas, brain abnormalities such as cortical dysplasias, astrocytomas, seizures, angiomyolipomas, renal cell carcinomas, rhabdomyomas, and/or cardiac arrhythmias
 - Cognitive/psychiatric: developmental delays/intellectual disabilities, autism spectrum disorder, anxiety, depression, self-injurious behaviors, and/or sleep difficulties
 - Genetic testing: clinical criteria available as well as genetic molecular sequencing for the *TSC1* and *TSC2* genes
 - Prevalence: approximately 1/5800 individuals
 - Symptom onset: variable (childhood to adulthood)
- *Urea cycle disorders* (autosomal recessive and X-linked)
 - Physical/medical: encephalopathy, seizures, behavioral abnormalities, hyperammonemia, protein aversion, and liver disease
 - Cognitive/psychiatric: developmental delay/intellectual disabilities, anxiety, mood disorders, and attention problems
 - Genetic testing: single-gene or panel-based testing based on biochemical profile
 - Prevalence: 1 in 35,000 births
 - Symptom onset: variable (childhood to adulthood)
- *Wilson disease* (autosomal recessive)
 - Physical/medical: build-up of excess copper in body causing liver disease (jaundice, chronic liver disease), movement disorders such as tremors, poor coordination, chorea, rigid dystonia, and/or Kayser-Fleischer ring (deposition of copper in membrane of cornea)

- Cognitive/psychiatric: mood changes, depression, neurotic behaviors, and/or intellectual deterioration
- Genetic testing: genetic molecular sequencing of *ATP7B* gene. Low serum copper and ceruloplasmin concentrations with elevated urinary copper excretion can also be expected
- Prevalence: approximately 1/30,000 individuals
- Symptom onset: late childhood to adulthood

Genetic testing

In the course of an evaluation, a patient's physical or medical findings may be suggestive of a specific underlying genetic syndrome (such as those described above). In these cases, genetic testing can be used to diagnose the underlying genetic condition which may have mental illnesses as a feature.

Large scale genome-wide association studies compare the genetic variants [single-nucleotide polymorphisms (SNPs)] identified among individuals to look for correlations with various complex medical conditions such as heart disease and mental illnesses. Individual SNPs are not diagnostically predictive of symptoms. These studies do not establish causation [7], and initial results were disappointing because genetic variants (often SNPs) with only small impact factors were identified. Recently polygenic risk scores have been used as predictive screening models that take into account these small impact changes. While these SNPs individually result in a small impact on risk, collectively they increase the overall risk for a particular condition. However, a limitation of these studies is that they are often based on predominantly Caucasian populations and may not be generalizable to all. Individuals with various forms of mental illnesses such as schizophrenia, psychosis, and bipolar disorder have been evaluated for common SNPs that may be considered predisposition factors. While some studies suggest a link between certain SNPs and specific mental illnesses [8], others do not [9]. Therefore, more studies are required to use polygenic risk scores for mental illness risk assessment.

Vignette 21.1

MZ is a 36-year-old man that presented to the Genetics clinic for more information regarding his diagnosis of bipolar disorder. MZ was diagnosed in his 20s but has a long history of mental health concerns. He has attempted suicide on multiple occasions and has struggled to maintain a relationship or job as an adult. His history is significant for multiple traumatic events including seeing his mother and sister perish in a fire and the effects of his father dying by suicide. He is otherwise generally healthy. His family history is significant for his father having bipolar disorder and other family members with suspected, but not documented, mental health illnesses including his estranged brother. MZ suspected his illness was inevitable because of his father's diagnosis of bipolar disorder and wanted more information about the risk for his future children.

The genetic counseling session included discussion of his personal journey, trauma, family history, and what strategies he has used so far to maintain his mental health. The “Jar Model” [10] was used to explain the multifactorial nature of mental illness, as well as protective tools to improve mental health. It also allowed him to understand the risk for his children to be affected. It was also explained that a long-term or more in-depth counseling is outside the scope of the genetic practice. He was offered a referral to a psychiatrist or psychologist to continue with a psychological assessment, and therapy as needed. At the conclusion of the Genetics session, MZ was provided with resources for local counseling services that could assist with providing support and work with him to establish a more cohesive treatment plan.

Management

The management of mental illness will primarily be based on the recommendations of psychologists, psychiatrists, and other healthcare providers. Some of the genetic disorders such as 22q11 deletion syndrome, which have an increased risk for mental illness, have specific guidelines based on the genetic disorder. According to the *Journal of Pediatrics* [11], there are various evaluations necessary when someone is initially diagnosed with 22q11 deletion syndrome including calcium, parathyroid, thyroid-stimulating hormone levels, complete blood count with differential, renal ultrasound, palatal evaluation, echocardiogram, and possible electrocardiogram. In addition to these an immunologic, ophthalmologic, developmental, audiologic, genetic, and mental health evaluations are all pertinent. Depending on the age of the child, these evaluations may need to be delayed or repeated at various intervals to assess for aspects of the disorder that become more prominent with age, including mental illnesses, that may not be apparent until the child is older.

In some cases, pharmacogenomic information may help to guide the management of medication choices for the treatment of mental illnesses. See Chapter 29, Pharmacogenomics, for a detailed discussion on the utility of pharmacogenomics in clinical practice. Approximately 90% of all drugs are metabolized by seven different cytochrome enzymes [12]; changes in those genes (*CYP2C19*, *CYP2D6*, etc.) may result in actionable medical management changes [13]. In some studies, there is greater tolerability when using pharmacogenomic testing but the efficacy is similar to standard of care [14,15]. While research on pharmacogenomics is advancing, guidelines are still lacking for effective implementation.

Pregnancy and the postpartum period may also be a difficult time for some women to manage their mental health. Up to 30% of women experience a mental illness, such as depression, during their pregnancy. In addition, some women and providers have concerns about patients continuing to take their medications during pregnancy due to fear of negative consequences for the fetus. Since controlled studies are not feasible in this setting, data has been unclear. However, the consensus is that the benefit to the mother and fetus by using medications to treat the mental illness, such as depression, typically out-weighs the risks to both the mother and fetus. For example, even women with untreated depression have an increased risk for adverse pregnancy outcomes such as low birth weight and/or preterm labor. These women may also be more likely to have a significant depressive episode and

poor health habits affecting their overall health such as poor diet and lack of sleep [16]. It is necessary to balance the risks of the medication and the risks of the untreated mental illness on a case by case basis since both could have an adverse effect on the fetus and mother.

The diagnosis and management of mental illnesses are often complex. Not only is there a significant stigma around the conditions, which may make an individual hesitate to seek treatment, but the path to diagnosis can also be complicated and frustrating for both patients and providers. In addition, the treatment is often a series of trial and error methods regarding medications, counseling, behavioral therapies, diet, exercise, and other approaches. This treatment helps patients to understand the etiology and complexities of mental illnesses, and as a result they can feel empowered and begin to advocate for themselves and their families. Genetic testing will undoubtedly improve with time to help guide the decisions that patients and providers might make to improve patient outcomes. Genetic counseling can be a valuable piece of the diagnostic process and management of these complex conditions.

Genetic counseling

The family history is a critical component of any genetic counseling session. In a family history related to mental health conditions, the provider should pay special attention to factors such as drug abuse, alcoholism, or estranged family members. These could be signs of possible undiagnosed mental health concerns, in some cases. Simply asking if anyone in the family has been diagnosed with a psychiatric disorder may not trigger the patient to think about depression, anxiety, panic attacks or even autism, which are relevant to assessing the family as a unit. In addition, documenting individuals with birth defects, intellectual disabilities, seizures, or rare medical conditions can be valuable to assessing whether an underlying genetic syndrome that may be related to the mental illness is present in the family.

Helping families understand the multifactorial nature of mental health can be a key to establishing the most effective mechanism for managing symptoms and using the Jar Model [10] can be a valuable tool available to providers for this purpose. The Jar Model is an analogy used to help families understand the complexities of mental illness by comparing a jar to an individual's mental health status. When the jar is filled as the result of the accumulation of various risk factors, it represents an active episode of illness or initial diagnosis (Fig. 21.1).

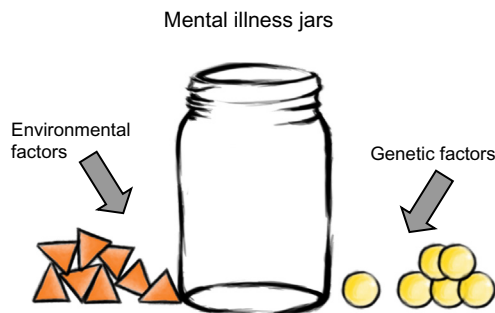


FIGURE 21.1 Mental illness jars. Source: From Peay H, Austin J. *How to talk with families about genetics and psychiatric illness*. WW Norton and Company; 2011. Copyright © 2011 by H. Peay and J. Austin. Used by permission of W. W. Norton & Company, Inc.

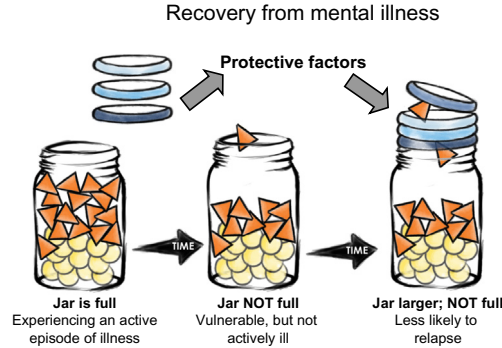


FIGURE 21.2 Recovery from mental illness.

Table 21.1 Empiric recurrence risk table.

Condition	Incidence	Recurrence risk (first-degree relatives affected)
Generalized anxiety [1,6,20]	9.9%–9%	10%–30%
Bipolar disorder [6,21]	0.6%–5%	4%–18%
Schizophrenia [6,21]	0.3%–1%	5%–16%
Major depression [6,21,22]	7%–20%	10%–25%

The risk factors that fill the jar are represented by different colored shapes with one shape representing genetic factors and another shape representing environmental/life experience factors. The genetic factors in the jar generally stay constant but the environmental factors can be added or removed from the jar over time. The last components of the jar model are protective factors that can be represented as rings added to the top of the jar. Examples of protective factors including medications, good social support network, counseling, sleep, diet, and other mitigating factors. These rings make the jar taller and therefore less likely to be filled resulting in an active episode (Fig. 21.2).

In many cases, it is not possible to provide a specific recurrence risk for the child of an individual with idiopathic mental illness to develop the same condition because of the complex and multifactorial nature of psychiatric disorders. While estimations of recurrence for first-degree relatives (Table 21.1) can be useful in some cases, it can also be critical to discussing that mental illnesses may represent a continuum of mental health findings in the general population or within a single family [17,18]. Finally discussing support resources in the unique context of the patient can provide valuable protective factors for sustaining a patient’s mental health. Studies suggest genetic counseling has a positive impact on patient outcomes, especially empowerment and self-efficacy using a clinically validated genetic counseling outcomes scale [19].

Points to remember

- Mental illnesses are multifactorial and complicated.
- Genetic counseling can improve patient outcomes.
- Genetic testing cannot be used for diagnosis or prediction of mental illness but may be valuable for guiding management or identifying an underlying genetic condition.
- Multiple genetic syndromes have mental illness as a feature.

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Renal disorders

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Introduction

Chronic kidney disease (CKD) is generally defined as either kidney damage or decreased kidney function for three or more months, irrespective of the cause. Glomerular filtration rate (GFR) is the best overall index of kidney function. The normal GFR in young adults is approximately 125 mL/min/1.73 m²; GFR <15 mL/min/1.73 m² is defined as kidney failure. Any patient requiring dialysis or transplantation for treatment of CKD is considered to be in “end-stage renal disease” (ESRD). About 30 million US adults are estimated to have CKD and most are undiagnosed. Patients with kidney disease may present with hematuria, proteinuria, hypertension, or flank pain; however, CKD may be asymptomatic in early stages. Hypertension and diabetes are considered the most common etiologies of CKD in the adult population. Kidney disease may first become evident on a routine basic metabolic panel. Based on initial findings, additional evaluation may include urine electrolytes, urinalysis, renal ultrasound, and/or renal biopsy.

Cystic renal diseases

Nonhereditary conditions should be considered in the differential diagnosis of polycystic kidney disease. When patients present with simple renal cysts, acquired cystic kidney should be considered. These are rare in children but the incidence increases with age. Acquired cystic renal disease can be seen in patients with ESRD, usually on dialysis. When cysts are bilateral and multiple, differentiation from hereditary disease may be difficult. In these situations, the distribution of renal cysts, presence or absence of family history, renal enlargement, and associated liver disease can be helpful for further differentiation. Nephronophthisis may present in young adults and is associated with small echogenic kidneys and renal failure. When the renal cysts are unilateral or asymmetric, the differential diagnoses may include multicystic dysplastic kidney, and benign and malignant cystic renal tumors including

Table 22.1 Types of cysts in different hereditary renal disorders.

Type of cysts	Associated disorder
Simple renal cysts	Acquired cystic kidney disease
Diffusely distributed bilateral cysts	Polycystic kidney disease [AD (autosomal dominant)/AR (autosomal recessive)], acquired cystic renal disease, lithium nephrotoxicity
Predominant medullary cysts	Medullary cystic renal disease, medullary sponge kidney
Predominant cortical cysts	Glomerulocystic disease
Renal cysts with solid/fatty tumors	Von Hippel-Lindau (VHL), tuberous sclerosis
Renal cysts with diabetes	Renal cysts and diabetes syndrome
Renal cysts with genitourinary, facial, or CNS anomalies	Oro-facial-digital syndrome

multilocular cystic nephroma and cystic renal cell carcinoma (RCC) as well as atypical autosomal dominant polycystic kidney disease (ADPKD) and localized cystic disease [1,2]. Differential diagnosis of cystic kidney disease in adulthood is summarized in Table 22.1. The most common form of cystic kidney disease in adulthood is ADPKD which is discussed here.

Autosomal dominant polycystic kidney disease

Overview

Polycystic kidney disease can be categorized into three major subtypes such as ADPKD, autosomal recessive (ARPKD), and syndromic (Joubert, Meckel, and Bardet-Biedel syndrome). ADPKD is usually adult-onset and is the most common potentially lethal Mendelian disorder and the most prevalent inherited progressive kidney disease. ESRD occurs as a result of ADPKD in up to 75% of patients by 70 years of age. Pathogenic variants in *PKD1* (chromosome 16p13.3) are responsible for almost 80% of cases of ADPKD, whereas ~15% of ADPKD cases are attributed to variants in *PKD2* (chromosome 4q22.1) and the remaining ~5%–10% of ADPKD cases are genetically unresolved or are due to rare variants in other loci. The PKD genes are expressed in a wide range of tissues beyond the kidney, and their expression is developmentally regulated in most of these tissues. Accordingly, other extrarenal manifestations can occur, including cysts in the liver, pancreas, seminal vesicles, and the arachnoid membrane, in patients with ADPKD [3].

Evaluation

ADPKD is a systemic disease and, in addition to the renal involvement, patients develop liver cysts and cardiovascular abnormalities including hypertension, left ventricular hypertrophy, aortic root dilatation, arterial aneurysms, heart valve abnormalities, and intracranial aneurysms (ICAs). Key points in history include early onset hypertension, abdominal fullness and pain, hematuria, and urinary tract infections (UTIs). These are usually first observed decades before the onset of renal insufficiency, sometimes even in childhood. Abdominal or renal imaging (most commonly renal ultrasound) can detect large bilateral cystic kidneys.

Specific ultrasound diagnostic criteria have been established to make the diagnosis of ADPKD among at-risk individuals, particularly those older than 15 years of age, who are from families without a known pathogenic variant in the PKD genes. These include:

- 15–39 years of age: at least three unilateral or bilateral cysts
- 40–59 years of age: two cysts in each kidney
- 60 years or older: four cysts in each kidney

Some asymptomatic patients at risk for ADPKD are from families with known and well-characterized pathogenic variants in the *PKD1* or *PKD2* locus. In this setting, testing for the known familial pathogenic variant is more definitive and may be more cost-effective than ultrasonography.

Genetic testing

PKD1 and *PKD2* are commonly associated with ADPKD; however, pathogenic variants in other genes including *GANAB* and *DNAJB1* are also found in a small subset of patients. Genetic testing for *PKD1* is complicated by several highly homologous pseudogenes. In general, patients with *PKD1* have a younger age of ESRD compared to *PKD2*. In individuals with truncating *PKD1* variants, the age of onset of ESRD is also earlier than in those with nontruncating pathogenic variants (55.6 years compared to 67.9 years). In patients with a high suspicion of ADPKD, a limited genetic panel is more appropriate and cost-effective. Although most cases are familial, in 10%–25% of patients, a positive family history is absent, posing a diagnostic challenge. These cases are explained by *de novo* disease in up to 10%–15% (3), missing parental medical records, germline or somatic mosaicism, or mild disease from hypomorphic *PKD1* and *PKD2* alleles (4). The penetrance in ADPKD is dependent on the age and the genotype of the individual. Multiple bilateral renal cysts are typically seen in almost 100% of older adults [2,3].

Vignette 22.1

PA is a 28-year-old woman who presents to her PCP with HTN and flank pain. Renal ultrasound reveals large bilateral kidneys with multiple cysts. She reports a family history of renal failure requiring hemodialysis in her father; however, limited information was available about other paternal family members. A genetic testing panel is ordered and reveals a nonsense single nucleotide variant in PKD1. Upon further questioning it was learned that the paternal grandmother had died because of a likely intracranial bleed. The following workup is indicated: echocardiogram to evaluate aortic dilation and brain MRI (magnetic resonance imaging)/MRA or CTA to evaluate ICAs. Risk factors for progressive renal disease include hyperlipidemia, hypertension, and metabolic syndrome and should be monitored periodically.

Management

Management of ADPKD includes reducing/preventing the risk of renal disease by optimal blood pressure control, prompt treatment of UTIs, avoidance of nephrotoxic agents, and management of ESRD.

Patients with ADPKD require ongoing surveillance to monitor the progression of renal disease. Surveillance following initial diagnosis include:

- CT or MRI examination of the abdomen with and without contrast enhancement,
- blood pressure examination to detect hypertension,
- measurement of blood lipid concentrations because hyperlipidemia is a correctable risk factor,
- urine studies to detect the presence of microalbuminuria or proteinuria, which in the presence of severe renal cystic disease indicates an increased likelihood of disease progression and mandates strict control of the blood pressure,
- echocardiography,
- head MRA or CT angiography to screen persons with a family history of ICAs.

Tolvaptan, a selective oral vasopressin V2-receptor antagonist, is the first pharmacological agent that has been approved for therapy to manage ADPKD. Since its initial approval in Japan, other countries such as Canada, Europe, South Korea, Switzerland, Hong Kong, Australia, and, most recently, the US have also approved the use of Tolvaptan in ADPKD. Various guidelines have been generated for guiding the selection of individuals with rapidly progressive disease that is likely to result in ESRD [4]. Factors indicating rapidly progressive ADPKD include total kidney volume (TKV)/age, rate of change of TKV, eGFR/age, rate of decline of eGFR, genotype, and family history.

Genetic counseling

If a known pathogenic variant has already been identified in an affected relative other at-risk family members can be tested. This becomes particularly important when screening adult family members for transplant.

Autosomal recessive polycystic kidney disease

Autosomal recessive polycystic kidney disease (ARPKD) is a rare form of polycystic kidney disease characterized by hepato-renal cysts, which usually manifest in utero or during the perinatal period. This condition is caused by pathogenic variants in *PKHD1*. The classic clinical presentation includes early onset disease with bilaterally enlarged kidneys and impaired renal function along with congenital hepatic fibrosis. While most present in early childhood, some rare cases may be present in late adolescence or adulthood. Signs of hepatic involvement including portal hypertension, thrombocytopenia or splenomegaly in an adult patient with cystic renal disease may be clinical indicators of adult ARPKD. Genetic testing for the *PKHD1* gene will help to establish the diagnosis.

Inherited glomerulopathies

Glomerular disease is caused by structural defects in the glomerular basement membrane (GBM) or the podocytes and can be inherited or acquired. They manifest in a variety of

ways, ranging in severity from asymptomatic urinary abnormalities to acute kidney injury or ESRD. The most common adult-onset inherited glomerular disease is Alport syndrome (AS). Diseases of the glomerulus can result in two different urinary and clinical patterns: nephritic and nephrotic. Nephritic kidney disease presents with hematuria with possible non-nephrotic range proteinuria. On the other hand, nephrotic syndrome (NS) presents with more than 3.5 g/d proteinuria, albumin less than 3.5 mg/dl, edema, and elevated cholesterol.

Glomerular diseases with hematuria

Patients presenting with primary glomerular hematuria have RBC casts or dysmorphic RBCs. These patients may have nephritic syndrome defined by the presence of hypertension and edema. Some of the common forms of glomerulonephritis belong to this group. Patients may also have proteinuria but not in the NS range. Serum complement levels may be useful in differentiating the underlying etiology of glomerulonephritis; complement levels are typically normal in anti-GBM disease and pauci-immune glomerulonephritis but low in immune complex-mediated glomerulonephritis [with the exception of immunoglobulin A (IgA) nephropathy]. IgA nephropathy is the most common chronic glomerulopathy caused by multiple factors including some ill-defined genetic factors.

Some of the nongenetic forms of glomerular disease include lupus nephritis, fibrillary glomerulonephritis, vasculitis, poststreptococcal glomerulonephritis, and staphylococcus-associated glomerulonephritis.

Genetic forms of glomerular disease with hematuria are described below.

Fibronectin glomerulopathy

Fibronectin glomerulopathy is a rare, AD, inherited, glomerular disease associated with the massive deposition of the glycoprotein fibronectin. It is estimated that pathogenic variants in the *FN1* gene are responsible for 40% of cases of fibronectin glomerulopathy. The cause of the remaining cases of this condition is unknown. Clinical characteristics include the onset of proteinuria between the ages of 20 and 40 years. This is followed by hypertension, microscopic hematuria, and slow progression to ESRD over the next 15–20 years. Complement levels are normal. There may be an association with hyperkalemic distal renal tubular acidosis. Diagnosis is typically by biopsy; however genetic testing may be used as a primary or supportive diagnostic modality. No specific treatment is available; however ACEI and ARBs may be used.

Complement factor 3 glomerulopathies

Abnormal regulation of the alternative complement pathway leads to dense deposit disease (DDD) and complement factor 3 glomerulonephritis (C3GN), both of which are rare forms of glomerulonephritis that affect both children and adults. DDD is typically a childhood disease; however, diagnosis may be extended into adulthood. The discovery of a lesion suggesting DDD or C3GN in an older adult should always initiate a search for a monoclonal

gammopathy. The prognosis of DDD is generally poor. Prognosis of C3GN is variable but tends to be better than DDD. Some individuals with C3GN will have biallelic or heterozygous pathogenic variants in one or more genes (including *C3*, *CD46*, *CFB*, *CFH*, *CFHR1*, *CFHR5*, *CFI*, and *DGKE*). At-risk family members of affected patients should undergo genetic testing if a pathogenic variant is known in the family. Asymptomatic individuals can have hypocomplementemia and an abnormal urinalysis or elevated serum creatinine. A kidney biopsy should be done to exclude DDD if there is clinical evidence of glomerular disease. C3GN recurs in nearly all grafts and is the predominant cause of graft failure in 50%–90% of transplant recipients [5].

MYH9-related nephritis

MYH9-related disorders (MYH9-RD) are characterized by large platelets and thrombocytopenia, both present from birth. Some individuals may present in early adulthood with progressive sensorineural hearing loss (SNHL), presenile cataracts, elevation of liver enzymes, and glomerular nephropathy. When macrothrombocytopenia is associated with kidney disease, the diagnosis of MYH9-RD should be suspected. MYH9-RD is inherited in an AD manner. Approximately 35% of affected individuals are simplex cases, half of them with a documented *de novo* pathogenic variant in *MYH9*. However, the penetrance and severity of kidney disease are very variable, making it challenging to make the diagnosis. Treatment is symptomatic.

Alport syndrome

Overview

AS, also referred to as hereditary nephritis, is an inherited progressive form of glomerular disease that is characterized by hematuria, progressive renal failure, SNHL, and ocular abnormalities. It is the second most common cause of monogenic renal failure—the first being ADPKD. Approximately 85% of AS is X-linked (XLAS), 14% is AR, and 1% is AD.

Evaluation

In the absence of treatment, renal disease progresses from microscopic hematuria (microhematuria) to proteinuria, progressive renal insufficiency, and ESRD in all males with XLAS, by about age 25 years, and in all males and females with ARAS. Progressive SNHL is usually present by late childhood or early adolescence. Nearly all individuals with ARAS develop early hearing loss, but other clinical features are similar to patients with XLAS. Ocular findings in AS can be pathognomonic and include anterior lenticonus, retinal thinning and macular holes, central or peripheral retinopathy, corneal endothelial vesicles, and recurrent corneal erosion. In individuals with ADAS, ESRD is frequently delayed until later adulthood, SNHL is relatively late in onset, and ocular involvement is rare. These individuals develop thin basement membrane nephropathy which is characterized by persistent hematuria, normal levels of proteinuria, and normal BP and renal function. Rarely, individuals with AS may have associated aortic disease or diffuse leiomyomatosis.

Historical clinical criteria for establishing a diagnosis of AS include any three of the following:

1. family history of macroscopic/microscopic hematuria or chronic renal failure
2. typical renal biopsy findings of AS
3. pathognomonic ocular findings (anterior lenticonus and macular flecks)
4. SNHL

Female carriers of XLAS have a variable clinical presentation that ranges from being asymptomatic to lifelong microscopic hematuria with progression to ESRD to development of renal failure at an early age. This variability is due to skewed X inactivation. However, almost all females (95%) have hematuria. Only about 15% develop ESRD by the age of 60 years [6].

Genetic testing

AS is caused by pathogenic variants in *COL4A3*, *COL4A4*, and *COL4A5* that result in abnormalities of the collagen IV α 345 network of basement membranes [7]. XLAS patients have a deficient α 5 chain due to pathogenic variants in *COL4A5*. Individuals with ARAS are either homozygous or compound heterozygous for variants in the *COL4A3* or *COL4A4* genes. Heterozygous variants in *COL4A3* or *COL4A4* genes lead to ADAS, which could be considered as a trait of an ARAS carrier. Several genotype-phenotype correlations such as earlier-onset of ESRD and risk of extrarenal manifestations have been described with these genes and hence genetic testing is recommended to predict prognosis.

Management

Medical management: Currently, there is no specific treatment for AS. Medications aimed at slowing the progression of renal disease including angiotensin-converting enzyme inhibitor or angiotensin receptor blockers are commonly used. Factors that worsen renal dysfunction such as uncontrolled hypertension should be avoided. Renal transplantation is the treatment of choice when a patient develops ESRD. Potential living related donors must be evaluated carefully for AS, preferably by genetic testing, in order to avoid nephrectomy in an affected, but asymptomatic individual. Routine treatment of SNHL with hearing aids and surgical intervention for cataracts and symptomatic leiomyomas can be pursued.

Surveillance

Surveillance of individuals with AS mainly includes periodic monitoring of renal function and associated ocular and hearing abnormalities. Follow up of all individuals having AS with a nephrologist every 6–12 months is recommended. Evaluation for aortic dilation should be considered for males with XLAS [8].

Genetic counseling

About 10%–15% of individuals with AS carry a *de novo* pathogenic variant or one of the parents may have gonadal mosaicism. In such families, there is no history of nephropathy in

other individuals. Molecular genetic testing for at-risk family members, prenatal testing for pregnancies at increased risk, and preimplantation genetic diagnosis are possible if the pathogenic variant(s) in the family are known.

Nephrotic syndrome

In NS, a variety of disorders cause proteinuria, often resulting in marked edema and hypoalbuminemia. Approximately 30% of adults with NS have an underlying systemic disease such as diabetes mellitus, amyloidosis, or systemic lupus erythematosus; the remaining cases are usually due to primary disorders including minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), and membranous nephropathy. Heavy proteinuria in patients without edema or hypoalbuminemia is more likely to be due to secondary FSGS. In children, MCD is the most common cause of NS, while FSGS accounts for more cases of NS in adults. Only 10%–25% of cases of NS in adults are due to MCD and hence therapy is begun after a diagnosis is made on kidney biopsy.

Genetic diagnosis is usually considered in patients with steroid-resistant NS (SRNS). The genetic predisposition to steroid-sensitive NS is not well understood. Detection rates of available genetic tests for SRNS depend on the age group and presence of a family history. Genes associated with SRNS include *NPHS1*, *NPHS2*, *TRPC6*, *CD2AP*, *PLCE1*, *INF2*, *WT1* (exons 8 and 9), and *ACTN4* (exons 1 to 10) genes. The most commonly associated gene is *NPHS2* [9]. Pathogenic variants in *COL4A3*, *COL4A4*, or *COLA5* are not unusual in adult patients with proteinuria and renal biopsy findings of FSGS [10]. Inherited FSGS can be broadly divided into two subtypes: (1) AD type (caused by gain-of-function variants in several different genes, the most common being *INF2*), characterized by proteinuria, progressive chronic renal disease, and FSGS and (2) AR type (caused by loss of function variants in *NPHS1* and *NPHS2*) and present in infancy or early childhood.

The increased susceptibility of African American (AA) patients to FSGS may be explained, at least in part, by genetic factors, although socioeconomic and environmental factors may also play a role. Variants in the apolipoprotein L1 (*APOL1*) gene, termed as G1 and G2, have been shown to confer an increased risk of developing FSGS, hypertension-associated kidney disease, or HIV nephropathy. However, the presence of *APOL1* risk alleles in AAs with FSGS lesions has no bearing on treatment decisions.

In adults, renal biopsy is the first step in the diagnosis of NS because it allows ruling out other causes of NS such as membranous nephropathy. Genetic testing should be strongly considered in any adult, regardless of age, with an “apparently” primary FSGS lesion on kidney biopsy and SRNS.

Genetic testing is confirmatory in establishing the diagnosis. Although the analysis of multiple genes may become time-consuming and expensive, the identification of pathogenic variants can help on several fronts including (1) avoidance of adverse effects of unnecessary steroid/immunosuppressive treatment, (2) encouraging living donor kidney transplantation, (3) prediction of prognosis depending on the genotype, and (4) availability of presymptomatic, prenatal, and preimplantation genetic diagnosis.

Common genetic disorders with renal involvement

Fabry disease

Overview

Fabry disease, the most prevalent lysosomal storage disorder, is a multisystem disease that affects kidneys, heart, blood vessels, peripheral nerves, skin, and eyes, among others. It is caused by pathogenic variants in *GLA* that encode lysosomal hydrolase, alpha-galactosidase A, a key enzyme of the glycosphingolipid metabolic pathway. The genomic location of *GLA* is on Xq22.1 and thus Fabry disease is inherited as an X-linked (XL) disorder. Hemizygous males are more severely affected than females. Female heterozygotes have a variable presentation that can range from being an asymptomatic carrier to a severe phenotype that resembles that seen in males.

Evaluation

Clinical manifestations often begin during childhood or adolescence. However, individuals with residual enzyme activity may present later in life. The classical manifestations of Fabry disease in affected males include the following: (1) acroparasthesias—painful burning sensation that occurs in the extremities which are precipitated by changes in temperature, exercise, and fatigue, (2) telangiectatic skin lesions or angiokeratomas that occur on the abdominal wall, groins, hips, thighs, and back, (3) renal involvement with proteinuria, polyuria, polydipsia, and progressive loss of renal function, (4) cardiac involvement with left ventricular hypertrophy, conduction abnormalities, heart failure, and mitral and aortic valve abnormalities, (5) corneal and lenticular opacities with a characteristic appearance of cornea on slit lamp examination called corneal verticillata, (6) vasculopathy that may manifest as transient ischemic attacks, or ischemic and hemorrhagic stroke, (7) gastrointestinal symptoms including abdominal pain, nausea, vomiting, diarrhea, and constipation, (8) heat, cold, and exercise intolerance, and (9) hypohidrosis or hyperhidrosis. In adulthood, progressive cardiovascular disease and stroke account for significant morbidity and mortality. The phenotype in females is highly variable but they can manifest with symptoms observed in the affected males. It has been estimated that 20% of females develop major cardiovascular, renal, or cerebrovascular complications. In addition to these classical presentations, atypical later-onset presentations that only involve the heart (cardiac variant) or the kidneys (renal variant) have been described.

Laboratory evaluation should include a urinalysis looking for proteinuria, assessment of renal function, electrocardiogram, and echocardiogram. MRI of the heart and brain may also be considered, particularly in females and patients who present with neurologic manifestations [11].

Genetic testing

In affected males, a diagnosis of Fabry disease is typically established by demonstrating low enzyme activity of alpha-galactosidase A in peripheral blood leukocytes. Affected males typically have less than 1%–3% of residual enzyme activity. Individuals with late-onset Fabry

disease may have enzyme activity that is much higher than 3% of the control values; a definitive diagnosis in such individuals may also require molecular genetic testing. Enzyme testing is not reliable for the identification of carriers or mildly affected females with the disorder. Sequencing can detect pathogenic variants in *GLA* in 95% of those with Fabry disease; 5% are caused due to copy number variation. Thus, a comprehensive test should include sequencing and analysis for deletion and duplication. Genetic testing not only is important to establish the diagnosis in a proband and help cascade testing but also has therapeutic implications. Individuals with specific pathogenic variants in *GLA* may be candidates for targeted therapy with migalastat.

Management

The treatment of Fabry disease typically involves an interdisciplinary approach that aims at preventing disease progression with the use of enzyme replacement therapy (ERT) and managing symptoms and complications of the disease.

ERT is typically initiated in all males with classic presentation soon after diagnosis. In males with atypical presentations and females, the initiation of ERT is typically based on the presence of clinical manifestations. Two formulations are available—agalsidase beta and agalsidase alpha. The formulation approved by the FDA in the United States, agalsidase beta, is typically administered as an intravenous infusion at a dose of 1 mg/kg every two weeks. ERT monitoring and dose adjustments are best handled by healthcare professionals who have experience in the management of this disorder. More recently, migalastat, a small-molecule pharmacological chaperone therapy that can be administered orally, has been approved for treatment of a subset of individuals with Fabry disease caused by specific variants that are amenable to such chaperone therapy. This could be considered as an alternative to ERT in individuals who would benefit from the treatment.

Other treatments like gabapentin, carbamazepine for acroparathesias, angiotensin convertase enzyme inhibitors or angiotensin receptor blockers to reduce proteinuria, medications to treat angina and heart failure, and symptomatic treatments for gastrointestinal disease are often required. Individuals with ESRD require hemodialysis and renal transplantation.

Genetic counseling

Fabry disease is inherited as an XL disorder. Thus, hemizygous males are more severely affected than females. Identifying a pathogenic variant in a family is of significant importance for monitoring as well as cascade testing.

Nail-patella syndrome

The nail-patella syndrome (NPS) is an AD disorder characterized by hypoplastic or absent patella, dystrophic fingernails and toenails, and dysplasia of elbows and iliac horns. NPS is caused by pathogenic variants in *LMX1B* that encodes a LIM-homeodomain type transcription factor that has an important role in limb and renal development [12]. Kidney

involvement typically manifests with proteinuria and hematuria and is observed in one-third to half of the affected individuals. Progression to ESRD occurs in about 5% of affected individuals. Electron microscopy of renal tissue typically shows an irregularly thickened GBM with a mottled appearance. More than 95% of affected individuals have nail abnormalities ranging from pits and ridges on the nails to hypoplasia and absence of nails. Skeletal abnormalities include dislocated, hypoplastic, or absent patella, bilateral conical projects from the ilium, scoliosis, and abnormalities of the foot such as talipes equino varus. The diagnosis is made based on clinical features and molecular genetic testing. The management is largely based on clinical manifestations and treatment of symptoms. There are no disease-specific therapies to delay the progression of renal disease.

Papillorenal syndrome

Papillorenal syndrome is a disorder that presents with renal and eye abnormalities [13]. The disorder has been recently discovered to be caused by pathogenic variants in *PAX2* that encodes a paired box type transcription factor. *PAX2*-related disorders present with a wide array of renal abnormalities including hypoplastic kidneys, renal hypodysplasia, FSGS, and other congenital anomalies of the kidney and urinary tract (CAKUT). The ocular abnormalities include optic nerve dysplasia and retinal colobomas. The diagnosis is typically made based on clinical presentation and molecular genetic testing. The treatment consists of management of symptoms and complications due to compromised renal function.

Congenital anomalies of the kidney and urinary tract

CAKUT is a term used to describe a range of abnormalities including multicystic dysplasia, renal agenesis and hypoplasia, cross-fused ectopia, duplications of the collecting system, mega-ureter, posterior urethral valves, and vesicoureteral reflux. CAKUT accounts for one-fifth of birth defects and is responsible for nearly half of all ESRD in children. With improved management, the overall survival has improved and thus the number of adults with these abnormalities has increased. It has been estimated that nearly one-fifth of individuals with CAKUT have a Mendelian disorder. As pathogenic variants in numerous genes that are required for the development of the kidneys and the urinary tract cause CAKUT, establishing a specific diagnosis often requires genome-wide molecular tests such as exome or genome sequencing and chromosomal microarray.

Hereditary renal cell carcinoma

Renal cell cancer, which accounts for 4% of all adult malignancies in the United States, may be sporadic or more infrequently (5%–8%) associated with a hereditary cancer syndrome. VHL, described in this chapter, is a hereditary cancer syndrome associated with an increased risk for renal cancer. Specifically, the cumulative risk for renal cell cancer is approximately 24%–45% for individuals with VHL. Clear cell kidney cancer is also associated with

chromosome 3 translocations, *PTEN* hamartomatous syndrome, and pathogenic variants in the *BAP1* gene as well as several of the genes encoding the proteins comprising the succinate dehydrogenase complex (SDHB/C/D) as described in more detail in Chapter 8, Cancer syndromes. Type 1 papillary kidney cancers arise in conjunction with germline pathogenic variants in *MET* and type 2 as part of hereditary leiomyomatosis and kidney cell cancer [(HLRCC); fumarate hydratase (FH) pathogenic variants]. Chromophobe and oncocytic kidney cancers are predominantly associated with Birt-Hogg-Dube (BHD) syndrome. Patients with TSC commonly have angiomyolipomas and rarely their malignant counterpart epithelioid angiomyolipomas. The targeted therapeutic options for kidney cancer associated with these diseases are starting to expand and are an area of active clinical research [14]. BHD and HLRCC are also described in Chapter 8, Cancer syndromes, while TSC is covered in Chapter 24, Skin disorders.

Von Hippel-Lindau syndrome

Overview

VHL is characterized by a predisposition to develop hemangioblastomas. While these tumors are predominantly benign, they may lead to significant morbidity or mortality. Hemangioblastomas may develop in the brain, spine, and retina. Individuals with VHL are also at increased risk to develop pancreatic and kidney cysts, tumors of the endolymphatic sac in the inner ear, tumors in the liver and lungs, and pheochromocytomas. In addition, there is an increased risk to develop malignancy, including clear cell renal carcinoma and pancreatic neuroendocrine tumors and adenocarcinoma.

The *VHL* gene is a classic tumor suppressor, and loss of the wild-type allele is found in hemangioblastomas, pancreatic neuroendocrine tumors, kidney cysts, and clear cell kidney cancer from patients with VHL. Approximately 20% of the cases of VHL are *de novo*, while the rest are inherited in an AD manner.

Evaluation

RCC that presents at a young age (generally before 47 years) or that occurs bilaterally and sometimes with hundreds of lesions in the kidney should prompt further evaluation for VHL. Likewise, the presence of spinal or cerebellar hemangioblastomas, pheochromocytomas, multiple renal or pancreatic cysts, neuroendocrine tumors of the pancreas or endolymphatic sac, or retinal angiomas is suggestive of VHL.

Clinical diagnostic criteria have been developed. An individual with a family history of VHL and who has one of the following has a clinical diagnosis of VHL: retinal angioma, spinal or cerebellar hemangioblastoma, adrenal or extra-adrenal pheochromocytoma, RCC, or multiple renal and pancreatic cysts. An individual without a family history would also meet clinical criteria for diagnosis if they have two or more of the following features: two or more hemangioblastomas in association with a visceral manifestation, such as multiple renal or pancreatic cysts, RCC, pheochromocytomas, or endolymphatic sac tumors, papillary cystadenomas of the epididymis or broad ligament, or neuroendocrine tumors of the pancreas.

A diagnosis of VHL can also be made via molecular analysis of the *VHL* gene.

Genetic testing

Genetic testing for VHL is available and includes sequencing and duplication/deletion analysis of the *VHL* gene. Approximately 89% of individuals with VHL have a pathogenic variant that will be identified by sequencing while 11% have been found to have a deletion or duplication.

Vignette 22.2

AP is a 35-year-old man who is referred to the genetics clinic with a history of bilateral kidney cancer. Pathology revealed clear cell renal carcinoma. Family history is significant for the history of pheochromocytoma in AP's brother. Sequencing of VHL revealed a pathogenic frame-shift variant. Subsequently, it is recommended that the patient have routine surveillance (as described in the "Management" section of the VHL section). As a pathogenic variant was identified in AP, cascade testing for family members was possible and the patient was encouraged to share this information with his siblings and other at-risk family members. Though he does not yet have children, he was informed that should he wish to discuss the option of pre-conception genetic diagnosis or prenatal testing in the future, options are available. The patient reports that two of his siblings have chosen not to be tested for the pathogenic variant in VHL. It was explained that first degree relatives of an individual with a known VHL pathogenic variant require regular surveillance to assess their risk. The recommendations for this screening are as follows:

- *Starting at age one year: Annual evaluation for neurologic symptoms, vision problems, and hearing disturbance; annual blood pressure monitoring; annual ophthalmology evaluation.*
- *Starting at age five years: Annual plasma or 24-hour urine for fractionated metanephrines; audiology assessment every two to three years; thin-slice MRI with contrast of the internal auditory canal in those with repeat ear infections.*
- *Starting at age 16 years: Annual abdominal ultrasound; MRI scan of the abdomen and MRI of the brain and total spine every two years [15].*

Management

Increased awareness has led to earlier diagnosis and intervention of VHL. Familial genetic screening, routine imaging, and an aggressive surgical approach to kidney tumors in early stage disease can help prolong the quality of life with low morbidity. Because these patients present with multifocal disease at an early age and the tumors vary in aggressiveness, every effort should be made to preserve kidney function through nephron-sparing approaches (partial nephrectomy, thermal ablative therapies, or observation) in patients with disease limited to the kidneys. However, in patients with locally advanced disease, the likelihood of recurrent disease and ESRD is much higher; thus, bilateral resection of the kidneys followed by kidney transplantation is a more accepted approach.

Genomic medicine in adult nephrology

Genomic medicine is being integrated into daily practice of medicine. In the practice of nephrology, until recently, genetic testing was limited largely to patients with pediatric-onset and/or familial renal diseases. The typical genetic evaluation involved the use of multigene panels to interrogate specific genes that are involved in causing a specific type of renal or urogenital abnormalities. However, recent work has shown that the pathogenic variants in single genes may be responsible for a significant proportion of renal failure. For example, in one study, exome sequencing (ES) was done to study a diverse cohort of 3315 patients with CKD, and a molecular cause for CKD was identified in nearly 10% of the patients [16]. This yield is similar to that observed for cancer, for which genomic diagnostics are routinely used. In another study [17], diagnostic yield of ES was investigated in a cohort of 114 Irish adult probands with CKD; the overall diagnostic yield was 37% (42 of 114) and variants in 29 distinct single genes were identified as a cause. Among these 29 monogenic disorders, 21 (72%) were detected only once, in a single proband, highlighting the genetic heterogeneity of Mendelian nephropathies.

Points to remember

- ADPKD is the most common cystic disease in adults.
- Individuals with nephritic and NS of unclear etiology may have an underlying genetic disorder.
- Female carriers of AS may have chronic microscopic hematuria.
- Fabry disease can be treated with ERT or Migalastat.
- Nearly 10% of ESRD can have a genetic etiology.
- When RCC is of early onset or bilateral, consider hereditary cancer syndromes such as VHL.

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Skeletal disorders

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Introduction

Some disorders of bones have a high prevalence. For example, osteoporosis and low bone mass, which are major causes of fracture in individuals over the age of 50 years, are estimated to affect 10 million and 40 million Americans, respectively [1]. The disorders of bone commonly encountered in clinical practice may occur due to disease processes that primarily target the skeleton (e.g., age-related osteoporosis) or from secondary causes such as chronic inflammation (e.g., rheumatoid arthritis), hormonal imbalances (e.g., Cushing syndrome, primary hyperparathyroidism, and diabetes mellitus), chronic kidney disease (e.g., renal osteodystrophy), and gastrointestinal disorders (e.g., celiac disease). In contrast to such common multifactorial disorders of bone, genetic skeletal disorders (GSDs), which *are constitutional errors of bone development* caused by pathogenic variants in single genes, are rare. The collective incidence of GSDs is estimated to be 1 in 5000 births [2]. GSDs are clinically heterogeneous and the clinical spectrum ranges from disorders that affect single or few skeletal elements (e.g., preaxial polydactyly type 1, split-hand-foot malformation type 4), disorders with multiple bone abnormalities (e.g., osteogenesis imperfecta [OI], Bruck syndrome), disorders with involvement of multiple organ systems (e.g., ciliopathies, lysosomal storage diseases), to perinatally lethal conditions (e.g., thanatophoric dysplasia). Moreover, constitutional bone disorders are highly genetically heterogeneous. Pathogenic variants in multiple genes can give rise to overlapping clinical phenotypes (e.g., many forms of short rib-polydactyly syndromes), and variants in the same gene can give rise to clinically distinct disorders (e.g., pathogenic variants in *COL2A1* can give rise to Stickler syndrome type 1, spondyloepiphyseal dysplasia congenita, and Torrance-type platyspondylic dysplasia, among others). Based on the clinical presentation, radiographic features, and genetic bases of disease, the most recent nosology and classification system categorized 461 GSDs into 42 groups [3]. To date, over 430 genes have been identified as causing these GSDs.

Most GSDs manifest during childhood. Providers involved in the care of adults typically encounter affected individuals in the following scenarios: (1) transition of care from the

pediatric facilities, (2) evaluation of an individual with a mild form of GSD that was undiagnosed during childhood, and (3) genetic counseling and testing for reproductive purposes. Adults with GSDs may present with one or more of the following symptoms: short stature, abnormal patterning of bones, bowing of long bones, kyphoscoliosis, increase or decrease in bone mineral density (BMD), recurrent fractures, limitation of mobility, musculoskeletal pain, as well as extraskeletal manifestations. The evaluation of an individual who is suspected to have a GSD should begin with detailed medical and family history to understand the pattern and the extent of skeletal involvement and the potential mode of inheritance. The physical examination should include measurement of total height, upper segment and lower segment length, arm span, and length of arms, forearms, hands, as well as lower limb segments. If shortening of extremities is observed, understanding whether this is because of shortening of the proximal (rhizomelia), middle (mesomelia), or distal (acromelia) segments, or combinations thereof (e.g., acromesomelia) could help narrow the diagnostic considerations. For example, an individual with shortening of the middle and/or proximal segments of the limbs is more likely to have a disorder that can be categorized within the mesomelic and rhizo-mesomelic dysplasia category. Skeletal surveys are very valuable in delineating bone abnormalities, understanding the pattern of bone involvement, and identifying classic radiographic features that are diagnostic hallmarks of a particular disorder. For instance, predominant involvement of bone in the region of the growth plate of the long bones (metaphyses) is classic for metaphyseal dysplasias; involvement of the metaphyses and the spine is classic for the disorders that are termed spondylometaphyseal dysplasias.

Whereas some GSDs can be typically diagnosed based on clinical and radiographic features (e.g., achondroplasia, OI, and Treacher Collins syndrome), the variable expressivity, clinical heterogeneity, and the significant overlap of phenotypic features between different groups of disorders often make the clinical diagnosis challenging. Moreover, in adults, the classic radiographic features that are typically observed during childhood may be absent. Thus, genetic testing is often required to make an accurate diagnosis. Appropriate diagnosis and management of GSDs require referral to specialized centers. Providing a detailed overview of the groups of GSDs and radiographic approaches for their diagnosis is beyond the scope of this chapter. Here, a general approach for the diagnosis and management of some of the commonly encountered GSDs in adults are presented.

GSDs that present with altered BMD

BMD is mineral content per unit area or unit volume of the bone. The areal BMD (aBMD) is measured at the lumbar vertebra, femoral neck, total hip, or the radius using dual-energy X-ray absorptiometry (DXA). Volumetric BMD is typically measured at the lumbar vertebra, radius, and tibia by quantitative computed tomography. Both areal and volumetric BMD have been used in clinical practice as surrogate markers of bone strength. The overall mineral content of bone is determined by the balance between the bone formation by osteoblasts and bone resorption by osteoclasts. Genetic disorders characterized by abnormalities in the function of osteoblasts or bone matrix can present with low BMD and altered bone

quality leading to significantly increased risk for fracture (e.g., OI). Similarly, pathogenic variants in genes that are required for the normal functioning of osteoclasts can result in disorders with increased bone density, decrease in bone turnover, altered bone quality, and increased fracture risk (e.g., osteopetrosis).

Disorders with decreased BMD

Many GSDs are characterized by decrease in BMD. Some examples include OI, Bruck syndromes types 1 and 2, osteoporosis-pseudoglioma syndrome, Cole–Carpenter dysplasia, spondylodysplastic type of Ehlers Danlos syndrome, cutis laxa, Mendelian forms of osteoporosis (e.g., *WNT1*-related osteoporosis), and disorders of connective tissue such as Marfan syndrome and Ehlers Danlos syndrome. In this section, we discuss OI, the most common genetic disorder that presents with low BMD and recurrent fractures.

Osteogenesis imperfecta

Overview

OI, also known as “brittle bone disease,” refers to a group of disorders that are characterized by significantly increased bone fragility [4]. The skeletal features of OI include low BMD, recurrent fractures, bone deformities, kyphoscoliosis, and short stature [5]. The extraskeletal features include blue sclera, dentinogenesis imperfecta (opalescent and brittle teeth), dental malocclusion, conductive or sensorineural hearing loss, pulmonary impairment, muscle weakness, and joint laxity. Whereas pathogenic variants in multiple genes can cause OI, a vast majority of OI is caused by pathogenic variants in two genes, namely *COL1A1* and *COL1A2*, which encode for pro α 1(I) and pro α 2(I) chains of type I collagen, the major matrix protein in bone. Type I collagen-related OI constitutes over 90% of all OI and has been classified based on clinically severity into one of the four “Sillence types”: nondeforming (type I, mild), perinatally lethal (type II, most severe), progressively deforming (type III, severe), and common variable (type IV, moderate). Majority of adults with OI encountered in clinical practice will be OI type I [5].

Evaluation

A diagnosis of type I collagen-related OI can often be made based on clinical features. The evaluation should focus on assessing the severity and type of OI. History of recurrent fractures with minimal or no trauma, recent history of long bone fractures, and worsening of prevalent vertebral fractures or occurrence of new vertebral fractures are typical indications for treatment with medications that increase BMD. Radiographs of the spine and DXA scans should be considered in all individuals. Chronic musculoskeletal pain is common and is caused by microfractures and generalized joint laxity and muscle weakness. Review of pain diaries and monitoring of intake of pain medications should be a part of routine evaluation. The prevalence of hearing loss increases with age and thus a baseline audiology evaluation should be conducted in all individuals; the evaluation should be repeated periodically based on symptoms and degree of hearing loss. Spirometry should be performed preferably in all individuals but especially in those with moderate-to-severe forms of the disorder. Clinicians should be aware that spirometry volumes are “normalized” to stature, and thus the “percent

predicted values” on spirometry underestimates the pulmonary involvement in OI [6]. A detailed oral examination is required to assess the degree of functional impairment due to dentinogenesis imperfecta and malocclusion of teeth, especially in individuals with more severe disease. Whereas there have been reports of aortic and arterial dissection, there is no consensus on cardiovascular surveillance in OI. Individuals with dyspnea, palpitations, or chest pain should undergo detailed cardiac evaluation. Individuals with OI types III and IV may have basilar invagination and compression of the brainstem or upper spinal cord; hence, recurrent headaches or features suggestive of compressive myelopathy should prompt further evaluation by imaging and referral to neurosurgery.

Genetic testing

In individuals with classic features of type I collagen-related OI (i.e., blue sclera, dentinogenesis imperfecta, multiple fractures, and hearing loss), sequencing and analysis of copy number variants (CNVs) in *COL1A1* and *COL1A2* should be performed. The majority of adults with OI have type I OI and hence the genetic testing should include assays to detect CNV. In individuals without pathogenic variants in *COL1A1* and *COL1A2*, panel-based testing can be used to interrogate other genes known to cause OI. When the phenotype is indistinguishable from other forms of skeletal dysplasia with recurrent fractures, or when skeletal features are associated with multisystem involvement, a genome-wide tool such as exome sequencing may be a more appropriate test.

Vignette 23.1

AB, a 26-year-old female who has a history of chronic musculoskeletal pain for many years, presents to the clinic with an acute-onset of back pain that is severe enough to interfere with daily activities. She states that there was no trauma to the back. Her medical history is significant for over 20 fractures during her lifetime. She states that her father has also had 20 or more fractures and thus she did not think this was unusual. Examination reveals bluish gray sclera, minimal bowing of the forearm, and mild scoliosis. There is tenderness over T7 vertebra.

The clinical presentation is consistent with OI type I. Management should focus on evaluation and treatment of acute vertebral pain as this is likely due to a vertebral fracture. Given the significant history of pain and the vertebral fracture, this individual will be a candidate for therapy with bisphosphonates. Spirometry, dental examination, audiology examination, and pain management should be instituted.

Management

The management of OI requires a multidisciplinary team including clinical geneticists, orthopedic surgeons, pain management specialists, endocrinologists, rehabilitation medicine experts, dentists, and otolaryngologists. The overall goals are to reduce fracture risk, increase mobility when possible, maximize function, optimize management of pain, and prevent pulmonary and cardiovascular complications. The primary healthcare providers should identify a team of physicians for coordination of care. Patients should be provided with a plan of action when they sustain fractures and information regarding precautions while undergoing

anesthesia. Currently, the medical therapy for OI to reduce fracture risk is largely limited to repurposing of medications that are used for the treatment of osteoporosis. Bisphosphonates, a class of medications that prevent bone resorption have become a standard of care in the treatment of OI, especially in children. Whereas the role of these medications in changing the natural history of the disorder is still debated [7], most experts would agree that children with the more severe forms of OI warrant treatment with this class of medications. The efficacy of bisphosphonates in adults has been less well established. Generally, adults with very low BMD, active history of fractures, and severe musculoskeletal pain are often candidates for treatment. Anabolic bone therapy with teriparatide has been shown to increase BMD and bone strength in adults with OI type I, but may not be similarly effective in severe forms of OI (types III and IV) [8]. Denosumab (anti-RANKL antibody) and setrusumab (antisclerostin antibody) are being evaluated in clinical trials.

Genetic counseling

Type I collagen-related OI (i.e., OI types I–IV) is transmitted in an autosomal dominant manner. Most severe forms are due to *de novo* pathogenic variants; however, in the mild form, that is, OI type I, up to 40% of probands can have a family history of the disorder. OI type I is caused by deficiency of qualitatively normal type I collagen. Thus, the variants that lead to haploinsufficiency of type I collagen include indels, nonsense and splice site variants, and CNVs that affect the reading frame. The triple helical regions of type I collagen chains have Glycine-X-Y repeats. OI types III and IV are caused by missense variants that result in glycine substitution; such variants have a dominant negative effect leading to posttranslational over-modification of assembled collagen. Hence, OI types III and IV is caused by production of qualitatively abnormal, overmodified type I collagen. Sequencing assays to detect CNV are typically able to detect the causative variant in more than 90% of individuals with type I collagen-related OI. It is important to note that the estimated rate of parental mosaicism in families with type I collagen-related OI had been previously estimated to as high as 15%; this is important for counseling regarding recurrence risks. However, this is likely to be an overestimate and such a high recurrence risk is at least in part due to the contribution of recessively inherited forms of OI. Regarding the other forms of OI, OI type V is transmitted in autosomal dominant manner, OI type XVIII is transmitted in an X-linked recessive manner, and the remaining forms (OI types VI to XVII) are autosomal recessive. If the pathogenic variant in the family is known, prenatal testing may be performed on fetal-derived tissues such as chorionic villus sampling or amniocentesis.

Disorders with increased BMD

GSDs that present with high bone density are characterized by defects in bone remodeling. Increase in osteoblastic activity (causing osteosclerosis) and/or decreased osteoclastic resorption (causing osteopetrosis) can lead to bones becoming thick, hard, and brittle. Examples for such conditions include osteopetrosis and related disorders (e.g., osteopetrosis late-onset form type 2, pcynodysostosis, and dysosteosclerosis), osteopoikilosis,

melorheostosis, Camurati–Engelmann diaphyseal dysplasia, LRP-5 related osteosclerosis, sclerostosis, and van Buchem-type endosteal hyperostosis.

Osteopetrosis

Overview

Osteopetrosis comprises a group of rare metabolic bone diseases characterized by impaired osteoclast activity or development. Absence of bone resorption leads to overly dense bone that is structurally brittle. The disruption of the normal remodeling of bone leads to fractures, bone deformities, dental abnormalities, mandibular osteomyelitis, and scoliosis. The expansion of bone into the marrow cavities and neural foramina results in secondary bone marrow failure and compression neuropathies, respectively. Most forms of osteopetrosis are childhood-onset diseases that are transmitted in an autosomal recessive manner. The form most likely to be encountered in adults is the autosomal dominant disorder, “osteopetrosis, late-onset form type 2” (OPTA2), which is caused by pathogenic variants in *CLCN7*. The latter condition is eponymously called Albers–Schönberg disease.

Evaluation

OPTA2 can present with variable severity [9]. The onset is typically during childhood and adolescence; however, individuals who manifest the first symptoms during the adult years and asymptomatic individuals have also been described. Fractures are the most common clinical feature. Dental abnormalities and osteomyelitis of the mandible can be observed. Osteosclerosis of the skull base can cause cranial nerve entrapment and visual loss; however, this feature is less commonly observed as compared to the autosomal recessive forms of osteopetrosis. Radiographs reveal osteosclerosis of the spine especially at the vertebral endplates (rugger-jersey spine). Sclerosis of iliac spine gives the appearance of “bone-within-bone.” Long bones can have transverse bands of sclerosis, perpendicular to the main axis.

Genetic testing

As there is significant overlap in phenotype between the various forms of osteopetrosis-related disorders as well as other sclerosing bone disorders, panel-based testing or exome sequencing would be appropriate to make the diagnosis.

Management

There are no disease-specific therapies for OPTA2. The management should be geared toward prevention and treatment of fractures, maintaining good oral hygiene to prevent osteomyelitis, and early recognition and treatment of entrapment neuropathies. Individuals with symptoms of visual impairment, headaches, and other cranial nerve compression should be promptly evaluated by imaging and referral to ophthalmology and neurosurgery.

Genetic counseling

OPTA2 is inherited in an autosomal dominant manner. Osteopetrosis with ectodermal dysplasia and immune defect is an X-linked disorder. The remaining forms of osteopetrosis including severe neonatal-onset, infantile-onset, and intermediate-onset forms are transmitted in an autosomal recessive manner.

GSDs with abnormal bone mineralization

Bone mineralization is the process by which calcium phosphate crystals are deposited on organic bone matrix. The mineralization of bone is a highly orderly process that is dependent on numerous factors including nature and structure of collagen matrix, calcium and phosphate flux, endocrine regulators, and paracrine factors. Disorders that are characterized by a decrease in bone mineralization can present with bone pain, bone tenderness, fractures, and bowing of long bones. The most common disorders that are characterized by decreased bone mineralization occur due to abnormal calcium, phosphate, and vitamin D metabolism; small bowel disease, malabsorption syndromes, biliary cirrhosis, nephrotic syndrome, Fanconi syndrome, secondary hyperparathyroidism, and tumor-induced osteomalacia are examples of such disorders. GSDs that manifest with decreased bone mineralization are rare. Some of the well-known disorders in this group include Mendelian forms of hypophosphatemia, hypophosphatasia, familial hyperparathyroidism types 1-4, and vitamin D-dependent rickets types 1 and 2.

Hypophosphatemia

Overview

Hereditary forms of hypophosphatemia can be transmitted in X-linked dominant, autosomal dominant, and autosomal recessive fashion. X-linked hypophosphatemia (XLH) is the most prevalent form [10]. These disorders are characterized biochemically by low plasma phosphate, renal phosphate wasting, and mild elevations of parathyroid hormone (PTH). Clinically, they present with short stature, rickets, osteomalacia, bowing of long bones, bone pain, musculoskeletal pain, and nephrocalcinosis. Here, the evaluation and management of adults with XLH will be discussed.

Evaluation

The phenotype of XLH is highly variable and ranges from isolated hypophosphatemia to severe disease with bowing of long bones, varus and valgus deformities of the knees, short stature, bone pain, and fractures. Early-onset osteoarthritis, enthesopathy (calcification of ligaments, joint capsules, tendons, and periarticular tissues), and osteophytes are highly prevalent and can cause significant musculoskeletal pain. Dental abnormalities including enlarged pulp chambers and spontaneous abscesses, and hearing loss are a part of the phenotype. Nephrocalcinosis can occur as a complication from treatment with oral phosphate and calcitriol supplements. Laboratory evaluation typically reveals hypophosphatemia, normal serum levels of calcium, normal or mildly elevated PTH levels, normal plasma 25-hydroxy vitamin D concentrations, and increased urinary phosphorus excretion (as quantified by calculation of

fractional renal tubular phosphate reabsorption). X-rays may show evidence of osteomalacia. The levels of phosphaturic hormone fibroblast growth factor 23 (FGF23) are typically elevated thus treatment with anti-FGF23 antibody has been shown to be an effective intervention.

Genetic testing

Whereas the diagnosis of XLH can be made based on clinical and biochemical features, genetic testing may be required for confirmation of diagnosis. XLH is caused by pathogenic variants in *PHEX*, which encodes for a neutral endopeptidase. About two-thirds of pathogenic variants are due to single-nucleotide variants while the remaining are due to deletions and duplications involving the gene. In cases where there is overlap with the phenotype with other forms of hypophosphatemia, a panel-based testing should be considered.

Vignette 23.2

BC, an 18-year-old female, presents to the clinic for evaluation of short stature, bowing of legs, significant back pain, and mild hearing loss. She has had little access to medical care and thus has not been evaluated for these complaints. Routine diagnostic evaluation reveals elevated alkaline phosphatase activity, modest elevation of PTH, and low phosphate levels in the blood. X-rays show radiolucencies in the femur which appear like “pseudofractures.” Urinary phosphorus levels are highly elevated.

The clinical presentation is highly consistent with XLH. Hypophosphatemia and phosphaturia are a classic biochemical findings in the disorder. The elevated alkaline phosphatase and the pseudofractures are markers of osteomalacia that results due to phosphate loss and defective mineralization.

Management

The overall goals of treatment in adults are to optimize pain, treat metabolic bone disease, prevent secondary hyperparathyroidism, treat fractures, and prevent nephrocalcinosis. Unlike in children, where treatment is recommended for all prior to fusion of epiphyses, the efficacy of treatment in adults is not well established. Calcitriol and neutral phosphate were the mainstay of treatment for many years. More recently, borsomab-twza, a monoclonal antibody that neutralizes FGF23, has been approved for treatment. In adults, treatment with this medication has been shown in few publications to improve fracture healing and decrease pain [11]. The treatment is typically indicated in symptomatic individuals with osteomalacia and pain, fractures, or those who are expected to undergo skeletal surgery.

Genetic counseling

XLH is inherited in an X-linked dominant manner; thus, both males and females can be affected.

Hypophosphatasia

Hypophosphatasia is caused by pathogenic variants in *ALPL*, which encodes tissue nonspecific alkaline phosphatase (TNSALP). Decreased activity of TNSALP in bone leads to accumulation of

inorganic pyrophosphate, a potent inhibitor of bone mineralization. The clinical manifestations of the hypophosphatasia are highly variable and range from still birth and perinatal lethality to mildly affected adults [12]. Adult hypophosphatasia typically presents with foot pain and stress fractures that heal poorly. Pseudofractures or “looser zones” in the hip and femur present with pain. Extracellular accumulation of inorganic pyrophosphate can cause arthropathy. Many adults report early loss of primary dentition as well as secondary dentition. In some individuals, adult hypophosphatasia can cause debilitating joint and skeletal pain. The diagnosis of hypophosphatasia can be made by clinical features and low alkaline phosphatase activity in plasma (although this has limited sensitivity), elevated blood pyridoxal phosphate (vitamin B6), or urinary phosphoethanolamine. Genetic testing is useful for confirmation. The management includes supportive treatment, pain management, and orthopedic interventions for fractures. Asfotase alfa, a formulation of nonspecific alkaline phosphatase with bone-specific targeting has been approved for the treatment of patients with perinatal/infantile- and juvenile-onset hypophosphatasia. The utility of such therapy in adults with mild forms of the disease is not yet established.

FGFR3-related chondrodysplasia

FGFR3-related chondrodysplasias are characterized by disproportionate short stature and rhizomelic shortening of extremities. This group of disorders includes achondroplasia, hypochondroplasia, thanatophoric dysplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), and camptodactyly, tall stature, and hearing loss syndrome (CATSHL).

Achondroplasia

Overview

Achondroplasia is a panethnic disorder and the most common inherited cause for disproportionate short stature. The estimated prevalence of the disorder is 1 in 20,000 live births. Nearly all individuals with achondroplasia harbor one particular gain of function, missense substitution in *FGFR3* that increases signaling through the receptor. The classic clinical features include macrocephaly with frontal bossing, midface hypoplasia, rhizomelic shortening of arms, short stature, brachydactyly, trident shape of the hands, and spinal abnormalities. The diagnosis is usually made at birth or early during life. The average adult height is around 4 ft. In addition to the skeletal abnormalities, obesity, obstructive sleep apnea, and spinal stenosis are major problems for adults with the disorder. Spinal stenosis may manifest with intermittent claudication pain, sensory deficits (numbness and pain), and motor symptoms (weakness, gait abnormalities, and limited walking endurance).

Evaluation

Radiographs reveal undertubulated and shortened long bones. In contrast to the normal pattern of widening of the interpediculate distance in the lumbosacral vertebrae, there is progressive narrowing. The iliac bones may appear flat with narrow sacroiliac notch. Individuals

with history of loud snoring, nocturnal awakenings, and daytime sleepiness should undergo detailed evaluations for obstructive sleep apnea. History suggestive of spinal stenosis should actively be sought.

Genetic testing

The diagnosis of achondroplasia is typically made by the characteristic clinical and radiographic features; genetic testing is often not required for the diagnosis. If genetic testing is required, targeted analysis should be performed for two variants that cause nearly all cases of achondroplasia, c.1138G > A (p.Gly380Arg), and c.1138G > C (p.Gly380Arg).

Management

In adults, the goal of management is to achieve normal body weight, identify and treat obstructive sleep apnea, and manage symptoms of spinal stenosis. The treatment of complications and symptoms as they arise is largely symptomatic. Recently, vosoritide, a recombinant C-type natriuretic peptide analog that stimulates endochondral ossification, has been evaluated in children with achondroplasia and has been shown to increase annualized growth velocity [13]. However, the effect of this medication on other skeletal manifestations of the disorder or its utility in prevention of complications of the spine has not yet been studied.

Genetic counseling

Achondroplasia is an autosomal dominant disorder. Nearly 80% of achondroplasia is a result of *de novo* variants. The risk for such *de novo* variants increases with advanced paternal age. About a fifth of individuals with the disorder have an affected parent. Homozygous achondroplasia results from transmission of the pathogenic variant from two affected parents. Homozygous achondroplasia is a severe skeletal disorder that resembles thanatophoric dysplasia and is perinatally lethal.

Points to remember

- GSDs are clinically and genetically heterogeneous.
- Due to the variable expressivity, clinical heterogeneity, and the significant overlap of phenotypic features between different groups of disorders, genetic testing is often required to make a diagnosis of GSDs in adults.
- OI is the most common Mendelian disorder that presents with recurrent fractures.
- The medical management of OI is largely limited to repurposing of medications used for treatment of low bone density and osteoporosis.
- Osteopetrosis is characterized by dense and brittle bones.
- XLH is an X-linked dominant disorder characterized by renal phosphate loss and decreased bone mineralization.
- Achondroplasia is the most common skeletal dysplasia that presents with disproportionate short stature.

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Skin disorders

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Introduction

The genodermatoses represent a large heterogeneous group of inherited disorders presenting with a myriad of skin findings, benign and malignant. Often, cutaneous manifestations are the presenting sign of many multisystem diseases—the ash leaf macules can signal a risk for cardiac rhabdomyoma in tuberous sclerosis, or multiple epidermoid inclusion cysts and pilomatricomas can predict colon cancer in Gardner syndrome. The recognition of dermatologic manifestations is therefore of utmost importance for the appropriate workup of associated abnormalities, surveillance of associated malignancies and the ongoing management of complex disorders often requiring a multidisciplinary approach. In this chapter, the commonly encountered genodermatoses are discussed.

Pigmentation disorders

The quantity and quality of pigment production determine the color of the skin. The melanosome is an intracytoplasmic organelle within the melanocyte where melanin is produced, processed and eventually transported to nearby keratinocytes. Alterations in embryogenic melanocyte migration or the melanin biosynthetic pathway lead to pigmentary alterations.

There are numerous inherited and acquired conditions characterized by altered pigmentation, thus the correct nomenclature is crucial in making the correct diagnosis. Hypopigmentation and hyperpigmentation are general descriptive terms meaning lightening or darkening of the skin, respectively, and are not always related to the melanin, that is, pigment, content in the skin. In the case of hypopigmentation, more precise are the terms hypomelanosis and amelanosis, meaning a reduction in, and an absence of melanin, respectively. Hypomelanosis may be a result of a decrease or absence of melanocytes, that is, melanocytopenic hypomelanosis. Examples include Waardenburg syndrome (WS) and piebaldism.

Alternatively, the number of melanocytes may be normal, and rather the melanocytes cannot synthesize melanin or transfer it to the surrounding keratinocytes properly, that is, melanopenic hypomelanosis. Examples of melanopenic hypomelanosis include oculocutaneous albinism (OCA) and the silvery hair syndromes. A similar concept exists in areas of hyperpigmentation. Melanocytes may be increased, as seen in melanocytic nevi or lentigines, or conversely, melanin content may be increased, as seen in ephileides (freckles).

In the setting of both hypo- and hyperpigmentation collectively, a term often used is dyschromatosis. The diagnosis that typically falls into this category includes dyschromatosis symmetrica hereditaria and dyschromatosis universalis hereditaria. Disorders of chromosomal instability such as Fanconi anemia and Nijmegen breakage syndrome (NBS) are in this differential diagnosis.

Oculocutaneous albinism

Overview

Albinism is characterized by partial or total absence of melanin within melanocytes of the skin, hair follicles and eyes, leading to diffuse pigmentary dilution. The seven subtypes of nonsyndromic OCA (Table 24.1) [1] are caused by pathogenic variants in genes required for melanin production and processing. All are inherited in an autosomal recessive (AR) manner.

Evaluation

Individuals with OCA1A are designated tyrosinase-negative, as they are unable to produce any melanin in the hair, skin, and irides. This leads to a severe, early-onset ocular phenotype, consisting of photophobia, nystagmus, strabismus, and decreased visual acuity. These patients often develop skin cancers in young adulthood. OCA1B and OCA2 have varying

Table 24.1 Nonsyndromic oculocutaneous albinism subtypes.

Type	Gene	Clinical characteristics
OCA1A	<i>TYR</i>	Most severe form with total absence of pigmentation: white skin, hair, blue irides; highest risk of skin cancer
OCA1B	<i>TYR</i>	Amount of pigmentation depends on activity level of tyrosinase preserved
OCA2	<i>P protein</i>	Pigmentation advances with age. Most common form in patients of African origin. <i>P</i> gene resides on chromosome 15. 1% of patients with Prader–Willi or Angelman syndrome have OCA2
OCA3	<i>TRYP1</i>	Reddish-bronze color of skin and hair
OCA4	<i>MATP/SLC45A2</i>	Most common form in Japan. Similar phenotype to OCA2
OCA5	Unknown	Very rare
OCA6	<i>SLC24A5</i>	Pigmentation exists on a spectrum
OCA7	<i>C10orf11</i>	Eye symptoms predominate

OCA, Oculocutaneous albinism.

levels of pigment, depending on the amount of tyrosinase activity. Similarly, their ocular involvement and risk of skin cancers exist on a spectrum.

The differential diagnosis of pigmentary dilution includes syndromic forms of OCA, including Hermansky–Pudlak syndrome (HPS) and Chediak–Higashi syndrome (CHS), which have other organ involvement. HPS is also characterized by a hemorrhagic diathesis and deposition within the lungs, GI tract and reticuloendothelial system. CHS presents with immunodeficiency leading to recurrent infections of the upper and lower respiratory tract, as well as the skin. Griscelli syndrome (GS), like CHS, presents with pigmentary dilution and is classified as a silvery hair syndrome. GS, though, is not thought of as a type of albinism due to the lack of visual alterations [1].

Genetic testing

The diagnosis of OCA is typically based on clinical findings, especially for OCA1A where there is a total absence in pigment; molecular genetic testing may be used to confirm or establish a diagnosis. Pathogenic sequence variants in *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5* may be discovered through sequence analysis.

Management

Individuals suspected of albinism should follow up with a dermatologist and ophthalmologist. The risk of both keratinocyte carcinoma and melanoma is increased and strict sun-protective behavior should be encouraged. Tinted and corrective lenses can help with vision and photophobia, while surgery and patching may be helpful for nystagmus and strabismus, respectively.

Genetic counseling

Heterozygotes, or carriers, are asymptomatic. Carrier testing for at-risk family members is possible if both the pathogenic variants in the affected individual have been identified.

Neurocristopathies

Overview

The process by which melanocytes migrate to their ultimate destination in the skin from the neural crest during embryogenesis is dependent upon complex interactions between numerous receptors and signaling ligands. Transmembrane KIT receptors on melanocytes facilitate this migration, and heterozygous pathogenic variants in *KIT* lead to piebaldism. Transcription factors also play an integral role in this sophisticated embryogenic process, and pathogenic variants in those such as *PAX3*, *MITE*, and *SOX10* lead to WS. Both of these neurocristopathies are most likely to be inherited in an autosomal dominant (AD) fashion.

Evaluation

In patients with congenital patches of depigmentation, it is most often due to a lack of melanocytes, as a result of failed migration during embryogenesis. In the case of piebaldism, the

depigmented areas tend to be symmetric, and do not increase in size or number over time. Within the areas of depigmentation on extremities, often there are islands of hyperpigmentation. Similar to WS, a white forelock of hair may be present, though heterochromia irides and sensorineural hearing loss are unlikely to be discovered in piebaldism.

There are multiple subtypes of WS and manifestations typically include congenital sensorineural hearing loss, as well as a pigment alteration of the iris (heterochromia iridum), hair (white forelock) and skin (depigmented patches). In addition, some subtypes may present with dystopia canthorum. In a patient suspected of having WS, audiology evaluation is essential. The differential diagnosis includes pigmentary mosaicism, which often presents around the time of birth, though can go unnoticed until adolescence. Clinically, the patches are hypopigmented, rather than depigmented, and often have feathered or ill-defined margins. Vitiligo may also be a consideration with depigmented patches, though this is very rarely found at birth.

Genetic testing

Identification of a pathogenic variant by sequence analysis with a multigene panel, including *PAX3*, *MITF*, *SLUG*, *SOX10*, *EDNRB*, and *EDN3*, depending on the clinical findings can confirm the diagnosis of WS.

Management

Symptomatic management is recommended.

Genetic counseling

The majority of individuals with WS and piebaldism have an affected parent. A careful clinical exam of parents is warranted, as they may have a milder phenotype. In women at risk for having a child with WS, folic acid supplementation in pregnancy is recommended due to a possible increased risk of neural tube defects.

Multiple lentiginos syndromes

Overview

Lentiginos are typically subcentimeter tan to brown macules. They may occur diffusely or in a localized or segmental distribution. Both, localized or extensive lentiginos may indicate a multisystem autosomal dominant disorder. Peutz–Jeghers syndrome (discussed in Chapter 13: Gastrointestinal disorders), Carney complex and Noonan syndrome with multiple lentiginos (NSMLs) are the three main inherited lentiginous syndromes. NSML was previously referred to as LEOPARD syndrome and cardiomyopathic lentiginos. Individuals previously diagnosed with NAME (nevi, atrial myxoma, myxoid neurofibroma, ephelides) or LAMB (lentiginos, atrial myxoma, mucocutaneous myxoma, blue nevi) likely fall under the name of Carney syndrome or complex [2].

Evaluation

While relatively uncommon, the recognition of the lentiginosis syndromes is important due to the multiplicity of systemic manifestations. While these skin findings may present early in life, they usually present around puberty [2]. Café au lait macules (CALMs) are frequently observed, usually preceding the appearance of lentigines [3]. Individuals with lentigines around the lips, eyelids, genitalia, and ears should be considered as deserving of evaluation for the other manifestations in these conditions.

Patients with NSML syndrome frequently display dysmorphic facies including widely spaced eyes and ptosis, in addition to pectus deformity and short stature. The diagnosis may be suspected in the first year of life with CALMs, hypertrophic cardiomyopathy and characteristic facies [3]. Evaluation in a patient suspected to have NSML syndrome should include an evaluation of growth parameters, a cardiac evaluation with echocardiogram and electrocardiography, an ophthalmologic exam, audiometry testing, and a renal ultrasound.

Carney complex may present early in life, though more commonly it is diagnosed in the second decade. In addition to lentigines and CALMs, as seen in NSML, affected individuals may also have epithelioid-type blue nevi. Myxomas are characteristic of NSML, involving multiple organ systems including the skin, breasts, oropharynx, bone, and heart. Cardiac involvement is responsible for more than 50% of mortality in this patient population [2,4]. In addition, adrenocortical disease as well as testicular tumors can be seen in affected individuals. As such, an echocardiogram, as well as imaging or biochemical screening for endocrine tumors, is necessary for their evaluation.

In addition to the major three lentiginosis syndromes, the differential diagnosis for the multiple lentigines includes xeroderma pigmentosum (XP), which can present with early lentigines in a photo-distributed pattern as well as chromosomal breakage syndromes such as Fanconi anemia and NBS presenting with a speckled like pigmentation. Familial lentiginosis typically is not associated with other syndromic findings. With lentigines limited to the genitals, Bannayan–Zonana–Ruvalcaba syndrome, which is allelic with Cowden syndrome, should be considered.

Genetic testing

Carney complex has an overall penetrance that exceeds 95% by the age of 50 years. The *de novo* rate is approximately 30% in Carney complex [5]. Single base substitutions and copy number variants in *PRKACA* and *PRKACB* have been described [4,6].

NSML has variable expressivity, and thus penetrance is difficult to determine. In patients suspected to have NSML, a multigene panel that includes *PTPN11*, *RAF1*, *BRAF*, and *MAP2K1* detects nearly 100% of variants [3].

Management

Any patient with the diagnosis of Carney complex should be followed closely on an annual basis at a minimum, which improves prognosis (Table 24.2). In the case of NSML, treatment

Table 24.2 Recommended surveillance and follow-up of patients with Carney complex [7].

Recommended test	Comments
Annual echocardiogram	Beginning in infancy; if patient was diagnosed with a cardiac myxoma at least once, cardiac imaging may be done biannually
Regular skin evaluations	
Blood tests to check for:	Annually, beginning in adolescence, as appropriate for the detection of GH and prolactin excess
• Serum GH	
• IGF-1	
• Urinary free cortisol	
Thyroid gland clinical exams	Annually. Ultrasound, if needed
Imaging may include:	
• Adrenal computed tomography for the detection of primary pigmented nodular adrenocortical disease	
• Pituitary MRI	
• MRI of brain, spine, chest, abdomen, retroperitoneum, pelvis for detection of psammomatous melanotic schwannomas	
Testicular examinations with ultrasound	Annually
Transabdominal ultrasound of the ovaries	Baseline examination; it may be repeated, as needed
Close monitoring of linear growth rate and annual pubertal staging	In prepubertal children

GH, Growth hormone; MRI, magnetic resonance imaging.

of cardiovascular anomalies, deafness, and cryptorchidism is the same as in the general population. For those affected by hypertrophic cardiomyopathy, there may be restrictions on some physical activities.

Genetic counseling

Family history may be falsely negative in the case of Carney complex and NSML, as early death may have occurred before the recognition of other symptoms or signs of disease. Due to the potentially fatal cardiac complications in at-risk family members, it is appropriate to consider molecular genetic testing in those circumstances. Women affected by NSML with hypertrophic cardiomyopathy or valve dysfunction are at risk for development or exacerbation of heart failure during pregnancy.

Photosensitivity disorders

Inherited conditions in which repair of UV radiation-induced DNA damage is inadequate are characterized by cutaneous and ocular photosensitivity and an increased risk of cutaneous and ocular malignancies.

Xeroderma pigmentosum

Overview

XP is an autosomal recessive disease divided into seven complementation groups based on genes whose products affect DNA repair. The majority of cases appear between 6 months and 3 years of age [8], though variants may present much later [9].

Evaluation

A diagnosis of XP is suspected in those with severe sunburn with blistering following minimal sun exposure. All patients with XP have numerous freckle-like brown or black macules. Ocular abnormalities, including photophobia and keratitis, develop in the anterior UV exposed portions of the eye. Cancer of the eye and skin as a result of UV exposure occur often in the first decade of life. In 25% of affected patients, progressive neurologic abnormalities, including microcephaly, ataxia, intellectual impairment, and sensorineural hearing loss have been reported.

Cockayne syndrome (CS), Fanconi anemia, and trichothiodystrophy (TTD) are allelic to various subtypes of XP and are included in the differential diagnosis. An XP/CS complex attributed to pathogenic variants in *ERCC2–5* exists, characterized by facial freckling, early skin cancers and intellectual disability, though the neural disease is characterized by dysmyelination rather than degeneration. Similarly, an XP/TTD complex exists as a result of *ERCC2* pathogenic variants and results in both phenotypic features of TTD and XP including ichthyosis and brittle hair, as well as increased frequency of skin cancers. While the multiple lentiginoses syndromes like Carney complex may be included in the differential, they lack the signs of photodamage such as poikiloderma. Blistering with minimal sun exposure also warrants consideration of porphyrias and systemic lupus erythematosus.

Genetic testing

A multigene panel consisting of *DDB2*, *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *POLH*, *XPA*, and *XPC* can be obtained to identify biallelic pathogenic variants.

Management

Preventative management includes strict photoprotection with sunscreen, photoprotective clothing, and sun avoidance behavior. Entire body skin exam with special attention to areas of sun exposure must be done every 3–12 months. Examination of the lids and anterior portions of the globe is also recommended.

Field therapy with 5-fluorouracil or imiquimod can be used for widespread actinic damage. Oral retinoids can be helpful in chemoprevention in those actively developing large numbers of skin cancers. Skin cancers are treated with electrodesiccation/curettage, surgical excision or Mohs surgery. Eye drops can be helpful in keeping the cornea moist. A corneal transplant can treat keratitis. Vitamin D supplementation may be warranted in those avoiding the sun with low serum concentrations. Magnetic resonance imaging (MRI) of the brain, nerve conduction studies, and audiometry testing may be recommended.

Genetic counseling

Heterozygote carriers have classically been thought to be unaffected, though some evidence suggests variants of the *POLH* gene mutated in XP variant have an increased risk of melanoma [10]. Oral retinoids must be used with caution in women with childbearing potential due to the risk of birth defects.

Rothmund–Thomson syndrome*Overview*

Rothmund–Thomson syndrome (RTS) is an autosomal recessive disease with pathogenic variants in a DNA helicase required for DNA replication, repair of UV light-induced DNA damage and telomere maintenance found in over half of affected patients [11].

Evaluation

Diffuse erythema of the face, buttocks and extensor surfaces of the arms and legs with poikiloderma—reticulated telangiectasia and areas of both hypo—and hyper-pigmentation with atrophy, progress until age five. Patients often have severe growth failure with a characteristic triangular configuration to their faces. Adults have an increased risk of squamous cell carcinoma and melanoma [12]. Cataracts typically appear early but can come in adulthood, and hypogonadism occurs in some [12]. Bony abnormalities characterize the disorder, with abnormalities in the thumb and distal extremities occurring most commonly. Osteosarcoma, most commonly in the tibia or femur occurs in roughly one-third of patients, highlighting the importance of surveillance for bone pain, swelling or an enlarging lesion on a limb. Baseline radiologic evaluations of long bones are recommended by age 3 years. As isolated hematologic abnormalities exist, a complete blood count with differential is recommended in the initial evaluation of those suspected of RTS.

The differential diagnosis includes other conditions characterized by poikiloderma, such as dyskeratosis congenita (DKC). DKC displays nail dystrophy, oral leukoplakia with the poikiloderma, and the majority of patients eventually develop bone marrow failure. Bloom syndrome also shows photosensitivity but will also demonstrate recurrent infections and chronic pulmonary disease. XP will show that poikiloderma, through the early onset of keratinocyte carcinoma before age 10 years, and keratitis have distinguishing features.

Genetic testing

In a patient with early-onset poikiloderma coupled with skeletal abnormalities and a strong suspicion for RTS, single-gene analysis of *RECQL4* may be performed in presence of such mutation shows relationship to risk of osteosarcoma.

Management

Following diagnosis, a baseline dermatologic evaluation, skeletal radiographic examination, complete blood count with differential and an ophthalmologic examination is advised. Calcium and vitamin D supplementation may be beneficial. Cataracts affecting vision require

surgical removal. Surveillance includes an annual examination monitoring growth, skin examination monitoring for cancer and ophthalmologic examination screening for cataracts. Most importantly, skeletal imaging must be obtained should there be any suspicion of osteosarcoma.

Genetic counseling

Heterozygote carriers are asymptomatic. The carrier frequency is unknown, though estimated to be low given the rarity of the disorder. Founder variants exist in western Austria and in the Mennonite population. Prenatal genetic testing is possible if *RECQL4* pathogenic variants are known, though ultrasound examination at 16–18 weeks of gestation may detect skeletal abnormalities.

The porphyrias

Overview

Porphyrin, a chemical precursor for hemoglobin synthesis, is a photosensitizer excited by visible light. When exposed to wavelengths 400–410 nm in the presence of oxygen, free radicals are generated causing cell and tissue damage. The porphyrias can be classified clinically into three groups, acute, cutaneous and rare recessive porphyrias; [13] all are characterized by pathogenic variants in genes encoding enzymes in the heme biosynthetic pathway. The porphyrias and their inheritance patterns are described in Table 24.3.

Evaluation

Hereditary porphyrias are characterized by acute neurovisceral symptoms, skin lesions, or both. The acute porphyrias—acute intermittent porphyria (AIP), variegate porphyria (VP), and hereditary coproporphyria (HCP)—present with sudden life-threatening crises that are infrequent and very difficult to diagnose. A prodrome is described with anxiety and insomnia, followed by severe abdominal pain commonly with nausea, vomiting, and constipation. Hyponatremia may lead to seizures, and in 20%–30% of patients, psychiatric manifestations such as depression, disorientation, hallucinations or paranoia are reported. Neuropathy can develop if porphyrinogenic drugs are used and manifests as pain and weakness leading to local paresis. VP and HCP may present with acute blistering of the skin. AIP never presents with skin lesions.

Algorithms for the diagnosis of acute porphyrias exist [14]. When any acute porphyria is suspected, urine porphobilinogen and total porphyrins are recommended. Elevated urinary porphobilinogen is the first-line test for the diagnosis of acute porphyria, as it is elevated in AIP, VP, and HCP [13]. To distinguish the type of acute porphyria, plasma fluorescence emission spectroscopy is helpful, as a peak at 624–628 nm will arise in VP. In remission, urine, fecal, and plasma porphyrin concentrations are generally normal in acute porphyrias, and

Table 24.3 The porphyrias.

Type	Inheritance	Gene	Enzyme deficiency	Clinical characteristics
Congenital erythropoietic porphyria	AR, XLR	<i>UROS, GATA1</i>	Uroporphyrinogen III synthase, GATA binding factor 1	Red urine in infancy, severe photosensitivity, splenomegaly, and hemolytic anemia
Erythropoietic protoporphyria	AD/AR, XLR	<i>FECH</i>	Ferrochelatase, δ -aminolevulinic acid synthase, erythroid-specific (ALAS2)	Most common porphyria in children; photosensitivity manifests as pain, burning; cholelithiasis, liver failure
Acute intermittent porphyria	AD	<i>HMBS</i>	Porphyrinogen deaminase	Acute abdomen and neuropsychiatric manifestations
Porphyria cutanea tarda	AD	<i>UROD</i>	Uroporphyrinogen decarboxylase	Most common porphyria in adults, presents in third to fourth decades; bullae on dorsal hands, milia in sites of sun exposure
Hereditary coproporphyria	AD	<i>CPOX</i>	Coproporphyrinogen oxidase	Neonatal jaundice and anemia; acute abdomen and neuropathy
Variegate porphyria	AD	<i>PPOX</i>	Protoporphyrinogen oxidase	Acute abdomen
δ -Aminolevulinic acid dehydratase porphyria	AR	<i>ALAD</i>	δ -Aminolevulinic acid dehydratase	Acute abdomen and neuropsychiatric manifestations
Hepatoerythropoietic porphyria	AR	<i>UROD</i>	Uroporphyrinogen decarboxylase	Erythrodontia, splenomegaly, and anemia

AD, autosomal dominant; AR, autosomal recessive.

fluorescence emission spectroscopy of plasma has the greatest sensitivity. DNA analysis is the gold standard.

The cutaneous porphyrias can be divided into those with bullous disease—VP, HCP, and porphyria cutanea tarda (PCT), and those with painful photosensitivity—erythropoietic protoporphyria (EPP) [13]. Bullous presentations include blisters and skin fragility on the dorsal hands and sun-exposed areas, resulting in milia and scarring. Facial hypertrichosis and hyperpigmentation are also frequently observed. PCT, the most common type seen in adults and second most commonly seen in children, may be sporadic in the setting of multiple susceptibility factors associated with PCT, or familial in the setting of positive family history. Sporadic and familial PCT are clinically indistinguishable, however the sporadic subtype presents earlier.

The best initial test for the diagnosis of cutaneous porphyrias is the plasma fluorescent spectrum and measurement of plasma porphyrins to differentiate between VP and PCT. Detailed algorithms are available [15]. EPP is the most common type seen in children and is characterized by burning, pain, and pruritis immediately following sun exposure beginning in childhood. Cholelithiasis may be seen in a hepatic ultrasound if suspected clinically. Markedly increased free erythrocyte protoporphyrin establishes the diagnosis.

Rare recessive porphyrias include congenital erythropoietic porphyria (CEP) and hepatoerythropoietic porphyria (HEP). CEP is the most frequent of the two and is characterized by severe photosensitivity and hemolysis; erythrodontia, osteodystrophia, and hypercellular bone marrow are present in almost all patients. Red fluorescent urine in diapers is a helpful clue, and early diagnosis is critical to avoid phototherapy for neonatal jaundice. HEP also presents with red urine and blistering skin, however biochemical findings resemble those in PCT. When a porphyria is suspected, a medical history regarding alcohol consumption, tobacco exposure, medication use and exposure to hepatotoxins is necessary. Laboratory evaluation in search of excess hepatic iron, hepatitis C and human immunodeficiency virus infection is also warranted.

Genetic testing

Once a diagnosis is established by biochemical methods, an analysis of respective genes may be obtained to confirm the diagnosis. Given the episodic nature of the porphyrias and the possibility of clinical and biochemical remission, genetic testing may be diagnostic. Furthermore, the majority of carriers of the autosomal recessive porphyrias are asymptomatic. When acute porphyria is suspected and there are cutaneous manifestations, *CPOX* and *PPOX* may be analyzed due to the overlapping biochemical findings in HCP and VP. If there are no cutaneous manifestations, *HMBS* may be added to this panel, to evaluate for AIP. If skin findings predominate without neurovisceral episodes, a multigene panel with *UROD*, *CPOX*, and *PPOX* may be obtained.

Management

Preventative measures in all porphyrias include avoiding porphyrinogenic drugs, alcohol, smoking, cannabis and hallucinogens, dieting and fasting. Treatment in the case of acute porphyrias should be started promptly. Pain management often requires opioid therapy. Fluid and electrolyte balance should be maintained, and adrenergic and central nervous system (CNS) symptoms managed closely so as to monitor for paralysis and impairment of consciousness. Intravenous heme administration is the treatment of choice.

The hallmark of preventative management of the cutaneous porphyrias is photoprotection. Beta carotene supplementation in EPP can increase tolerance to sunlight. Hemolytic anemia in HEP and CEP can be treated with blood transfusion. Weekly iron and porphyrin depletion and/or chloroquine can be used to treat PCT. Screening for diabetes mellitus and hepatocellular cancer annually is generally agreed upon for PCT. Hepatic failure in EPP may require liver transplantation. Allogeneic bone marrow transplant is the only curative treatment in CEP.

Genetic counseling

Carriers, regardless of symptomatology, should be counseled to maintain a healthy diet, avoid alcohol, smoking, and porphyrinogenic drugs. Patients with both symptomatic and

latent diseases have increased risks of hypertension, hepatocellular carcinoma and chronic renal failure [13].

Neurocutaneous disorders

The neurocutaneous disorders, often called the phakomatoses, include neurofibromatosis type 1 (NF1) and tuberous sclerosis complex (TSC). Like most genetic tumor syndromes, they are inherited in an autosomal dominant fashion. In both NF1 and TSC, the first recognized manifestation is often in the skin, with CALMs and ash leaf macules, respectively, presenting in the first year of life.

Neurofibromatosis type 1

Overview

NF1 is caused by pathogenic variants in the *NF1* gene. This protein acts as a tumor suppressor, negatively regulating the Ras-mitogen-activated protein kinase pathway, thereby regulating cellular growth and proliferation [16].

Evaluation

The diagnosis of NF1 is typically made based on criteria set forth by the National Institutes of Health (NIH) (Table 24.4) [17]. A full-body skin examination can reveal the majority of clinical criteria. Special attention must be paid to the distribution of any cutaneous findings, as the occurrence of mosaicism is well described in NF1. CALMs are found in over 90% of affected individuals. Multiple CALMs are very common in the normal population, and thus a minimum of six CALMs are required for diagnosis. Tan macules in the axillae and inguinal folds represent intertriginous freckling, and by puberty patients will develop fleshy, skin-colored papules, called neurofibromas. A thorough skin examination may also reveal plexiform neurofibromas (PNFs), pathognomonic for NF1, which are typically congenital though not often recognized until adolescence. PNFs are benign tumors of the nerve sheath, and present variably, from hyperpigmented patches or plaques to a saggy plaque described as a

Table 24.4 The National Institutes of Health diagnostic criteria for neurofibromatosis type 1.

Two or more of the following present:	Six or more café au lait macules, >5 mm in prepubertal individuals, >15 mm in postpubertal individuals
	Two or more neurofibromas of any type, or one plexiform neurofibroma
	Axillary or inguinal freckling
	Optic glioma
	Two or more Lisch nodules
	Osseous lesion, for example, sphenoid wing dysplasia or thinning of long bone cortex
	First-degree relative with neurofibromatosis type 1 by the above criteria

Table 24.5 Major clinical features of NF1.

Cutaneous	Cutaneous neurofibromas, café au lait macules, freckling, plexiform neurofibromas, nevus anemicus, and juvenile xanthogranulomas
Ocular	Lisch nodules, choroidal nodules, glaucoma, and optic gliomas
Skeletal	Macrocephaly, hypertelorism, sphenoid wing dysplasia, dysplasia of long bone cortex, scoliosis, osteopenia, short stature, and pectus deformity
Cardiovascular	Hypertension, pulmonic stenosis, and renal artery stenosis
Neurologic	Unidentified bright objects on magnetic resonance imaging, learning difficulties, seizures, intellectual impairment, aqueductal stenosis, and hydrocephalus
Malignancy	Juvenile myelomonocytic leukemia, CNS tumors, rhabdomyosarcoma, breast cancer, and malignant peripheral nerve sheath tumors

“bag of worms.” Given their origin from the nerve sheath, these may cause pain, focal neurologic deficits or deformity.

The emergence of the myriad of clinical features in patients with NF1 (Table 24.5) over time is well described. CALMs appear in the first few years of life, followed by intertriginous freckling, Lisch nodules, and optic gliomas, until puberty, when cutaneous neurofibromas emerge. By age 8 years, 97% of affected individuals will meet NIH criteria for NF1, and by age 20 years 100% will meet criteria.

The differential diagnosis in the evaluation of neurofibromatosis includes conditions with freckling or multiple CALMs. Aside from mosaic NF1, unilateral freckling, or lentiginos, may be found in a large nevus spilus or partial unilateral lentiginosis, both of which do not cross the midline. CALMs and freckling may also be seen in Legius syndrome, which lacks the tumors and noncutaneous findings, for example, neurofibromas, Lisch nodules, gliomas, etc., found in NF1 [18]. Watson syndrome, which is allelic with NF1 presents with CALMs, freckling, neurofibromas and Lisch nodules in addition to pulmonic stenosis. Constitutional mismatch repair deficiency syndrome, defined by pathogenic variants in DNA mismatch repair genes, may also have CALMs, axillary freckling, and neurofibromas, with an increased risk of malignancy. Multiple CALMs are also found in Fanconi anemia, which can display areas of depigmentation and hyperpigmentation.

Genetic testing

In NF1, loss of function pathogenic variants occurs nearly anywhere within the *NF1* gene, found on chromosome 17q11.2.

Though typically a clinical diagnosis, molecular genetic testing for the identification of a pathogenic variant in *NF1* can be pursued in those (usually children) in whom NF1 is suspected but who do not yet fulfill the criteria, or in those with a tumor, for example, optic glioma, and a diagnosis would immediately affect management. Prenatal or preimplantation diagnosis in affected families is also a consideration [18].

A multigene panel can be considered, especially if Legius syndrome is considered in the differential diagnosis, given the significant phenotypic overlap. However, the detection

frequency of pathogenic variants in *NF1* on a panel using genomic DNA sequencing alone only detects between 60% and 92% of pathogenic variants [19,20].

Management

A multidisciplinary approach is required for the management of NF1. The American Academy of Pediatrics maintains an excellent guideline for the management and supervision of children with NF1 over time [21]. Monitor for the development of malignant peripheral nerve sheath tumors and/or painful or disfiguring neurofibromas. Rapid growth, increased firmness or persistent pain in an existent PNF warrants positron emission tomography-computed tomography (PET-CT) imaging. Surgical excision of any symptomatic or disfiguring neurofibromas or PNFs has been the mainstay, although medical treatments with MEK inhibitors are currently being studied for inoperable or health-threatening PNFs [22].

Careful attention to blood pressure should be paid, as pheochromocytomas and renal artery stenosis may present as hypertension, although benign essential hypertension is the most common etiology of hypertension in NF1 patients. Breast cancer is a particular concern for women under 50, as the risk in those with NF1 is five times higher than those without; this is coupled with higher mortality rates than the general population [23]. A reduction in bone mineral density compared with the general population has been well established in both pediatric and adult patients with NF1. Monitoring density and vitamin D3 supplementation should be considered.

Genetic counseling

NF1 is inherited in an autosomal dominant manner. While the penetrance of NF1 approaches 100%, nearly half of patients possess *de novo* variants [24] and the disease carries widely variable expressivity. All first-degree family members should undergo a clinical evaluation. If a pathogenic variant has been identified, prenatal testing for pregnancies at increased risk is possible.

Tuberous sclerosis complex

Overview

TSC, another multisystem tumor predisposition syndrome classically known for its neurocutaneous hamartomas, is caused by pathogenic variants in the *TSC1* or *TSC2* gene. Similar to other tumor predisposition syndromes, it is inherited in an autosomal dominant pattern, though somatic mosaicism is described. Two-thirds of individuals with TSC have a *de novo* variant [25].

Evaluation

TSC should be suspected in individuals with either one major clinical feature or two or more minor features listed in Table 24.6, or with the identification of a heterozygous pathogenic variant in either *TSC1* or *TSC2* by molecular genetic testing [26].

Table 24.6 Clinical features of TSC.

Major features	Angiofibromas (> 2) or fibrous cephalic plaque Cardiac rhabdomyoma Cortical dysplasias, including tubers and cerebral white matter migration lines Hypomelanotic macules (3 > 5 mm in diameter) Lymphangioliomyomatosis Shagreen patch Subependymal giant cell astrocytoma Subependymal nodules Ungual fibromas (> 1) Detailed dermatologic exam Detailed dental exam
Minor features	“Confetti” skin lesions (numerous 1–3-mm hypopigmented macules scattered over regions of the body) Dental enamel pits (> 3) Intraoral fibromas (> 1) Multiple renal cysts Nonrenal hamartomas Retinal achromic patch

Similar to NF1, the skin often represents the presenting system of involvement. Hypomelanotic macules or patches, ranging from 1 to 12 cm in size, are typically the first presenting sign. “Confetti” or “guttate” hypopigmented macules, though seen less frequently, are likely the most specific pigmentary manifestation. In a patient with multiple hypomelanotic macules, a full skin exam should be obtained. If Wood’s lamp confirms hypopigmentation and identifies three or more hypomelanotic macules, an ophthalmologic exam and a cranial MRI should be obtained. If retinal hamartomas or depigmentation, brain tubers, migrational defects, subependymal nodules, or cardiac rhabdomyomas are revealed TSC is likely, especially in the setting of a seizure history. If at this point in time the patient does not meet clinical criteria, genetic testing can be considered, though sensitivity is only 85%. Alternatively, the patient could be monitored annually with mucocutaneous and ophthalmologic exams, with the consideration of renal ultrasounds evaluating for renal angiomyolipomas or repeat brain imaging.

By 2 years of age, smooth skin-colored papules called angiofibromas tend to appear bilaterally and symmetrically on the central face. A larger variant of the angiofibroma is the fibrocephalic plaque, a firm pink-brown slow-growing plaque that occurs on the face and scalp and often presents before other features of TSC. The shagreen patch, a type of collagenoma, often presents as skin-colored plaques with accentuated follicular ostia in the lumbosacral region. Later into adolescence and adulthood, ungual fibromas, also known as Koenen tumors, develop. Many adults with TSC develop intraoral fibromas commonly found on the gingiva, in addition to dental enamel pits, so a thorough oral exam is necessary. Seizures occur in nearly 70% of infants with TSC, and typically present as early as 3 months of age. The earliest seizures are called hypsarrhythmia, manifesting as massive flexion of the limbs,

trunk and head lasting for several seconds. TSC-associated neuropsychiatric disorders are not infrequent and range from varying degrees of intellectual disabilities to autism, attention deficit disorder and other psychiatric conditions.

The differential diagnosis in considering the manifestations of TSC includes benign skin findings commonly found in the general population. A nevus depigmentosus is not infrequently seen in healthy infants, and similarly, hypopigmented patches following lines of Blaschko can represent pigmentary mosaicism. If there was preceding inflammation or a wound, postinflammatory hypopigmentation may result and can mimic hypomelanotic macules. Furthermore, the distinction must be made between hypo- and de-pigmentation, as a truly depigmented patch would represent vitiligo or a neurocristopathy like piebaldism or WS. Smooth muscle hamartomas, presenting as skin-colored hypertrichotic patches that accentuate with rubbing can resemble the shagreen patch. Facial angiofibromas can occur in Birt–Hogg–Dube syndrome, and if they are seen in addition to collagenomas, hypomelanotic macules, and benign or malignant neuroendocrine tumors, multiple endocrine neoplasia type 1 should be suspected.

Genetic testing

Concurrent gene testing of *TSC1* and *TSC2* may be performed. If no pathogenic variant is detected, mosaicism should be suspected [27].

Management

Recommendations following the 2012 International TSC Consensus Conference are summarized in [Table 24.7](#) for both initial and ongoing evaluation and management. There are ongoing clinical trials showing benefits in both topical and systemic therapy with mammalian target of rapamycin (mTOR) inhibitors for many organ systems involved in TSC.

Genetic counseling

TSC is inherited in an autosomal dominant manner. Nearly 75% of cases are sporadic, and the penetrance is 100%. Pathogenic variants in *TSC2* are more common in patients with TSC [28]. *TSC2* pathogenic variants produce a more severe phenotype than *TSC1* pathogenic variants; *TSC2* variants confer a greater risk for renal malignancy [29], intellectual disability, autistic disorder and infantile spasms [30]. If a pathogenic variant has been identified in an affected family member, prenatal testing for pregnancies at increased risk and preimplantation genetic diagnosis are possible. Prenatal screening can be helpful in the diagnosis and management of fetal cardiac rhabdomyomas.

Disorders of the epidermis

The maturation of the epidermis resulting in differentiation, cornification and ultimately desquamation is impaired in this group of hereditary disorders.

Table 24.7 Surveillance and management recommendations for TSC [26].

Specialty area or organ system	Recommendations in patients with suspected or newly diagnosed TSC	Recommendations in patients diagnosed with definite or possible TSC
Genetics	Obtain a three-generation family history to assess for additional family members at risk In questionable TSC cases, offer genetic testing or counseling	Offer genetic testing and family counseling if not done previously to individuals of reproductive age
Neurologic	MRI of the brain to assess for: • Tubers • Subependymal nodules • Migrational defects • SEGAs Evaluate for TAND In infancy, educate parents to recognize infantile spasms Baseline EEG • If abnormal, follow up with 24-h video EEG to assess for subclinical seizure activity	MRI of the brain every 1–3 years in asymptomatic TSC patients <25 years to assess for SEGAs. Surgical and medical management exist Screen for TAND at least annually Routine EEG if known or suspected seizure activity • Vigabatrin is a first-line therapy for infantile spasms. • Anticonvulsants and surgical management of other seizure types follow that of other epilepsies
Renal	MRI of the abdomen to assess for: • Presence of angiomyolipoma • Renal cysts Blood pressure to screen for hypertension Determine glomerular filtration rate GFR to assess renal function	MRI of the abdomen to assess for progression of angiomyolipoma and renal cystic disease every 1–3 years throughout lifetime • Embolization and medical management with mTOR inhibitors are used to treat angiomyolipomas Assess blood pressure at least annually Assess renal function at least annually
Dermatologic	Detailed dermatologic exam	Perform a detailed dermatologic exam annually • Rapidly changing or symptomatic TSC skin lesions can be treated with laser, surgical excision, or topical mTOR inhibitor
Dental	Detailed dental exam	Perform a detailed dental exam every 6 months, with panoramic radiographs by age 7 • Symptomatic dental and oral lesions can be treated with surgical excision or curettage

(Continued)

Table 24.7 (Continued)

Specialty area or organ system	Recommendations in patients with suspected or newly diagnosed TSC	Recommendations in patients diagnosed with definite or possible TSC
Ophthalmologic	Detailed ophthalmologic evaluation, including dilated fundoscopy to assess for retinal lesions and visual field deficits	Perform annual ophthalmologic evaluation in patients with previously identified ophthalmologic lesions or vision symptoms at baseline
Cardiovascular	Consider fetal echocardiography to detect individuals with a high risk of heart failure after delivery, when rhabdomyomas are identified via prenatal ultrasound Obtain ECG in pediatric patients, especially if younger than 3 years of age Obtain electrocardiogram in all patients to assess for underlying conduction defects	Obtain an echocardiogram every 1–3 years in asymptomatic pediatric patients until the regression of cardiac rhabdomyomas is documented Obtain an ECG every 3–5 years in asymptomatic patients in all ages to monitor for conduction defects
Pulmonary	Baseline PFT • Pulmonary function tests • Six-minute walk test HRCT in females 18 years or older to assess for LAM Counsel on smoking risks and estrogen use in adolescent and adult females	Clinically screen for LAM assessing for shortness of breath and exertional dyspnea annually Obtain HRCT every 5–10 years in asymptomatic individuals at risk (females >18) • If known LAM, increase HRCT to every 2–3 years and PFT annually • mTOR inhibitors may be used to treat LAM patients Counsel on smoking risks and estrogen use in adolescent and adult females

ECG, Echocardiogram; EEG, electroencephalogram; HRCT, high-resolution chest computed tomography; LAM, lymphangioleiomyomatosis; MRI, magnetic resonance imaging; PFT, pulmonary function testing; SEGA, subependymal giant cell astrocytoma; TAND, TSC-associated neuropsychiatric disorder; TSC, tuberous sclerosis complex.

Nonsyndromic ichthyosis

Overview

The ichthyosis can be divided into syndromic and nonsyndromic types [31]. Nonsyndromic Ichthyosis includes ichthyosis vulgaris (IV), recessive X-linked ichthyosis (RXLI), keratino-poathic ichthyosis (KI), and autosomal recessive congenital ichthyosis (ARCI). The majority of ichthyosis will present in the neonatal period, and all by adulthood.

Evaluation

Ichthyosis vulgaris is by far the most common inherited disorder of keratinization [32]. Fine larger scales are noted predominantly on extensor surfaces typically in infancy, while hyper-linear palms develop over time. Patients have an increased risk of atopic dermatitis and keratosis pilaris. Often attributed to dry skin with general improvement over time, IV typically goes undiagnosed. Males present with RXLI within the first several months of life with varying degrees of scaling over the entire body. Scales tend to be larger than those seen in IV, and often appear dark and unwashed. Hypogonadism and/or cryptorchidism may be observed. A slit lamp exam reveals corneal opacities in 50% of affected adult males, though they do not affect vision [33].

Ichthyosis defined by keratin pathogenic variants are classified under the KI and include epidermolytic ichthyosis and superficial epidermolytic ichthyosis.

ARCI typically presents in the neonatal period, often in the setting of being born with a thick membrane-like coating called a collodion. The ARCI is a collective term for genetically distinct ichthyosis with variable overlap in phenotypes. Features typically persist through adulthood; pruritis and varying degrees of hyperpyrexia and heat intolerance predominate symptoms, depending on severity. The classic phenotype is that of lamellar ichthyosis, which presents a large plate-like scale predominantly over the face, trunk, and extremities.

Table 24.8 Genes associated with nonsyndromic ichthyosis.

Ichthyosis	Gene/Protein	Comments
Ichthyosis vulgaris	<i>FLG</i> /profilagrin	Diagnosis often made clinically. Semidominant inheritance.
Recessive X-linked ichthyosis	<i>ARSC1</i> /steroid sulfatase	Female carriers unaffected, gene is at distal tip of X chromosome, escaping X-inactivation.
Epidermolytic ichthyosis	<i>KRT1</i> /keratin 1; <i>KRT10</i> /keratin 10	Mostly autosomal dominant, rarely autosomal recessive.
Superficial epidermolytic ichthyosis	<i>KRT2</i> /keratin 2; <i>KRT10</i> /keratin 10	
Autosomal recessive congenital ichthyosis	<i>ABCA12</i> , <i>ALOX12B</i> , <i>ALOXE3</i> , <i>CAS14</i> , <i>CERS3</i> , <i>CYP4F22</i> , <i>LIPN</i> , <i>NIPAL4</i> , <i>PNPLA1</i> , <i>SDR9C7</i> , <i>SLC27A4</i> , <i>TGM1</i>	A multigene panel is recommended. <i>ABCA12</i> typically results in the most severe harlequin phenotype.

Genetic testing

Genes associated can be found in [Table 24.8](#). Diagnosis is often made in infancy.

Management

Frequent application of emollients to the skin is the mainstay of treatment for all ichthyosis. For more severe presentations, supportive care with the prevention of infection, dehydration and overheating with sufficient caloric intake is necessary. Consultation with a dermatologist regarding further treatment with keratolytic agents and retinoids is warranted [\[34\]](#). Consultation with an ophthalmologist for management of ectropion is also recommended.

Genetic counseling

Reduced steroid sulfatase activity seen in RXLI results in low placental production of estrogens. Elevated urinary sulfated steroid levels are found in mothers of individuals with RXLI, and are associated with prolonged labor and failure to dilate.

Darier disease*Overview*

Darier disease (DD), also referred to as keratosis follicularis, is marked by crusted papules and plaques in a seborrheic distribution and is inherited in an autosomal dominant manner.

Evaluation

Patients with DD typically present in adolescence with a waxy crusted scale on the scalp, forehead, nasolabial folds, mid-chest and upper back. Exacerbations occur with friction and heat. Some patients may have punctate keratoses on the palms and soles. A phenotype called acrokeratosis verruciformis may occur and is characterized by thin verrucoid papules on the dorsal hands. Nail changes are common, displaying V-shaped nicking, subungual thickening, and streaks of discoloration. An examination of the oral and anogenital mucosa can be fruitful, showing white verrucous plaques.

Patients with DD are often afflicted by neuropsychiatric disease, including learning disability in almost half of the patients [\[35\]](#), as well as schizophrenia and bipolar disorder.

Genetic testing

While often diagnosed clinically coupled with classic histopathologic findings on skin biopsy, single-gene testing for pathogenic variants in *ATP2A2*, which encodes the sarcoplasmic/endoplasmic reticulum calcium ATPase type 2 can be obtained. *De novo* rates exceed 50% in some reports [\[36\]](#). The disease has a high penetrance and variable expressivity [\[37\]](#). Linear or segmental forms exist, demonstrating postzygotic mosaicism.

Management

Primary management is best served by a dermatologist and is focused around infection control and often systemic retinoids. A combination of laser and topical treatments can be helpful adjuncts. A thorough neurologic and psychiatric review of systems can be helpful to screen

Hailey—Hailey Disease

Overview

Inherited in an autosomal dominant pattern, Hailey—Hailey disease (HHD) is also referred to as familial benign pemphigus.

Evaluation

Typically presenting just before the second decade of life, individuals with HHD develop recurrent vesicles and erosions in the intertriginous areas of the neck, axillae, and groin. Often buildup of superficial crust can form granular vegetations. Affected areas tend to be irritated, pruritic and are very uncomfortable. Stress and heat often exacerbate the condition leading to infections and scarring, and ultimately many patients experience poor quality of life as a result.

Genetic testing

While the diagnosis is typically established with a skin biopsy, single-gene testing for pathogenic variants in *ATP2C1* can be helpful for confirmation. HHD has complete penetrance, variable expressivity and a *de novo* rate approaching 30% [38].

Management

A number of therapies are established in treating HHD, including avoiding precipitating factors like wearing loose-fitting clothing and treating bacterial or candidal superinfection. Medical management of HHD is best provided by a dermatologist. Topical calcineurin inhibitors and corticosteroids are often used, though the promise is in emerging therapy with botulinum toxin injections and oral naltrexone [39,40].

Vascular anomalies

Most vascular diseases of the skin are evident at birth. In general, these conditions will be diagnosed in the pediatric clinic and are characterized by asymmetric overgrowth of the head, trunk, and/or extremities. Genetic testing for many vascular syndromes is not yet available, as many are mosaic conditions without described family histories.

PI3K-related overgrowth spectrum

An emerging group of syndromes characterized by regional tissue overgrowth caused by mosaic postzygotic somatic variants encoding proteins in the PI3K-AKT signaling pathway [41] includes CLOVES (congenital lipomatous overgrowth, vascular malformations, epidermal nevi, scoliosis/skeletal/spinal anomalies), megalencephaly-capillary malformation (M-CM) syndrome, fibroadipose hyperplasia and hemimegalencephaly. Patients with regional overgrowth should undergo a thorough physical exam for any associated abnormalities, as syndromes within this category exist on a spectrum.

Due to substantial allelic heterogeneity, optimal diagnosis requires fresh skin or surgical samples from affected tissue. Ultra deep (> 1000-fold) next-generation sequencing for molecular diagnosis of PI3K-related overgrowth spectrum (PROS) yields a diagnosis rate of 66% [42]. Blood and buccal swabs can detect *PIK3CA* pathogenic variants in M-CM patients, though with a significantly lower diagnostic rate. Management of these complex multisystem diseases are likely best suited for an experienced multispecialty vascular anomalies center

RASA1-related disorders

RASA1-related disorders are autosomal dominant conditions marked by multiple small capillary malformations and an increased prevalence of fast-flow vascular anomalies in the skin, muscle, bone, spine, and brain. Similar to other emerging paradigms as we learn more about the genetics of vascular anomaly syndromes, these conditions may exist on a spectrum, and include diseases such as capillary malformation-arteriovenous malformation syndrome (CM-AVM) and *RASA1*-related Parkes Weber syndrome.

Patients found to have multiple small (1–2 cm) pink to tan, round to oval patches thought to be CMs, AVMs or arteriovenous fistulas of the soft tissue, bone, and brain with or without overgrowth should be suspected of having a *RASA1*-related disorder [43].

Genetic testing for *RASA1* pathogenic variants in patients for whom the diagnosis is uncertain. Molecular genetic testing via sequence analysis and gene-targeted deletion/duplication of the *RASA1* gene detects nearly 100% of pathogenic variants [44]. In those with epistaxis and telangiectasias associated with multifocal CMs, a multi-gene panel to include *EPHB4* is warranted. If CMs are of cosmetic concern, pulsed dye laser can be of use, though incomplete removal and recurrence over time are likely outcomes.

Points to remember

- OCA is characterized by hypopigmentation of the skin and hair, with characteristic ocular findings.
- The white forelock is the most common hair pigmentary anomaly seen in Waardenburg syndrome and piebaldism. It may present at birth or appear later, and it may become normally pigmented over time.
- In patients with early onset of multiple lentiginoses, especially in the centropalpebral area, a detailed family history in the setting of a cardiac and gastrointestinal evaluation is necessary.
- Poikiloderma, sparse hair, cataracts along with dental and skeletal abnormalities characterize RTS. Osteosarcoma is the most commonly reported malignancy, with a prevalence of 30%.
- Mitigation of susceptibility factors when applicable and photoprotection in the case of the cutaneous porphyrias are the mainstays of treatment.
- Ichthyoses typically present early in life, often in the neonatal period.

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Access to Genetic Services

Billing and reimbursement

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Introduction

In 1989 the first edition of the Manual on Reimbursement for Medical Genetics Services was published. At the same time, the reimbursement system for healthcare services was in a transition as the Centers for Medicare and Medicaid Services (CMS) promulgated the resource-based relative value scale. Moreover, during this time, the American Medical Association (AMA) refined the Current Procedural Terminology (CPT) as a coding system for professional services and physician-provided procedures and the World Health Organization developed the International Classification of Diseases (ICD) coding system for diagnostic terminology. Under the American College of Medical Genetics and Genomics (ACMG) auspices, the second edition of the manual was published in 2002. While medical geneticists and genetic counselors provide quality services that will improve the health care and lives for their patients, many are still unfamiliar with the requirements for documentation and billing for these services. It is important to have a well-developed billing and reimbursement program and to implement the principles of documentation and billing, so as to be reimbursed appropriately.

This chapter will discuss billing and reimbursement for clinical adult genetic services as well as laboratory services and includes attention to Medicare policy. Note that these are general guidelines and one's specific clinical practice environment may have different or additional requirements. Moreover, following these general principles does not guarantee that readers will receive optimal reimbursement.

Referral to a clinical genetic service

All clinical services involve getting a referral from a provider, scheduling the patient encounter, providing a clinical service (in outpatient or inpatient settings), documenting the service, and submitting a bill in the correct manner. Note, in the language of the business of medicine, a bill is referred to as a "claim." In medical genetics, the flow of service usually starts by getting a referral from another provider (either a primary care provider or a specialist). Sometimes, the patients can self-refer themselves for a genetic evaluation. It is important to

obtain both the referring provider's name and identification number. The identification number required may vary based on the patient's third-party payer. A provider needs to be enrolled with the patient's payer. Note that different payers have different standards for enrolling providers and most require board certification in the specialty. As of October 1, 2018, Medicare has established a physician specialty ID code for Medical Genetics and Genomics specialists (D3) to enroll as providers [1].

Prior authorization may sometimes be necessary. The third-party payer for each patient should be contacted prior to the encounter to determine eligibility and coverage. After the eligibility and coverage is ensured, patient information is registered for the encounter into the billing system including patient demographic information and information about the patient's payer.

CPT coding for clinical services

There are many types of clinical genetic services, including inpatient consultations, outpatient consultations, outpatient office visits (nonconsultative), and genetic counseling encounters. Encounters may be new patient encounters or follow-up encounters. There are CPT codes for all of these types of services.

CPT is the coding system used to report physician services and procedures [2]. The purpose of the coding system is to provide a uniform coding language that accurately describes medical, surgical, diagnostic, and laboratory services, thus providing an effective means of communication between providers and third-party payers. The system is updated annually by the AMA. A new publication is released each November, and the codes become effective on January 1 of the following year. The codes within the CPT manual are organized into six sections according to the types of services or procedures provided.

The section of CPT termed Evaluation and Management (E&M) codes comprise codes 99201–99499. These are generic clinical service codes in the sense that all physicians and other qualified healthcare providers may use them to report clinical services. Medical geneticists' services are typically reported using these codes. E&M services are subclassified according to the level of service provided.

Important terminologies

It is essential to define the correct terminologies when rendering clinical care. The E&M code submitted for billing must be supported by the service documented in the patient's chart for that service.

Office or outpatient services

Office or outpatient services are provided in the physician's office or in an outpatient or other ambulatory facility.

Inpatient services

Services that are provided to hospital inpatients.

New patient

A new patient is one who has not received any professional services from the physician or another physician of the same specialty who belongs to the same group of practice, within the past 3 years.

Consultation

A consultation is a type of service provided by a physician whose opinion or advice regarding evaluation and/or management of a specific problem is requested by another physician or other appropriate source. A written or verbal request for a consultation must be documented in the patient's chart and the consultant's opinion must be communicated by written report to the requesting physician or other appropriate source.

Office visit

An office visit is a service provided to an outpatient that does not meet the criteria for a consultation.

Documentation

Based on evaluation and management

Evaluation and management (E&M) documentation needs to be followed correctly. There are two sets of E&M documentation guidelines that are approved by CMS and the AMA: 1995 and 1997 [3]. The ACMG recommends that geneticists adopt the 1997 documentation guidelines, which may provide the most protection during an audit [4].

The only significant difference between the 1995 and 1997 documentation guidelines is within the physical examination requirements [5,6]. Within the examination requirements, there are two major differences:

1. The 1997 documentation guidelines recognize 14 different "system/body areas" within the multisystem examination. The 1995 documentation guidelines recognizes the 14 "system/body areas" as well as an additional three "body areas": head (including face), back (including spine), and each extremity, which are commonly evaluated during a medical genetics examination.
2. The 1997 documentation guidelines list the actual elements within each "system/body areas" to determine the level of examination. For example, in the "eye-system/body area," three elements may be documented: inspection of conjunctive and lids, examination of pupils and irises, and ophthalmoscopic examination of optic discs. The 1995 documentation guidelines do not list specific elements with each "system/body area."

Medical record documentation is particularly important to medical geneticists because they typically bill for a higher level of service (level 4 or 5 CPT code). Payers are more likely to demand medical record documentation for medical geneticists to support claims if the provider consistently bills for a higher level of service.

Most payers require that the request for a consult be documented in order for the provider to submit a claim for consultation, and a copy of the patient note must be sent to the provider who requested the consultation. In order to achieve consistent appropriate documentation, it is recommended to use templates for history, examination, and decision-making.

Documentation of history taking

There are four levels of history taking: problem focused, expanded problem focused, detailed, and comprehensive. Each level of history taking is determined by four history components: chief complaint, history of present illness, review of systems, and medical, family, and social histories. These must be documented in the medical record.

Documentation of examination

There are four levels of examination: problem focused, expanded problem focused, detailed, and comprehensive. As mentioned above, there are a number of elements required for each of the four levels of the general multisystem examination according to the 1997 documentation guidelines. “Areas” refer to the body or organ areas.

- Problem focused requires documentation of one to five elements
- Expanded problem focused requires at least six elements
- Detailed requires at least 12 elements from two or more areas
- Comprehensive requires at least 12 elements from nine areas

Documentation of medical decision-making

According to the CPT manual and the 1995 and 1997 Documentation Guidelines, there are four levels of medical decision-making: straightforward, low complexity, moderate complexity, and high complexity. The level of complexity is determined by its three components: number of diagnoses/management options, amount/complexity of data and medical records reviewed, and risk. The CPT manual and CMS documentation guidelines provide a matrix for determining the level of medical decision-making based on the components documented in the record.

Payer auditors recognize that electronic health records (EHRs) make it possible to “copy and paste” information from one note to another and are concerned when notes seem “cloned.” It is important to ensure that information one documents for a specific visit has been collected at that visit and is pertinent to the reason for the visit.

Based on time

Every E&M code has a “typical” face-to-face time spent with the patient in an outpatient or inpatient setting. One can bill based on time if greater than 50% of the time of the visit was spent in counseling and/or coordination of care. One needs to document the time spent face to face with the patient and document the counseling and/or care coordination in the note.

Note: Readers should be aware that at the time of the writing of this chapter, CMS has proposed changing the documentation requirements for E&M coding in order to reduce the burden on providers. This has led to hundreds of stakeholder comments back to CMS and the AMA has also been working on a proposal for simplifying E&M documentation. It is likely that changing the guidelines will take more than a year. Consequently, readers should check up on the status of this CMS proposal, as it may lead to significant changes in documentation requirements, which providers would need to comply with.

New payment models

Another aspect of CMS reimbursement for clinical services involves its Quality Performance Programs, established under the Medicare Access and CHIP Reauthorization Act, which are considered value-based payment programs, which provide opportunities for increased reimbursement [7]. There are two parts to this Program, merit-based incentive payment system, and alternative payment models program. These apply to providers seeing Medicare patients and have complex requirements, which are best discussed by genetics providers with their practice group or hospital.

Billing for clinical services

This includes preparing the bill, submitting the bill, and dealing with denials.

Bill preparation

Preparing a bill must include documentation of the reason for the visit, that is, the medical necessity of the visit, plus the service provided.

Diagnosis/diagnoses

At present, use the ICD-10 coding system. Document “diagnosis” to the highest level of specificity. This sometimes means just listing symptom(s) or physical abnormalities.

Note that most payers’ computer processing systems can only accept up to three or four ICD codes for a claim. Any additional codes are discarded.

Service provided

Use CPT code(s), which correspond to the level of service documented in the record, following the guidelines described above. Another option is to select the code based on time. Every E&M code has a “typical” face-to-face time spent with the patient in an outpatient or inpatient setting. One can bill based on time if greater than 50% of the time of the visit was spent in counseling and/or coordination of care. One needs to document the time spent face to face with the patient and document the counseling and/or care coordination in the note.

Many EHRs include an option for the EHR to select a CPT code based on its analysis of the note. One needs to carefully review the proposed code before simply accepting it, as the provider is ultimately responsible for the coding. Some providers are cautious and may “downcode” a visit, submitting a code that is for a lower level of service than is documented in the note. This is looked upon by payers’ auditors just as negatively as “up-coding,” and might lead to penalties. Note that Medicare does not cover nor pay claims for consultations (CPT codes 99241–99245 and 99251–99255). Claims need to be submitted as Office Visits (99201–99205) or inpatient visits (99231–99233). Many state Medicaid programs no longer cover consultation codes either, so one needs to check with the state Medicaid program in order to bill correctly.

Billing for genetic counselor services

There is a CPT code for genetic counseling by a genetic counselor, 96040, which is a time-based code. The charge can be billed in increments of 30 minutes and can be submitted in multiples as long as time is documented. Note that CPT rules indicate that additional instances of time-based codes must extend past 50% of the expected time in the code descriptor.

Some institutions have used incident to billing, in which genetic counselors bill for services through their supervising physician. Medicare permits “billing of services and supplies incident to the services of a physician” (Medicare Carriers Manual Sec. 2050.1). This policy applies to nonphysician practitioners. Readers are recommended to discuss with their practice plan or health system whether this is an option for their practice.

Moreover, genetic counselors are not recognized as providers who can enroll with many payers. This has led some institutions to submit bills for genetic counseling as facility fees. One should discuss models for billing genetic counseling services with their institution’s billing and compliance departments.

Billing for telehealth services

Many medical specialties including clinical genetics have started using telehealth services to increase access of care to their patients. Further discussion on telegenetics can be reviewed in Chapter 26, Telegenetics and other care models.

Claims and denials

Submitting a claim

A bill must be submitted based on the concept of “clean” claim. Clean claim means that every relevant box must be completed on the claim form for the insurance plan to process the claim. Any error on the claim may cause payment to be delayed or denied, including electronic claims.

Reasons for denial

A denial is a formal report back to the physician’s billing office stating that payment for the claim is denied. Denials can take many different forms, including referral denials, prior authorization denials, denials for tests or procedures, or denials of payment. There are many possible reasons, and the reason should be stated in the denial. Infrequently denial is due to genetics not being a covered service under the contract covering the patient. Often this is due to errors in the information provided in the claim such as incorrect patient demographic information, incorrect plan policy number, prior authorization not being obtained or documented for the visit, ICD code not being appropriate for the service (lack of medical necessity), more than one provider seeing the patient on the same day and each submitting a claim with the same ICD code), or incorrect CPT code (e.g., consultation code submitted to Medicare, or code for a new patient visit and payer considers it an established patient visit, claim submitted late as most payers require claim be submitted within 90–180 days of the date of service).

Appealing denials

A denial letter should state the reason for denial and cite the specific policy reason. Note that most payers publish their coverage policy on their websites. To appeal denials, one should start with checking the published policy to see whether it is congruent with the policy stated in the denial. Payers all should have an established appeal process and it should be described in the denial. Typically, they first request copies of the documentation. This is followed by setting up a peer-to-peer phone call to discuss the denial, where the provider will have the opportunity to address the issue and make a case for why the service is valid and the claim should be accepted. It is important for the provider to be collegial during a peer-to-peer call. If the denial is sustained after a peer-to-peer call, the payer should have a next step in the appeal process, which can be followed by the provider.

Billing for genetic laboratory services

Genetic testing is an important part of clinical genetic services. The billing and reimbursement ecosystem for laboratory genetics is similar to that for clinician services but has important differences, which providers need to understand.

As for clinical genetic services, billing for genetic tests require a CPT code for the test which is performed, and an ICD code for the indication for the test. As for clinical services, the CPT

code indicates what service/procedure is performed on/for the patient, and the ICD code indicates the “diagnosis”/clinical problem for which the test/procedure is being performed (medical necessity). ICD coding for laboratory genetics should parallel the method described above regarding clinical service billing. However, unlike clinical genetic services, which have a limited set of CPT codes, there is a dazzling array of CPT codes for genetic tests, and it is important to understand the nuances involved.

CPT codes for genetic tests

Genetic tests are listed in the Molecular Pathology section of the CPT manual. Tier 1 molecular pathology procedures are specific to one gene and represent tests that are performed frequently, nationally. Tier 2 molecular pathology procedures are tests for genes that are performed in lower volume nationally than are Tier 1 procedures. Tier 2 codes are grouped by the level of technical resources and interpretive work involved. There are nine levels of Tier 2 codes. Each Tier 2 code includes a list of genes that can number more than 100. Thus, Tier 2 codes are not specific. Next-generation sequencing panel tests are listed as genomic sequencing procedure codes in CPT. Typically, the code specifies a minimum number of genes that should be included in the panel and includes examples of genes that should be included in the panel but typically do not specify an upper limit number of genes.

Coverage policies

Private payers’ coverage policies for molecular pathology codes may be published; however, there are many new tests added to CPT on an ongoing basis and payers’ process for establishing coverage is lengthy and may lag. Some payers have technology evaluation centers that assist the payer in making coverage decisions. In general, payers make coverage decisions about molecular pathology codes based on three criteria: analytic validity, clinical validity, and clinical utility. It is important to understand these criteria, especially when seeking authorization for a test or during an appeal of a denial.

- Analytical validity refers to how well the test predicts the presence or absence of a change or variant in the gene being tested.
- Clinical validity regards how definite the changes in the gene being tested is related to the presence, absence, or risk of a specific disorder or disease.
- Clinical utility has several definitions, but one definition is that a test has clinical utility if the result, positive or negative, will lead to a change in management of the patient. Others have proposed a more stringent definition for clinical utility, requiring that there is published evidence that the result changes patient outcomes. Since most genetic disorders are rare, there is limited literature about clinical outcomes and thus this standard could effectively preclude most genetic tests. The ACMG in 2015 presented a position statement regarding clinical utility for genetic tests, which broadened the concept to include not only change in management of the patient being tested but also considers benefit to family members [8].

Medicare has two forms of coverage policies: national coverage determinations (NCDs), which are made and applied nationwide, and local coverage determinations (LCDs), which are made by individual Medicare administrative contractors (MACs) and apply to states in the MAC's region. The process for developing NCDs and LCDs is spelled out by law and should be transparent and include opportunity for stakeholder public comments before a proposed NCD or LCD is finalized. In the recent instance of the NCD for cancer next generation sequencing testing, stakeholder input led to changes in the final NCD [9]. Once finalized, NCDs and LCDs are published and can be found on the Internet.

Reimbursement of genetic testing

In general, private payers do not publish their payment policy, so laboratories may not know how much they will be paid even for a covered test, until they submit a claim to a payer. Medicare is more transparent and publishes its clinical laboratory fee schedule (CLFS) annually, so labs can see what they should receive as payment for specific molecular tests [10].

The Protecting Access to Medicare Act is in the process of being implemented and includes the most extensive reform of the Medicare CLFS [11]. Many stakeholders are concerned about the valuation process for most laboratory tests and feel that it will lead to significant decreases in reimbursement for tests, including genetic and genomic tests. Laboratory geneticists should follow this issue closely and provide comments to CMS when appropriate.

Prior authorization

In many instances, payers require prior authorization before a genetic test can be performed. When seeking prior authorization, review the payer's coverage policy to be sure that the prior authorization request includes all the information that the payer's policy requires.

Denials of laboratory service claims

There are many reasons why a laboratory claim may be denied. Most often it is because prior authorization was not obtained, or the test is not a covered service.

Denials are often a problem with Tier 2 molecular pathology CPT codes, as an individual Tier 2 code applies to dozens of different gene "analytes," and the payer is not able to determine which gene is being tested and why. Another reason for lack of coverage is because the code for the test is too new for the payer to have established a coverage policy. Often a payer's policy may not be up to date with regard to evidence published since the time of the original policy decision. This can be discussed on an appeal.

Appeals

A general approach as discussed in the Clinical Service section, adapting to possible issues described above should be followed when appealing a denial of a laboratory service claim. Unfortunately, these appeals can sometimes be unsuccessful, and patients are left with the option of paying out of pocket or not receiving testing.

Points to remember

- Implement the principles of documentation and billing.
- Use either 1995 or 1997 documentation guidelines and templates for history, examination, and decision-making; understanding the differences between them may make one more suitable than the other for different types of cases.
- Make sure the E&M code submitted for billing is supported by the service documented in the patient chart for that service.
- Use CPT code 96040 for genetic counseling service provided by a Genetic Counselor.
- Keep in mind that Medicare does not cover nor pay claims for Consultations (CPT codes 99241–99245 and 99251–99255). Those claims need to be submitted as Office Visits (99201–99205).
- Tier 1 molecular pathology procedures are specific to one gene and represent tests that are performed frequently, nationally while Tier 2 codes are tests for genes that are performed in lower volume grouped by level of technical resources.
- Appeal denials, look for the reason and policy.
- When in doubt, check with the billing and coding staff at your medical center.

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Telegenetics and other care models

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Introduction

Provision of services remotely by healthcare professionals referred to as telehealth (TH) has emerged as an important healthcare delivery model to address the growing shortage of clinical expertise. TH is defined as remote health care involving the exchange of medical information from one site to another via interactive electronic communications to improve a patient's health [1]. It has been widely used in various medical disciplines, such as dermatology, cardiology, radiology, psychiatry, and others including clinical genetics, which we refer to as “telegenetics.” Telegenetics involves electronic communication between the patient and the provider, as well as between providers, and this relatively new concept of healthcare delivery promises to improve accessibility to genetic care. Different modalities of delivery include synchronous videoconferencing and asynchronous electronic consultations.

Advances in human genomics and genetics are revolutionizing health care and ushering in a new era of genetic information-based precision or personalized medical care. However, there are challenges that currently limit accessibility to this genetic information. Despite a widespread need for genetic testing, and a growing need for clinical and medical genetic services, there is currently a shortage of genetics professionals to support the appropriate acquisition and application of genetic information for patient care.

However, even though there are perceived advantages and acceptance by other specialties of medicine, telemedicine (TM) implementation has yet to carry over significantly to the field of medical genetics. There are different reports on the implementation of telegenetic services around the world [1–3]. In the United States, the Heartland Regional Genetics Network

(Heartland) (<https://www.heartlandcollaborative.org>) is a network of genetics professionals, policy leaders, laboratory personnel, and family advocates working together to promote and improve quality genetic services. Heartland has created a toolkit to help with developing tele-genetic services.

Accomplishing telegenetic services is not always an easy process. This chapter focuses on two areas: (1) current literature that describes the use of TM/TH in adult genetics and (2) the crucial information necessary to accomplish that use. The current literature describing telegenetics includes genetic evaluations (family history, risk, diagnostic testing, and counseling); genetic service providers; provider and patient satisfaction with telegenetics; and remote monitoring of patients with adult genetic disorders. Secondly, the focus is on telegenetic equipment and technology, support of rural health providers, E-consults and video-consults, commercial use of telegenetics, standards, and regulatory policy, and billing and reimbursement.

Use of Telemedicine/Telehealth in adult genetics

Although there are reports of telegenetic services across the country and the world, provision of such services appears to be predominantly for pediatric patients, with only a minority of services available for adult patients. Medical care through TM has been provided by health-care professionals associated with major medical schools, private companies, and government agencies with funding obtained from private sources or grants. There are limited data regarding the indications for genetic evaluations, the number of cases, reported outcomes, and which professionals provided telegenetic services. Limited literature regarding telegenetic services for adults has been focused on the comparison of face-to-face genetic counseling in the office setting with remote video TM counseling. The primary outcomes that have been assessed include satisfaction and indications for evaluation.

Genetic evaluation

The majority of studies that evaluated family history, risk, testing, and counseling compared person to person services with telegenetic services. Telegenetic services were provided in two ways: from a clinic base site to an outlying “remote” clinic ($n = 15$) or from a clinic base site to the actual patient home ($n = 6$). All of the remote site genetic evaluations were video telegenetics. The majority of remote clinic evaluations were for cancer genetics ($n = 12$ sites, $n = 482$ cases): three breast/ovarian ($n = 255$) sites [4–7], two colon cancer ($n = 27$) sites [8,9], two breast/ovarian/colon cancer ($n = 81$) site [10,11], one breast/ovarian/colon cancer and melanoma ($n = 48$) site [12], and four nonspecific oncology ($n = 71$) sites with a family history of cancer [8,13,14]. In addition, there was one remote clinic service site that provided genetic evaluation and counseling for Huntington disease (HD) ($n = 11$) [15] and two remote sites for familial glaucoma ($n = 1117$) [16,17].

There were six studies that provided genetic evaluation from the clinic base to the actual patient home. Three were video telegenetics and three were by telephone, with a majority

(five of the six) for cancer genetic evaluation ($n = 534$): one for video oncology genetics ($n = 34$) [18,19], two for telephone colon cancer ($n = 451$) [20,21], one for colon cancer ($n = 18$) that compared telephone to video [9], one for video colon cancer ($n = 15$) and BRCA/OV ($n = 16$) [22]. The last patient home site involved video telegenetics for cardiogenetic evaluation ($n = 17$) [18,19].

Thus, the majority of adult telegenetic evaluation studies focused on cancer genetics.

Genetic service providers

There is some variation across sites in the types of providers offering telegenetic services, including independent genetic counselors, medical geneticists, and teams of genetic counselors and medical geneticists providing care together. They are sometimes supported by registered nurses, technicians, research coordinators, or health educators at the base site, remote site, or both. Genetic testing companies employ both physicians and genetic counselors to guide patients, physicians, and other health professionals. These genetic professionals provide information about appropriate tests and report results.

For cancer genetic evaluation, there were four clinic base sites where MDs provided adult telegenetic cancer counseling/diagnosis services, three that included both MD and genetic counselors, and three where genetic counselors alone provided service. The majority of providers at remote cancer sites were genetic counselors ($n = 6$), genetic nurses ($n = 2$) outreach nurses ($n = 2$), and clinic staff ($n = 3$).

At the base clinic HD site, a genetic counselor was present and at each of the two familial glaucoma base clinics there was an MD present and a retina reader present at one. At the remote HD site, a genetic counselor and local MD who had undergone training were present, and at the familial glaucoma sites, there were ocular technicians, an orthoptist and orthoptist trained nurse and a health educator.

As is apparent from these studies, there is no standard format for how telegenetics services should be provided. Individual facilities have set up TH clinics based on their provider resources and funding.

Provider and patient satisfaction

Even though there is no standardized protocol for telegenetic practice, it is important to note that patients and provider satisfaction has been reported as excellent. In 18 of the studies listed earlier, there was no difference between patient satisfaction between person to person and telegenetic care. Three of the studies reported that patients preferred telegenetics [5,14,23]. As might be expected, the data suggest that patients appreciated the convenience of telegenetic services, not having to travel to a remote base site, and not having to bear the cost of the travel [4–6,8,12,18,19,23]. Patients felt less anxious and less depressed after the remote telegenetic interaction [4–7,14,18,19,23].

In two studies, although the patients rated the remote service highly, about 30% of patients would have preferred face-to-face consultation. [4,11]. In the Buchanan study, telegenetic participants were older, unemployed, had a lower income, and were less familiar

with computer operations. In-person participants were significantly more likely (89% vs 79%) to attend their appointment. Race, income, and familiarity with computer operations were significantly linked to attendance.

Genetic counselor satisfaction indicated the need for improvement in only one study, the Otten study, with video telegenetics at home. The genetic counselors felt there was insufficient verbal communication and frequent technical problems. Genetic counselors indicated there needed to be a technical improvement.

With a better understanding of these challenges, providers can strategize ways to mitigate issues with equipment and interaction with patients. In general, though it appears that for the adult genetic disorders evaluated, video remote evaluation is a positive addition to health care.

TM use for remote patient monitoring

Once a patient has had a genetic evaluation and counseling, geneticists usually monitor their patients. The major literature regarding video telegenetic services for remote patient monitoring of adult genetic disorders focused primarily on care for individuals with neurogenetic disorders at home. Three followed patients with HD ($n = 22$) (five followed patients with Parkinson disease ($n = 219$), and three followed patients with a diagnosis of amyotrophic lateral sclerosis ($n = 147$) [24–26]. There were two reports of adults with childhood onset genetic disorders, cystic fibrosis, and hemophilia being monitored via telegenetic services.

Different remote monitoring strategies have been employed, such as evaluation of motor performance in one HD study [27] and three Parkinson studies [28–32] and evaluation of patient cognition for HD and Parkinson's disease using the Montreal Cognitive Assessment scale and other standardized assessment [28,33,34]. Constantinescu and Griffin evaluated dysarthria in Parkinson's disease and Tedesco evaluation swallowing in HD.

The first remote monitoring study that included adults with childhood onset disorders is cystic fibrosis ($n = 10$). In-person versus remote evaluation of the 3 minute step test that promotes aerobic fitness and survival was completed. The second study involving patients with inherited hemophilia ($n = 1923$), involved Haemtrack, an electronic home treatment diary, which monitored bleeding onset, severity, pain, and treatment outcomes. The compliance rate was 78%.

The utility of telegenetic remote monitoring of home care appears to be high as does patient satisfaction. For example, in one study involving evaluation of motor scores for patients with HD, good reliability between in-person versus remote testing with excellent test-retest reliability was observed [27]. Similarly, provider satisfaction for the two joint Montreal Cognitive Assessment studies was high, indicating adequate reliability and internal consistency, and a strong predictive value for patients with mild cognitive impairment. In that study, patients reported a positive experience with the rater and using the web-based video conferencing tool. They were also very positive about (1) the reduced burden on their caregivers and (2) the time saved without the need for commuting and waiting in offices. Provider satisfaction for both the Parkinson's Montreal Cognitive Assessment studies

indicated moderate satisfaction and reliability between in-person versus video remote evaluation and the two Montreal Cognitive Assessment studies could be successfully completed in persons with mild cognitive impairment [33].

Early findings of the utility of video telegenetic for remote patient monitoring are encouraging.

Equipment and technology for telegenetic services

Equipment

TM and other digital tools have been, and are currently, employed in many medical settings in different ways. Their use in adult genetics is evolving, as public interest in the testing, diagnosis, and monitoring of genetic disorders to guide maintenance of health has increased. Video contact for the provision of genetic services via stationary computers, laptop computers, tablets, I-PADs, smart phones, and smart watches with video calling and recording capability has increased exponentially. Equipment capable of video transmission, including computers, I-Phones, Android phones, tablets (Skype, Google + Hangouts), and IPAD minis with clip on bluetooth microphones are required for telegenetic services. This equipment must be available at both the base (healthcare professional) site and the remote (patient) site. Equipment at the remote site may be at a local healthcare facility, or even in a patient's home.

Software applications (commonly known as apps)

Apps related to TH for remote care coordination and management of digital health tools are now also available. One example is mobile health (mHealth). *mHealth* is the practice of medicine and public health supported by mobile devices, such as mobile phones, tablet computers and personal digital assistants, and wearable devices such as smart watches. This form of medical care is used for health services, information, and data collection. mHealth has worked with the Marfan Foundation, the Lupus Research Alliance, as well as with the providers at the University of Minnesota offering TH care for Parkinson disease. An online survey reported on the perception of use of mhealth technologies among families of patients affected with Down syndrome, Williams syndrome, and 22q11.2 deletion syndrome. The responses ($n = 166$) indicated that 80% of parents had an interest in m-health applications to (1) save time—49%, (2) be more involved in disease management—49%, and (3) be able to access updated information on research and on-going clinical studies—66%. Access to the Internet was by mobile phone for 47% and by tablet for 34% [35].

Other genetic disorders have mobile health apps available. It is hoped that this will result in improved care coordination, management, and research, as information is gathered using wearables and TH devices to get a better picture of daily activities that may affect the disease. Collaboration with patients and providers around the world will provide a better view of what are the best practices and new approaches to treatment.

Use of telegenetic services to support rural health providers

Increased use of telegenetics in adult disorders has the potential to benefit rural populations. In the recent survey of TM use by Dhar et al. (unpublished data) aimed at genetic professionals who saw adult genetics cases, 31.9% used telegenetics. Physicians were 64% of this group and genetic counselors were 36%. Individuals in rural settings may lack access to genetics professionals due to a dearth of providers in their area, logistically challenging drive time to appointments, and financial barriers.

Project echo as a model

A big challenge of genetic care is that nongenetic providers may not be aware of resources available for provision of genetic testing and/or counseling, including telegenetic services. A recent study of physicians' awareness and use of genetic services in Texas indicated that although half of them reported being aware of genetic testing and services in their area, over two-thirds rarely or never referred to genetic specialists or genetic counselors. Very few reported awareness of TM/telegenetics services [36]. Project Extension for Community Healthcare Outcomes (ECHO) began at the University of New Mexico School of Medicine as a telementoring service helping primary care providers manage patients with Hepatitis C. It links expert specialist teams at an academic "hub" with primary care clinicians in local communities in a hub and spoke model (<https://echo.unm.edu>). Together, using teleconferencing technology on a regular basis, primary care clinicians from multiple sites present patient cases to the specialist teams and to each other, discuss new developments relating to their patients, and determine treatment at a local site. This model has been replicated and applied to many other medical conditions around the world. In the United States, ECHO type projects are found in 46 states, with 164 ECHO hubs and 402 programs that include different medical specialties (<https://echo.unm.edu/locations/global>).

The "Identifying & Managing Patients at Risk for Cancer ECHO" CME series was started in Utah to improve access for rural and urban clinicians working with patients/families at increased risk for cancer to medical genetic cancer specialists (<https://physicians.utah.edu/echo/clinical-support-areas/past-offerings>). Teleconferencing technologies were used to facilitate (1) an effective "knowledge network" for advanced practice clinicians, family physicians, and others providing care to patients with a personal/family history of cancer and (2) enhance their knowledge of current genetic testing and screening for cancer syndromes.

Although Project ECHO is in 46 states and other countries, there do not appear to be links that identified any other adult genetic hubs [37,38].

E-consults and videoconsults

Telephone consults have long been the basis for consultation among health professionals. Secure, Health Insurance Portability and Accountability Act (HIPAA)-compliant electronic consults (E-consults) and videoconsults can now be easily used and provide additional visual information at the time of the consult.

The use of e-consults and videoconsults between medical genetics professionals and other physicians and health professionals is a positive way to guide referral or care for individuals with adult genetic disorders. A 2018 survey report of medical genetics professionals (58 physicians and 11 genetic counselors) indicates that 39% of the medical genetics professionals used e-consults. It was not clear whether the e-consults were genetics professional to genetic professional or genetics professional to other health professionals. (Dhar et al, unpublished data). A study of genetic professionals in Europe reported that while videoconferencing was available to 24%, 9% used them for patient counseling. The rest presumably was a physician to healthcare provider [3].

An international survey of healthcare delivery practices for a specific adult genetic disorder focused on HD Specialty Clinics taking part in ENROLL HD. Over half (52.2%) of the 231 clinics in Europe, North America, Latin America, and Oceania responded. Of these, 106 clinics responded to the question of videoconferencing with 23.6% using videoconferencing/TM. Three options were presented. Among the 25 sites, TM use was 45.8% for patient consults, 62.5% with other clinicians, and 25% listed as other [39].

Thus, while we do not currently have good data on a physician to physician and physician to healthcare consultations for adult genetic disorders, it would appear that in some cases, that use is increasing.

Commercial use of telegenetics

In a growing trend, genetic testing companies employ both physicians and genetic counselors to guide physicians and other health professionals, and in some cases patients themselves. These genetic professionals are qualified and certified to provide information about appropriate tests as well as results, and in some cases provide pretest genetic counseling, review and order genetic testing and offer posttest genetic counseling. For example, commercial genetic testing laboratories offer various forms of genetic counseling services via telephone and/or videoconferencing, from answering routine questions about the genetic test ordering process to full-fledged genetic counseling sessions. This has been described by Phillips et al. as a “hybrid model” for commercial laboratories [40]. There are also examples of nonlaboratory based companies employing physicians and genetics professionals including genetic counselors primarily for the purpose of remote provision of pretest and posttest genetic counseling (e.g., Genome Medical and PWN Health). These services are primarily directed at the assessment and delivery of adult genetic services such as hereditary cancer genetic testing (e.g., *BRCA1*, *BRCA2*) and carrier testing where personal and family history are the key components of current genetic testing criteria. The growing demand for clinical genetic testing and persisting shortage of genetics professionals has also led to the evolution of a patient-initiated testing model. In this model, individuals who have a personal or family history suspicious for a genetic condition (e.g., hereditary breast and ovarian cancer) are able to initiate an online request for an appropriate physician-ordered genetic test. This might be driven by individuals facing months-long wait times for an appointment with their

local genetics provider, fruitless efforts to engage their primary care professional in ordering genetic testing on their behalf, or geographical barrier to genetic services providers, among others. In this patient-initiated model, individual requests for testing are reviewed remotely in the context of patient provided clinical information and pretest counseling is provided via telephone or videoconference (telecounseling). If appropriate, a physician orders genetic testing for the patient, and posttest genetic telecounseling is provided. While this evolving paradigm is available for certain forms of diagnostic genetic testing, it is also being implemented for individuals who seek to understand their underlying genetic risks for disease through preventive genetic screening, thought they have no particular personal or family history concerning for a genetic condition. There is currently a dearth of published information on the number of interactions related to specific adult genetics tests by e-consult, by telephone, or by videoconferencing from these commercial entities, and to what degree this is impacting access to genetics professionals and clinical genetic testing services.

It will be interesting to see how incorporating genetic counselors in commercial, or private companies changes the landscape of telegenetics. In 2018 a study by Wolff et al showed that genetic counselors employed by commercial laboratories increased from 21% in 2011 to 42% in 2015. In a 2016 Professional Status Survey conducted by the National Society Genetic Counselors, 1524 respondents counsel patients while 508 respondents do not. Approximately 50% of the respondents who do not counsel patients work for commercial laboratories ($n = 252$), while only 6% of the respondents who do counsel patients reported that they work for commercial laboratories ($n = 97$).

Standards and regulatory policy applicable to TM for genetic services

An overarching concept regarding a clinician choosing to use TM to provide genetic service is that the service provided via TM should be equivalent to the service that would have been provided in person. For this to be true, there are standards that should be met [https://www.americantelemed.org/resource_categories/practice-guidelines/ (accessed 10.08.19)]. One needs to ensure that the equipment meets technical standards delineated in guidelines from the American Telemedicine Association (ATA) to ensure that the connectivity and bandwidth can ensure a high-quality connection without interruption during the encounter, and high-definition video imaging and audio facilities. Similarly, peripheral devices including examination scopes and stethoscopes need to meet these standards. Additionally, the internet connectivity, TM software, and equipment must be secure and HIPAA compliant. It is necessary that the provider conduct the telegenetics encounter from a physical location which is secure and ensures that the virtual encounter meets HIPAA standards.

Obtaining a history of present illness, medical history, review of systems, and a family and social history can effectively be done via TM; however, one needs to be aware of the limitations of physical examination via TM. There are many peripheral devices that enable a physical examination of a patient via TM, including digital ophthalmoscopes, otoscopes,

dermatoscopes, and high-definition cameras allowing detailed examination of the external body, and digital stethophones. These facilitate an extensive virtual physical exam; however, one cannot palpate the patient. This limitation should be taken into account when deciding to see a patient via TM.

Licensure

One needs to ascertain that TM is included in the State's definition of the practice of medicine. This should be easily found on the State Medical Licensing Board's web site. Regarding licensure, some states require an additional license for practicing TM. All this information is available on the State Medical Board's site. If one is using TM to see patients in another state rather than the state in which they are licensed then they will need to be licensed in that other state as well. There is an effort under way to make obtaining licenses in another state less difficult, called the Interstate Medical Licensure Compact (IMLC). At the time of this writing, only 24 states and 1 territory participate in the IMLC, but it is expected that more will join, so it is best to check on the updated compact when planning to develop a TM service [<https://imlcc.org/> (accessed 10.08.19)].

It is not clear whether genetic counselors are required to be licensed across states to practice telegenetic counseling, but it seems reasonable to ensure that genetic counselors are licensed in originating states, when licensure exists in that state, and distant sites when providing telegenetic counseling.

Privileging and credentialing

If the site where the patient is or the site where the practitioner is, or both, is a hospital or hospital outpatient facility, one will need to be credentialed and have privileges from the hospital/hospital outpatient facility in accordance with standards set by The Joint Commission (TJC) [<https://www.foley.com/en/insights/publications/2018/02/telemedicine-credentialing-by-proxy-what-hospitals> (accessed 10.08.19)]. If one is going to be providing TM services to multiple sites that come under TJC accreditation, it is good to know that TJC allows facilities to use a "deemed" process for credentialing and privileges, which can streamline the paperwork and time spent to obtain privileges at those facilities. If TM is performed from an office or clinic to another office or clinic, credentialing and privileging is not likely to be needed, but it is best to check with the facilities to be sure.

Documentation for TM services

One documents the encounter in the same way as for any other clinical service, documenting the history, physical, assessment and plan/recommendations. It is a good practice to indicate in the note that the visit was conducted via TM. One needs to determine ahead of time which medical record is the primary record for the patient encounter; it could be the medical record/electronic health record (EHR) at the originating site or the record at the distant site. If one is to document in the originating site's record system, one needs to have

obtained appropriate access to that system. In either case, it is a good idea to be sure that there is a copy of the documentation in both records, realizing that one is a copy and not the “official” record.

If the encounter is a consultation, one needs to send a copy of the document to the provider who requested the consultation, in accordance with current procedural terminology (CPT) standards.

Billing and reimbursement for TM services

Note that these are general guidelines and one’s specific clinical practice environment may have different or additional requirements. Additionally, following these general principles does not guarantee that readers will receive reimbursement.

Synchronous (live) TM services

Physician services

Reimbursement for clinical services is affected by two issues: a payer’s coverage policy and its payment policy. Coverage establishes what service(s) the payer will pay for, while payment policy establishes how much the payer will reimburse for a covered service. Medicare has been expanding its coverage for synchronous TM services. There are still some geographic areas for which Medicare will not cover; so it is best to check on the limitations when one is planning a new TM service.

Medicaid programs differ from state to state regarding coverage and payment for TM. The ATA web site maintains a list of state coverage policies [<https://www.americantelemed.org/initiatives/2019-state-of-the-states-report-coverage-and-reimbursement/>] (accessed 10.08.19)].

The veterans administration (VA) has adopted TM extensively in a wide variety of specialties, including genetics. The VA recently reported that it has conducted a total of 1 million TM encounters. The VA only requires a providing physician to be licensed in one state [<https://www.telehealth.va.gov/>] (accessed 10.08.19)].

Private payers differ regarding coverage and paying for TM services. More payers are covering TM, but if the patient has concerns about possible out of pocket expenses, the patient should check with their payer before setting up a TM visit. In cases where the payer will not cover the TM service, patients may still regard the service to be valuable/convenient enough that they may be willing to pay out of pocket for the visit.

Genetic counselors

Although there is a CPT code, 96040, for genetic counseling provided by a genetic counselor, many payers, including Medicare, do not recognize Genetic Counselors, even licensed genetic counselors, as providers who can bill for service in person, let alone by TM. Advocacy regarding Medicare recognition of genetic counselors has led to the introduction of bills in both houses of the Congress in 2018 to rectify this situation.

Billing for synchronous TM services

One submits a claim (bill) for a TM service using one or more ICD-10 codes indicating the diagnosis and a CPT code indicating the type of service, usually an evaluation and management (E&M) code with the addition of the -95 modifier to the E&M code, to specify that the service was provided by synchronous TM. One should also append the -95 modifier to the genetic counseling CPT code, 96040 if counseling is done by TM. Many other medical services can be coded using the -95 modifier. The CPT manual appendix P lists all of these services and their CPT codes.

Reimbursement

If the patient's payer covers TM services, one should get paid if the claim has been coded and submitted properly. One should be aware however that not all payers reimburse the service at the same level as for the equivalent CPT code if the service had been provided in person. This discrepancy has led to the passage of "parity" laws in many states, to ensure that TM services are paid at the same rate as they would have paid for the service in person. At the time of this writing, 35 states have passed Parity laws, and 3 have bills pending. If a claim is denied, one can appeal the denial, as described in the chapter on billing and reimbursement (see Chapter 25: Billing and Reimbursement).

Asynchronous TM services (store and forward)

As discussed earlier, interprofessional internet consultation (frequently referred to as e-consults), and many forms of remote patient monitoring can also be used to provide genetic care. Effective January 1, 2019, there are CPT codes for many of these asynchronous TM services. Medicare has established coverage and payment policy for these services, but Medicaid and private payers may not have yet established policies for these. Additionally, Medicare established a HCPCS code, G2012, for a brief nonface-to-face virtual check-in with an established patient. As with the interprofessional consult code, there is a co-pay for this service, and patient verbal consent for this service needs to be documented. At some point it is likely that there will be a CPT code for this service for patients covered by private payers. Centers for Medicare and Medicaid Services (CMS) also published a HCPCS code G2010 for remote evaluation of prerecorded patient information. It anticipates that a patient can capture and send photographs or videos to a provider and the provider would review the images and render a medical decision back to the patient. Once again, verbal consent by the patient is necessary. If a CPT code is developed for this service, private payers might cover and pay for it as well.

Conclusions

Telegenetics for genetic evaluation and counseling of adult genetic disorders and monitoring patients after diagnosis over time is a service that is growing. The majority of adult telegenetic cases seen for evaluation, diagnosis, and counseling are cancer genetic cases. Currently, the

majority of adult telegenetic cases followed over time after diagnosis are adult neurogenetic degenerative disorders. As this chapter demonstrates there is increasing interest in use of TM and other digital tools to increase access to genetic services and changes in governmental and private payers' policies are now supportive of adoption of these service models.

Points to remember

- Telegenetics involves electronic communication between the patient and the provider, as well as between providers and may improve access to genetic care. When providing telegenetic care, secure, HIPAA-compliant equipment and Apps should be used to protect patient privacy.

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Ethical, Legal, and Social Implications

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Introduction

Ethical, legal, and social challenges are present at every step of the genetic testing process. First, when recommending testing, a clinician must decide what type of test to order. That decision should take into account that although genome-wide tests may provide more accurate and complete information, they also are more likely to identify genetic variants unrelated to the symptoms or risks that are the clinical purpose for ordering the test. The clinician must then obtain the patient's informed and voluntary consent to conduct testing, which requires disclosure of the results the patient can expect to receive, the risks, benefits, and limitations of testing for the patient, and the implications of testing for the patient's genetic relatives. The patient should also be informed of his or her right to decline the test and to opt out of analysis of certain genetic variants, as well as the genetic privacy and anti-discrimination laws that are in place to protect against the inappropriate access and use of the patient's test results. Finally, after testing is conducted, the clinician will communicate the results to the patient and, with the patient's consent, may facilitate communication of the result to the patient's at-risk relatives. This chapter discusses these issues in the order in which they usually arise during the testing process.

Throughout the discussion, it is important to appreciate that there is a difference between ethical conduct and lawful conduct. Sometimes healthcare professionals may find it ethically necessary to take legal risks, and sometimes simply complying with legal standards is not sufficient from an ethical perspective. This chapter considers both legal requirements and ethical guidelines relevant to common Ethical, Legal, and Social Implications (ELSI) issues in genetic testing but does not attempt to resolve them where they are in tension. Furthermore, the laws that are discussed in this chapter and their implementing regulations are complex and frequently revised. For these reasons, any questions concerning the application of these laws and regulations to specific situations should be discussed with an attorney. For resources that are generally relevant to ELSI issues, see [Table 27.1](#).

Table 27.1 ELSI resources.

Topic	Resources
General ELSI	• World Health Organization: https://www.who.int/genomics/elsi/resources/en/
Decision-making and informed consent	• NIH: https://ghr.nlm.nih.gov/primer#testing • Genetic Alliance: http://www.genesinlife.org/testing-services/testing-genetic-conditions
Disclosure of results	• ACMG: https://www.acmg.net/docs/standards_guidelines_for_the_interpretation_of_sequence_variants.pdf • ACMG: https://www.nature.com/articles/gim2016190
Genetic privacy and discrimination	• NIH: https://www.genome.gov/10002077/genetic-discrimination/; https://www.genome.gov/27561246/privacy-in-genomics/; https://www.genome.gov/27552194/ • GINAhelp.org: http://ginahelp.org/ • American Medical Association: https://www.ama-assn.org/delivering-care/precision-medicine/genetic-discrimination

ACMG, American College of Medical Genetics and Genomics; ELSI, Ethical, Legal, and Social Implications; GINA, Genetic Information Nondiscrimination Act; NIH, National Institutes of Health.

Decision-making and informed consent

Identifying the decision maker

Informed consent is an important aspect of genetic testing. Only capacitated adult patients can give informed consent. When adults lack decision-making capacity, legally authorized surrogates can make decisions on their behalf. A surrogate should use substituted judgment to make decisions based on what he or she thinks the patient would decide given the patient’s previously stated preferences, values, and beliefs. If there is no evidence of what the patient would have wanted or if the patient has never been capacitated to make decisions for himself or herself, then the surrogate decision maker should act in the best interest of the patient.

State law governs who the appropriate surrogate decision maker is for an incapacitated patient. In all states, if the patient has been declared legally incompetent and has appointed a legal guardian, then that person is authorized to make all medical decisions on the patient’s behalf. If there is no legal guardian, but the patient has completed a Medical Power of Attorney (MPOA), then the MPOA is the legally authorized representative. In the absence of either of these legal documents, physicians should look to whomever is the legally authorized surrogate according to state statute (e.g., in Texas, the surrogacy hierarchy is as follows: spouse, adult children, parents, and next of kin) [1].

It is important to note that capacity is a threshold concept and that even patients who are not capacitated to make medical decisions for themselves should be included in the decision-making process, commensurate with their cognitive ability. For example, adult patients with mild intellectual disability may not be able to legally consent to genetic testing if they lack decisional capacity, but the test should be explained to them, they should be

asked about their preferences and concerns, and their assent should be sought whenever possible.

Process of informed consent

Regardless of whether the decision maker is the proband or a legally authorized surrogate, that person must give informed consent for genetic testing. Assuming the decision maker is a patient, in order to give informed consent, the patient must have enough information to understand the risks and benefits of testing, as well as any available alternatives, and he or she must make a voluntary decision to have the test performed. Informed consent should be documented, but it is not a signed sheet of paper. Rather, it is a process of communication between patient and clinician, where the patient has an opportunity to ask questions and make decisions based on his or her own values and beliefs [2]. Often, the process of decision-making takes place over time and culminates in the patient's written authorization, which is documented on the consent form. The consent form, in turn, should summarize the key information that was communicated.

State laws vary on whether specific informed consent is needed for genetic testing or if such testing is covered under the general consent to treat that all patients sign when receiving medical care or registering with a health care institution. When specific consent is required, there is also variation in what information needs to be included in the consent form [3,4]. From an ethical perspective, however, patients have an autonomy-based right to decide whether they want genetic testing, and they cannot exercise this right unless they have an adequate understanding of the following information [5].

Type of test

First, patients must understand what test is to be performed, as well as the purpose of the test and why it is being performed. It is also essential that patients understand how the test will be carried out and the physical risks associated with that method. For example, will it involve a blood draw or the collection of a saliva sample, or will the test be conducted using discarded tissue postsurgery?

Types of genetic results

Genome-wide tests may yield a higher diagnostic rate for complex conditions and can uncover *de novo* genetic variants [6]. However, because genome-wide tests produce large amounts of data, they may generate unwanted information, such as risk alleles for untreatable adult-onset genetic disorders. Patients need to not only be told what test will be performed, but they need to understand the pros and cons of that test, as well as what types of results they can expect to receive (see Disclosing results section later).

They also need to understand what different types of results mean. For example, they should be told what it means to get a positive result, a negative result, or a result identifying a variant of unknown significance, in their particular circumstances, including the fact that a negative result may not necessarily mean that there is no genetic contribution to their disease.

They should also be informed that the results might include false positives or false negatives. If the laboratory plans to re-analyze test results as new knowledge is gained, then that should be explained to patients, along with information about who will pay for re-analyses [7].

Psychosocial risks associated with testing

There may be emotional or psychosocial risks associated with genetic testing, and patients should be warned of these during the informed consent process. For example, one study suggests that patients who test positive for Huntington disease (HD) are at increased risk of major depression, compared to those who test negative for HD and the general population [8]. However, other studies of at-risk populations have not shown any significant increase in depressive symptoms or anxiety, even after learning about an increased risk of developing Alzheimer's disease, another untreatable adult-onset condition [9]. Nonetheless, individuals vary in how they respond to genetic information and need to understand the potential negative, as well as positive, emotional impacts that such information may have on them.

Retention of genetic information

If the specimen used for genetic testing is going to be retained, either for clinical purposes or for future research, then that should also be discussed with the patient. Moreover, if the genetic data that are generated by the test are going to be stored or shared for research purposes, the patient should be notified and given the opportunity to opt out. Data that are shared as part of research funded by the National Institutes of Health (NIH) must comply with the NIH Genomic Data Sharing Policy, which requires informed consent for future use and broad data sharing, even if the data come from clinical specimens and even if they are de-identified [5].

Risks for family members

Finally, test results might provide health information, not only about patients but also about their genetic relatives. Patients should be informed of the possibility that the test can provide information about their relatives' risk of developing particular conditions or of having affected children [10].

Declining and retracting consent

Some patients choose not to have genetic testing because of concerns about genetic discrimination [11]. Patients need to understand who will receive their test results and should be informed of the ways in which their privacy will be protected. Laws that protect against genetic discrimination (see Privacy and Discrimination later) should be explained to patients, including what circumstances they do and do not cover [11].

Patients have a right to decline or change their mind about genetic testing, even after a consent document is signed. One reason patients may decline testing is because they do not want to know their genetic risk of disease. The "right not to know" is firmly established in

medicine, medical ethics, and genetics, especially in the area of predictive genetic testing. Individuals vary in their desire for predictive genetic information; some want all the information they can get so that they can better plan for the future, while others worry that such information could cause anxiety, fear, or depression or may be used against them in discriminatory ways. The paradigmatic case involves predictive DNA testing of asymptomatic individuals with a family history of HD. Even though that test has been available since 1993, the majority of at-risk individuals choose not to take it [12]. The right not to know is not absolute [13], but it generally protects individuals from receiving a genetic test without their informed consent.

Vignette 27.1

ST is a 24-year-old with mild intellectual disability (ID). When ST was 5 years old, she underwent Fragile X testing and chromosomal microarray analysis (CMA), consistent with the standard of care, but neither revealed any information that could explain her symptoms [14]. ST's brother is recently married, and he and his wife would like to have children. However, he is concerned about the likelihood that his children might have ID. ST's brother wants ST to undergo genome-wide (whole-exome) testing with the hope of identifying a potential genomic explanation for ST's ID. The patient's brother talked to their parents about this and ST's parents believe it would be a good idea. They bring ST to a genetics clinic and explain that they want ST to undergo genome-wide testing.

The first thing the physician should do in this case is to assess whether ST has capacity to make her own decisions about genomic testing. If so, then the physician should discuss the testing with ST and obtain her informed consent. If the patient lacks decision-making capacity, then the physician must determine who is ST's legally authorized surrogate decision maker (in this case, probably her parents). In either case, the physician should obtain informed consent for the testing and carefully describe the risks, benefits, and alternatives.

Disclosure of results

Genetic findings

In addition to obtaining consent for testing, clinicians must be prepared to manage the genetic information generated by testing. Genetic findings associated with the purpose for ordering the test are referred to as *primary findings*. For example, identification of a *MLH1* pathogenic variant in an individual with a family history of Lynch syndrome, as a result of testing to determine whether the person has a germline pathogenic variant associated with that condition, is a primary finding. However, genome-wide tests generate not only information about the genes that are relevant to the symptoms or risks that are the clinical purpose for ordering such tests. They also generate information about genes that play no known role in those symptoms or risks. In some cases, a laboratory may report findings, including interpretations of genes or genomic loci, that are unrelated to the clinical purpose for ordering a genetic test. When this happens, the findings are known as *secondary findings*.

Primary and secondary findings are typically categorized by their clinical significance. Many laboratories categorize genetic variants as pathogenic, likely pathogenic, variants of unknown significance (VUS), likely benign, or benign [15]. Determining the pathogenicity of a variant is heavily dependent on the level of evidence available to associate the variant with a particular health outcome in a specific population. For many variants, perhaps most, there is not enough information to determine their pathogenicity: these variants are known as VUSs. The appropriateness of reporting VUSs is a matter of debate. On the one hand, there is concern that some clinicians and patients will wrongly believe that VUSs are explanatory of symptoms or predictive of risk, when they are not. On the other hand, reporting VUSs may be worthwhile in the long-run because, as medicine progresses, data may later reveal that what was previously a VUS is actually a pathogenic variant with clinical implications. If VUSs are reported, the patient should understand that they are not currently explanatory or predictive, but as the field progresses, researchers may learn new things that change the interpretation of particular findings.

Vignette 27.2

RV is a 39-year-old woman who seeks evaluation in your genetics clinic due to a personal history of hypertrophic cardiomyopathy (HCM). She also reports a family history of HCM in her sister who died a sudden cardiac death at age 19y. She has one son, aged 16 years and although he is currently asymptomatic, she is concerned that he might also develop HCM. You suggest that she undergo genetic testing and explain the implications of possible results for herself and her family members, particularly her son. The patient consents to genetic testing and is found to have a variant of unknown significance (VUS). She is now requesting that her son be tested for the VUS.

In this case, the patient's VUS has no clinical significance yet for either her or her son since it is not known whether the variant that was identified in her is the true cause of her disease. In the absence of informative genetic test results, her son should be assumed to be at 50% risk, and standard clinical screening guidelines should be followed. However, there is no clinical utility associated with conducting genetic testing of her son to determine whether he has the VUS.

Some pathogenic variants are associated with poor health outcomes that are *medically actionable*, which means there are medical interventions that can help prevent or decrease the risk of these outcomes. For example, an individual who has a pathogenic variant associated with an increased risk for colon cancer can undergo more frequent colorectal screening to identify and remove polyps that might become cancerous. When a genome-wide test is ordered, it is possible to identify genomic risks for some medically actionable conditions that are unrelated to the purpose for ordering the test. The question thus arises: do clinicians have an obligation to order the analysis of secondary genes associated with medically actionable conditions? Do laboratories have an obligation to analyze those genes and report their findings as a matter of routine practice? [16,17]

The American College of Medical Genetics and Genomics (ACMG) recommends that when clinical genomic tests are ordered, laboratories should analyze and report any

pathogenic or likely pathogenic variants identified in 59 genes associated with medically actionable conditions, including *BRCA1*, *BRCA2*, *KCNQ1*, and *LDLR* [18,19]. The ACMG also recommends that, before ordering genomic tests, clinicians should discuss with their patients that those tests may generate secondary findings and give them the opportunity to opt out of the analyses of genes that are unrelated to the clinical purpose for ordering the tests [20].

Disclosing results

When analyses are complete, the results must be disclosed to patients. Studies suggest that most clinicians believe they do not have adequate knowledge about genetics or the necessary training regarding how to interpret genetic results or counsel patients [21]. Certified genetic counselors are trained in the communication of genetic information to patients, and it is important that patients receive appropriate pre- and post-test counseling from these or other trained healthcare professionals (see Chapter 4: Genetic counseling for adults). However, clinicians still must know enough about genetics to recognize when testing might be appropriate, provide general guidance to their patients and identify when referral for counseling is in the best interests of the patient.

Impact on family dynamics

There are distinct ethical concerns associated with the disclosure of genetic test results to a patient's genetic relatives when those results indicate that they are at risk of disease or having affected children. The patient should be strongly encouraged to communicate such findings to the at-risk relatives [10], and the clinician or genetic counselor should be prepared to assist the patient by providing a letter to share with relatives that explain the test results, their implications for family members, and recommendations for testing or screening. The clinician or genetic counselor may also offer to discuss the test results with any at-risk family member, with the patient's consent.

When a patient refuses to disclose test results to at-risk relatives, however, there is some debate about whether the clinician has a “duty to warn” those individuals. The American Society of Human Genetics has suggested that clinicians may make an unauthorized disclosure to an at-risk genetic relative in the event the patient refuses to make such disclosure; it is likely that serious, imminent, and foreseeable harm to the relative will occur; the relative is identifiable; and the disease for which the relative is at genetic risk is either preventable or treatable or early monitoring is likely to decrease the risk [10]. However, there is no legal consensus regarding a duty to warn in the United States [22], and unauthorized disclosure of a patient's test results may be a violation of the Health Insurance Portability and Accountability Act (HIPAA), a US federal law that governs the clinical use, storage, and disclosure of patients' *protected health information*, which includes genetic information.

There are numerous cases in which testing relatives may help categorize the pathogenicity of a genetic variant identified in a proband. In cases in which a family member (e.g., an uncle) is tested to help categorize a proband's variant, laboratories will often addend the results of the family member on the proband's report. In circumstances like this, it is

important to remember that the clinician who orders testing establishes a clinician–patient relationship with the proband’s relative and the clinician has an obligation to protect the confidentiality of that patient’s information. Thus, if it is foreseeable that the patient’s results will be included in the proband’s report, the clinician has an obligation to disclose this to the patient before testing to evaluate whether the patient is willing to share the results with the proband.

Genetic information and medical records

There are also ethical issues regarding the placement of a patient’s genetic information in the medical record. Studies have found that patients sometimes request that their genetic results be excluded from their medical record [21,23]. Such requests create a difficult situation for clinicians because genetic findings may have important clinical implications. When faced with a request to exclude genetic test results from the medical record, the clinician should try to understand the patient’s reasons. It may be that the patient is concerned about genetic discrimination and is not aware that there are federal and state laws in the United States that prohibit genetic discrimination in certain contexts. The patient also may not appreciate that if he or she is seen by other clinicians who can access only the information in the medical record, the omission of the patient’s genetic information might lead the clinicians to overlook diagnoses, order unnecessary tests, or recommend less effective treatments [24].

Importantly, HIPAA recognizes patients’ legal right to access their protected health information as long as it is maintained by their clinicians (or those who conduct services for clinicians) and regardless of whether it is placed in their medical record. This means that patients have a right to access their genetic information maintained by their clinicians as well as their genetic information maintained by clinical laboratories that perform testing and analyses on behalf of clinicians. The Office of Civil Rights within the US Department of Health and Human Services, which enforces HIPAA, has interpreted the scope of genetic information that patients have a right to access to include not only test reports but also the full gene variant information generated by the test [25].

Privacy and discrimination

Although the terms *genetic privacy* and *genetic discrimination* are often discussed interchangeably, these concepts are distinct. Genetic privacy concerns access to information. Dignitary harms can occur when patients’ genetic information is accessed without their explicit or implicit permission. Genetic discrimination, on the other hand, concerns the unfair or unequal use of such information after it has been accessed. Financial, physical, and psychological harms can occur when individuals are treated differently solely on the basis of their genetic information, regardless of whether it was appropriately accessed [26]. Studies indicate that individuals are generally worried about the privacy of their genetic information and its potential use by others to discriminate against them [27]. Vignette 27.3 raises important questions related to *genetic privacy* and *genetic discrimination*.

Vignette 27.3

FK is a 42-year-old woman who comes to the genetics clinic because her sister who was recently diagnosed with ovarian cancer was found to have a pathogenic variant in BRCA2. FK was counseled that she might also be at risk to be a carrier of this pathogenic variant. The patient is reluctant to proceed with genetic testing and expresses concerns that the genetic information could possibly lead to discrimination for her.

Health Insurance Portability and Accountability Act of 1996

In the United States, there is a complex web of federal and state laws that address unauthorized access to and discriminatory uses of genetic information. The major federal statute that addresses health information privacy is HIPAA, which generally prohibits the disclosure of patients' protected health information—including genetic information—without the patients' authorization. These prohibitions do not apply, however, where they would unnecessarily interfere with patient care or other important public benefits. Clinicians are therefore permitted to disclose and access patients' health information without their authorization when necessary to treat them, obtain payment for services, and support certain healthcare operations activities, such as quality assessment and auditing activities.

Affordable Care Act

The Affordable Care Act recently closed a major loophole in these protections by prohibiting both group and individual health insurers from denying coverage based on an individual's pre-existing condition. This means that health insurers cannot use an individual's manifested health conditions—including conditions with a genetic basis—as a reason for denying coverage to that person [11].

Americans with Disabilities Act

The Americans with Disabilities Act (ADA) also addresses discrimination against individuals with manifested health conditions in employment, public services, and public accommodations contexts, where such conditions qualify as *disabilities*. According to the ADA, a disability includes any physical or mental impairment that substantially limits one or more major life activities, such as performing manual tasks. The ADA prohibits employers from discriminating against individuals with disabilities who, with or without reasonable accommodation, can perform the essential functions of employment positions. Employers are also generally prohibited from conducting medical examinations or making inquiries of employees as to whether they have a disabling condition unless the examinations or inquiries are job related.

Genetic Information Nondiscrimination Act

Individuals may face discrimination not only on the basis of manifested genetic conditions. Asymptomatic individuals may also face discrimination on the basis of genetic information that indicates or is perceived to indicate their risk for health conditions. In 2008 Congress passed the Genetic Information Nondiscrimination Act (GINA) to address these concerns.

GINA limits access to and use of certain genetic information—which is defined to encompass information related to genetic testing and other services provided to patients and their families—by health insurers and employers. Specifically, GINA prohibits health insurers from using genetic information to set premiums, requesting or requiring genetic testing prior to an individual's enrollment, and purchasing genetic information for underwriting purposes. Similarly, employers are generally prohibited from requesting, requiring, or purchasing genetic information about employees or their family members or using this information to make decisions related to hiring, firing, promoting, and compensating employees. Applying these rules, FK's positive test results for a *BRCA1* mutation may not be used to discriminate against Lauren under GINA.

GINA goes a long way toward protecting asymptomatic individuals against genetic discrimination in health insurance and employment contexts. However, according to the NIH, GINA does not apply to individuals who receive their insurance through the Federal Employees Health Benefits, the Veteran's Health Administration, the US Military (TRICARE), and the Indian Health Service, although some of these programs have internal policies that protect against genetic discrimination [28]. GINA also does not apply to life, disability, or long-term care insurers, or providers of other resources or services, such as public housing [11].

Over the years, many states have passed laws that supplement or provide greater protections than federal genetic privacy and antidiscrimination laws. The NIH reports that 41 states have adopted protections to safeguard the privacy of genetic information and genetic test results [29]. Moreover, 24 states have passed laws regulating genetic discrimination by life, disability, or long-term care insurers. In 2011 California passed the most comprehensive antidiscrimination statute to date (CalGINA), which prohibits genetic discrimination in housing, mortgage lending, and state-funded programs. Perhaps in part due to these legal protections, there are few known instances of genetic discrimination in the United States [27]. However, clinicians should generally be aware of the potential discriminatory downstream uses of patients' genetic information and their ethical and legal obligations to respect patients' genetic privacy.

Points to remember

- Genetic testing should not be performed without informed consent.
- It is important during the informed consent process to explain, among other things, the type of test that is recommended, what results the patient can expect to receive, what different types of results mean, the risks and benefits of testing for the patient, and implications of testing for the patient's genetic relatives.
- Genetic variants are generally categorized as pathogenic, likely pathogenic, variants of unknown significance, likely benign, or benign.
- Patients have a legal right to access their genetic information maintained by clinicians and clinical laboratories.

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- Clinicians generally may disclose patients' genetic information for treatment, payment, or healthcare operation purposes.
- In the United States, there is a complex web of federal and state laws that address unauthorized access to and discriminatory uses of genetic information.

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Future Directions

Precision medicine in the genomic era

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Introduction

The current approaches to disease prevention and treatment have had a remarkable impact on the lives of affected individuals. For example, over the last two-to-three decades, the overall death rates due to cancer and cardiovascular disease (CVD) have decreased by nearly 30% [1,2]. It has been estimated that almost half of the reduction in mortality due to CVDs is because of improved therapies. However, in spite of such significant advances, the current approaches in clinical investigation and clinical care are far from ideal. The treatments for most disorders tend to embrace the “one-size-fits-all” approach wherein it is assumed that a diverse group of individuals with varying risk factors are likely to respond similarly to a therapeutic intervention. Such simplistic and reductionistic views ignore the impact that genetic heterogeneity, environmental factors, and the diverse pathophysiological mechanisms have on disease progression and responses to therapy.

Precision medicine is a broad term used for the approach to disease prevention and treatment that incorporates variations in an individual’s genetic, lifestyle, and environmental factors. The term “precision medicine” is sometimes used interchangeably with “personalized medicine.” However, rather than seeking to tailor treatments specific to each individual, as the term “personalized” implies, precision medicine aims to organize individuals into subgroups according to characteristics such as disease susceptibility and treatment response. Hence, this approach directs treatment and prevention toward those who stand to benefit most and away from those who might experience adverse effects. Whereas the concept of precision medicine is not new, the recent advances in (1) sequencing technologies to assess genomic variation, (2) “omic” methodologies to understand disease mechanisms, (3) electronic medical record systems that allow sharing of phenotypic data, and (4) novel computational methods that are able to analyze “big data” have ushered the incorporation of precision medicine into clinical investigation and clinical care. Numerous platforms including genomics, transcriptomics, lipidomics, metabolomics, epigenomics, and microbiomics

have been used to implement the promise of precision medicine. Whereas it would be beyond the scope of this chapter to detail the many approaches used in precision medicine, we provide a brief overview of some clinically relevant aspects, especially involving the use of genomic information.

Precision medicine initiative

The idea of precision medicine received a major impetus in 2015, when the 44th President of the United States of America, Barack Obama, announced the launch of a Precision Medicine Initiative (PMI). The mission of the PMI was *“To enable a new era of medicine through research, technology, and policies that empower patients, researchers, and providers to work together toward development of individualized care.”* [3] The initiative provided a vision to revolutionize the prevention, diagnosis, and treatment of disease. A central goal of the initiative was to harness the power of the genome to create a new taxonomy of disease. The PMI focused on precision oncology as one of the immediate fields of focus, with the long-term goal of understanding the variations that are important for the treatment of more common diseases. In order to carry out the initiative’s ambitious goals, the National Institutes of Health created the Precision Medicine Initiative Cohort Program (PMI-CP). Among other tasks, the program’s purpose was to establish a diverse research cohort of one million Americans. The “All of Us” cohort, as the group would come to be called, became a pivotal component of the PMI. Using data from a large group of individuals, the “All of Us” research programs aim to understand the role of genetic and environmental factors and interactions between the two in disease causation, discover biomarkers for disease, identify causes of individual differences in response to medications, develop targeted therapies, and empower patients with information to improve their health.

Applicability of precision medicine in the clinic

In this section, we highlight the clinical applicability of precision medicine in three diverse groups of disorders—Mendelian disorders, cancer, and CVD.

Precision medicine in Mendelian disorders

The use of genotypic information to predict disease severity, identify individuals who are at increased risk to develop complications, and customize surveillance and treatments regimens has been an integral component of the practice of clinical genetics. For example, individuals with osteogenesis imperfecta, who have glycine substitution mutations in *COL1A1* and *COL1A2*, have more severe, bone disease than individuals with nonsense mutations and are thus more likely to be treated with long-term bisphosphonate therapy (Chapter 23: Skeletal disorders). Individuals with congenital long QT syndrome caused by pathogenic variants in *KCNQ1* are more likely to benefit from therapy with beta-blockers as compared to those with pathogenic variants in *SCN5A* (Chapter 9: Cardiac disorders). Inhalational

anesthetics and depolarizing skeletal muscle relaxants are contraindicated during anesthesia in individuals with pathogenic variants in *RYR1* that cause malignant hyperthermia. Individuals with pathogenic variants in the 5' or 3' regions of the *APC* gene can have an attenuated form of polyposis syndrome and can thus be managed with less aggressive strategies (Chapter 13: Gastrointestinal disorders). The threshold for aortic surgery based on proximal aortic root diameters in connective tissue disorders is influenced by genotype and family history of dissection (Chapter 10: Hereditary connective tissue disorders). While the use of genotypic information in the management of Mendelian disorders is not new, a recent exciting advancement has been the development of medications to target-specific genotypes. In the following paragraphs, we will highlight two well-known genotype-specific therapies.

Fabry disease is caused by pathogenic variants in *GLA* that encodes the enzyme alpha-galactosidase A. Migalastat is a chaperone that binds to the active site and stabilizes the enzyme allowing for its transport from the endoplasmic reticulum into the lysosome, the site of action of the enzyme. Migalastat has been approved by the Food and Drug Administration (FDA) for treatment of Fabry disease in a subset of adults with specific variants in *GLA*. Preliminary studies have shown that migalastat treatment can reduce the deposition of globotriaosylceramide in the kidneys, decrease left ventricular hypertrophy, and improve gastrointestinal symptoms [4]. As expected, individuals with nonsense mutations or other missense mutations that are not amenable to the chaperoning activities of migalastat are not candidates for therapy.

Duchenne muscular dystrophy is caused by pathogenic variants in the *DMD* gene, which encodes for dystrophin. The complex structure of the *DMD* gene prevents translation if variants are present in specific locations of the gene. Eteplirsen is an antisense oligonucleotide that can induce specific exon skipping during premessenger RNA splicing. This approach can lead to correct reading of the open reading frame and thus partially restore the expression of dystrophin. This therapy has been approved by the FDA only for patients who have a confirmed mutation that is amenable to exon 51 skipping based on an increase in dystrophin in skeletal muscle observed in some patients treated with the medication [5].

Precision oncology

Cancer is a genetic disease characterized by numerous somatic mutations that drive tumorigenesis and metastasis. Targeting key pathways that are preferentially active in cancer has been a well-studied approach. Thus not surprisingly, precision oncology was a prominent aspect of PMI; in fact, one of the strategies of PMI was to leverage insights gained through precision oncology research to elucidate the pathophysiology of chronic diseases [3].

One of the goals of precision oncology is to use genomic and protein biomarkers for the appropriate diagnosis, risk stratification, and treatment strategies including surveillance measures and risk-reducing surgeries. Individuals with early-onset cancers, multiple primary cancers, and other affected family members are more likely to have a Mendelian form of cancer. An accurate molecular diagnosis in such families would be pivotal in instituting appropriate surveillance measures. More recently, genomic data are being used to detect

cancer at an earlier stage even in non-Mendelian forms of cancer. For example, CancerSEEK, a multianalyte blood test that assays for “driver mutations” and protein biomarkers that are associated with common cancers, has been shown to have an overall median sensitivity of 70% to detect cancer in individuals with nonmetastatic disease [6].

Genomic and biomarker data are also increasingly being used for tailoring treatment regimens for cancers. For example, for breast cancer, many biomarker assays such as recurrence score (RS), the breast cancer index, predictor analysis of microarray 50, and EndoPredict have been validated for clinical use [7]. One of the more widely used and well-validated biomarker assays, Oncotype Dx 21-gene RS, is used to help identify patients who are most and least likely to derive benefit from adjuvant chemotherapy. While somatic tissue testing for specific types of cancer has been in practice for some time, more recently, testing for a wide range of solid tumors independent of the tissue type has been developed. In 2017 the US FDA approved two gene panels (MSK-IMPACT and F1CDx) for assessing pathogenic variants in solid tumors. These tests can be performed on formalin-fixed tissue samples from biopsy and can identify potential variants that could be targeted [8]. For example, in unresectable solid tumors that have deficient mismatch repair, pembrolizumab can be used as therapy, irrespective of the cell or origin of the tumor.

Precision medicine in CVDs

CVD, a prototypic multifactorial and common disease, affects more than one in three Americans and is the number one cause of death in the United States [9]. Traditional approaches to treatment of CVD comprise preventive efforts, lifestyle modifications, and therapy aimed at modifying a shared risk phenotype such as dyslipidemia and hypertension. The current treatment strategies do not typically address the marked genetic heterogeneity in the population or the diverse mechanisms causing CVD. Individuals with diverse genetic and environmental risk factors are treated as though they belong to a homogenous population who are likely to respond to the same class of medications. With estimates projecting that by 2035, nearly 45% of the US population will have some form of CVD, greater precision in phenotyping, and individual risk-based treatment strategies are of great importance [9]. Precision medicine can offer a new approach to the treatment and prevention of CVD. Genomic information can be a valuable addition to the clinical and standard diagnostic studies to help develop more appropriate and targeted prevention and treatment options. For example, the utility of pharmacogenomic information in adjusting the dosage of anticoagulants such as warfarin or selection of antiplatelet medications after percutaneous coronary interventions has been discussed in Chapter 29, Pharmacogenomics. Here, we focus on the use of genomic data for the risk assessment in CVD.

Some of the Mendelian forms of CVD have a phenotype that overlaps with early-onset disease in the general population. A classic example of such a disorder is heterozygous familial hypercholesterolemia (FH) due to pathogenic variants in *LDLR*. Individuals with heterozygous FH have nearly a 20-fold risk of developing coronary artery disease as compared to the general population [10]. Early diagnosis and genotyping individuals with

elevated low-density lipoprotein cholesterol (LDL-C) can help tailor a therapeutic strategy that can reduce the adverse cardiovascular outcomes. In contrast to the general population, the majority of individuals with heterozygous FH do not achieve target goals of LDL-C with standard therapy with statins. Most require additional therapy with ezetimibe or inhibitors of proprotein convertase subtilisin kexin 9 such as alirocumab and evolocumab. While successful treatment of monogenic disorders such as FH has an impact on patients and their families, the vast majority of CVD are multifactorial disorders that exhibit polygenic inheritance [11]. Therefore, developing strategies for risk stratification requires considering the collective small effect sizes conferred by numerous common genetic variants and the development of genome-wide risk scores (GRSs). The development and validation of GRS has been challenging due to limitations in the number and effect sizes of the incorporated genetic variants and the use of small nondiverse populations. However, more recently, revisions of existing GRS with use of additional genetic variants have been shown to improve the predictive ability, thus demonstrating the validity of the approach wherein genomic information can be used to complement traditional risk factors for a more accurate risk assessment in CVD [12].

Pharmacogenomics

Another facet of precision medicine is pharmacogenomics, which studies the effects of genetic and genomic variation on one's response to medications. A detailed discussion of the clinical applications of pharmacogenomics is provided in Chapter 29, Pharmacogenomics.

Direct-to-consumer genetic testing and precision medicine

The promise of precision medicine extends beyond the formal structure of medical institutions. Direct-to-consumer (DTC) genetic testing, which enables individuals to have access to some of their genetic information, has experienced tremendous growth since it first came on the stage in 1996. Individuals who undergo DTC genetic testing may come into the clinic expecting a more “precise treatment plan” based on their genomic information. For example, in the Impact of Personal Genomics Study survey, nearly 6% of individuals who underwent DTC testing reported that they either started a new medication or had altered the medication that they were previously prescribed [13].

However, there have been concerns about the validity, reliability, reproducibility, and reporting of DTC genetic tests. With no regulatory oversight, it is not known how well all of the platforms can detect specific genotypes and how these are used to predict the disease risk. For example, in one study, samples from five individuals were sent for testing at two different DTC companies. While there was concordance in correctly calling the genotypes, the interpretation was significantly different. The results from two companies were in disagreement for over half of the 13 diseases for which the risk was provided [14]. In 2013, the US FDA placed restrictions on marketing and information that can be reported from genomic information available through DTC. Currently, the FDA allows DTC companies to report on

the genetic risk for a limited number of diseases, carrier status for certain recessive disorders, three common *BRCA1* and *BRCA2* pathogenic variants found in individuals of Ashkenazi Jewish heritage, and two particular pathogenic variants in *MUTYH*.

In the near future, it would be reasonable to assume that the number of disorders for which genetic risk can be reported and the number of companies that offer such testing will increase. An important role for the physician would be to counsel patients regarding the evidence behind the utility and validity of DTC genetic tests, educate them on matters of privacy, and the possibility of receiving inconclusive results with regard to one's own or one's family's risk for developing a particular disease [15].

Challenges and future directions

In spite of the promise of personalized medicine, there are financial, technical, practical, and ethical considerations that pose a challenge to its widespread implementation. With the complexities of healthcare delivery systems in the United States, it may be difficult to get insurance companies to pay for such testing. Many payers require validated evidence that such testing is likely to make a meaningful change in clinical care. Many of the testing platforms that use “omic” data evolve at a rapid pace and the payers typically have lag periods before accepting that such tests are indeed useful. Developing targeted therapies involves conducting clinical studies in particular subset of individuals and typically such studies are costly. Once approved, as the target population may be small, the pharmaceutical industry may price these medications exorbitantly, thus, reducing the access to the medications. The technical challenges include inability to determine accurate linkage of the biomarker and genomic data with phenotype, housing the large amount of data generated from the analyses, and the need to constantly update and reanalyze the data as new information becomes available [16]. One of the primary practical challenges in implementation is regarding how genomic and other data would be incorporated into clinical care and who would be responsible for management guided by the information. If pharmacogenomic data were ordered by a cardiologist on a patient with heart disease and HIV infection specifically looking for variants that can guide dosing of an antiplatelet agent, but the testing also revealed the presence of the HLA-B*5701 allele that precludes treatment with abacavir for HIV, who would be responsible to make sure this individual is not started on abacavir for his HIV? Most physicians and other healthcare providers would have limited knowledge about many of the specialized tests and analyses. It would be unreasonable to expect even specialists to know about the genomic and transcriptomic advances in their own specialties. As with many genome-wide testing strategies, an ethical issue to consider includes how a test may reveal information that can adversely impact individuals if they were to know their risk for psychiatric disorders or diseases for which there are no treatments. Discrimination based on the risk for disease is also a concern; though the Genetic Information Nondiscrimination Act prevents discrimination based on the genetic information in procuring health insurance or employment, such protections are not afforded to life, disability, or long-term care insurances.

In summary, precision medicine has the potential to significantly impact the way we diagnose and treat individuals with various disorders. When wondering whether it would be worthwhile to implement this idea in spite of many obstacles, we could all benefit from being reminded of Victor Hugo's wise words "No one can resist an idea whose time has come."

Points to remember

- The practice of medicine is moving away from a "one-size-fits-all" approach and becoming more individualized.
- The PMI has fostered incorporation of genomic information into everyday medical practice.
- Individualized treatment is now being used in many cancer treatment regimens, as well as in the management of CVDs, such as FH.

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Pharmacogenomics

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Introduction

Pharmacogenomics studies the effects of genetic factors on an individual's response to a drug or medication. Although the term “pharmacogenomics” remains relatively new, physicians have for centuries observed interindividual differences in drug responses. Pythagoras in the 6th century, for example, first noted the toxic effects of fava beans in some individuals (later identified as those deficient in glucose-6-phosphate dehydrogenase). In 1968 Vesell and Page documented the differences in antipyrine metabolism in monozygotic and dizygotic twins. As our knowledge of the human genome grows, observational studies like these have now given way to targeted-sequencing and genome-wide association studies leading to the discovery of hundreds of genetic polymorphisms affecting drug responses in individuals.

These genetic polymorphisms often affect one of the two important features of a pharmaceutical compound: pharmacokinetics or pharmacodynamics. *Pharmacokinetics* describes how a drug is metabolized by an individual and includes its absorption, conversion to its active metabolites and its subsequent elimination from the body. Various cytochrome P450 CYP alleles may affect a drug's effect through changing its activation or deactivation. The clinical effects for the drug ondansetron (Zofran), for instance, can be significantly affected by a person's *CYP2D6* genotype with certain alleles increasing the metabolism of the drug to its inactive compounds (e.g., *CYP2D6*1*). Other alleles decrease its metabolism (e.g., *CYP2D6*4*), thereby prolonging its therapeutic effects.

Pharmacodynamics describes how well a drug is able to preferentially exert its therapeutic effects while minimizing harmful side effects. A variant in a drug target may change its binding affinity to the drug and thus affect its efficacy. In cancer therapy, the tumor genome may encode unique targets to allow therapeutic targeting and mitigation of the toxic side effects. The drug trastuzumab (Herceptin), for example, is designed to target the human epidermal growth factor receptor 2, which is genetically amplified in certain breast cancers. Targeting of this specific moiety allows for signal blockade and cessation of tumor growth.

[Fig. 29.1]

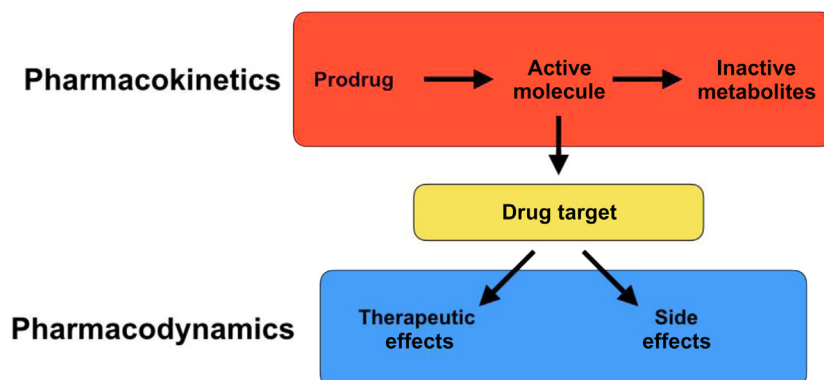


FIGURE 29.1 Pharmacokinetics and pharmacodynamics.

Clinical applications of pharmacogenomics

With advances in molecular technologies, the use of pharmacogenomics has permeated medical practice. The following examples illustrate the utility of pharmacogenomics in various disorders.

Mendelian disorders

One of the most exciting examples of pharmacogenetic data-driven therapeutic development has been the introduction of targeted drugs for cystic fibrosis (CF). CF is an autosomal recessive condition caused by biallelic, loss-of-function *CFTR* variants, which lead to progressive lung disease, gastrointestinal malabsorption, and failure to thrive. *CFTR* variants are classified according to their pathological effects. The variant *CFTR* G551D (c.1652G > A, p. Gly551Asp) is an example of a “gating variant,” as the mutant *CFTR* protein is able to be transported to the cell surface but does not open and close normally [1]. Gating mutations are the target of *CFTR* potentiators, drugs that are able to increase *CFTR* channel gating to enhance chloride transport. The potentiator Ivacaftor, introduced in 2012, was found to improve lung function and weight gain in patients both homozygous and compound heterozygous for the G551D variant [2].

Only around 4%–5% of patients with CF carry the G551D variant; however, the most common pathogenic variant is *CFTR* Δ F508 (c.1521_1523delCTT, p.Phe508del). This particular allele is found in ~75% of patients with clinical disease and leads to the production of a misfolded protein that becomes retained in the endoplasmic reticulum (ER) and degraded before it is able to reach the cellular membrane. In 2011 the first *CFTR* “corrector” Lumacaftor was introduced. Correctors are compounds able to sterically interact with the aberrant *CFTR* protein and induce a more normal three-dimensional structure, allowing for its increased release from the ER and transport to the cell surface [3]. While Lumacaftor led to small but measurable improvements in channel activity in patients homozygous for the

Δ F508 variant, the combination of a corrector (Lumacaftor) and a potentiator (Ivacaftor) appears to have a more significant improvement in lung function and other manifestations of the disease [4,5]. The combination drug Orkambi (Lumacaftor/Ivacaftor) has been met with intense excitement as a significant milestone in the treatment of CF. Although the gains in CFTR transport activity are still somewhat modest, it is hopeful that this targeted approach will continue to lead to more efficient treatments in the future.

Cancer

Nowhere would pharmacogenomics appear more powerful than in the field of oncology. Here, a drug must interact with two genomes, and thus its effects are influenced not only by inherited or germline mutations but also by tumor-specific or somatic gene changes. With some exceptions, a common paradigm is to view germline mutations as heavily influencing drug metabolism and toxicity while somatic variants as strongly impacting clinical efficacy. An understanding of both genomes is required for optimizing tumor response, treatment regimens, and ultimately long-term survival.

One of the best known examples of tumor-specific therapeutics is the drug imatinib and its use in chronic myeloid leukemia (CML) [6]. More than 95% of CML cases are associated with a reciprocal translocation and subsequent fusion between the *ABL* tyrosine kinase gene on chromosome 9 and the breakpoint cluster gene (*BCR*) on chromosome 22 [t(9;22)]. The resultant chimeric oncogene, *BCR-ABL*, encodes a constitutively active tyrosine kinase, which further activates multiple downstream processes eventually leading to the malignant transformation of hematopoietic cells. Imatinib functions as a BCR-ABL specific tyrosine kinase inhibitor. The drug binds to the inactive conformation of the protein tyrosine kinase domain and blocks ATP binding, inhibiting conversion of the enzyme to its active form. By exploiting this particular molecular target, imatinib is able to induce a complete hematologic remission in greater than 95% of patients. Imatinib also inhibits tyrosine kinase for platelet-derived growth factor receptor A (PDGFRA) and stem cell growth factor receptor also known as proto-oncogene c-Kit. Thus, imatinib has also been used as an adjuvant therapy for gastrointestinal stromal tumors with *c-KIT* and *PDGFRA* mutations; in some studies, the response to imatinib has been shown to be dependent on the specific genotypes [7].

Therapeutic compounds have been developed targeting tyrosine kinase domains in several other oncogenes including somatic mutations in *EGFR*, the *EML4-ALK* fusion gene, and the V600E variant of *BRAF*.

In addition to tumor-specific targets, some chemotherapeutics take advantage of tumor-specific defects by creating a “synthetic lethal” scenario. Hereditary breast and ovarian cancers, for example, are commonly associated with germline variants in *BRCA1* and *BRCA2*. Both gene products play an important role in the stable repair of double-strand breaks induced by DNA-damaging agents or breaks due to stalled replication forks. The proteins accomplish this task by facilitating repair via homologous recombination, a relatively error-free process. Loss-of-function variants in *BRCA1* or *BRCA2* thus force the cell to rely on other, often more error-prone repair mechanisms such as a single-strand break repair

facilitated by poly (ADP-ribose) polymerases (PARPs). These enzymes are the target of a recently developed class of drugs known as PARP inhibitors (Olaparib, Niraparib, Rucaparib). In the presence of pathogenic variants in *BRCA1* or *BRCA2*, PARP inhibitors prevent a tumor cell from utilizing the backup single-stranded repair mechanisms leading to highly unstable DNA products and increased apoptosis. PARP inhibitors have been approved for the treatment of patients with confirmed germline or somatic mutations in *BRCA1* or *BRCA2*, and their improved efficacy over other treatment options has been a groundbreaking advancement.

While targeted therapeutics offer the opportunity for more efficient tumor treatment, an intimate knowledge of germline mutations in the metabolism of chemotherapeutics is equally as important. 6-Mercaptopurine (6-MP), for instance, is used in the treatment of leukemia for its ability to inhibit the formation of purine nucleotides during cellular replication and synthesis. The drug is inactivated by the enzyme thiopurine methyltransferase; however, nonsynonymous variants within the *TMPT* gene may lead to excessive level of 6-MP and life-threatening toxicity in both homozygous and heterozygous individuals [8].

Cardiovascular medicine

Cardiovascular medicine is one field where the clinical outcome of modern pharmacogenetics has been closely evaluated with randomized controlled trials and incorporated into clinical practice guidelines [9].

Warfarin has long been used for the prophylaxis and treatment of thromboembolic events. Dosing of this medication, however, has always been notoriously difficult given its narrow therapeutic window and high side-effect profile. Coumarins such as warfarin work by inhibiting vitamin K epoxide reductase (*VKORC1*), the enzyme that controls the formation of vitamin K-dependent clotting factors. Variants within the *VKORC1* gene have been linked to abnormal drug responses within the general population and at the extreme end, warfarin resistance in rare families. The noncoding variant c. – 1639G > A has been most strongly associated with increased sensitivity to warfarin therapy. This is postulated to occur because the single-nucleotide polymorphism (SNP) alters a transcription factor-binding site leading to lower protein expression and thus a requirement for lower dosing [10]. Warfarin response also appears to depend heavily on its metabolism by the P450 2C9 enzyme system (*CYP2C9*) in the liver. Two polymorphisms within this gene, *CYP2C9**2 (c.430C > T, p.Arg144Cys) and *CYP2C9**3 (c.1075A > C, p.Ile359Leu), have been strongly associated with decreased enzyme activity and abnormal warfarin metabolism. Based on the multiple lines of evidence, there now exist suggested guidelines for warfarin dosing based on *VKORC1* and *CYP2C9* genotypes [11].

The P450 CYP enzymes also play an important part in the pharmacogenetics of platelet aggregation inhibitors such as Clopidogrel. These medications belong to a class of compounds called thienopyridines that selectively and irreversibly inhibit platelet P2RY₁₂ receptors involved in ADP-mediated aggregation. In order to exert its therapeutic effects, clopidogrel must first be metabolized to its active metabolite by CYP enzymes, the most important of which appears to be *CYP2C19*. A significant portion of individual variation in

clopidogrel response appears to be due to a single loss-of-function variant denoted *CYP2C19**2 (c.681G > A, p.Pro227 =). The presence of this allele has been shown to lead to decreased drug activity and an increased risk for myocardial infarction, death, and stent thrombosis in patients undergoing percutaneous coronary intervention (PCI) [12]. Because of these findings, there are now recommendations for clopidogrel dosing in patients undergoing PCI based on *CYP2C19* genotyping, although broad acceptance of these guidelines remains incomplete [13]. There are now efforts put forth into understanding how best to incorporate pharmacogenetic information into anticoagulant therapy. Large clinical trials have been approved, for instance, to examine the effects of prospective *CYP2C19* (ADAPT—Assessment of Prospective CYP2C19 Genotype Guided Dosing of Antiplatelet Therapy in Percutaneous Coronary Intervention, NCT02508116 and TAILOR-PCI—Tailored Antiplatelet Therapy Following PCI, NCT01742117) and *VKORC1* genotyping (COAG—Clarification of Optimal Anticoagulation Through Genetics, NCT00839657; GIFT—Genetics Informatics Trial of Warfarin to Prevent DVT, NCT01006733; EU-PACT—European Pharmacogenetics of Anticoagulant Therapy—Warfarin, NCT 01119300).

Several large-scale clinical trials including JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin), PRINCE (PRavastatin Inflammation CRP Evaluation), CARDS (Collaborative Atorvastatin Diabetes Study), and ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial) examined the effects of various polymorphisms on the response to 3-hydroxymethyl-3-methylglutaryl coenzyme A reductase inhibitors (statins) and identified candidate SNPs in genes such as apolipoprotein E (*APOE*), *APOB*, lipoprotein A (*LPA*), *SLCO1B1*, *ABCG2*, and *LDLR*. Follow-up studies and meta-analyses have further singled out SNPs in *APOE* and *LPA* as those most closely associated with an enhanced reduction in LDL levels with statin therapy though the mechanism behind these effects remains unclear. What has also emerged from these studies is a clear role for polymorphisms within the gene *SLCO1B1* in statin-induced myopathy. The gene encodes an organic anion transporter responsible for transporting compounds such as bilirubin and statins into the liver for detoxification. A common polymorphism in this gene (c.521T > C, p. Val174Ala) has been shown to reduce the uptake and transport of statins into the liver [14]. This leads to elevated plasma levels and an increased risk for myopathy although this effect may be mitigated by close clinical observation and careful dose titration in at-risk individuals [15]. Variants in *SLCO1B1* are now the focus of the Integrating Pharmacogenetics In Clinical Care (I-PICC NCT02871934) trial. The trial seeks to evaluate the impact of *SLCO1B1* genotyping on compliance, reduction in LDL levels and statin-related myopathy.

Mental health and pain management

Because of the complexity of psychiatric disorders and the poorly understood mechanism of many psychotropic drugs, applying pharmacogenetic principles to psychiatric disorders can be challenging yet important discoveries have been made in the field.

The dopamine D2 receptor is the primary target for most antipsychotics. The receptor is encoded by the *DRD2* gene, and the functional polymorphisms within its promoter region

have been shown to alter gene expression and subsequently the efficacy of drugs targeting this pathway [16]. Numerous SNPs within *DRD2* have been proposed to alter antipsychotic response in patients including –141C Ins/Del (c.-141delC, rs1799732), A-241G (c.-241A > G), and Ser311Cys (c.1167C > G, p.Ser311Cys). Among them, the best described is the *DRD2-141delC* variant, which reduces the expression of the D2 receptor and leads to decreased efficacy of psychotropics.

Similar polymorphisms have also been studied in relation to the serotonergic system and in particular the serotonin transporter SLC6A4, which is encoded by the *5HTT* gene and transports serotonin back into presynaptic neurons to terminate its effects. The variant 5-HTTLPR, is a repeat length polymorphism involving insertion/deletion of a 44-bp segment located upstream of the transcription start site of the *5HTT* gene [17]. The longer of the repeat polymorphisms is associated with increased transcription of the transporter and, as a result, serotonin uptake. Several large-scale studies have since demonstrated that patients carrying the “long allele” are twice as likely to respond to treatment with selective serotonin reuptake inhibitors (SSRIs) and less likely to suffer from side effects than patients with the “short allele” [18].

Genetic variants in the members of the cytochrome P450 family of enzymes also play an important role in psychiatric illnesses. For instance, antipsychotics such as risperidone, aripiprazole, haloperidol, and chlorpromazine are all metabolized by CYP2D6. Nonfunctional or low-functional alleles of *CYP2D6* put carriers at risk for drug accumulation and increase the likelihood for side effects such as tardive dyskinesia and extrapyramidal symptoms.

Pharmacogenetic considerations also play an important role in chronic pain management and analgesia. Opioid receptor agonists, for example, have been some of the mainstays of pain management for centuries with genetic variation thought to play an important role in individual response [19]. One well-studied polymorphism in the micro-opioid receptor gene (*OPRM1*), c.118A > G facilitates enhanced drug binding and increases the risk for spinal analgesia and respiratory depression. Most of the pharmacologic activity of opioid agonists, however, is influenced by pharmacokinetic factors given that they must be converted from prodrugs to active compounds and then de-activated and cleared. Codeine, for instance, undergoes *o*-demethylation to morphine (its active compound) via the action of CYP2D6 before its inactivation via glucuronidation by UGT2B7 or UGT1A1. The functionality of alleles in either of these enzymes therefore has a significant impact on the pharmacokinetics of codeine and other opioid analgesics.

Clinical resources and adaptation of pharmacogenomics

As pharmacogenomic testing becomes more widely available and used, it becomes critical for healthcare providers to have access to resources that would help in using the genotypic information in the management of patients. The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Pharmacogenomics Knowledgebase (PharmGKB) have led the efforts in compiling clinical and research data that have been made publicly available in an

Table 29.1 Select examples of the type of pharmacogenomic biomarkers that the FDA uses in drug labeling.

Drug	Biomarker	Labeling information	Label recommends testing prior to drug use
Abacavir	Presence of HLA-B*5701 allele	Lactic acidosis, and severe hepatomegaly can occur in patients who carry the HLA-B*5701 allele	Yes
Clopidogrel	CYP2C19	Patients with genetically reduced CYP2C19 function have diminished antiplatelet response, and higher cardiovascular event rates following myocardial infarction than do patients with normal CYP2C19 function	No
Carbamazepine	Presence of HLA-B*1502 allele	Serious dermatologic reactions can result in individuals with the HLA-B*1502 allele	Yes in patients of Asian ancestry
Cetuximab	Presence of mutations in RAS	Use not recommended for the treatment of patients with colorectal cancer who have mutated RAS in their tumors	Yes
Ivacaftor	At least one p.G551D variant in CFTR	Indicated for treatment of individuals with CF with specific genotype	Yes

effort to improve incorporation of pharmacogenomic data in clinical practice. These efforts will be an additional tool to help select appropriate medications, adjust drug dosages, and prevent adverse drug reactions in *individual patients*. Moreover, the FDA provides a number of resources on pharmacogenomics, including a list of biomarkers involved in drug labeling and information on which drugs may warrant genetic testing before prescribing (<https://www.fda.gov/drugs/science-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling>) (Table 29.1).

Challenges and future directions

In 2015 when President Barack Obama introduced the “Precision Medicine Initiative,” an important part of this initiative, was expanding the role of pharmacogenomics in the care of patients. Despite our rapidly growing knowledge of pharmacogenomics, the complete integration of this information into clinical practice remains challenging.

A prominent part of this difficulty has been simply the daunting amount of information that can be gained from the pharmacogenomic studies. There are now hundreds of identified “pharmacogenes” and within each gene may be many important polymorphisms. With the complexity comes a need for not only centralized resources and infrastructures that can make this information useful and available to everyone involved in caring for a patient, but also practitioners capable and comfortable with interpreting this information. Many

providers, however, have received limited, if any training in genomics and pharmacogenetics. And, on a broader scale, very few hospital systems have mechanisms in place through their electronic medical records systems or otherwise to make pharmacogenomic information accessible for providers. Even in medical centers that have made a concerted effort to integrate pharmacogenomics through “pilot programs,” there can be challenges. Insurance reimbursement practices, for instance, vary greatly often covering only the cost of a single gene or polymorphisms when expanded panel may prove to be more cost effective and clinically effective over time.

Efforts to use pharmacogenomics have also been limited by a relative lack of easily available testing especially at the time when drug dosing decisions are being made such as during PCI. With the advances in next-generation sequencing and our expanded knowledge of the human genome, however, dozens of commercial laboratories have begun to offer pharmacogenomic testing and a test covering all known pharmacogenomic variants a priori has become possible. The need for genetic counseling has also greatly increased as our ability to conduct these tests grows.

In some cases, the benefits of performing pharmacogenomic testing are not easily appreciated. One example is chronic pain management, where very few individuals receive pharmacogenomic testing as part of their care. The outcome of pain management is difficult to assess objectively and quantitatively, yet ineffective treatment can lead to perpetuating non-productive cycles and significant physical and social impairments as well as poor quality of life. Pharmacogenomics has great potential in optimizing therapy quickly and effectively, may contribute to reducing the long-term costs to the health system, and potentially curtail the growing problem of opioid dependence in the United States.

The inconsistent integration of pharmacogenomic data across clinical practices has led to a recognition of the need to have clear clinical guidelines for practitioners [20]. Recently the CPIC has been established as an attempt to compile clinical and research data, as well as establish drug and allele-specific recommendations. Made up of both industrial and academic representatives, the consortium has now published consensus guidelines on the use of dozens of different drug-gene combinations. The PharmGKB has also been established as a way to collect and curate information on pharmacogenomic data [21]. The site is publicly accessible and seeks to provide curated data in a clear and easily understood manner. Finally, the US Food and Drug Administration has compiled a list of all medications with pharmacogenomic considerations, and in some cases, the labeling itself contains specific actions to be taken based on the genetic information.

Points to remember

- Pharmacokinetics describes how a drug is metabolized by an individual.
- Pharmacodynamics describes how well a drug is able to specifically exert its therapeutic effects.
- Online resources for providers can be extremely helpful and include the CPIC, the PharmGKB, and the US Food and Drug Administration sites.

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Gene therapy and gene editing

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Introduction

Gene therapy is the treatment of diseases by delivery of nucleic acids (either DNA or RNA), and it has the premise to cure genetic diseases by acting at the level of their primary defects. Progress in genomic sequencing is leading to the identification of an exponential number of genes responsible for Mendelian diseases [1]. With this rate of disease-gene discovery, it is conceivable that the genes responsible for all Mendelian diseases will be identified in the near future. This knowledge is a requirement for the development of gene therapy. Moreover, information about expression patterns, tissues requiring the gene function and whether the disease results from loss or gain of function in the mutated gene are needed.

Although initially conceptualized for the treatment of Mendelian disorders, gene therapy has been applied to a broad range of acquired diseases including cancer, infectious, autoimmune, and cardiovascular diseases. Nowadays, the successful treatment of blood cancers using chimeric antigen receptor-modified T cells is considered one of the major successes of gene transfer technologies [2]. Like previous advancement in medicine (e.g., bone marrow and organ transplantations), early clinical gene therapy approaches have led to a mixture of encouraging and disappointing clinical outcomes, including major setbacks such as the death of a patient affected by ornithine carbamoyltransferase (OTC) deficiency and the development of leukemia in patients with X-linked severe combined immunodeficiency (X-SCID) [3,4]. Nevertheless, clinical gene therapy has matured tremendously thereafter, accumulating significant clinical successes that are prompting the development of gene therapy for a growing number of Mendelian diseases. Moreover, continuous progress is being made toward optimization of available strategies and more effective and precise treatments that incorporate genome editing tools.

This chapter does not comprehensively depict the wide range of gene therapy approaches developed so far, but rather it focuses on the most representative examples that have reached the clinic in patients with Mendelian diseases.

Gene therapy strategies and vectors

In gene therapy, delivery of the genetic material is mostly performed with virus-based vectors that retain the natural capacity of viruses to deliver DNA or RNA to the infected cells

but are deleted of the harmful viral coding sequences that are replaced with the therapeutic gene of interest. There are four main strategies in gene therapy: (1) *gene replacement* or *gene augmentation*, which is the addition of a normal copy of the mutated gene, is best suited for diseases due to loss-of-function mutations; (2) *gene reprogramming*, which is based on the inhibition of the expression of a mutated gene to avoid the deleterious consequences of mutated proteins exerting gain of toxic functions; (3) *gene supply*, which is the addition of a gene that is not mutated or directly implicated in the disease pathogenesis but the expression of which can prevent or arrest disease progression; and (4) *gene repair* or *genome editing*, which seeks to correct mutant sequences in the genomic DNA by delivering chimeric proteins composed of a DNA sequence-specific binding domain and an endonuclease capable of inducing DNA site-specific double-strand breaks (DSBs) and a template encompassing the wild-type sequence to be used as a substrate for repair by homology-directed repair (HDR). Each of these strategies can be applied in *ex vivo* or *in vivo* approaches. Among various viral-based vector systems, lentiviral and adeno-associated virus (AAV) vectors have shown the greatest clinical success for *ex vivo* and *in vivo* gene delivery, respectively.

Ex vivo gene therapy

In *ex vivo* approaches, cells taken from the patient are genetically modified outside the body and then reinjected to repopulate the organism with gene-corrected progeny (Fig. 30.1). Although gamma-retroviral vectors have been shown to be effective in delivering and integrating therapeutic genes into the genome of target cells, earlier clinical trials with gamma-retroviral vectors reported delayed occurrence of T-acute lymphoblastic leukemia and myelodysplasia in at least three clinical trials (X-SCID, Wiskott–Aldrich syndrome, and chronic granulomatous disease) [4–7]. Gamma-retroviral vector integration sites are concentrated near the 5' ends of transcription units, and the strong enhancer activity of the viral long terminal repeat (LTR) elements of gamma-retroviral vectors induced activation of nearest oncogenes leading to clonal expansion of transformed cells [4]. Nevertheless, despite these serious adverse events, these initial trials showed that gene therapy could offer significant clinical benefit and improve patient survival in otherwise life-threatening diseases.

In contrast to X-SCID, patients with adenosine deaminase (ADA)-SCID treated with gamma-retroviral vectors have not developed events of insertional carcinogenesis for reasons that are not entirely clear. ADA-SCID patients underwent a gene transfer protocol based on a nonmyeloablative chemotherapy regimen given prior to the gene therapy to make space for genetically corrected hematopoietic stem cells (HSCs). This protocol resulted in sustained lymphoid reconstitution with gene-corrected T cells, improvement of immune functions, and effective metabolic detoxification without leukemic transformation events [8]. This favorable outcome led to the approval of a gamma-retroviral vector-based gene therapy for ADA-SCID in the European Union [9].

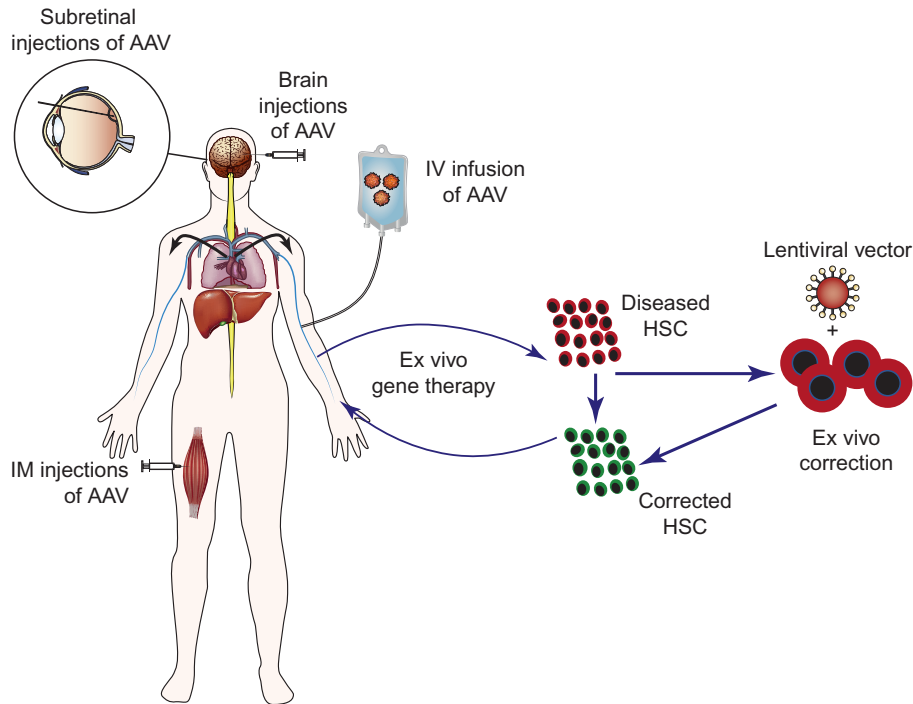


FIGURE 30.1 Gene therapy and genome editing can be performed in humans by either *ex vivo* or *in vivo* approaches. The former involves the removal of target cells from the patient and their culture *in vitro* followed by the correction of the genetic defect by viral vectors encoding the gene of interest or the genome editing tools (e.g., ZFN, TALEN, CRISPR/Cas9) and DNA donor sequence and reinfusion back into the patient. The *in vivo* approach involves the injection of vectors delivering the therapeutic gene or the genome editing tools and DNA donor sequence into the patient. According to the tissue to be targeted, the injections can be performed by different routes such as systemic intravenous administration, local brain administrations (e.g., intracerebral, intracerebroventricular, and intracisternal injections), retinal (subretinal or intravitreal injections), and muscle (intramuscular) injections.

The recognition of the mechanism underlying the insertional mutagenesis induced by gamma-retroviral vectors led to the design of self-inactivating gamma-retroviral and lentiviral vectors lacking the harmful viral LTR sequences and incorporation of alternative mammalian promoters to drive transgene expression [10]. These adverse events also promoted a shift toward lentiviral vectors, which have shown a better preclinical safety profile. Moreover, compared to gamma-retroviral vectors, substantially higher levels of stable HSC gene transfer have been obtained in clinical trials with lentiviral vectors. Lentiviral vector-mediated gene therapy to provide the deficient gene product has been successfully used in a growing number of patients affected with other severe combined immunodeficiencies, Wiskott–Aldrich syndrome [11], and hemoglobinopathies such as thalassemia and sickle cell disease [12,13]. In these trials, engraftment of transduced HSC required preparative myeloablation, akin to

HSC transplantation from healthy donors. However, in contrast to transplantation from healthy donor, in HSC gene therapy, the infused cells are the patient's own cells, and therefore, there is no risk of major complications, such as rejection or graft-versus-host disease.

Clinical applications of *ex vivo* gene therapy have been mostly focused on gene delivery to autologous HSC to treat congenital immunodeficiencies and hematological disorders. However, because genetically corrected myeloid cells can cross the blood–brain barrier and differentiate into microglia, *ex vivo* gene therapy has been successfully applied for the treatment of the neuronal manifestations in two inborn errors of metabolism, namely X-linked adrenoleukodystrophy and metachromatic leukodystrophy [14,15]. Prevention of neurodegeneration was achieved when the gene therapy was started in the pre- or very early-symptomatic stage of the disease [16].

Besides HSC, skin cells genetically corrected by a gamma-retroviral vector have been grown to obtain large transgenic epidermal grafts to replace the affected skin of a 7-year-old child with a severe form of epidermolysis bullosa. After almost 2 years of follow-up, the only child treated thus far seemed to have made a full recovery with no blistering [17].

In vivo gene therapy

In vivo gene therapy is based on the direct administration in the organism of a vector encoding the therapeutic gene (Fig. 30.1). According to the tissue to be targeted, the gene therapy vector can be delivered by different routes such as systemic intravenous administration, local brain administrations (e.g., intracerebral, intracerebroventricular, and intracisternal injections), retinal (subretinal or intravitreal injections), and muscle (intramuscular) injections (Fig. 30.1). Wild-type AAV is not pathogenic in humans, and based on their safety and efficacy, AAV vectors are emerging as the most promising gene delivery vectors for *in vivo* applications. Multiple available AAV vector serotypes with different tissue tropisms allow for efficient gene delivery in multiple cell types. Since the first clinical trial in 1994, there have been over 100 gene therapy trials with AAV vectors mainly involving gene transfer to the eye, central nervous system, and liver.

AAV vectors can accommodate sequences up to 4.5–5 kb in size. The large gene size has been a major obstacle for hemophilia A and Duchenne muscular dystrophy (DMD), but the smaller versions of the genes encoding functionally active proteins has been developed to overcome this obstacle.

The lack of antibodies against AAV-specific capsids is a common criterion for enrollment into clinical trials because these antibodies prevent vector-mediated gene transfer. However, following exposures to wild-type AAVs, a significant proportion of individuals ranging from 10% to 80% depending on the serotypes develop humoral immunity against the viral capsids [18]. Switching capsids may provide a solution for increasing patients' access to gene therapy. Strategies such as plasmapheresis, pharmacological modulation of the B cell, and/or T-cell activation and others might offer alternative solutions to overcome the neutralizing antibody response.

AAV vectors elicit only a weak activation of the innate immune response. However, recent studies suggested that the innate immune response may occur with high doses of the vector, which might affect the safety of this approach. Severe toxicities in piglets and juvenile non-human primates were observed following the administration of high doses of an AAV9 variant [19]. Furthermore, a clinical trial for DMD was placed on hold by the Food and Drug Administration (FDA) after a report of platelet and red blood cell count reductions, transient renal impairment, and complement activation occurring several days after a patient received high dose of an AAV9 intravenously. Nevertheless, this patient did not show signs of bleeding, clotting abnormalities, or liver dysfunction, recovered from the event, and the trial was resumed [20]. Together, these data indicate that careful monitoring and management of the innate immune response should be included in clinical protocols. Nevertheless, based on the clinical/benefit ratio, high-dose systemic AAV delivery remains promising particularly in life-threatening diseases such as spinal muscular atrophy (SMA).

A T-cell response to AAV capsid occurring several weeks after the intravenous injections of AAV vectors was first reported in hemophilia B gene therapy trial. Specifically, approximately 4 weeks after vector infusion, an asymptomatic rise in liver transaminases [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] peaking at about 4–6 weeks and gradually returning to normal levels over the ensuing 8 weeks without any medical intervention was observed concomitantly with loss of transgene [Factor IX (FIX)] expression [21] (Fig. 30.2). Later, transaminase elevations were also observed in systemic AAV gene therapy for hemophilia A and SMA [22,23]. This response was accompanied by the expansion of capsid-specific T cells, and transient application of high-dose glucocorticosteroids has been shown to control liver enzymes. Moreover, steroids appeared to result in rapid disappearance of the capsid-reactive T cells from the circulation and long-term expression of the therapeutic gene [24].

In humans, transduction of postmitotic or slowly replicating adult tissues results in vector genome persistence and long-term expression of the therapeutic gene for >7 years after a

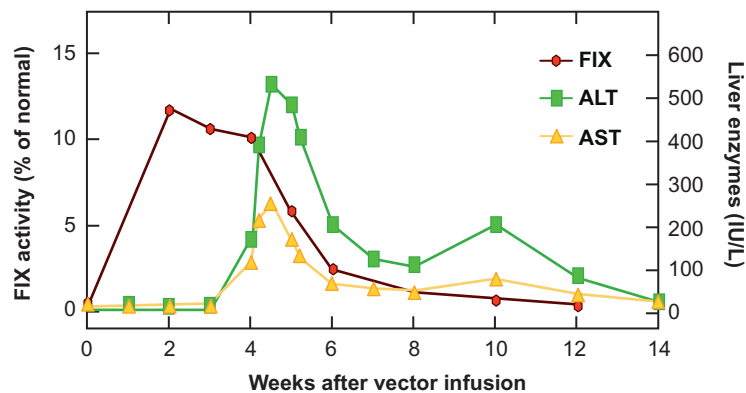


FIGURE 30.2 Subject in the first liver-directed AAV-mediated hemophilia B clinical trial showed therapeutic levels of FIX (10%–12%) for 4 weeks and then FIX levels declined with a concomitant asymptomatic transient transaminase elevation due to a CTL-immune response. *With permission from [21].*

single AAV vector administration [24,25]. In liver-directed approaches, participants recruited to trials have been largely limited to adults for whom the liver is fully developed, such that the effective hepatocyte transduction is not lost in mitotically active and growing liver tissue. However, experiments in animal models with partial hepatectomy after AAV administration or neonatal injections of AAV resulted in loss of transgene expression indicating that AAV vector genomes do not integrate in significant proportion of the host liver genome and transgene expression is lost in actively replicating cells [26]. While a single administration of AAV vector at the appropriate therapeutic dose may be still sufficient to achieve a lifelong correction in diseases with low therapeutic threshold such as hemophilia, vector dilution is a major challenge for the treatment of several diseases requiring more robust levels of transgene expression or early interventions in life. Vector dilution with secondary loss of transgene expression has also been a major hurdle for the development of gene therapy for cystic fibrosis because cells of the airway epithelium have elevated turn-over [27].

The risks of insertional carcinogenesis and germline transmission are low with AAV vectors because their genome remains episomal and does not integrate into the host genome. However, a minor fraction of AAV genomes integrate into the host genome [28]. Following natural infection with wild-type AAV viruses, there is a low level of AAV integration estimated at 0.1% for chromosome 19-specific locus [29]. Therefore, there is a hypothetical risk of cancer development following AAV gene therapy if AAV vector integration results in loss- or gain-of-function mutations altering cell homeostasis. Clonal integration of wild-type AAV2 has been found in a small percentage of hepatocellular carcinoma (HCC) samples, which have upregulated known cancer driver genes [30]. Moreover, in preclinical studies, insertional mutagenesis by AAV gene transfer has been reported in few studies following intravenous vector delivery to newborn mice, which developed HCC because of the insertion of viral genomes into the *Rian* locus encoding for several regulatory noncoding RNAs [31,32]. Although the human miRNA counterparts are also overexpressed in HCC, the locus of AAV integration in the mouse genome is not conserved in humans [31,32]. Moreover, several long-term studies including larger animal models such as dogs and nonhuman primates have not raised concerns about AAV liver genotoxicity thus far [33]. Nevertheless, the number of AAV-treated subjects remains small, and careful follow-up and surveillance in injected subjects are warranted.

Gene therapy for hereditary blindness

The eye offers several advantages as a target organ for gene therapy: (1) it can be easily accessed by subretinal injection with low vector doses, (2) it is enclosed and relatively immune privileged given the presence of a blood – retinal barrier, (3) retinal cells do not proliferate after birth, and thus, a single injection of nonintegrating AAV vector can result in long-lasting expression of the therapeutic gene, and (4) it can be investigated in great details by noninvasive methods. Subretinal injections of AAV2 vectors have improved visual function in patients with Leber congenital amaurosis (LCA) due to mutations in the *RPE65* gene [34–36]. However, the long-term follow-up of these patients has provided conflicting

outcomes. Although patients from two trials experienced progressive decline in visual function [37,38], a cohort from one of the three AAV2-based trials in LCA continues to show clinical benefits after a similar follow-up period of at least 3-year postinjection [39]. There is no clear explanation for such different outcomes, but variations in manufacturing, expression cassette, or adjuvant immunomodulatory regimens have been proposed [40]. Following the phase I/II trials, a randomized controlled phase III gene therapy trial showed that patient mobility, a measure of functional vision, and visual function were significantly improved in subjects receiving injections of AAV2 encoding *RPE65* in both eyes [41]. Based on these outcomes, the FDA approved this as the first gene therapy product in the United States [42].

Encouraging clinical data with improved visual acuity and retinal sensitivity have also been observed in an AAV2 clinical trial for choroideremia, another degenerative disease of the retina leading to blindness [43], and AAV are being pursued in clinical trials for several inherited diseases of the retina (e.g., achromatopsia, LCA 2, Leber hereditary optic neuropathy, X-linked retinoschisis, and X-linked retinitis pigmentosa).

Gene therapy for hemophilias

Hemophilia A and B, which occur due to deficiency in factor VIII (FVIII) and FIX, respectively, present with bleeding diathesis and spontaneous bleedings in the severe forms. Untreated patients rarely survived beyond 10 years with death occurring due to intracranial or other life-threatening bleedings. Hemarthroses leading to chronic arthropathy with significant deformities is a common complication in untreated patients. The availability of factor concentrate for treatment and prophylaxis has substantially improved survival and its associated complications [44]. Clotting factor replacement requires repeated intravenous infusions. Replacement factor therapy needs interval dosing, and bleeds might occur in the periods most distant from the peaks achieved after the infusion when levels of the clotting factors are insufficient. Both hemophilia A and B are ideal candidates for gene therapy because even a small increase in factor levels can substantially ameliorate the bleeding phenotype. For these reasons, they have been the benchmark for the investigation of virtually all gene therapy vectors developed so far.

Liver-directed gene transfer by AAV8 resulted in sustained FIX expression at 1%–6% of activity in severe hemophilia B patients during a 6-year follow-up period [24,25]. These FIX levels resulted in a concomitant 90% decrease in FIX concentrate. Another trial using a novel engineered AAV capsid expressing the FIX Padua transgene with higher catalytic activity resulted in even higher mean FIX activity of approximately 34% in the 10 enrolled subjects, thus enabling termination of clotting factor prophylaxis and near elimination of bleedings and factor use [45].

The size of the *FVIII* gene (7 kb) has been a challenge for the development of AAV-based gene therapy because of the limited cargo capacity of AAV vectors. To overcome the size limitation, either a B-domain-deleted or truncated *FVIII* has been developed in conjunction with adjustments of regulatory sequences to reduce the size of the expression cassette. The infusion of an AAV5 encoding a B-domain-deleted *FVIII* resulted in sustained normalization

of FVIII activity over a period of 1 year in participants who received the high dose and reduced bleedings and reduction in factor VIII usage [23]. At least three more hemophilia A gene therapy trials using different capsids (AAV8, AAV6, novel AAV8-based capsids) and promoters (NCT03001830, NCT03003533, and NCT03061201) are currently in the recruitment phase. Although newly developed clotting factors and nonclotting factor-based therapies have substantially improved hemophilia treatment [46], they require vascular access and results in peaks and troughs of activity, which do not always prevent bleedings. In contrast, gene therapy providing sustained expression of the clotting factor offers the potential for a cure by a single intravenous administration.

The promising results on liver-directed gene therapy for hemophilias have led to the development of gene therapy for several inborn errors of liver metabolism, including OTC deficiency (NCT02991144), homozygous familial hypercholesterolemia (NCT02651675), and Crigler–Najjar syndrome (NCT03466463 and NCT03078881), which have long been considered as targets of gene therapy.

Gene therapy for SMA

Like most neurodegenerative disorders, SMA, which is caused by mutations in the *survival motor neuron 1* (*SMN1*) gene, has only been treated symptomatically until recently when treatments affecting the underlying disease process have become available [47]. Patients with SMA typically present with muscle atrophy and respiratory failure and die before 2 years of age. Gene therapy has significantly changed the early course of the disease and improved motor function in 15 children (0.9- to 7.9-month olds) administered intravenously with high doses of AAV9 expressing the *SMN1* [22]. Like in the hemophilia trial with intravenous AAV administrations, SMA patients receiving AAV9 intravenously had ALT increases that were 10–30 times above the upper limit of the normal range, but none of these events was accompanied with abnormal liver function and all were controlled with prednisolone treatment [22]. All patients remained healthy, and most of the patients of the high-dose cohort (11 of 12) attained motor milestones that were not achieved in any of the patients from the historical cohorts.

Besides SMA, long-term expression from AAV gene transfer to nondividing cells such as neurons has been exploited in other neurologic diseases, such as Parkinson disease, other neurodegenerative diseases, and several lysosomal storage disorders [48]. Several trials focused on localized and invasive brain injections to achieve gene transfer to neuronal cells. However, to overcome the invasiveness of these injections, systemic administration of AAV9 that can cross the blood-brain barrier to transduce the brain has also been investigated [48].

Gene therapy for DMD

There have been great efforts toward the development of gene therapy for DMD. Although conceptually similar to hemophilias and inherited blindness, gene therapy for DMD turned out to be much more challenging. The first obstacle is the size of the dystrophin gene that

exceeds the packaging capacity of the AAV vector. Truncated versions of the dystrophin cDNA based on the genetic deletions in patients with the milder form of Becker muscular dystrophy were shown to almost completely prevent disease in the DMD mouse models and ultimately led to the design of smaller but functional versions of the dystrophin gene that can fit into AAV vectors [49]. The second problem is the challenge of delivering the gene to the great amount of muscle tissue distributed all over the body, including internal sites such as the heart and the diaphragm that are of utmost importance for disease complications. Treating a single muscle or a group of muscles by intramuscular injections can only improve the function of the treated muscle. Although this may improve the quality of life, it will neither slow down the disease progression nor reduce mortality. The only gene therapy clinical trial completed involved intramuscular injection of an AAV5 vector in six patients, and while the vector injection was safe, none of the patients expressed significant levels of microdystrophin [50], likely because of immune responses against dystrophin and/or AAV capsids. Nevertheless, the favorable results from high-dose systemic AAV therapy clinical trials in SMA and the encouraging preclinical data in the murine and canine models generated new enthusiasm for systemic DMD gene therapy with three ongoing clinical trials (NCT03368742, NCT03375164, and NCT03362502) differing in AAV serotypes, types of promoters, vector doses, and patient inclusion criteria [49].

Genome editing

Genome editing can be performed either *ex vivo* or *in vivo* and has the potential to overcome several limitations of gene replacement therapy. First, genotoxicity due to ectopic activation of nearby proto-oncogenes or knock-out of tumor suppressor genes should theoretically not occur with on-target editing. Second, in contrast to gene replacement therapy, genome editing allows physiological regulation of the expression of the corrected gene. Third, autosomal dominant diseases due to gain-of-function mutations cannot be treated by gene replacement therapy, whereas they are amenable to genome editing strategies. Fourth, genome editing is maintained in proliferating cells and overcomes the dilution effect observed with replacement gene therapy by nonintegrating vectors.

Genome editing is based on zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9), which combine specific DNA-recognition sequences with a nuclease that generates DSB in the DNA. The efficiency and flexibility in developing guides to target sites are a major advantage of CRISPR/Cas9 over other nucleases. Following DSB, repair is mediated by either nonhomologous end joining (NHEJ) or HDR that repairs DNA accurately in the presence of a donor sequence. NHEJ repairs the DSB by joining the two ends of the DSB often introducing small insertions or deletions (indels) at the site of DSB, which generally inactivates gene function. When there are two DSBs instead of one, NHEJ can generate deletions, inversion, or translocations. Therefore, NHEJ can be used to repress the expression of aberrant proteins. Compared to NHEJ, HDR is less efficient and

requires a donor DNA template that directs accurate repair of the DSB. Depending on the DNA template and the location of the homology arms, HDR can introduce changes in a single nucleotide or affect larger regions that ultimately result in correction of a mutation or site-specific insertion of new sequences. Genetic diseases caused by loss-of-function mutations or deficient gene expression could be reverted by HDR-mediated gene correction or targeted addition of therapeutic transgenes. The wild-type copy of the mutated gene can be integrated into the endogenous locus, which is expected to result in more physiological regulation of gene expression [51]. Another option is to drive integration of the therapeutic gene into “safe harbors” that allow expression of the gene. For example, targeted introduction of therapeutic genes downstream of the highly active albumin promoter in hepatocytes has shown promise in animal models (Fig. 30.3) [52]. Based on these results, three *in vivo* clinical trials are ongoing for ZFN-mediated insertion into the albumin locus of hepatocytes of *FIX* gene for hemophilia B (NCT02695160), α -L-iduronidase gene for mucopolysaccharidosis I (NCT02702115), and iduronate-2-sulfatase gene for mucopolysaccharidosis II (NCT03041324).

In preclinical studies, *in vivo* genome editing has been largely performed by multiple AAV vectors delivering the components of the editing machinery to the target cells (Fig. 30.3)

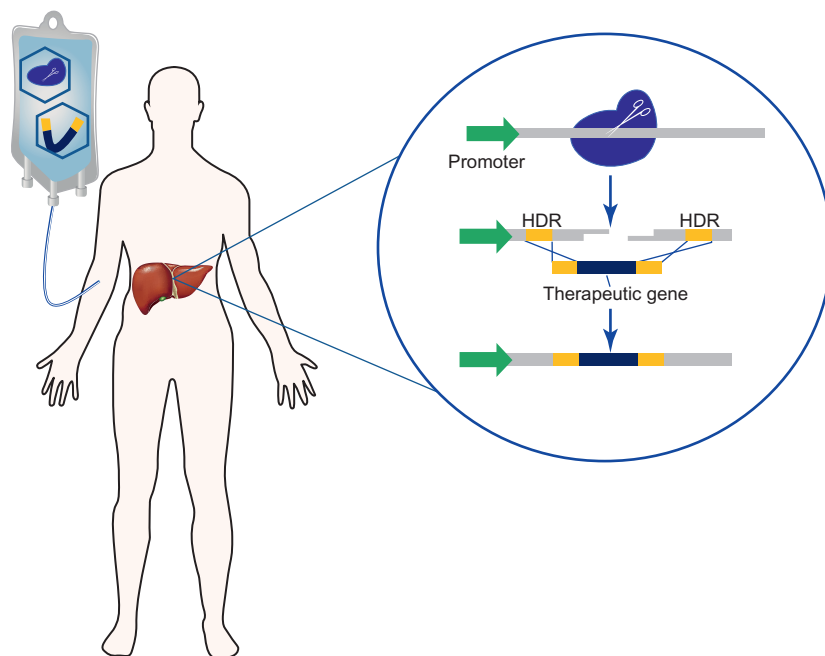


FIGURE 30.3 In vivo liver genome editing performed by intravenous co-delivery of multiple AAV vectors including the donor DNA sequence and the genome editing tools (ZFNs, TALENs, or CRISPR/Cas9), which induce DSB in the genome that stimulates HDR for replacement of a mutated gene or targeting gene insertion at a specified locus. WT, wild type; HDR, homology-directed repair.

[53]. Promising alternatives to AAV vectors are lipid nanoparticles (LNPs), which can deliver drugs or nucleic acids to various tissues. LNPs were used to deliver CRISPR/Cas9 mRNA to murine liver, in combination with an AAV vector that contained the HDR-template repair and a guide RNA expression cassette [54]. However, the long-lasting expression of the editing machinery delivered by the AAV can lead to DNA damage, improper gene editing, and deleterious cellular effects that ultimately might predispose to cancer. Although not observed in preclinical animal models, these consequences are serious concerns.

Another major obstacle for therapeutic applications of genome editing is the specificity of the genome editing that might have serious effects on clinical safety. Off-target cleavage is a known limitation of genome editing tools, and indels at off-target sites have been repeatedly reported [55]. Improvements in guide-design and new generation of CRISPR/Cas9 with higher specificity are critical for efficient on-target cleavage and low levels of off-target cleavage [56,57]. Moreover, a better understanding of the factors that influence CRISPR/Cas9-mediated genome editing is required. Two independent recent reports showed that CRISPR/Cas9-mediated genome editing can lead to p53-mediated stress response and cell-cycle arrest [58,59]. Although there have been no correlations between CRISPR/Cas9 editing and tumorigenesis, these findings raise concerns that CRISPR/Cas9 genome editing may be biased toward tumor-prone cells with p53-deficient activity. When cultured *ex vivo* for genetic correction, cells with p53 dysfunction might undergo selection, and while such clonal selection could be identified and eliminated by screening prior to infusions into patients, it cannot be avoided during *in vivo* gene correction.

Despite these concerns, genome editing has tremendous potential and is rapidly advancing into the clinic. Besides *in vivo* approaches for integration into the albumin locus, ZFNs have been used *ex vivo* to disrupt CCR5 expression in human T cells or HSCs to make them resistant to HIV infection [60]. To boost fetal hemoglobin, ZFNs to disrupt the enhancer of *BCL11A* gene that suppresses fetal hemoglobin production [61] are under investigation in transfusion-dependent β -thalassemia (NCT03432364). Moreover, TALENs- and CRISPR/Cas9-edited T cells are under investigations in patients with leukemia and solid tumors [62].

The range of applications of genome editing is wide, and recent attractive applications have been reported in a proof-of-concept study in cells carrying chromosomal abnormalities. Using a ZFN to target chromosome 21, a large genomic fragment containing X inactivation-specific transcript was inserted in induced pluripotent stem cells from a patient with trisomy 21 to induce silencing of the extra chromosome 21 [63]. This approach resulted in trisomy rescue that was stable in differentiated cells and throughout cell divisions. However, applying this strategy to the whole organism is a major challenge. Although correction of brain and developmental defects still appear objectives too far to be reached, tissue-restricted trisomy rescue could be feasible by bone marrow transplantation.

Current status, future challenges, and conclusions

Gene therapy and genome editing medicines are made by precise assembly of protein and nucleic acid components and are among the most complex drugs developed so far. They are

derived from viruses that have evolved for their ability to transfer foreign genetic information into target cells. Several years ago, this feature led visionary scientists to engineer recombinant viral vectors for delivery of therapeutic genes into diseased cells. In the recent years, clinical gene therapy is finally showing its potential for long-lasting benefits in at least a subset of human diseases. These successes justify pursuing gene therapy for a growing number of disease targets. However, several challenges from genotoxicity to the immune responses against viral vectors and higher efficiency of both gene transfer and genome editing remain to be addressed.

In conclusion, the recent successes in clinical gene therapy make not too far a future with newborn diagnosis of genetic diseases followed by early gene therapy performed prior to the onset of disease manifestations and before irreversible tissue damage is established. It is an amazing testimony of human ingenuity having converted infectious agents into drugs with applications in potentially thousands of debilitating diseases.

Points to remember

- Gene therapy is the treatment of diseases by delivery of nucleic acids and has potential for the treatment of genetic and nongenetic diseases.
- Gene therapy can be performed in either *ex vivo* or *in vivo*. Lentiviral and AAV vectors have shown the greatest clinical potential for *ex vivo* and *in vivo* gene delivery, respectively.
- In *ex vivo* approaches, patient's cells are genetically modified outside the body and then injected into the patient. This approach was mostly focused on gene delivery to autologous HSC to treat congenital immunodeficiencies and hematological disorders.
- *In vivo* gene therapy is based on the direct administration by different routes of administration of a vector delivering the therapeutic gene to the target tissue. *In vivo* gene therapy with AAV vectors resulted in the long-term correction in inherited blindness, hemophilias, and SMA. Based on the promising results in these disorders, it is currently under investigation in several genetic disorders.
- *Genome editing* seeks to correct mutant sequences in the genomic DNA and can be applied in *ex vivo* or *in vivo*. Based on the preclinical efficacy data, genome editing strategies have been rapidly translated into clinical trials that are currently ongoing.

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Index

Note: The page numbers followed by “*f*” and “*t*” refer to figures and tables, respectively.

A

- Abnormal bone mineralization, GSD with, 375–377
 - hypophosphatasia, 376–377
 - hypophosphatemia, 375–376
- Absence of heterozygosity (AOH), 47
- Achondroplasia, 377–378
- Acquired cystic renal disease, 353–354
- Acquired immunodeficiency, 231–233, 232*t*, 239
- Acrokeratosis verruciformis, 400
- Acroparasthesias, 361
- Acute aortic dissection, 130–131
- Acute intermittent porphyria, 390*t*
- Acute lymphoblastic leukemia
 - definition of, 228
 - syndromes associated with, 228*t*
- Acute myeloid leukemia, 217–218
- Acute recurrent pancreatitis, 201–202
- Adeno-associated virus (AAV) vectors, 299, 463–464, 465*f*, 466–471
 - AAV2-based trials in LCA, 468–469
 - B-domain-deleted FVIII, 469–470
 - for Duchenne muscular dystrophy, 470–471
 - in hemophilia B gene therapy trial, 467, 467*f*
 - for hemophilias, 469–470
 - hepatocellular carcinoma (HCC) samples, 468
 - for hereditary blindness, 468–469
 - for spinal muscular atrophy, 470–471
- Adenomatous polyposis syndromes, 189–192, 190*t*
- Adrenal cortex tumors, 87*t*
- Adrenal glands, genetic disorders affect
 - bilateral macronodular adrenal hyperplasia (BMAH), 181–182
 - Carney complex, 180–181
 - congenital adrenal hyperplasia (CAH), 179–180
- Adrenal rest tumors, 180–181
- Adult genetics practices, 3, 6
 - genetic care for adult patients, 5
 - medical education and practice for students, 5
 - training, genetics residency programs, 5
- Adult hypophosphatasia, 376–377
- Adult-onset cerebellar ataxia, 323
- Agalsidase alpha, 362
- Agalsidase beta, 362
- Albers–Schönberg disease, 374
- Albinism, 382
- Albright’s hereditary osteodystrophy (AHO), 176
- Aldolase A deficiency, 274–275
- Allogeneic bone marrow transplant, congenital erythropoietic porphyria, 391
- Allogeneic hematopoietic stem cell transplant, 227
- Alpha-1 antitrypsin deficiency (AATD), 325–327
- α -synuclein, 309–310, 317
- Alpha thalassemia (α thal), 225*t*
- Alport syndrome (AS), 356–360
- Altered bone mineral density, GSD with, 370–375
 - decreased bone mineral density, disorders with, 371
 - increased bone mineral density, disorders with, 373–374
 - osteogenesis imperfecta (OI), 371–373
 - osteopetrosis, 374–375
- Alzheimer disease, 53, 174–175, 254–255, 313, 316–317
 - sporadic form of, 317
- Ammonia, 261
- Amniocentesis, 75–76, 80
- Amniotic fluid (AF), 75–76
- Amsterdam II criteria, 186–187
- Amyotrophic lateral sclerosis (ALS), 312–313
- Anderson–Fabry disease, 110–111
- Angelman syndrome, 16
- Angiofibromas, 170, 395–396
- Angiokeratomas, 361
- Angiotensin convertase enzyme inhibitors, 362

- Angiotensinogen convertase enzyme inhibitor (ACEI)-associated angioedema, 242
- Angiotensin receptor blocker (ARB), 130–131, 362
- Anhydramnios, 75–76
- Anterior pituitary tumors, 170
- Anti-androgen therapies, 180
- Antibody deficiency, 232*t*, 233–239
- autosomal dominant hyper IgE syndrome, 237–239
 - B-cell deficiencies, 233
 - Bruton X-linked agammaglobulinemia (BXLA), 236–237
 - common variable immunodeficiency (CVID), 231, 233–235
 - selective IgA deficiency, 235–236
- Antithrombin III deficiency, 220, 220*t*
- Aortic root, Marfan syndrome, 130–131
- Aortopathies, 78
- Aortopathy gene panel testing, 132
- ApoE4* allele, 53, 317–318
- Apolipoprotein E (*APOE*), 457
- Apolipoprotein L1 (*APOL1*) gene, 360
- Argininosuccinate lyase deficiency, 265–266
- Argininosuccinate synthase 1 deficiency, 265–266
- Arrhythmias, 23–24, 116
- Arrhythmogenic right ventricular cardiomyopathy (ARVC), 113*t*, 114–115
- Arteriovenous malformations (AVMs), 334–336
- ARVC. *See* Arrhythmogenic right ventricular cardiomyopathy (ARVC)
- ARX gene, 257–258
- Aryl hydrocarbon receptor interacting protein gene (AIP), 171
- Asfotase alfa, 376–377
- Assisted reproductive technologies, 93, 95, 102, 107–108, 152
- Ataxia, 306–308, 323
- spinocerebellar ataxia type 2, 307*f*
- Ataxia-telangiectasia (AT), 239–240
- Atherosclerotic diseases, 23–24
- Atopic dermatitis, 237–239
- ATP7B* gene, 206
- Attention-deficit hyperactivity disorder (ADHD), 343
- Attenuated familial adenomatous polyposis (AFAP), 190*t*, 192
- Autism, 339
- Autism spectrum disorder (ASD), 49–50, 256–257
- Autoinflammatory diseases (AIDs), 244–246
- familial Mediterranean fever (FMF), 244–246
- Autosomal dominant hyper IgE syndrome (AD-HIES), 237–239
- Autosomal dominant (AD) inheritance, 12, 14–15, 118, 136, 164
- Autosomal recessive ataxias, 306–308
- Autosomal recessive disorders, 72, 77–78
- Autosomal recessive (AR) inheritance, 12, 15, 35, 142
- Azoospermia, 54
- B**
- Bannayan–Riley–Ruvalcaba syndrome, 105–107, 193*t*
- Bannayan–Zonana–Ruvalcaba syndrome, 385
- BAP1* tumor predisposition syndrome, 92
- BAPoma, 92
- Bardet–Biedl syndrome, 16–17, 344
- Barth syndrome, 298
- Bartter syndrome, hypocalcemia with, 174–175
- BAV. *See* Bicuspid aortic valve (BAV)
- B-cell deficiencies, 233
- B-cell lymphoproliferative disorders, 242
- Beals syndrome, 143
- Becker muscular dystrophy (BMD), 316
- Bernard–Soulier syndrome, 217*t*
- Beta carotene, porphyrias, 391
- β -cell dysfunction, 164–167
- β -human chorionic gonadotropin (β -hCG), 73–74
- β thalassemia, 225*t*
- Bicuspid aortic valve (BAV), 133, 136–137
- management, 137
 - medical, 137
 - surgical, 137
 - surveillance, 137
- NOTCH1*, 136
- SMAD6*, 136
- Bilateral adrenalectomy, 181–182
- Bilateral breast cancer, 97–99
- Bilateral macronodular adrenal hyperplasia (BMAH), 181–182

- Bilateral risk-reducing mastectomy (RRM), 99–100
- Billing and reimbursement
- for clinical services
 - appealing denials, 413
 - bill preparation, 411–412
 - claim submission, 413
 - denial, 413
 - for genetic counselor services, 412
 - for telehealth services, 412
 - for genetic laboratory services, 413–416
 - coverage policies, 414–415
 - CPT codes, 414
 - genetic testing, reimbursement of, 415–416
 - appeals, 415–416
 - clinical laboratory fee schedule (CLFS), 415
 - denials of laboratory service claims, 415
 - prior authorization, 415
 - telemedicine (TM), 426–427
 - asynchronous TM services, 427
 - synchronous (live) TM services, 426–427
- Bipolar disorders, 341–342, 344–347
- Birt-Hogg-Dube (BHD) syndrome, 93–94, 252, 363–364, 396
- Bladder cancers, 87*t*
- Bleeding disorders, 213–218
 - clotting factor deficiencies, 215–216
 - platelet disorders, 216–217, 217*t*
 - RUNX1 familial platelet disorder, 217–218
 - Von Willebrand disease (VWD), 213–215
- Bone cancers, 87*t*
- Bone marrow failure disorders, 218–219
 - Fanconi anemia (FA), 218
 - telomere disorders, 219
- Bone marrow transplantation, 219, 299
- Bone mineral density (BMD), 370–371
- Bradykinetic movement disorders, 306
- BRAF* (V600E) mutation, 187–188
- BRCA1* gene mutations, 54, 96–98, 455–456
- BRCA2* mutations, 54, 96–98, 199, 455–456
- BRCAPRO model, 97–98
- Breakpoint cluster gene (BCR), 455
- Breast cancer, 85–86, 87*t*, 106, 394, 448, 453
 - BRCA1* and *BRCA2* variants in, 27–28
 - incidence of, 96
 - predisposition, 44
- Brittle bone disease. *See* Osteogenesis imperfecta (OI)
- Brugada syndrome, 23–24, 118–119, 120*t*
- Bruton agammaglobulinemia, 233
- Bruton tyrosine kinase (BTK), 236–237
- Bruton X-linked agammaglobulinemia (BXLA), 236–237
- Bullous presentations, porphyrias, 390
- C**
- Café au lait macules (CALMs), 385, 392–393
- Café-au-lait spots, 178, 305
- Calcium metabolism, genetic disorders of
 - CASR, 174–175
 - familial hyperparathyroidism, 174, 174*t*
 - familial hypocalciuric hypercalcemia, 175
 - familial isolated hyperparathyroidism, 175–176
 - hyperparathyroidism-jaw tumor syndrome (HPT-JT), 174
- Calcium-sensing receptor (CASR), 173, 175
 - disorders of, 174–175
- Cancer, 447. *See also* Hereditary cancer syndromes
 - of eye and skin, 387
 - pharmacodynamics, 453
 - pharmacogenomics, 455–456
- Capillary malformation-arteriovenous malformation syndrome (CM-AVM), 402
- Carbamoylphosphate synthase 1 deficiency, 265–266
- Carbamylglutamate, 265–266
- Cardiac disorders, 109
 - cardiomyopathy, 109–115
 - ARVC, 114–115
 - definition of, 109
 - dilated cardiomyopathy, 112–114
 - genes associated with, 113*t*
 - hypertrophic cardiomyopathy, 109–111
 - idiopathic, 109
 - LV noncompaction, 115
 - peripartum, 115
 - restrictive, 115
 - inherited arrhythmogenic disorders (IADs), 116–122
 - Brugada syndrome, 118–119, 120*t*

- Cardiac disorders (*Continued*)
 - catecholaminergic polymorphic ventricular tachycardia, 119, 120*t*
 - familial hypercholesterolemia, 119–121
 - genes associated with, 120*t*
 - long QT syndrome, 116–117, 120*t*
 - short QT syndrome, 118, 120*t*
 - tranythretin amyloidosis, 122–124
- Cardiac myxomas, 180–181
- Cardiolipin, 298
- Cardiomyopathic lentiginosis, 384
- Cardiovascular disease (CVD), 23–24, 119–121, 445
 - precision medicine in, 448–449
- Cardiovascular medicine, pharmacogenomics, 456–457
- Carney complex (CNC), 180–181, 384–386
 - recommended surveillance and follow-up of patients with, 386*t*
- Carney–Stratakis syndrome, 197, 198*t*
- Carney triad, 198*t*
- Carrier screening, 71–73
 - assays used for, 72–73
 - definition of, 71–72
 - limitations of, 73
 - pan-ethnic expanded carrier screening, 72
 - practical considerations, 73
 - sequential carrier screening, 72
 - targeted carrier screening, 72
- CASQ2 gene, 119
- Catecholaminergic polymorphic ventricular tachycardia (CPVT), 119, 120*t*
- Catecholamines, 101
- CDH1* gene mutation, 196–197
- CDKN1B*, 172
- CDKN2A* gene, 95–96
- Cell-free DNA (cfDNA) screening, 74–75, 80
 - limitations of, 75
 - practical considerations, 75
 - SNP-based analysis, 74–75
- Cellular deficiency and combined
 - immunodeficiencies, 232*t*
- Cellular immunodeficiencies, 239
- Cerebellar atrophy, 239–240
- Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), 319–320, 344
- Cerebrotendinous xanthomatosis, 271*t*
- Ceruloplasmin, 207
- Cervical cancer, 87*t*
- CFTR modulators, 328
- CFTR* variants, 454
- Charcot–Marie–Tooth (CMT) disease, 12, 44, 286, 313
- Chediak–Higashi syndrome (CHS), 383
- Chloroquine, porphyrias, 391
- Chompret criteria, 102–103
- Chondrocalcinosis, 175
- Chorionic villus sampling (CVS), 75–76, 80
- Chromosomal abnormalities, 73, 251
- Chromosomal analysis, 26–27
- Chromosomal aneuploidies, 13
- Chromosomal microarray (CMA), 26–27, 44–48, 54–55, 76, 256, 343
 - copy number variants, 47
 - microarray-based comparative genomic hybridization (aCGH), 47
 - single-nucleotide polymorphism (SNP)-based microarrays, 47
- Chromosomal translocations, 13–14
- Chromosomal variation, 13–14
- Chromosome banding techniques, 43
- Chromosome microdeletion/duplication syndromes, 342–343
 - 15q13.3 deletion, 342
 - 16p11.2 deletion syndrome, 343
 - 17p11.2 deletion syndrome, 343
 - 22q11.2 deletion syndrome, 343
- Chromosome-specific banding pattern, 45–46
- Chronic kidney disease (CKD), 353, 366
- Chronic myeloid leukemia (CML), 455
- Chronic obstructive pulmonary disease (COPD), 325
- Chronic sensory-motor axonal polyneuropathy, 278
- Chronic sensory-motor demyelinating neuropathy, 278
- C1 inhibitor (C1INH) angioedema, 243–244
- C1 inhibitor (C1INH), deficiency of, 242–243
- Cirrhosis, 209

- Citrin deficiency, 264
 c-KIT protein (CD117), 197
 Clinical Pharmacogenetics Implementation Consortium (CPIC), 458–461
 ClinVar, 24
 Clotting factor deficiencies, 213, 215–216
 CMA. *See* Chromosomal microarray (CMA)
 CNVs. *See* Copy number variants (CNVs)
 Cobalamin C disease, 270–273
 Cobalamin metabolism disorders, 270–273, 271*t*
 Cockayne syndrome (CS), 387
 Codeine, 458
 Coenzyme Q10, 269
COL3A1 gene, 133–134
COL2A1, 143
 Colon cancers, 44, 85, 87*t*, 98–99
 Colorectal cancer (CRC), 23, 107, 185–195, 188*t*
 Combined immunodeficiencies, 234
 Common variable immunodeficiency (CVID), 231, 233–235
 Complement deficiencies, 232*t*, 242–244
 definition of, 242
 hereditary angioedema (HAE), 242–243
 Complement factor 3 glomerulonephritis (C3GN), 357–358
 Complete androgen insensitivity syndrome (CAIS), 158–159
 Confined placental mosaicism (CPM), 75–76
 Congenital adrenal hyperplasia (CAH), 153, 179–180
 definition of, 179
 Congenital anomalies of the kidney and urinary tract (CAKUT), 363
 Congenital bilateral absence of the vas deferens (CBAVD), 149–150, 327
 Congenital contractural arachnodactyly (CCA). *See* Beals syndrome
 Congenital erythropoietic porphyria (CEP), 390*t*, 391
 Congenital hepatic fibrosis, 356
 Congenital unilateral absence of the vas deferens (CUAVD), 149–150
 Constitutional mismatch repair deficiency syndrome, 393
 Coombs-negative hemolytic anemia, 205
 Coomb's test, 222, 224
 Copy neutral SVs, 13
 Copy-neutral variation, 44
 Copy number gains, 13
 Copy number losses, 13
 Copy number variants (CNVs), 13, 44, 46, 53–54, 76, 236–238, 241, 372–373
 CoQ10 (ubiquinone), 296
C9orf72-related amyotrophic lateral sclerosis, 50
C9orf72-related disease, 312
 Coronary artery disease (CAD), 27–28, 119, 121
 transthyretin amyloidosis, 123–124
 Costello syndrome, 110
 Cowden syndrome (CS), 105–107, 385
 CPT2 deficiency, 276
 CRISPR/Cas9-mediated genome editing, 473
 Cryptorchidism, 176
 C282Y mutations, 208
 Curaçao diagnostic criteria, 335
 Current Procedural Terminology (CPT), 407–409, 426
 consultation, 409
 definition, 408
 evaluation and management (E&M) codes, 408
 genetic counselor services, 412
 inpatient services, 409
 new patient, 409
 office/outpatient services, 408
 office visit, 409
 synchronous TM services, 427
 Cushing's syndrome, 171, 178, 180–182
 Cutaneous lichen amyloidosis (CLA), 172
 Cutaneous porphyrias, 390–391
 CYP21A2, 180
CYP2D6, 458
 Cystathionine β -synthase (CBS) deficiency, 143
 Cystic fibrosis (CF), 61, 79, 149–150, 327–329, 454–455
 causes, 327
 congenital bilateral absence of the vas deferens (CBAVD), 149–150
 Cystic fibrosis transmembrane conductance regulator (*CFTR*), 149–150
 Cystic renal cell carcinoma (RCC), 353–354
 Cystic renal diseases, 353–356, 354*t*

- Cystic renal diseases (*Continued*)
 autosomal dominant polycystic kidney disease, 353–355
 autosomal recessive polycystic kidney disease, 354, 356
- Cytogenetic testing, 44–48, 52*t*
 chromosomal microarray, 47–48
 fluorescence in situ hybridization, 46–47
 karyotyping, 45–46
- D**
- Danon disease, 110–111
 Darier disease (DD), 400
 Deferoxamine, 209
 δ -Aminolevulinic acid dehydratase porphyria, 390*t*
- Dementias
 Alzheimer disease, 316–318
 sporadic form of, 317
 frontotemporal dementia, 318–319
 onset of, 316
- Dense deposit disease (DDD), 357–358
- Developmental delays, 256, 321, 339
- Developmental disabilities, 62. *See also*
 Intellectual disability (ID)
- Diabetes mellitus (DM), 207
 monogenic causes of, 163–168
 maturity-onset diabetes of young, 164
 maturity-onset diabetes of young 2, 165*t*, 167
 maturity-onset diabetes of young 3, 164–168, 165*t*
 types of, 163
- Diagnostic DNA technologies, 26–27
 chromosomal analysis, 26–27
 clinical cytogenetic analysis, 26–27
 DNA sequencing, 27
- Diamond Blackfan anemia, 219
- DICER1*-related pleuropulmonary blastoma
 familial tumor and dysplasia syndrome, 94–95
- Diffuse large B-cell lymphoma (DLBCL), 228
- Diffuse-type gastric cancer, 196
- Digenic inheritance, 16–17
- DiGeorge syndrome. *See* 22q11.2 deletion syndrome (22q11.2 DS)
- Dilated cardiomyopathy (DCM), 12, 112–114, 113*t*
- Direct-to-consumer (DTC) genetic testing, 54, 449–450
- Disopyramide, 111–112
- Disorders of sex development (DSD), 156–161
 45,X/46,XY mixed gonadal dysgenesis, 159–160
 46,XX, 156–158
 46,XY, 158–159
 genes affecting gonadal differentiation, 160–161
- DMPK* gene, 79
- DNA banking, 39–40
- DNA-based testing, 21–22, 22*f*
- DNA, molecular architecture of, 9–10
- DNA sequencing technology, 27, 43, 48–50, 52*t*
 exome sequencing (ES), 49–50
 panel testing, 48–49
 single-gene testing, 48
- Documentation
 billing and reimbursement, 409–411
 based on time, 411
 evaluation and management (E&M), 409–410
 for TM services, 425–426
- Dopamine D2 receptor, 457–458
- Doublecortin (DCX), 321
- Down syndrome (DS), 44–45, 62, 251, 254–255
 health screening in adults, 255*t*
- D-Penicillamine, 206–207
- Dravet syndrome, 321–322
- Dual-energy X-ray absorptiometry (DXA), 370–371
- Dual molecular diagnoses, 18
- Duchenne muscular dystrophy (DMD), 447, 466
in vivo gene therapy, 470–471
- Duodenal cancer, 192
- Dysferlin (DYSF), 315
- Dysfibrinogenemia, 220
- Dyskeratosis congenita (DKC), 388
- Dysplastic cells, 187
- Dystrophin, 447
- Dystrophinopathies, 274
- E**
- Early infantile epileptic encephalopathies, 321
- E-cadherin, 196

- Ectopia lentis, 128, 131–132
- Ehlers–Danlos syndromes (EDS)
- classical, 141–142
 - clinical classification of, 137, 138*t*
 - definition of, 137
 - hypermobile, 137–141, 138*t*, 140*t*
 - vascular, 133–134
- Electronic consults (E-consults), 422–423, 427
- 11 β -hydroxylase deficiency, 153
- ELSI. *See* Ethical, legal, and social implications (ELSI)
- Emery–Dreifuss muscular dystrophy, 12
- Encephalopathy, 261, 267
- Endocrine disorders, 163
- adrenal glands, genetic disorders affect
 - bilateral macronodular adrenal hyperplasia (BMAH), 181–182
 - Carney complex, 180–181
 - congenital adrenal hyperplasia (CAH), 179–180
 - calcium metabolism, genetic disorders of
 - CASR, 174–175
 - familial hyperparathyroidism, 174, 174*t*
 - familial hypocalciuric hypercalcemia, 175
 - familial isolated hyperparathyroidism, 175–176
 - hyperparathyroidism-jaw tumor syndrome (HPT-JT), 174
 - diabetes mellitus, monogenic causes of, 163–168
 - Gs-alpha subunit (GNAS), 176
 - McCune–Albright syndrome, 178–179
 - pseudohypoparathyroidism-related disorders, 176–178
 - multiple endocrine neoplasia syndromes, 168
- Endometrial cancer, 23, 87*t*, 188*t*
- Endovascular stenting, 131
- End-stage renal disease (ESRD), 353–358, 362–363, 365
- Entero-pancreatic neuroendocrine tumors, 170–171
- Epidermis, disorders of, 396–401
- Darier disease (DD), 400
 - Hailey–Hailey disease (HHD), 401
 - non syndromic ichthyosis, 399–400
- Epidermolytic ichthyosis, genes associated with, 399*t*
- Epilepsy, 267–268, 320–322
- Episodic hyperammonemia, 272
- Erythropoietic protoporphyria (EPP), 390–391, 390*t*
- ES. *See* Exome sequencing (ES)
- Esophageal cancer, 87*t*
- ESRD. *See* End-stage renal disease (ESRD)
- Ethical, legal, and social implications (ELSI), 431, 435–437
- Affordable Care Act, 439
 - Americans with Disabilities Act (ADA), 439
 - decision maker identification, 432–433
 - decision-making and informed consent, 432–435
 - declining and retracting consent, 434–435
 - family dynamics, impact on, 437–438
 - genetic discrimination, 438–441
 - genetic information and medical records, 438
 - Genetic Information Nondiscrimination Act (GINA), 439–441
 - genetic privacy, 438–441
 - Health Insurance Portability and Accountability Act of 1996, 439
 - informed consent
 - defined, 432
 - genetic results, types of, 433–434
 - psychosocial risks associated with testing, 434
 - retention of genetic information, 434
 - primary findings, 435–436
 - resources, 432*t*
 - secondary findings, 435–436
- Ethnicity-based carrier screening, 21–22
- Euchromatin, 45–46
- Evaluation and management (E&M)
- documentation, 408–410
 - based on time, 411
 - examination, 410
 - history taking, 410
 - medical decision-making, 410
 - medical record documentation, 410
 - 1997 documentation guidelines, 409–410
- Evolocumab, 448–449

- Exocrine pancreatic insufficiency, 327
 Exocrine tumors, 199
 Exome Aggregation Consortium, 24
 Exome sequencing (ES), 49–50, 54–55, 235, 238, 240–241, 245, 366
 Exon skipping therapy, 316
 Expanded carrier screening, 73
 Extension for Community Healthcare Outcomes (ECHO), 422
- F**
- Fabry disease, 278–280, 344–345, 361–362, 447
 enzyme replacement therapy for, 279
 Factor V Leiden, 215
 thrombophilia due to, 220, 220*t*
 Familial adenomatous polyposis (FAP), 185, 190*t*, 192, 200*t*
 Familial atypical multiple mole melanoma (FAMMM) syndrome, 95–96, 200*t*
 Familial benign pemphigus. *See* Hailey–Hailey disease (HHD)
 Familial hemiplegic migraine, 322
 Familial hypercholesterolemia (FH), 119–121
 genes associated with, 121*t*
 LDL receptor mutations in, 27–28
 Familial hyperparathyroidism, 175–176
 Familial hypocalciuric hypercalcemia (FHH), 171, 175
 Familial interstitial pneumonia (FIP), 330
 Familial isolated hyperparathyroidism (FIHP), 171, 175–176
 Familial isolated pituitary tumor syndrome (FIPA), 171
 Familial lentiginosis, 385
 Familial Mediterranean fever (FMF), 244–246
 MEFV gene mutation, 244–245
 Familial medullary thyroid cancer (FMTC), 172–173
 Familial MEN1, 169, 171
 Familial pancreatic cancers (FPCs), 193*t*, 199
 Familial polyposis syndrome, 5
 Familial pulmonary fibrosis, 330–331
 Family health history, 24–26
 Fanconi anemia, 382, 385, 387, 393
 Fascioscapulohumeral (FSHD) dystrophies, 315
 Fatty acid oxidation disorders, 261
FBN1 gene, 128–131
FBN2 gene, 143
 Female infertility, 151–152
 congenital adrenal hyperplasia (CAH), 153
 45,X (Turner syndrome), 151–152
 Kallmann syndrome, 154
 recurrent spontaneous abortion, 155–156
 Fetal hemoglobin (HbF), 224–225
FGFR3-related chondrodysplasias, 377–378
 Fibroblast growth factor 21 (FGF21), 293
 Fibroblast growth factor 23 (FGF23), 375–376
 Fibroids, 100
 Fibronectin glomerulopathy, 357
 15q13.3 deletion, 342
 Filamin A, 321
 First trimester screening, 73–74
 Fluorescence in situ hybridization (FISH), 26–27, 44–47, 76, 256
 FMR1 gene, 252–253
 Focal segmental glomerulosclerosis (FSGS), 360
 45,X/46,XY Mixed gonadal dysgenesis (MGD), 159–160
 46,XX disorders of sex development, 156–158
 46,XY disorders of sex development, 158–159
 47,XXY (Klinefelter syndrome), 148–149
 47,XYY (XYY syndrome), 149
 Fragile X-associated primary ovarian insufficiency, 253–254
 Fragile X-associated tremor and ataxia syndrome (FXTAS), 50, 56, 253–254, 308–309
 Fragile X syndrome, 72–73, 77–78, 251–253
 Frameshift variant, 13
 Friedreich ataxia (FA), 50, 308
 Frontotemporal dementia (FTD), 50, 312, 318–319
 Fukutin-related protein (FKRP), 315
 Functional neuroendocrine tumors, 199–200
FXN gene, 308
- G**
- Gabapentin, 362
 Gamma-retroviral vector, 464–466
 Gardner syndrome, 381
 Gastric cancer, 87*t*, 188*t*, 195

- gastrointestinal stromal tumors (GISTs), 197–199, 198*t*
- hereditary diffuse gastric cancer, 196–197
- nongenetic risk factors, 195
- Gastrinoma, 170
- Gastrointestinal disorders, 185
 - diseases of the colon
 - adenomatous polyposis syndromes, 189–192, 190*t*
 - hamartomatous polyposis syndromes, 192–195, 193*t*
 - hereditary CRC and polyposis syndromes, 185–195
 - Lynch syndrome, 186–189, 188*t*
 - diseases of the liver, 205–210
 - etiologies of, 205
 - hereditary hemochromatosis, 207–210
 - Wilson disease, 205–207
 - diseases of the pancreas
 - definition of, 199
 - hereditary syndromes, increased risk for, 200*t*
 - pancreatic cancer, 199–201
 - recurrent pancreatitis, 201
 - diseases of the stomach
 - gastric cancer, 195
 - gastrointestinal stromal tumors (GISTs), 197–199, 198*t*
 - hereditary diffuse gastric cancer, 196–197
 - nongenetic risk factors, 195
- Gastrointestinal stromal tumor (GIST), 195, 197–199, 198*t*
- Gaucher disease, 309–310
- Generalized joint hypermobility (GJH), 137–139, 141
- Gene replacement, 463–464
- Gene reprogramming, 463–464
- Gene structure, 10*f*, 11–12
- Gene supply, 463–464
- Gene therapy, 216
 - ex vivo* approaches, 464–466, 465*f*
 - in vivo* gene therapy, 465*f*, 466–471
 - for Duchenne muscular dystrophy, 470–471
 - for hemophilias, 469–470
 - for hereditary blindness, 468–469
 - for spinal muscular atrophy, 470
 - genome editing, 471–473, 472*f*
 - strategies and vectors, 463–464
- Genetic and genomic variants
 - impact of, 14
 - types of
 - chromosomal variation, 13–14
 - indel variants, 13
 - large SVs, 13
 - single-nucleotide variant (SNV), 12–13
- Genetic counselling for adults
 - case management for adults, 39–40
 - definition of, 31
 - finding healthcare providers with genetics expertise, 31–32
 - genetics evaluation and testing in adults,
 - considerations for, 37–38
 - insurance coverage, 37–38
 - testing coordination, 38
 - principles of, 32–37
 - contracting, 33
 - elements of pedigree, 34
 - incomplete family history information, 34
 - patient education and counselling, 35–37
 - personal history, 34
 - resources for patients and clinicians, 32, 33*t*
- Genetic counselor, 28
- Genetic discrimination, 438–441
- Genetic evaluation of adult
 - approach to the patient
 - genome-first approach, 22, 24
 - phenome-first approach, 21–24
 - diagnostic DNA technologies, 26–27
 - chromosomal analysis, 26–27
 - clinical cytogenetic analysis, 26–27
 - DNA sequencing, 27
 - family health history, 24–26
 - follow-up and management, 28–29
 - genetic and genomic contribution to disease
 - monogenic disease, 27–28
 - polygenic disease, 28
- Genetic Information Nondiscrimination Act (GINA), 36, 439–441, 450
- Genetic privacy, 438–441
- Genetic resources for patients and clinicians, 32, 33*t*

- Genetic skeletal disorders (GSDs), 369–370
 with abnormal bone mineralization, 375–377
 hypophosphatasia, 376–377
 hypophosphatemia, 375–376
 with altered BMD, 370–375
 decreased BMD, disorders with, 371
 increased BMD, disorders with, 373–374
 osteogenesis imperfecta, 371–372
 osteopetrosis, 374–375
FGFR3-related chondrodysplasias, 377–378
 achondroplasia, 377–378
 characterization, 377
- Genetic testing in adults, 37–38, 43
 cytogenetic testing, 44–48, 52*t*
 chromosomal microarray, 47–48
 fluorescence in situ hybridization, 46–47
 karyotyping, 45–46
 direct-to-consumer (DTC) testing, 54
 DNA sequencing tests, 48–50, 52*t*
 exome sequencing (ES), 49–50
 panel testing, 48–49
 single-gene testing, 48
 genome sequencing, 53–54
 next-generation sequencing, 50–51
 nucleotide repeat expansions, 50
 principles and practical approach to, 44, 45*f*
 variant interpretation, 51–53
- Genome editing, 463–464, 465*f*, 471–473, 472*f*
 Genome-first approach, 22, 22*f*, 24, 27
 Genome sequencing (GS), 53–55, 235, 238, 240–241, 245
 Genome-wide association studies (GWAS), 28
 Genome-wide risk scores (GRSs), 448–449
 Genome-wide sequencing technique, 54–55
 Genome-wide testing technologies, 51–53
 Genomic architecture, 9
 Genomic hybridization technologies, 342
 Genotoxicity, 471, 473–474
 GJB1 (Connexin-32) gene, 313–314
 Glanzmann thrombasthenia, 217*t*
 Glomerular basement membrane (GBM), 356–357
 Glomerular diseases with hematuria
 Alport syndrome, 358–360
 complement factor 3 glomerulonephritis (C3GN), 357–358
 dense deposit disease (DDD), 357–358
 fibronectin glomerulopathy, 357
 IgA nephropathy, 357
 MYH9-related disorders, 358
 Glomerulonephritis, 357
 Glucagonomas, 170
 Glucokinase (*GCK*), 167
 Glucose-6-phosphate dehydrogenase deficiency, 222*t*
 GM2 gangliosidosis, 271*t*
 Gonadotropin-releasing hormone (GnRH) deficiency, 154
 G-protein coupled receptor (GPCR), 174–176, 178
 Gray platelet syndrome, 217*t*
 Gregor Mendel's laws of inheritance, 43
 Griscelli syndrome (GS), 383
 Growth and differentiation factor (GDF15), 293
 Growth hormone (GH) deficiency, 176
 Growth hormone (GH)-secreting tumors, 170–171
 Gs-alpha subunit (GNAS), 176
 McCune–Albright syndrome, 178–179
 pseudohypoparathyroidism-related disorders, 176–178
 G6PD deficiency, 222–223
 G6PD hemolytic anemia, 222
 GWAS. *See* Genome-wide association studies (GWAS)
- ## H
- Haemophilus influenzae*, 233, 235, 237
 Hailey–Hailey disease (HHD), 401
 Hamartomas, 174
 Hamartomatous polyposis syndromes, 185, 192–195, 193*t*
 Hemangioblastomas, 364
 Hematological disorders, 213
 bleeding disorders, 213–218
 clotting factor deficiencies, 215–216
 platelet disorders, 216–217, 217*t*
 RUNX1 familial platelet disorder, 217–218
 Von Willebrand disease (VWD), 213–215
 women with, 216
 bone marrow failure disorders, 218–219

- Fanconi anemia (FA), 218
- telomere disorders, 219
- hematological malignancies, 227–229
 - lymphoid neoplasms, 228–229
 - myeloid neoplasms, 227, 227*t*
- red cell defects, 221–227
 - hemoglobinopathies, 224, 225*t*
 - red cell enzyme defects, 221–222, 222*t*
 - red cell membrane defects, 223–224, 223*t*
 - sickle cell disease and related syndromes, 224–226
 - thalassemias, 226–227
- thrombophilia, 219–221, 220*t*
- Hematological malignancies, 227–229
 - lymphoid neoplasms, 228–229
 - myeloid neoplasms, 227, 227*t*
- Hematopoietic stem cells (HSCs), 464–466
- Hematopoietic stem cell transplantation, 237
- Hemochromatosis, 207–210
 - biochemical HFE hemochromatosis and nonexpressing C282Y homozygotes, 209
 - clinical HFE hemochromatosis, 208
- Hemoglobin C (HbC) disease, 226
- Hemoglobin E (HbE) disease, 226
- Hemoglobinopathies, 72–73, 224, 225*t*
- Hemolytic anemia, 221, 274–275
- Hemolytic uremic syndrome, 270–272
- Hemophilia A, 215–216, 466, 469
- Hemophilia B, 215, 469
 - AAV vectors, 467, 467*f*
 - genetic testing, 215–216
- Hemophilias, *in vivo* gene therapy, 469–470
- Hepatobiliary cancers, 87*t*, 188*t*
- Hepatocellular cancer, 391
- Hepatocyte nuclear factor 1 α (HNF-1 α), 164–167
- Hepatocyte nuclear factor 1 β (HNF-1 β), 164–168
- Hepatocyte nuclear factor 4 α (HNF-4 α), 164, 167
- Hepatoerythropoietic porphyria (HEP), 390*t*, 391
- Hepatomegaly, 327
- Hereditary angioedema (HAE), 242–243
- Hereditary breast and ovarian cancer syndrome (HBOC), 96–100
 - BRCA1* and *BRCA2*, 96
 - risk of cancers associated with, 97*t*
- Hereditary cancer syndromes
 - BAP1* tumor predisposition syndrome, 92
 - Birt Hogg Dube (BHD) syndrome, 93–94
 - cancers with their associated genes, 87*t*
 - DICER1*-related pleuropulmonary blastoma
 - familial tumor, 94–95
 - dysplasia syndrome, 94–95
 - evaluation for, 86–91
 - diagnostic testing, 86
 - family history, 86
 - scoring tools, 86
 - tumor characteristics, 86
 - familial atypical multiple mole melanoma syndrome, 95–96
 - hereditary breast and ovarian cancer syndrome, 96–98
 - hereditary leiomyomatosis and renal cell carcinoma, 100–101
 - hereditary paraganglioma and pheochromocytoma, 101–102
 - Li–Fraumeni syndrome, 102–104
 - personal and family history features of, 85–86
 - prostate cancer syndrome, 104–105
 - PTEN* hamartoma tumor syndrome, 105–107
 - sporadic *vs.* inherited, 85
- Hereditary colon cancer syndrome, 35
- Hereditary connective tissue disorders, 127
 - musculoskeletal and skin manifestations, conditions with, 137–143
 - classical Ehlers–Danlos syndrome, 141–142
 - Ehlers–Danlos syndromes, 137, 138*t*
 - hypermobile Ehlers–Danlos syndrome, 137–141, 140*t*
 - Stickler syndrome, 142–143
 - phenotypic overlap, conditions with, 143–144
 - Beals syndrome, 143
 - homocystinuria, 143
 - Shprintzen–Goldberg syndrome, 144
 - primary vascular phenotype/aortopathy, conditions with, 128–137
 - bicuspid aortic valve, 133
 - heritable thoracic aortic disease, 135–136
 - Loeys–Dietz syndrome, 131–133, 133*t*
 - Marfan syndrome, 128–130, 129*t*
 - vascular Ehlers–Danlos syndrome, 133–135
- Hereditary coproporphyrria, 390*t*

- Hereditary diffuse gastric cancer (HDGC), 196–197
- Hereditary elliptocytosis, 223*t*
- Hereditary hemorrhagic telangiectasia (HHT), 334–336
causes, 334
characterization, 334
screening, 195
- Hereditary leiomyomatosis, 363–364
- Hereditary leiomyomatosis and renal cell carcinoma (HLRCC), 100–101
- Hereditary nephritis. *See* Alport syndrome (AS)
- Hereditary pancreatitis (HP), 201–204
- Hereditary papillary renal cell cancer syndrome, 100
- Hereditary prostate cancer syndrome, 104–105
- Hereditary pyropoikilocytosis, 223*t*
- Hereditary spherocytosis, 223*t*
- Hereditary thrombocytopenia with risk of malignancy, 217–218
- Heritable thoracic aortic disease (HTAD), 135–136
- Hermansky–Pudlak syndrome (HPS), 383
- Heterochromatin, 45–46
- Heteroplasmy, 284, 295, 299–300
- Heterotaxy, 329
- Heterozygous familial hypercholesterolemia (FH), 448–449
- HEXA gene, 345
- Hexosaminidase A deficiency, 345
- Hexosaminidase A enzyme activity for Tay–Sachs disease, 72–73
- HFE*-related hereditary hemochromatosis, 208–210
- High microsatellite instability (MSI-H), 186–187
- Hirschsprung's disease, 172
- Histones, 11
- HIV infections
precision medicine in, 450
retroviral therapy for, 297–298
- Hodgkin lymphoma (HL), 229
- Homocystinuria, 143, 267
- Homology-directed repair (HDR), 463–464, 471–472
- Homozygous familial hypercholesterolemia (HoFH), 119–121
- Human genome
DNA, molecular architecture of, 9–10
gene structure, 10*f*, 11–12
mitochondrial genomes, 11
nuclear genomes, 11
- Human Genome Project, 9, 43
- Humoral immunodeficiency, 231–234, 240
- Huntington disease (HD), 50, 310–311, 345, 418, 434
- Hutchinson–Gilford progeria syndrome, 12
- Hyperammonemia, 79, 261–265
urea cycle disorders, 261–262, 263*t*
- Hyperandrogenism, 180
- Hypercalcemia, 163, 174
- Hypercalciuria, 175, 177
- Hyperglycemia, 163–164
- Hyperhidrosis, 361
- Hyper IgE syndrome. *See* Autosomal dominant hyper IgE syndrome (AD-HIES)
- Hyperimmunoglobulin M syndrome, 234
- Hyperkalemia, 320
- Hyperkinetic movement disorders, 306
- Hyperlipidemia, 356
- Hypermobile Ehlers–Danlos syndromes, 137–141, 138*t*, 140*t*
- Hypermobility spectrum disorder (HSD), 140
- Hyperparathyroidism, 170–173
- Hyperparathyroidism-jaw tumor syndrome (HPT-JT), 171, 174
- Hyperphenylalaninemia, 79
- Hyperpigmentation, 381–382, 388, 390
- Hyperthyroidism, 178
- Hypertrophic cardiomyopathy (HCM), 109–111, 113*t*
- Hypoalbuminemia, 360
- Hypocalcemia, 163, 177
- Hypocalciuria, 175
- Hypo-complementemia, 357–358
- Hypogonadism, 176
- Hypohidrosis, 361
- Hypomagnesemia, 175
- Hypomanic episode, 341
- Hypomelanosis, 381–382
- Hypophosphatasia, 376–377
- Hypophosphatemia, 179, 375–376

Hypopigmentation, 381–382, 388, 395

Hypospadias, 156–157

Hypothyroidism, 152

I

ICD-10 coding system, 411, 427

Ichthyosis vulgaris

evaluation, 399

genes associated with, 399*t*

Idebenone, 296

Idiopathic familial pulmonary fibrosis (IPF),
330–331

IgA nephropathy, 357

Immune complex-mediated glomerulonephritis,
357

Immunologic disorders, 231–233

antibody deficiency, disorder with, 233–239
autosomal dominant hyper IgE syndrome,
237–238

B-cell deficiencies, 233

Bruton agammaglobulinemia, 236–237

common variable immunodeficiency (CVID),
233–235

selective IgA deficiency, 235–236

autoinflammatory diseases (AIDs), 244–246

familial Mediterranean fever (FMF), 244–246

complement deficiencies, 242–244

definition of, 242

hereditary angioedema (HAE), 242–243

T-cell deficiency, disorders with, 239–242

ataxia-telangiectasia (AT), 239–240

22q11.2 deletion syndrome (22q11.2 DS),
240–242

Implantable cardiac defibrillator (ICD), 111–115,
117–119

Imprinting, 16

Inborn errors of metabolism (IEM), 261–266

hyperammonemia, 261–265

urea cycle disorders, 261–262, 263*t*

neuropsychiatric symptoms, 270–273

cobalamin metabolism disorders, 270, 271*t*

peripheral neuropathy, 276–280

in adulthood, 276, 277*t*

chronic sensory-motor demyelinating
neuropathy, 278

Fabry disease, 278

rhabdomyolysis, 274–276

dystrophinopathies, 274

Limb-Girdle Muscle Dystrophy, 274

LPIN1 deficiency, 274

metabolic-genetic etiology, 274

metabolic myopathies, 274

Pompe disease, 274

RYR1-related disorders, 274

stroke, 267–269

thromboembolic, 267

Infertility, 147

both male and female

congenital adrenal hyperplasia (CAH), 153

Kallmann syndrome, 154

recurrent spontaneous abortion, 155–156

disorders of sex development (DSD), 156–161

45,X/46,XY mixed gonadal dysgenesis, 159

46,XX, 156–157

46,XY, 158

genes affecting gonadal differentiation,
160–161

female, 151–152

male

cystic fibrosis (CF), 149

Klinefelter syndrome (KS), 148

obstructive azoospermia, 149

XYY syndrome, 149

Y chromosome microdeletions (YCMDs),
150–151

Inherited arrhythmogenic disorders (IADs),
116–122

Brugada syndrome, 118–119, 120*t*

catecholaminergic polymorphic ventricular
tachycardia, 119, 120*t*

familial hypercholesterolemia, 119–121, 121*t*

genes associated with, 120*t*

long QT syndrome, 116–117, 120*t*

short QT syndrome, 118, 120*t*

Inherited diseases, gene therapy for

ex vivo approaches, 464–466, 465*f*

gene therapy, defined, 463

genome editing, 471–473, 472*f*

in vivo gene therapy, 465*f*, 466–471

for Duchenne muscular dystrophy, 470–471

Inherited diseases, gene therapy for (*Continued*)

- for hemophilias, 469–470
- for hereditary blindness, 468–469
- for spinal muscular atrophy, 470

strategies and vectors, 463–464

Inherited episodic ataxias, 306

Inherited glomerulopathies, 356–360

glomerular diseases with hematuria, 357

Alport syndrome, 358–360

complement factor 3 glomerulonephritis (C3GN), 357–358

dense deposit disease (DDD), 357–358

fibronectin glomerulopathy, 357

IgA nephropathy, 357

MYH9-related disorders, 358

nephrotic syndrome, 360

Inherited thrombophilia, 219–220

Initiator codon, 12

Insulin, 163–164, 167

Insulinomas, 170

Intellectual disability (ID), 34–35, 46, 48–50, 55,

62, 142–143, 249–252, 306–307, 322

causes of, 249–250, 250*t*

classification, 249–250

nonsyndromic, 257

autism spectrum disorder, 257

types, 257

syndromic, 252–257

Down syndrome, 254–255

fragile X syndrome, 252–253

22q11.2 deletion syndrome (22q11.2 DS), 256–257

Intelligence quotient (IQ) scores, 249

Interdigital xanthomata, 120–121

Interstitial lung diseases (ILDs), 330

Intracranial aneurysms (ICAs), 354

Intravenous immunoglobulin (IVIG), 233, 237, 240

Intravenous (IV) L-arginine therapy, 296

Invasive ductal carcinoma, 98

In vitro fertilization/intracytoplasmic sperm

injection (IVF/ICSI), 150, 152, 154, 156, 159

In vitro fertilization with preimplantation genetic testing, 71*In vivo* gene therapy, 465*f*, 466–471

for Duchenne muscular dystrophy, 470–471

for hemophilias, 469–470

for hereditary blindness, 468–469

for spinal muscular atrophy, 470

Iron-deficiency anemia, 187

Isochromosome, 254

Ivacaftor, 459*t***J**

Jervell and Lange–Nielsen syndrome, 117

Job syndrome. *See* Autosomal dominant hyper IgE syndrome (AD-HIES)

Juvenile polyposis, 106, 335

Juvenile polyposis syndrome (JPS), 193*t*

K

Kallmann syndrome, 154–155

Karyotyping, 45–46, 54–55, 76

analysis, 11, 26–27, 44

banding patterns, 45–46

indications for, 46

KCNH2 gene, 116

KCNQ1 gene, 116

Kearns Sayre syndrome (KSS), 287*t*, 294

Keratolytic agents, 400

Keratosis follicularis. *See* Darier disease (DD)

Kidney cancer, 87*t*

Klinefelter syndrome (KS), 44–45, 54–55, 148–149, 252

Koenen tumors, 395–396

Krabbe disease, 278

Krebs cycle, 293

L

Lactate, 286

Lactic acidemia, 267–268

Lactic acidosis, 44, 267

Lafora body disease, 321

LDLR gene, 121, 121*t*

L-dopa, 309–310

LDS. *See* Loews–Dietz syndrome (LDS)

Leber congenital amaurosis (LCA), 468–469

Leber hereditary optic neuropathy (LHON), 287*t*, 294, 299

Left ventricular hypertrophy, 23, 110

- Left ventricular noncompaction (LVNC), 115
 - Legius syndrome, 393
 - Leigh syndrome, 285–286
 - mtDNA mutations in *MT-ATP6* and *MT-TL1*, 285
 - Lentigines, 384
 - Lentigines, atrial myxoma, mucocutaneous
 - myxoma, blue nevi (LAMB), 384
 - Lentiviral vector-mediated gene therapy, 465–466
 - Leukemia, 87*t*
 - Levodopa, 35
 - Lewy body (α -synuclein) aggregation, 317
 - Lewy body dementia, 318
 - Lhermitte–Duclos disease, 106
 - Li–Fraumeni syndrome (LFS), 102–104, 228*t*
 - cancer penetrance, 103
 - characterization, 102
 - Limb-girdle muscular dystrophies (LGMDs), 274, 315–316
 - Lipid nanoparticles (LNPs), 472–473
 - Lipomas, 170
 - Lipoprotein A (*LPA*), 457
 - Liver diseases, 205–210, 325–326
 - hereditary hemochromatosis, 207–210
 - Wilson disease, 205–207
 - Liver transplantation, 122–123, 209
 - Liver tumor, 74
 - LMNA* gene, 12, 112–113, 113*t*
 - Lobular breast cancer, 196
 - Loeys–Dietz syndrome (LDS), 23, 55, 78, 128, 131–133, 133*t*
 - Long-chain fatty acids oxidation disorders, 276
 - Long QT syndrome (LQTS), 116–117, 120*t*
 - Low-density lipoprotein cholesterol (LDL-C), 448–449
 - LRRK2 gene mutations, 309–310
 - Lung cancer, 87*t*
 - environmental carcinogens, 336–337
 - genetic testing, 336–337
 - non-small cell lung cancer (NSCLC), 336–337
 - risk factors, 336–337
 - small cell lung cancer (SCLC), 336–337
 - Lung cysts, 94
 - Lymphoid neoplasms
 - Diffuse large B-cell lymphoma, 228
 - Hodgkin lymphoma, 229
 - non-Hodgkin lymphoma, 228
 - Lymphoma, 87*t*, 238
 - Lynch syndrome, 23, 35, 86, 87*t*, 99, 186–189, 200*t*
 - lifetime risk and management, 188*t*
- ## M
- Male infertility
 - congenital adrenal hyperplasia (CAH), 153
 - cystic fibrosis (CF), 149–150
 - congenital bilateral absence of the vas deferens (CBAVD), 149–150
 - Kallmann syndrome, 154
 - Klinefelter syndrome (KS), 148–149
 - obstructive azoospermia, 149
 - recurrent spontaneous abortion, 155–156
 - XXY syndrome, 149
 - Y chromosome microdeletions (YCMDs), 150–151
 - Marfan syndrome (MFS), 23, 44, 55, 78, 128–130
 - cardinal manifestations of, 128
 - FBNI* gene, 128
 - management, 130–131
 - medical, 130–131
 - pregnancy, 131
 - surgical, 131
 - surveillance, 130
 - Revised Ghent criteria, 128, 129*t*
 - skeletal features of, 128
 - Massively parallel sequencing (MPS). *See* Next-generation sequencing (NGS)
 - Maternally inherited diabetes and deafness (MIDD), 287*t*
 - Maternal screening for fetal aneuploidy, 73–75
 - cell-free DNA (cfDNA) screening, 74–75
 - limitations of, 75
 - massively parallel shotgun sequencing with counting of sequenced fragments, 74–75
 - practical considerations, 75
 - single nucleotide polymorphism-based analysis, 74–75
 - multiple marker screening, 73–74
 - first trimester screening, 73–74
 - second trimester serum screening, 74
 - sequential and integrated screening, 74

- Maternal serum alpha-fetoprotein (MSAFP), 74
- Maturity-onset diabetes of the young (MODY), 164
- genetic classification of, 164, 165*t*
 - MODY1, 167
 - MODY2, 167
 - MODY3, 164–167
- McArdle disease, 274–275
- McCune–Albright syndrome (MAS), 171, 176, 178–179
- Medium-chain triglycerides, 275–276
- Medullary thyroid carcinoma (MTC), 172–173
- Meglitinides, 168
- MEK inhibitors, neurofibromatosis type 1, management of, 394
- Melanoma, 87*t*, 94, 107
- Melanopenic hypomelanosis, 381–382
- MELAS. *See* Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELASs)
- Membranous nephropathy, 360
- Menin, 170, 172
- Mental health and pain management, pharmacogenomics, 457–458
- Mental illnesses, 339–342
- bipolar disorders, 341–342
 - generalized anxiety disorder, 340
 - genetic conditions, increased risk for
 - chromosome microdeletion/duplication syndromes, 342–343
 - single-gene Mendelian disorders, 344–347
 - major depressive disorder, 340
 - recovery from, 350*f*
 - schizophrenia, 341
- MENX, 171
- Metabolic disorders. *See* Inborn errors of metabolism (IEM)
- Metabolic myopathies, 274
- Metachromatic leukodystrophy, 271*t*, 272, 278, 466
- Metformin, 269
- for diabetes, 297–298
- Methylation-based studies, 50
- MFS. *See* Marfan syndrome (MFS)
- Microalbuminuria, 356
- Microarray-based comparative genomic hybridization (aCGH), 47
- Microhematuria, 358–359
- Midodrine, 141
- Migalastat, 447
- Migraine headache, 319, 322
- Minimal change disease (MCD), 360
- Mitochondrial deoxyguanine kinase (DGUOK), 284–285
- Mitochondrial disorders
- evaluation, 285–294
 - genetic counseling, 299–300
 - genetic testing, 294–299
 - during critical illness, management, 297–298
 - emerging therapies, 298–299
 - management, 295–298
 - laboratory testing, 286–293
 - mtDNA, unique features of, 284–285
 - respiratory chain (RC), 283–284
 - components of, 283
 - reduced cytochrome *c*, 283–284
 - reduced nicotinamide adenine dinucleotide (NADH), 283–284
- Mitochondrial DNA depletion syndromes, 295
- Mitochondrial encephalomyopathy, 44
- Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELASs), 267–269, 287*t*, 294, 296, 345
- Mitochondrial genomes, 11, 50–51
- Mitochondrial heteroplasmy, 16*f*, 50–51
- Mitochondrial homoplasmy, 16*f*
- Mitochondrial inheritance, 15–16, 16*f*
- Mitochondrially inherited diabetes and deafness (MIDD), 294
- Mitochondrial neurogastrointestinal encephalopathy (MNGIE), 287*t*, 299
- Mitochondrial recessive ataxia syndrome (MIRAS), 287*t*
- Mitochondrial red flag symptoms, 285–286
- Mitochondrial replacement therapy, 299
- Mitochondrial thymidine kinase (TK2), 284–285, 298–299
- Mitochondrial tRNA genes, 294
- Mitomycin C, 218
- Mixed gonadal dysgenesis, 153

- Mobile health (mHealth), 421
- Modes of inheritance, 14–18
 - autosomal dominant, 14–15
 - autosomal recessive, 15
 - complex, 17, 17*f*
 - digenic, 16–17, 17*f*
 - imprinting, 16
 - mitochondrial, 15–16, 16*f*
 - X-linked (XL), 15
- Mohs surgery, 387
- Molecular cytogenetic techniques, 43
- Monosomies, 44–45
- Mosaicism, 45–46, 152
- Motor neuron and neuromuscular diseases, 311–316
 - amyotrophic lateral sclerosis, 312–313
 - muscular dystrophies, 314–315
 - peripheral nerves, 312
 - peripheral neuropathies, 312–314
 - acquired forms, 313
 - genetic forms, 313
- Movement disorders, 306–311
 - ataxia, 306–308, 307*f*
 - spinocerebellar ataxia type 2, 307*f*
 - classification, 306
 - Huntington disease, 310–311
 - Parkinson disease, 309–310
- Moyamoya-like cerebrovascular disease, 135
- mTOR inhibitors, tuberous sclerosis complex (TSC), 396
- MT-TL1* mutations, 294
- Muir–Torre syndrome. *See* Lynch syndrome
- Multicystic dysplastic kidney, 353–354
- Multilocular cystic nephroma, 353–354
- Multiple bilateral renal cysts, 355
- Multiple endocrine neoplasia 2 (MEN2), 172–173
- Multiple endocrine neoplasia 2A (MEN2A), 172–173
- Multiple endocrine neoplasia 2B (MEN2B), genetic testing, 173
- Multiple endocrine neoplasia (MEN) syndromes, 168
 - familial medullary thyroid cancer, 172–173
 - MEN2A, 172–173
 - MEN2B (MEN3), 172–173
 - multiple endocrine neoplasia 4, 171–172
 - multiple endocrine neoplasia type 1 (MEN1), 169–171
 - MEN1-like phenotypes, 171
 - multiple endocrine neoplasia 2 (MEN2), 172–173
 - multiple endocrine neoplasia 2A (MEN2A), 172–173
 - multiple endocrine neoplasia 2B (MEN2B), genetic testing, 173
- Multiple endocrine neoplasia type 1 (MEN1), 169–171, 174*t*, 193*t*
 - evaluation, 169–170
 - neuroendocrine tumors of gastrointestinal system, 170
 - parathyroid tumors, 169–170
 - pituitary tumors, 170
 - prevalence of clinical manifestations of, 169, 169*t*
 - MEN1-like phenotypes, 171
- Multiple lentigines syndromes, 384–386
- Multiple marker screening, 73–74
 - first trimester screening, 73–74
 - second trimester serum screening, 74
 - sequential and integrated screening, 74
- Multiple myeloma, 87*t*
- Multiplex ligation-dependent probe amplification (MLPA), 55, 256
- Muscular dystrophies, 314–315
- Mutational burden, 18
- MUTYH*-associated polyposis (MAP), 185, 190*t*
- MYBPC3* gene, 110–111
- Myelodysplastic syndrome, 87*t*
- MYH7* gene, 110–111
- MYH9*-related disorders (MYH9-RD), 217*t*, 358
- Myoclonic epilepsy, myopathy, sensory ataxia (MEMSA), 287*t*
- Myoclonic epilepsy with ragged red fibers (MERRFs), 287*t*, 294, 321
- Myopathy, 267
- Myotonia, 66
- Myotonic dystrophy (DM), 79, 315–316
- Myotonic dystrophy type 1 (DM1), 50, 65–66, 315
- Myotonic muscular dystrophy, 316
- Myxomas, 385

N

- N*-acetylglutamate synthase (NAGS) deficiency, 265–266
- Nail-patella syndrome (NPS), 362–363
- Neonatal hyperparathyroidism, 174–175
- Neonatal Marfan syndrome, 129–130
- Neoplasia of pancreaticoduodenal neuroendocrine cells, 170
- Nephritic kidney disease, 356–357
- Nephroblastomas, 174
- Nephrocalcinosis, 175, 177, 375–376
- Nephrolithiasis, 175
- Nephronophthisis, 353–354
- Nephrotic syndrome (NS), 356–357, 360
- Nerve conduction velocity (NCV), 312
- Neurocristopathies, 383–384
- Neurocutaneous disorders, 392–396
 - neurofibromatosis type 1 (NF1), 392–394, 393*t*
 - differential diagnosis in, 393
 - National Institutes of Health diagnostic criteria for, 392*t*
 - tuberous sclerosis complex, 394–396, 395*t*, 397*t*
- Neurocutaneous hamartomas, 394
- Neuroendocrine duodeno-pancreatic tumors, 172
- Neuroendocrine tumors, 87*t*
- Neurofibromas, 392–393
- Neurofibromatosis type 1 (NF1), 197, 198*t*, 305, 392–394, 393*t*
 - differential diagnosis in, 393
 - National Institutes of Health diagnostic criteria for, 392*t*
- Neurologic disorders, 305–306
 - CADASIL, 319–320
 - dementias
 - Alzheimer disease, 316–318
 - frontotempora dementia, 318–319
 - onset of, 316
 - motor neuron and neuromuscular diseases, 311–316
 - amyotrophic lateral sclerosis, 312–313
 - muscular dystrophies, 314–315
 - peripheral nerves, 312
 - peripheral neuropathies, 313–314
 - movement disorders, 306–311
 - ataxia, 306–308, 307*f*
 - classification, 306
 - Huntington disease, 310–311
 - Parkinson disease, 309–310
- neurogenetic disorders, 306
- paroxysmal disorders, 320–323
 - epilepsy, 320–321
 - headache, 322–323
- Neuronal ceroid lipofuscinosis, 321
- Neuropathy, ataxia, retinitis pigmentosa (NARP), 287*t*
- Neuropsychiatric symptoms, 270–273
 - cobalamin metabolism disorders, 270, 271*t*
- Nevi, atrial myxoma, myxoid neurofibroma, ephelides (NAME), 384
- Next-generation sequencing (NGS), 9, 24, 26–27, 43, 50–51, 76–77, 104, 153, 167–168, 249, 251, 414, 460
- Niemann–Pick type C, 271*t*, 272
 - vertical ophthalmoplegia in, 305
- Nijmegen breakage syndrome (NBS), 240, 382
- 9p21 locus, 123–124
- Nitrogen-scavenging agents, 265–266
- Nonalcoholic fatty liver disease (NAFLD), 205–206
- Nonalcoholic steatohepatitis (NASH), 205
- Nonclassic congenital adrenal hyperplasia (NCAH), 179–180
- Non-*HFE*-related hereditary hemochromatosis, 207–208
- Non-Hodgkin lymphoma (NHL), 228
- Nonhomologous end joining (NHEJ), 471–472
- Noninvasive prenatal screening (NIPS). *See* Cell-free DNA (cfDNA) screening
- Noninvasive prenatal testing (NIPT). *See* Cell-free DNA (cfDNA) screening
- Nonobstructive azoospermia (NOA), 148
- Nonsyndromic ichthyosis, 399–400, 399*t*
- Nonsyndromic intellectual disability (NS-ID), 249–250, 257
 - autism spectrum disorder, 257
 - types, 257
- Nonsynonymous variants, 12–13
- Noonan syndrome, 59–60, 74–75, 110
- Noonan syndrome with multiple lentigines (NSMLs), 384–386

- NOTCH3 gene, 319
- NSAIDs, 141–142
- Nuclear genomes, 11
- Nucleosomes, 11
- Nucleotide repeat expansions, 50
- O**
- Obstructive azoospermia, 149
- Obstructive lung disease
- AAT deficiency (AATD), 325–327
 - cystic fibrosis (CF), 327–329
 - causes, 327
 - primary ciliary dyskinesia (PCD), 329–330
 - causes, 329
- Oculocutaneous albinism (OCA), 381–383
 - nonsyndromic OCA, subtypes of, 382, 382*t*
- OMIM database, 28
- Oncocytic hybrid tumor, 93
- Oncocytic kidney cancers, 363–364
- Oogenesis, 155
- Open neural tube defects (ONTDs), 74
- Opioid receptor agonists, 458
- Opioid therapy, porphyrias, 391
- Oral glucose tolerance test (OGTT) testing, 164, 167
- Organic acidemias, 261, 267
- Orkambi (Lumacator/Ivacaftor), 454–455
- Ornithine carbamoyltransferase deficiency, 463
- Ornithine transcarbamylase (OTC) deficiency, 261–262, 264–266
- Osteogenesis imperfecta (OI), 74–75, 371–373, 446–447
- Osteoma cutis, 176
- Osteopetrosis, 374–375
- Osteoporosis, 369
- Osteosarcoma, 388
- Ovarian cancer, 87*t*, 188*t*
- Ovarian cystadenomas, 180–181
- Oxidation and phosphorylation (OXPHOS), 267, 283–285, 293–295, 298
- P**
- Pancreatic adenocarcinoma, 200
- Pancreatic cancer, 87*t*, 95–96, 188*t*, 199–201
 - hereditary syndromes, increased risk for, 200*t*
 - nongenetic risk factors, 199
- Pancreatic neuroendocrine tumors, 364
- Pancreatitis, 175
 - definition of, 201
- Pancytopenia, 218
- Panel-based testing, 55, 257
- Panel testing, 27, 48–49, 92
 - advantages of, 49
- Pan-ethnic expanded carrier screening, 72
- Papillorenal syndrome, 363
- Paragangliomas (PGLs), 101–102, 198–199
- Parathyroid carcinoma, 173, 175–176
- Parathyromatosis, 175–176
- Parental genetic disease and reproduction, 78–80
 - aortopathies, 78
 - collagen disorders, 78
 - cystic fibrosis, 79
 - Marfan syndrome, 78
 - myotonic dystrophy, 79
 - phenylketonuria and inborn errors of
 - metabolism, 79
 - skeletal dysplasias, 79
 - Turner syndrome, 78
- Parkinson disease (PD), 309–310, 420
- Paroxysmal disorders, 320–323
 - epilepsy, 320–321
 - headache, 322–323
 - migraine, 322
- Paroxysmal myotonic myopathy (PROMM), 315
- Pauci-immune glomerulonephritis, 357
- PCSK9 gene, 121, 121*t*
- Pearson syndrome (PS), 287*t*
 - single large deletions of mtDNA, 285
- Peripartum cardiomyopathy, 115
- Peripheral neuropathy, 276–280, 312–314
 - acquired forms, 313
 - in adulthood, 276, 277*t*, 312–314
 - chronic sensory-motor demyelinating neuropathy, 278
 - Fabry disease, 278
 - genetic forms, 313
- Personalized medicine, 445–446, 450
- Peutz–Jeghers polyps (PJP), 106, 192, 193*t*, 384
- P450 2C9 enzyme system (CYP2C9), 456–457
- P450 CYP enzymes, 456–457

- Phagocytic cell defects, 232*t*
- Phakomatoses. *See* Neurocutaneous disorders
- Pharmacodynamics, 453, 454*f*
- Pharmacogenomics, 348
- biomarkers, 459*t*
 - clinical applications of, 453–458
 - cancer, 455–456
 - cardiovascular medicine, 456–457
 - Mendelian disorders, 454–455
 - mental health and pain management, 457–458
 - clinical resources and adaptation of, 458–459
 - pharmacodynamics, 453, 454*f*
 - pharmacokinetics, 453, 454*f*
 - pilot programs, 459–460
 - precision medicine, 449
- Pharmacokinetics, 453, 454*f*
- Phenome-first approach, 21–24, 22*f*, 27
- Phenylalanine hydroxylase deficiency, 79
- Phenylketonuria, 79, 345–346
- Pheochromocytomas (PCCs), 101–102, 172–173, 198–199, 364
- Phosphoglycerate kinase deficiency, 274–275
- Photoprotection, cutaneous porphyrias, 391
- Photosensitivity disorders, 386–389
- Rothmund–Thomson syndrome (RTS), 388–389
 - xeroderma pigmentosum (XP), 385, 387–388
- Piebaldism, 381–384
- Pigmentation disorders, 381–386
- hyperpigmentation, 381–382
 - hypopigmentation, 381–382
 - multiple lentigines syndromes, 384–386
 - neurocristopathies, 383–384
 - oculocutaneous albinism, 382–383
- PI3K-related overgrowth spectrum (PROS), 401–402
- Pituitary adenomas, 170–172
- PKP2* gene, 114
- Plakophilin 2, 114
- Platelet-derived growth factor receptor A (PDGFRA), 455
- Plasma-derived/recombinant factor replacement product, 216
- Platelet disorders, 216–217, 217*t*
- Pleuropulmonary blastomas (PPBs), 94–95
- PMP22 duplication, 313–314
- Pneumothorax, 93–94, 332
- Poikiloderma, 387–388
- POLG-related disorders, 346
- Polycystic kidney disease, 353–354
- Polygenic disease, 27–28
- Polygenic risk scores (PRS), 28
- Polyhydramnios, 79
- Polymerase chain reaction (PCR), 72–73
- Polyostotic fibrous dysplasia (FD), 178
- Poly (ADP-ribose) polymerases (PARPs), 455–456
- inhibitors, 455–456
- Polyposis syndromes
- adenomatous polyposis syndromes, 189–192, 190*t*
 - hamartomatous polyposis syndromes, 192–195, 193*t*
- Pompe disease, 110–111, 274–275
- Porphyria cutanea tarda (PCT), 390, 390*t*
- Porphyrias, 389–392, 390*t*
- Prader–Willi syndrome, 16, 250, 252
- Precision medicine, 446–449
- in CVDs, 448–449
 - defined, 445–446
 - direct-to-consumer (DTC) genetic testing, 449–450
 - HIV infection, 450
 - in Mendelian disorders, 446–447
 - pharmacogenomics, 449
 - Precision Medicine Initiative (PMI), 446
 - precision oncology, 447–448
- Precision Medicine Initiative (PMI), 446, 459
- Precision oncology, 447–448
- Preconception and prenatal care, 71
- maternal screening for fetal aneuploidy, 73–75
 - cell-free DNA (cfDNA) screening, 74–75
 - multiple marker screening, 73–74
 - parental genetic disease and reproduction, 78–80
 - aortopathies, 78
 - collagen disorders, 78
 - cystic fibrosis, 79
 - Marfan syndrome, 78
 - myotonic dystrophy, 79

- phenylketonuria and inborn errors of metabolism, 79
- skeletal dysplasias, 79
- Turner syndrome, 78
- preimplantation genetic testing, 76–78
 - limitations of, 77–78
 - PGT-A for aneuploidy, 76–77
 - PGT-M for monogenic disorders, 77–78
 - PGT-SR for structural rearrangements, 76–77
 - practical considerations, 77–78
- prenatal diagnostic genetic testing, 75–76
 - amniocentesis, 75–76
 - chorionic villus sampling, 75–76
 - genetic testing modalities, 76
- reproductive carrier screening, 71–73
 - assays used for, 72–73
 - definition of, 71–72
 - limitations of, 73
 - pan-ethnic expanded carrier screening, 72
 - practical considerations, 73
 - sequential carrier screening, 72
 - targeted carrier screening, 72
- Pregnancy
 - management of
 - classical EDS, 142
 - hypermobility EDS, 141
 - Loeys–Dietz syndrome (LDS), 133
 - Marfan syndrome (MFS), 131
 - vascular Ehlers–Danlos syndrome (vEDS), 134
 - mental illness, 348–349
- Preimplantation genetic screening (PGS), 149, 154, 156, 159
 - BAP1* tumor predisposition syndrome, 93
 - Cowden syndrome (CS), 107–108
 - DICER1*-related pleuropulmonary blastoma
 - familial tumor and dysplasia syndrome, 95
- Preimplantation genetic testing (PGT), 76–78
 - in vitro* fertilization with, 71
 - limitations of, 77–78
 - PGT-A for aneuploidy, 76–77
 - PGT-M for monogenic disorders, 77–78
 - PGT-SR for structural rearrangements, 76–77
 - practical considerations, 77–78
- Preimplantation genetic testing for aneuploidy (PGT-A), 76–77
- Preimplantation genetic testing for monogenic disorders (PGT-M), 77–78, 80
- Preimplantation genetic testing for structural rearrangements (PGT-SR), 76–77
- Prenatal diagnostic genetic testing, 75–76
 - amniocentesis, 75–76
 - chorionic villus sampling, 75–76
 - genetic testing modalities, 76
- Primary ciliary dyskinesia (PCD), 329–330
- Primary CoQ10 deficiency, 296
- Primary familial GIST syndrome, 198*t*
- Primary hyperparathyroidism, 169–170, 172, 175
- Primary immunodeficiencies (PIDs), 231, 239–241
 - Bruton agammaglobulinemia, 236–237
 - classification of, 232*t*
 - common variable immunodeficiency (CVID), 233–235
 - selective IgA deficiency, 235–236
- Primary mitochondrial diseases (PMDs), 285, 296
- Primary pigmented nodular adrenal cortical disease (PPNAD), 180–181
- Primary sclerosing cholangitis, 205
- Primary spontaneous pneumothorax (PSP), 332–333
 - etiologies of, 332*t*
- Progressive ataxia, palatal tremor (PAPT) syndrome, 287*t*
- Progressive external ophthalmoplegia (PEO), 286, 287*t*
- Progressive myoclonic epilepsy (PME), 321
- Progressive osseous heteroplasia (POH), 176
- Prolactinomas, 171, 178
- Prostate cancer, 87*t*, 97, 99, 188*t*
- Protein C deficiency, 220, 220*t*
- Protein S deficiency, 220, 220*t*
- Proteinuria, 278, 356–357, 360–361
- Proteus-like syndrome, 105
- Prothrombin deficiency, 220*t*
- Prothrombin time (PT), 214
- Proto-oncogene c-Kit, 455
- Pseudofractures, 376–377
- Pseudo-hypertrophy, 315
- Pseudohypoparathyroidism-related disorders, 176
- Pseudohypothyroidism related disorders, 176

Pseudopseudohypoparathyroidism (PPHP),
176–177

Psychiatric disorders, 339, 457
genetic condition, increased risk for mental
illnesses, 342–351
chromosome microdeletion/duplication
syndromes, 342–343
single-gene Mendelian disorders, 344–347
mental health conditions, 339–342
bipolar disorders, 341–342
generalized anxiety disorder, 340
major depressive disorder, 340
schizophrenia, 341

PTEN hamartoma tumor, 193*t*

Ptoxis, 312

Pulmonary arterial hypertension (PAH), 333–334

Pulmonary disorders

lung cancer, 336–337
obstructive lung disease
AAT deficiency, 325–327
cystic fibrosis (CF), 327–329
primary ciliary dyskinesia (PCD), 329–330
restrictive lung disease, 330–331
spontaneous pneumothorax, 332–333
vascular disorders, 333–336
hereditary hemorrhagic telangiectasia,
334–335
pulmonary arterial hypertension (PAH),
333–334

Pyrimidine 5' nucleotidase (cN-III) deficiency,
222*t*

Pyruvate kinase deficiency, 222*t*

Q

Quadruple screening, 74

Quantitative fluorescence PCR, 76

R

Raloxifene, 99–100

RASA1-related disorders, 402

RASA1-related Parkes Weber syndrome, 402

Recessive X-linked ichthyosis, genes associated
with, 399*t*

Recurrent pancreatitis, 201

Recurrent spontaneous abortions (SAB), 155

Red cell defects, 221–227

hemoglobinopathies, 224, 225*t*

red cell enzyme defects, 221–222, 222*t*

red cell membrane defects, 223–224, 223*t*

sickle cell disease and related syndromes,
224–226

sickle hemoglobin, 224–225

thalassemias, 226–227

Red cell enzyme defects, 221–222

Red cell membrane defects, 223–224, 223*t*

Reed syndrome. *See* Hereditary leiomyomatosis
and renal cell carcinoma (HLRCC)

Refsum disease, 308–309

Regorafenib, 198–199

Reimbursement of genetic testing, 415–416

appeals, 415–416

clinical laboratory fee schedule (CLFS), 415

denials of laboratory service claims, 415

prior authorization, 415

Renal cell carcinoma (RCC), 87*t*, 106, 252, 363–365

Renal disorders, 353

cystic renal diseases, 353–356, 354*t*

autosomal dominant polycystic kidney
disease, 354–355

autosomal recessive polycystic kidney
disease, 356

genetic disorders with renal involvement
congenital anomalies of the kidney and
urinary tract, 363

Fabry disease, 361–362

nail-patella syndrome (NPS), 362–363

papillorenal syndrome, 363

genomic medicine in adult nephrology, 366

hereditary renal cell carcinoma, 363–365

inherited glomerulopathies, 356–360

glomerular diseases with hematuria, 357

nephrotic syndrome, 360

types of cysts, 354*t*

Renal tumor, 94

Restrictive cardiomyopathy (RCM), 115

Restrictive lung disease, 330–331

Rhabdomyolysis, 274–276, 293

dystrophinopathies, 274

Limb-Girdle Muscle Dystrophy, 274

LPIN1 deficiency, 274

- metabolic-genetic etiology, 274
- metabolic myopathies, 274
- Pompe disease, 274
- RYR1-related disorders, 274
- RIDDLE syndrome, 240
- Risk-reducing salpingo-oophorectomy (RRSO), 99–100
- RNA sequencing, 53–54
- Robertsonian translocation, 13–14, 254
- Rothmund–Thomson syndrome (RTS), 388–389
- Rubinstein–Taybi syndrome, 250
- RUNX1 familial platelet disorder, 217–218
- RYR1-related disorders, 274
- RYR2* gene, 119
- S**
- Sanger sequencing, 48–49, 167–168
- Sarcoma, 87*t*
- Schizophrenia, 341
- SCN5A* gene, 112–113, 113*t*, 116–117
- Sebaceous neoplasms, 188*t*
- Secondary hypoglobulinemia, 235
- Second trimester serum screening, 74
- Second wind phenomenon, 274–275
- Seizures, 61, 320, 339, 395–396
 - topiramate for, 297–298
- Selective IgA deficiency, 235–236, 241
- Senile systemic amyloidosis, 122
- Sensorineural hearing loss (SNHL), 358
- Sensory ataxia, neuropathy, dysarthria, ophthalmoplegia (SANDO), 287*t*
- Septal ablation, 111–112
- Serrated polyposis syndrome (SPS), 190*t*
- Sertoli–Leydig cell tumor, 94, 180–181
- 17-hydroxyprogesterone (17OHP), 179–180
- 17p11.2 deletion syndrome, 343
- Severe combined immunodeficiency, 236
- Short QT syndrome (SQTS), 118, 120*t*
- Shprintzen–Goldberg syndrome, 144
- Shwachman–Diamond syndrome, 219
- Sickle- α thalassemia, 225*t*
- Sickle-beta thalassemia, 225*t*
- Sickle cell anemia, 43
- Sickle cell disease and related syndromes, 224–226
- Sickle hemoglobin (HbS), 224–225, 225*t*
- Sickle trait, 224–225
- Simple virilizing CAH, 179
- Single-nucleotide polymorphism (SNP), 74–75, 331, 347, 456
- Single-nucleotide polymorphism (SNP)-based microarrays, 47
- Single-nucleotide variant (SNV), 12–13, 44, 54
- Single-site variant testing, 202
- 16p11.2 deletion syndrome, 343
- 6-Mercaptopurine (6-MP), 456
- Skeletal disorders. *See* Genetic skeletal disorders (GSDs)
- Skeletal dysplasias, 79
- SKI* gene, 144
- Skin cancers, 87*t*, 387
- Skin disorders, 381
 - epidermis, disorders of, 396–401
 - Darier disease (DD), 400
 - Hailey–Hailey disease (HHD), 401
 - nonsyndromic ichthyosis, 399–400
 - neurocutaneous disorders, 392–396
 - neurofibromatosis type 1, 392–394
 - tuberous sclerosis complex, 394–396
 - photosensitivity disorders, 386–389
 - Rothmund–Thomson syndrome (RTS), 388–389
 - xeroderma pigmentosum, 387–388
 - pigmentation disorders, 381–386
 - hyperpigmentation, 381–382
 - hypopigmentation, 381–382
 - multiple lentigines syndromes, 384–386
 - neurocristopathies, 383–384
 - oculocutaneous albinism, 382–383
 - porphyrias, 389–392, 390*t*
 - vascular anomalies, 401–402
 - PI3K-related overgrowth spectrum, 401–402
 - RASA1-related disorders, 402
- SLC2A1-related epilepsy, 322
- SLCO1B1* genotyping, 457
- Sleep apnea, 62
- Small cell lung cancer (SCLC), 336–337
- Small intestine cancer, 188*t*
- Smith–Magenis syndrome, 250, 252, 343
- SMN1* gene, 50

Smooth muscle disorders. *See* Heritable thoracic aortic disease (HTAD)

Southern blotting, 72–73

Spermatogenesis, 155

Spinal muscular atrophy (SMA), 467

Exon 7 of SMN1 gene for, 72–73

in vivo gene therapy, 470

Spinal stenosis, 377–378

Spinocerebellar ataxias, 50, 307–308

Splenectomy, 224, 227

Spontaneous pneumothorax, 93–94, 252, 332–333

STAT3 mutations, 238

Statins, 122–124

Stickler syndrome, 142–143

Stop codons, 12–13

Stop-gain variants, 12–13

Streptococcus pneumoniae, 233, 235, 237

Stroke, 267–269

thromboembolic, 267

Stroke-like episodes, 44

Sudden cardiac death (SCD), 23–24, 110, 116, 119

Superficial epidermolytic ichthyosis, genes associated with, 399*t*

Survival motor neuron 1 (*SMN1*) gene, 470

Syndromic intellectual disability, 249–250, 252–257

Down syndrome, 251, 254–255, 255*t*

fragile X syndrome, 252–253

22q11.2 deletion syndrome (22q11.2 DS), 256–257

Systemic lupus erythematosus, 360

T

Tafamidis, 123

Tamoxifen, 99–100, 242–243, 336

Tangier disease, 278

Tay–Sachs disease, 72–73, 345

T-cell deficiency, disorders with, 239–242, 251
ataxia-telangiectasia (AT), 239–240
22q11.2 deletion syndrome (22q11.2 DS), 239–242

Telangiectatic skin lesions, 361

Telegenetics, 417

commercial use of, 423–424

equipment and technology for, 421

to support rural health providers, 422

Telehealth (TH)

in adult genetics, 418–421

genetic evaluation, 418–419

genetic service providers, 419

provider and patient satisfaction, 419–420

billing for, 412

defined, 417

E-consults and videoconsults, 422–423

software applications, 421

Telemedicine (TM), 32, 417–418

adult genetics, 418–421

billing and reimbursement for, 426–427

asynchronous TM services, 427

synchronous (live) TM services, 426–427

equipment, 421

remote patient monitoring, adult genetics, 420–421

standards and regulatory policy to, 424–426

documentation, 425–426

licensure, 425

privileging and credentialing, 425

Telomeres, 46–47

Testicular cancer, 87*t*

Testicular sperm extraction (TESE), 148–149

Testosterone therapy, 148–149

Tezacaftor, 328

TGF- β signaling pathway, 78, 128, 130–132

Thalassemias, 226–227

Thin basement membrane nephropathy, 358

Thoracic aortic aneurysm disease, 127, 133–134

1000 Genomes Project, 24

3 β -hydroxysteroid dehydrogenase deficiency, 153

Thrombocytopenia, 217–218, 251

Thromboembolic strokes, 267

Thrombophilia, 219–221, 220*t*

Thyroid cancer, 87*t*, 105–106, 173

Tissue nonspecific alkaline phosphatase (TNSALP), 376–377

TK2 deficiency, 298–299

Tolvaptan, 356

Total pancreatectomy with islet autotransplantation, 204

Toxic gain-of-function, 325
 Toxic loss-of-function mechanism, 325
 Transcription, 10*f*, 11
 Transient neonatal hypoglycemia, 167
 Transitioning to adult care
 Down syndrome, 62
 education and employment, 62–64, 63*t*
 health insurance, 64–65
 medical decision-making and guardianship, 65–66
 Noonan syndrome, 59–60
 psychosocial considerations, 60–62
 resource for specific genetic conditions, 60*t*
 transition planning, 66–68
 Transition planning, 66–68
 Translation, 10*f*, 11–12
 Transthyretin (TTR) amyloidosis, 115, 122–124
 Trastuzumab (Herceptin), 453
 Trichothiodystrophy (TTD), 387
 Trientine, 206–207
 Triheptanoin, 276
 Trisomies, 44–45
 Trisomy 13, 44–45, 73–75
 Trisomy 18, 44–45, 73–75
 Trisomy 21, 13, 44–45, 73–74, 252, 254
 Tuberous sclerosis complex (TSC), 346, 394–396
 Turner syndrome (TS), 13, 44, 78, 151–152
 21-Hydroxylase deficiency, 153
 22q11 deletion syndrome, 348
 22q11.2 deletion syndrome (22q11.2 DS), 74–75, 239–242, 256–257, 343
 22q11.2 microdeletion syndrome, 252
 Type 1 Brugada ECG, 118
 Type I collagen-related OI, 372–373
 Type 1 diabetes mellitus (DM), 168
 Type 1 papillary kidney cancers, 363–364
 Type 2 diabetes mellitus (DM), 163, 177
 Tyrer–Cuzick model, 97–98

U

Ungual fibromas, 395–396
 Uniparental disomy, 14, 50
 Uniparental isodisomy, 47
 Unverricht–Lundborg disease (ULD), 321
 Upper motor neuron (UMN), 311–312

Urea cycle, 261–262, 262*f*
 Urea cycle disorders (UCDs), 261–266, 263*t*, 346
 Urinary tract cancer, 188*t*
 Urinary tract infections (UTIs), 354–355
 Usher syndrome, 16–17
 Uterine leiomyomas, 100

V

Valproic acid, 266, 269, 297–298
 Variants of uncertain significance (VUS), 51–53, 72–73
 Variants of unknown significance (VUS), 14, 436
 Variegate porphyria, 390*t*
 Vascular anomalies, 401–402
 PI3K-related overgrowth spectrum, 401–402
 RASA1-related disorders, 402
 Vascular dementia, 316
 Vascular disorders, 333–336
 hereditary hemorrhagic telangiectasia (HHT), 334–336
 pulmonary arterial hypertension (PAH), 333–334
 Vascular Ehlers–Danlos syndrome (vEDS), 78, 133–134
 Vasculopathy, 361
 Vasoactive intestinal polypeptide-omas (VIPomas), 170
 Velocardiofacial syndrome (VCFS). *See* 22q11.2 deletion syndrome (22q11.2 DS)
 Ventricular arrhythmias, 114
 Ventricular fibrillation (VF), 111–112
 Ventricular tachycardia (VT), 111–112
 Verapamil, 111–112
 VHL syndrome. *See* Von Hippel–Lindau (VHL) syndrome
 Videoconsults, 422–423
 Vitamin B12 deficiency, 270–272
 Vitamin B12 metabolism, disorders of, 270
 Vitamin D, 140, 387
 Vitamin E deficiency, 308–309
 Vitamin K epoxide reductase (VKORC1), 456
 Vitamin supplements and cofactor therapy, 297
 Von Hippel–Lindau (VHL) syndrome, 100–101, 193*t*, 364–365
 Von Willebrand disease (VWD), 213–215
 Von Willebrand factor (VWF), 213–215

W

Waardenburg syndrome (WS), 381–384, 396
 Warfarin, 319, 448, 456
 Watson syndrome, 393
 West syndrome, 321
 Whole-exome sequencing (WES), 26–27, 35, 38, 76, 251, 257, 293
 Whole-genome sequencing (WGS), 26–27, 175–176, 293
 Whole transcriptome sequencing (RNAseq), 293
 Williams syndrome, 252
 7q11.23 FISH, 46–47
 Wilms tumor 1 (*WT1*) variants, 160
 Wilm’s tumors, 174
 Wilson disease, 205–207, 271*t*, 272, 346–347
 Wiscott–Aldrich syndrome, 237, 465–466

X

Xanthoma, 120–121
 Xeroderma pigmentosum (XP), 385, 387

X-linked adrenoleukodystrophy, 271*t*, 272, 278, 466
 X-linked Alport syndrome (XLAS), 358
 X-linked ataxia, 308
 X-linked Duchenne muscular dystrophy (DMD), 315–316
 X-linked genetic disorders, 72
 X-linked hypophosphatemia (XLH), 375–376
 X-linked (XL) inheritance, 15
 X-linked ornithine transcarbamylase (OTC) deficiency, 79–80
 X-linked severe combined immunodeficiency (X-SCID), 463–464

Y

Y chromosome microdeletions (YCMDs), 150–151

Z

Zinc finger nucleases (ZFNs), 471–473
 Zinc supplementation, 272

HANDBOOK OF CLINICAL ADULT GENETICS AND GENOMICS

A Practice-Based Approach

Editor-in-Chief

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Handbook of Clinical Adult Genetics and Genomics: A Practice-Based Approach provides an overview of genetic disorders that are commonly encountered in adults and facilitates the translation of genetic and genomic information into clinical practice. Expert chapter authors supplement foundational knowledge with case-based strategies for the evaluation and management of genetic disorders in each organ system and specialty area. Topics discussed include employing genetic testing technologies, genetic counseling, medical genetics referrals, personalized medicine, opportunities and challenges of gene therapy, and diagnostic and treatment criteria for cardiovascular, connective tissue, developmental, endocrinological, gastrointestinal, hematological, immunological, metabolic, mitochondrial, neuropsychiatric, pulmonary, reproductive, and skeletal disorders, as well as cancer. In this book, students, physicians, medical residents in various specialties, genetic counselors, and translational researchers will discover case-based approaches and descriptions of disorders in the context of presenting symptoms and clinical scenarios they are likely to encounter in practice.

Finally, *Handbook of Clinical Adult Genetics and Genomics* also offers a practical framework for developing an adult genetics clinic or incorporating adult genetics into an existing practice, by addressing infrastructure, billing, counseling, telegenetics, and various challenges unique to the practice of adult clinical genetics.

Key Features

- Employs clinical case studies to demonstrate how to evaluate, diagnose, and treat adult patients with genetic disorders
- Offers a practical framework for establishing an adult genetics clinic; addressing infrastructure, billing, counseling, and challenges that are unique to adult clinical genetics
- Features chapter contributions from authors at leading institutions in the USA

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