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limited to, variable penetrance, which may be significantly impacted by family history (thus, those individuals with germline pathogenic variants detected via population screening in the absence of a classic family history may have significantly lower risks of disease), consensus of which genes should be included, management of VUS, and practi cal issues of implementation including insurance coverage, counseling related to the limitations of GINA, the role of primary care providers in ordering, and concerns about the possibility of worsening socioeco nomic and racial disparities that already exist in genetic testing. THERAPEUTIC INTERVENTIONS BASED

ON GENETIC RISK FOR DISEASE Specific treatments are available for a number of genetic disorders. Strategies for the development of therapeutic interventions have a long history in childhood metabolic diseases; however, these principles have been applied in the diagnosis and management of adult-onset diseases as well (Table 480-2). Hereditary hemochromatosis is usually caused by pathogenic variants in HFE (although other genes have been less commonly associated) and manifests as a syndrome of iron overload, which can lead to liver disease, skin pigmentation, diabetes mellitus, arthropathy, impotence in males, and cardiac issues (Chap. 426). When identified early, the disorder can be managed effectively with therapeutic phlebotomy. Therefore, when the diagnosis of hemochro matosis has been made in a proband, it is important to counsel other family members in order to minimize the impact of the disorder. Preventative measures and therapeutic interventions are not restricted to metabolic disorders. Identification of familial forms of long QT syndrome, associated with ventricular arrhythmias, allows early electrocardiographic testing and the use of prophylactic antiar rhythmic therapy, overdrive pacemakers, or defibrillators. Individuals with familial hypertrophic cardiomyopathy can be screened by ultra sound, treated with beta blockers or other drugs, and counseled about the importance of avoiding strenuous exercise and dehydration. Those with Marfan’s syndrome can be treated with beta blockers or angioten sin II receptor blockers and monitored for the development of aortic aneurysms. The identification of

germline abnormalities that increase the risk of specific types of cancer is rapidly changing clinical manage ment. Identifying family members with pathogenic variants that pre dispose to FAP or Lynch syndrome leads to recommendations of early cancer screening and prophylactic surgery, as well as consideration of chemoprevention and attention to healthy lifestyle habits. Similar principles apply to familial forms of melanoma as well as cancers of the breast, ovary, and thyroid. There has been a significant growth in the number of molecularly directed therapies for genetic diseases including transthyretin stabiliz ers for TTR-associated cardiac amyloid; poly (ADP-ribose) polymerase (PARP) inhibitors for treatment of BRCA1/2 and PALB2-associated breast, ovarian, prostate, and pancreatic cancer; and medications for Duchenne's muscular dystrophy that can either promote exon skip ping or allow bypass of nonsense mutations. Gene therapy either by replacement, such as in spinal muscular atrophy, or in sickle cell dis ease, increasing production of fetal hemoglobin or hemoglobin A, is an exciting area with tremendous opportunities (Chap. 483). The field of pharmacogenetics identifies genes that alter drug metabolism or confer susceptibility to toxic drug reactions. Pharmaco genetics seeks to individualize drug therapy in an attempt to improve treatment outcomes and reduce toxicity. Examples include thiopurine methyltransferase (TPMT) deficiency, dihydropyrimidine dehydro genase deficiency, malignant hyperthermia, and glucose-6-phosphate deficiency. Despite successes in this area, it is not always clear how to incorporate pharmacogenetics into clinical care. For example, although there is an association with CYP2C6 and VKORC1 genotypes and war farin dosing, there is no evidence that incorporating genotyping into clinical practice improves patient outcomes compared with clinical algorithms. Although the role of genetic testing in the clinical setting continues to evolve, such testing holds the promise of allowing early and more targeted interventions that can reduce morbidity and mortality. Rapid technologic advances are changing the ways in which genetic testing

is performed. As genetic testing has become less expensive and tech nically easier to perform, there has been significant expansion of its use. This has created both challenges and opportunities. It is critical that physicians and other health care professionals keep current with advances in genetic medicine in order to facilitate appropriate referral for genetic counseling and judicious use of genetic testing, as well as to provide state-of-the-art, evidence-based care for affected or at-risk patients and their relatives.

CHAPTER 481 ■ ■FURTHER READING ACMG Board of Directors: Direct-to-consumer genetic testing: Mitochondrial DNA and Heritable Traits and Diseases
A revised position statement of the American College of Medical Genetics and Genomics. Genet Med 18:207, 2016. Anya ER et al: The Goldilocks conundrum: Disclosing discrimination risks in informed consent. J Genet Couns 31:1383, 2022. Dewey FE et al: Distribution and clinical impact of functional variants in 50,726 whole-exome sequences from the DiscovEHR study. Science 354:aaf6814, 2016. Food and Drug Administration. FDA direct to consumer tests. Available at https://www.fda.gov/medical-devices/in-vitro-diagnostics/ direct-consumer-tests. Accessed January 1, 2024. Hampel H et al: A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: Referral indications for cancer predisposition assess ment. Genet Med 17:70, 2015. Miller DT et al: ACMG SF v3.2 list for reporting of secondary find ings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med 25:100866, 2023. Robson ME et al: American Society of Clinical Oncology policy state ment update: Genetic and genomic testing for cancer susceptibil ity. J Clin Oncol 33:3660, 2015. Splinter K et al: Effect of genetic

diagnosis on patients with previously undiagnosed disease. N Engl J Med 379:2131, 2018. Turnbull C et al: Population screening requires robust evidencegenomics is no exception. Lancet 403:583, 2024. Karl L. Skorecki, Bruce H. Cohen

Mitochondrial DNA

and Heritable Traits

and Diseases Mitochondria are cytoplasmic organelles whose major function is to generate ATP by the process of oxidative phosphorylation under aerobic conditions. This process is mediated by the respiratory elec tron transport chain (ETC) multiprotein enzyme complexes I–V and the two electron carriers, coenzyme Q10 (CoQ10) and cytochrome c, located in the inner mitochondrial membrane. Other cellular processes to which mitochondria make a major contribution include apoptosis (programmed cell death) and additional cell type–specific functions (Table 481-1). The efficiency of the mitochondrial ETC in ATP pro duction is the major determinant of overall body energy balance and thermogenesis. In addition, mitochondria are the predominant source of reactive oxygen species (ROS), whose rate of production relates to the delicately balanced coupling of ATP production to oxygen consumption in health and disease. Given the centrality of oxidative phosphorylation to the normal activities of almost all cells, it is not surprising that mitochondrial dysfunction can affect almost any organ system (Fig. 481-1). Until recently, it was thought that disruption of

TABLE 481-1 Functions of Mitochondria All Cells and Tissues Oxidative phosphorylation Free radical production Calcium homeostasis Apoptosis (programmed cell death) PART 16 Genes, the Environment, and Disease Tissue- or Cell-Specific Cholesterol metabolism Amino and organic acid metabolism Fatty acid beta oxidation Sex steroid synthesis Heme synthesis Hepatic ammonia detoxification Neurotransmitter metabolism energy production was the source of the pathophysiology in those with mitochondrial dysfunction, but recent evidence suggests that free radi cal production and the redox state of the mitochondria may play a role as well. Thus, physicians in many disciplines might encounter patients with mitochondrial diseases and should be aware of their existence and characteristics. The integrated activity of an estimated 1500 gene products is required for normal mitochondrial biogenesis, function, maintenance, and integrity. Aside from the 37 genes that comprise the mitochondrial Heart Conduction disorder Wolff-Parkinson-White syndrome Cardiomyopathy Skeletal muscle Weakness Fatigue Myopathy Neuropathy Oxidative phosphorylation Subunits Nuclear DNA Brain Seizures Myoclonus Ataxia Stroke Dementia Migraine Mitochondrial DNA Nuclear DNA Inner ear Sensorineural hearing loss Colon Pseudo obstruction FIGURE 481-1 Dual genetic control and multiple organ system manifestations of mitochondrial disease. (From DR Johns: Mitochondrial DNA and disease. N Engl J Med 333:638, 1995. Copyright © 1995, Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.)

DNA (mtDNA) molecule, the remaining 1400+ gene products are encoded by nuclear genes (referred to as nDNA) and thus follow the rules and patterns of nuclear genomic inheritance (Chap. 479). These nuclear-encoded proteins are synthesized in the cell cytoplasm and imported to their location of activity within the mitochondria through a complex biochemical process. This process includes unfolding of the nuclear-encoded protein, attachment to a chaperone protein that shut tles

it through a specific channel to a specific mitochondrial location, and detachment from the chaperone followed by assembly with other mtDNA- and nDNA-encoded proteins. In addition, the mitochon dria contain their own small genome consisting of numerous copies (polyploidy) per mitochondrion of a circular, double-strand mtDNA molecule comprising 16,569 nucleotides. This mtDNA sequence (also known as the “mitogenome”) might represent the remnants of endo symbiotic prokaryotes from which mitochondria are thought to have originated. The mtDNA sequence contains a total of 37 genes, of which 13 encode mitochondrial protein components of the ETC (Fig. 481-2). The remaining 22 tRNA- and 2 rRNA-encoding genes are mitochondriaspecific and dedicated to the process of translating the 13 mtDNAencoded proteins. The mtDNA itself replicates constantly, independent of cell division, and requires its own unique polymerase, referred to as polymerase gamma (polγ), which is encoded by the nuclear gene POLG, disorders of which are discussed in Chaps. 460 and 480. However, mutations in POLG can disrupt the endonuclease function of polγ, resulting in somatic mutations in the mtDNA that endure with future replication. Unless this mutation occurs and is propagated in the oocyte, it is not heritable. Mutations in POLG can also affect the polymerase function of polγ that results in decreased replica tion of the mtDNA and can lead to mtDNA depletion. This dual nuclear and mitochondrial genetic control of mitochondrial function results in unique and diagnostically challenging patterns of inheritance. The current chapter focuses on heritable traits and diseases related to the mtDNA com ponent of the dual genetic control of mitochondrial function. The reader is referred to Chaps. 460 and 479 for consideration of mitochondrial disease originating from mutations in the nuclear genome. The former include (1) disorders due to mutations in nuclear genes directly encoding structural components or assembly factors of the oxidative phosphoryla tion complexes, (2) disorders due to mutations in nuclear genes encoding proteins indirectly related to oxidative phosphorylation, (3) mtDNA deple tion syndromes (MDSs) character ized by a reduction of mtDNA copy number in affected tissues without mutations or rearrangements in the mtDNA, and (4) disorders due to mutations in nuclear genes that dis rupt normal mitochondrial dynamics (biosynthesis, mitophagy, fission, and fusion). Eye Optic neuropathy Ophthalmoplegia Retinopathy Liver Hepatopathy ATP Kidney Fanconi’s syndrome Glomerulopathy Pancreas Diabetes mellitus Blood Pearson’s syndrome The classic physical structure of the mitochondria is that of a thread-like organelle, which under fixed condi tions, such as observed with immu nohistochemical stains or electron microscopy, has a submarine shape and measures about 1 μm in length. However, in the living state, mito chondria comprise a network, with the mitochondrial shape being highly

I II III FIGURE 481-2 Maternal inheritance of mitochondrial DNA (mtDNA) disorders and heritable traits. Affected women (filled circles) transmit the trait to their children. Affected men (filled squares) do not transmit the trait to any of their offspring. variable based on the cell type, and manifests a complex and everchanging syncytial form, with continuous appearance and disappear ance of budding structures (representing mitochondrial fission) and reorganization of separate mitochondria (representing mitochondrial fusion). Although mitochondrial number per cell type appears in the medical literature, this is no longer considered as a reliable expression of actual functional mitochondrial volume or mass. Although the presence of mitochondria has been known for

> 150 years, and knowledge of their respiratory function was pro posed ~100 years ago, the initial description of an illness linked to mitochondrial dysfunction was only made in 1962. The presence of mtDNA was also only reported in the 1960s, and it was not until 1988 that the first mutations in the mtDNA causing human illness were described. These included the demonstration of a large-scale mtDNA deletion causing Kearns-Sayre syndrome (KSS) and the discovery of a point mutation in ND4, an mtDNA-encoded complex I gene, causing Leber's hereditary optic neuropathy (LHON). Following these two discoveries, >400 pathogenic mtDNA mutations or deletions have been reported. MITOCHONDRIAL DNA STRUCTURE

AND FUNCTION As a result of its circular structure and extranuclear location, the rep lication and transcription mechanisms of mtDNA differ from the cor responding mechanisms in the nuclear genome, whose nucleosomal packaging and structure are more complex. Specifically, mitochondria have their own transcription system, and the mtDNA itself replicates independently of cellular replication. Because each cell contains many copies of mtDNA and because the number of mitochondria can vary during the lifetime of each cell, mtDNA copy number is not directly coordinated with the cell cycle. Thus, vast differences in mtDNA copy number are observed between different cell types and tissues and during the lifetime of a cell. Another important feature of the mtDNA replication process is a reduced stringency of proofreading and replication error correction, leading to a greater degree of sequence variation compared to the nuclear genome. Some of these sequence variants are silent polymorphisms that do not have the potential for a phenotypic or pathogenic effect, whereas others may be considered pathogenic mutations. There are some mutations that may be consid ered ecogenetic, as they typically remain silent, meaning they do not cause disease, unless an external event occurs. One classic example is seen in a common (1:800) mutation in the mitochondrial 12S rRNA gene, m.A1555G, which is associated with hearing loss that is rapidly exacerbated by exposure to normal dosages of an aminoglycoside anti biotic. Because mtDNA replication is independent of cellular replica tion, the percentage of mutant mtDNA copies tend to increase with age, especially in cells that are terminally differentiated (nonreplicative) at birth such as neurons and myocytes, which may explain some features of mitochondrial dysfunction with aging. With respect to transcription, initiation can occur on both strands and proceeds through the production of an intronless polycistronic precursor RNA, which is then processed to produce the 13 individual mRNA and 24 individual tRNA and rRNA products. The 37 mtDNA genes comprise fully 93% of the 16,569 nucleotides of the mtDNA in what is known as the coding region. The control region, which is

contained in the D-loop, consists of ~1.1 kilobases (kb) of noncod ing DNA and is thought to have an important role in replication and transcription initiation.

■ ■MATERNAL INHERITANCE AND LACK OF RECOMBINATION In contrast to homologous pair recombination that takes place in the nucleus, mtDNA molecules do not undergo recombination, such that mutational events represent the only source of mtDNA genetic diver sification. Moreover, it is only the maternal DNA that is transmitted to the offspring. The fertilized oocyte degrades the paternal mito chondria involving the ubiquitin proteasome system and autophagy that takes place on the inner membrane of the oocyte. However, additional studies suggest that human

spermatozoa do not contain intact mtDNA and are missing TFAM, the mitochondrial transcrip tion factor necessary for mtDNA transcription. Thus, although moth ers transmit their mtDNA to both their sons and daughters, only the daughters can transmit the inherited mtDNA to future generations. Accordingly, mtDNA sequence variation and associated phenotypic traits and diseases are inherited exclusively along maternal lines, meaning both sons and daughters have equal chances of having symptomatic disease, with the only significant exception being LHON, as described below. CHAPTER 481 Mitochondrial DNA and Heritable Traits and Diseases
The phenotypic expression, including age of onset and the specific pattern and severity of organ dysfunction, of a pathogenic mtDNA mutation may vary greatly, even within families. Because of this com plex relationship between mtDNA mutations and disease expression, sometimes it is difficult to recognize the maternal pattern of inheri tance at the clinical or pedigree level. However, evidence of paternal transmission can almost certainly exclude an mtDNA genetic origin of phenotypic variation or disease; conversely, a disease affecting both sexes without evidence of paternal transmission strongly suggests a heritable mtDNA disorder (Fig. 481-2). ■ ■MULTIPLE COPY NUMBER (POLYPLOIDY),

HIGH MUTATION RATE, HETEROPLASMY,

AND MITOTIC SEGREGATION Each aerobic cell in the body has multiple mitochondria, often num bering many hundreds or more in cells with extensive energy produc tion requirements. Furthermore, the number of copies of mtDNA within each mitochondrion varies from several to hundreds; this is true of both somatic as well as germ cells, including oocytes in females. In the case of somatic cells, this means that the impact of most newly acquired somatic mtDNA mutations is likely to be very small in terms of total cellular or organ system function; however, because of the manyfold higher mutation rate during mtDNA replication, numerous different mutations may accumulate with aging of the organism. It has been proposed that the total cumulative burden of acquired somatic mtDNA mutations with age may result in an overall perturbation of mitochondrial function, contributing to age-related reduction in the efficiency of oxidative phosphorylation and increased production of damaging ROS. Because certain mtDNA (and nDNA) mutations may result in electron leak within the ETC, the ROS damage may rise to a level causing increased susceptibility to somatic mtDNA damage and disease expression. The accumulation of such acquired somatic mtDNA mutations with aging may contribute to age-related diseases, such as metabolic syndrome and diabetes, cancer, and neurodegen erative and cardiovascular disease in any given individual. However, somatic mutations are not carried forward to the next generation, and the hereditary impact of mtDNA mutagenesis requires separate consid eration in the female germline. The multiple mtDNA copy number within each cell, including the maternal germ cells, results in the phenomenon of heteroplasmy, in contrast to the much greater uniformity (homoplasy) of somatic nuclear DNA sequence. Heteroplasmy for a given mtDNA sequence variant or mutation arises as a result of the coexistence within a cell, tissue, or individual of mtDNA molecules bearing more than one version of the sequence variant (Fig. 481-3). The importance of the heteroplasmy phenomena to the understanding of mtDNA-related

PART 16 Genes, the Environment, and Disease Cell division Mutated mtDNA Mitochondrial bottleneck Wild-type mtDNA Nondividing cell FIGURE 481-3 mtDNA genetic bottleneck and changes of heteroplasmy level throughout the lifetime. Each oocyte can inherit a different proportion of mutated mtDNA molecules from maternal mitochondria. When cells divide (shown in pink),

heteroplasmy levels in each daughter cell can either increase, decrease, or stay approximately the same. Once inherited, mtDNA mutations can continuously “clonally expand” throughout life, even in nondividing cells (shown in green, blue, and yellow). If one genotype is copied more frequently than another, it will change the overall proportion of different genotypes within the cell over time. The direction of this change can be influenced by selection for or against a particular mtDNA variant (shown in blue and yellow). When a mutated mtDNA molecule has a replicative advantage, the level will increase during life and possibly exceed the biochemical threshold, and thus contribute to the age-related pathologies or the aging process (shown in the blue box). (Reproduced from W Wei, PF Chinnery: Inheritance of mitochondrial DNA in humans: Implications for rare and common diseases. J Intern Med 2020; 287:634.) mitochondrial diseases is critical. The coexistence of mutant and nonmutant (wild-type) mtDNA and the variation of the mutant load, which can be thought of as the percentage of mutant mtDNA mol ecules within a specific cell, tissue, organ, or organism, contribute to the expression of a phenotype among individuals from the same maternal sibship. At the level of the oocyte, the percentage of mtDNA molecules bearing each version of the polymorphic sequence vari ant or mutation depends on stochastic events related to partitioning of mtDNA molecules during the process of oogenesis itself. Thus, oocytes differ from each other in the degree of heteroplasmy for that sequence variant or mutation. In turn, the heteroplasmic state is car ried forward to the zygote and to the organism as a whole, to varying degrees, depending on mitotic segregation of mtDNA molecules during organ system development and maintenance. For this reason, in vitro fertilization, followed by preimplantation genetic diagnosis (PGD), is not as predictive of the genetic health of the offspring in the case of mtDNA mutations as in the case of mutations and subsequent diseases occurring in the nuclear genome. Similarly, the impact of somatic mtDNA mutations acquired during development and subse quently also shows a wide spectrum of variability. In general, a higher mutant load will result in a more severe and earlier phenotypic presen tation. However, measuring heteroplasmy in one tissue (lymphocytes from blood or urine sediment containing kidney and bladder epithe lial cells, for example) may not represent the percentage of mutant heteroplasmy in the tissue or organs most affected, such as the cardiac atrioventricular node or brain. Furthermore, the threshold of mutant heteroplasmy that results in clinical illness may vary depending on the specific mutation.

Mitotic segregation refers to the unequal distribution of wild-type and mutant versions of mtDNA molecules during all cell divisions that occur during prenatal development and subsequently throughout the lifetime of an individual. The phenotypic effect or disease impact will be a function not only of the inherent disruptive effect (pathogenicity) on the mtDNA-encoded gene (coding region mutations) or integrity of the mtDNA mol ecule (control region mutations) but also of its distribution among the multiple copies of mtDNA in the various mitochondria, cells, and tissues of the affected individual. Thus, one consequence can be the generation of a bottleneck due to the marked decline in given sets of mtDNA variants, pathogenic and non pathogenic, consequent to such mitotic segre gation. It is postulated that the main effects of this bottleneck occur between the primordial germ cell state and the primary oocyte stage of development. Heterogeneity arises from differences in the degree of heteroplasmy among oocytes of the transmitting female, together with subsequent, probably random, mitotic segregation of the pathogenic muta tion during tissue and organ development and throughout the lifetime of the individual offspring. The actual expression of disease is believed to primarily depend on a threshold percentage of mitochondria whose function is disrupted by mtDNA mutations. This in turn confounds hereditary transmission patterns and hence genetic diagnosis of pathogenic heteroplasmic mutations. Generally, if the

proportion of mutant mtDNA is <60%, the individual is unlikely to be affected, whereas proportions exceeding 90% likely result in clinical disease. One notable exception is LHON, in which these mutations are present either in 100% mutant homoplasmy, which causes the disease expression, or 100% wild-type homoplasmy. It is not understood why this specific phenotype and the several known mtDNA alleles that result in LHON behave in this manner. Homoplasmy Heteroplasmy mtDNA randomly replicated Through aging/ selection for replication Selection against replication ■ ■HOMOPLASMIC VARIANTS AND

HUMAN mtDNA PHYLOGENY In contrast to classic mtDNA diseases, most of which have clinical onset during childhood and are the result of heteroplasmic mutations as noted above, during the course of human evolution, certain mtDNA sequence variants have drifted to a state of homoplasmy, wherein all of the mtDNA molecules in the organism contain the new sequence variant. This arises due to a “bottleneck” effect followed by genetic drift during the very process of oogenesis itself (Fig. 481-3). In other words, during certain stages of oogenesis, the mtDNA copy number becomes so substantially reduced that the particular mtDNA species bearing the novel or derived sequence variant may become the increas ingly predominant, and eventually exclusive, version of the mtDNA for that particular nucleotide site. All of the offspring of a woman bearing an mtDNA sequence variant or mutation that has become homoplas mic will also be homoplasmic for that variant and will transmit the sequence variant forward in subsequent generations. Considerations of reproductive fitness limit the evolutionary or population emergence of pathogenic homoplasmic mutations that are lethal or cause severe disease in infancy or childhood. Thus, with a number of notable exceptions (e.g., as noted mtDNA mutations causing LHON; and see below), most homoplasmic mutations are considered to be neutral markers of human evolution, which are useful and interesting in the population genetics analysis of shared maternal

ancestry but have little significance in human phenotypic variation or disease predisposition. More important is the understanding that this accumulation of homoplasmic mutations occurs at a genetic locus that is transmit ted only through the female germline and that lacks recombination. In turn, this enables reconstruction of the sequential topology and radiating phylogeny of mutations accumulated through the course of human evolution since the time of the most recent common mtDNA ancestor of all contemporary mtDNA sequences, some 200,000 years ago. The term haplogroup is usually used to define major branching points in the human mtDNA phylogeny, nested one within the other, which often demonstrate striking continental geographic ancestral partitioning. At the level of the complete mtDNA sequence, the term haplotype is usually used to describe the sum of mutations observed for a given mtDNA sequence and as compared to a reference sequence, such that all haplotypes falling within a given haplogroup share the total sum of mutations that have accumulated since the most recent common ancestor and the bifurcation point they mark. The remaining observed variants are private to each haplotype. Con sequentially, the human mtDNA sequence serves with high fidelity as a molecular prototype for a nonrecombining locus, and its variation has been extensively used in phylogenetic studies. Moreover, the mtDNA mutation rate is considerably higher than the rate observed for the nuclear genome, especially in the control region, which contains the displacement loop, or D-loop, in turn comprising two adjacent hypervariable regions (HVR-I and HVR-II). Together with the absence of recombination, this amplifies drift to high frequencies of novel haplotypes that are highly partitioned across geographically defined populations. Despite extensive research, it has not been well established that such haplotype-based partitioning has a significant influence on human health

conditions. However, mtDNA-based phylogenetic analysis can be used both as a quality assurance tool and as a filter in distinguishing neutral mtDNA variants comprising human mtDNA phylogeny from potentially deleterious mutations. Parkinsonism, aminoglycoside-induced deafness LS, MELAS, multisystem disease Cardiomyopathy PEO, LHON, MELAS, myopathy, cardiomyopathy, diabetes and deafness MITOCHONDRIAL DNA DISEASE The true prevalence of mtDNA disease is difficult to estimate because of the phe notypic heterogeneity that occurs as a function of heteroplasmy, the challenge of detecting and assessing heteroplasmy in different affected tissues, and the other unique features of mtDNA func tion and inheritance described above. It is estimated that at least 1 in 200 healthy humans harbors a pathogenic mtDNA mutation with the potential to causes disease but that heteroplasmic germline pathogenic mtDNA mutations actually result in clinical disease in ~1 in 5000 individuals. LHON Cyt b Myopathy, cardiomyopathy, PEO Myopathy, lymphoma Myopathy, MELAS Cardiomyopathy LHON LS, ataxia, chorea, myopathy PEO Myopathy, PEO ECM PEO Myoglobinuria, motor neuron disease, sideroblastic anemia PPK, deafness, MERRF-MELAS Cardiomyopathy myoclonus The true disease burden relating to mtDNA sequence variation will only be known when the following capabilities become available: (1) ability to distin guish a completely neutral sequence variant from a true phenotype-modify ing or pathogenic mutation, (2) accu rate assessment of heteroplasmy that can be determined with high fidelity, and (3) a systems biology approach (Chap. 499) to determine the network of epistatic interactions of mtDNA sequence variations with mutations in the nuclear genome. FIGURE 481-4 Mutations in the human mitochondrial genome known to cause disease. Disorders that are frequently or prominently associated with mutations in a particular gene are shown in boldface. Diseases due to mutations that impair mitochondrial protein synthesis are shown in blue. Diseases due to mutations in protein-coding genes are shown in red. ECM, encephalomyopathy; FBSN, familial bilateral striatal necrosis; LHON, Leber’s hereditary optic neuropathy; LS, Leigh’s syndrome; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; MILS, maternally inherited Leigh’s syndrome; NARP, neuropathy, ataxia, and retinitis pigmentosa; PEO, progressive external ophthalmoplegia; PPK, palmoplantar keratoderma; SIDS, sudden infant death syndrome. (From S DiMauro, E Schon: Mitochondrial respiratory-chain diseases. N Engl J Med 348:2656, 2003. Copyright © 2003, Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.)

■ ■OVERVIEW OF CLINICAL AND PATHOLOGIC FEATURES OF HUMAN mtDNA DISEASE Given the vital roles of mitochondria in all nucleated cells, it is not surprising that mtDNA mutations can affect numerous tissues with pleiotropic effects. More than 200 different disease-causing, mostly heteroplasmic mtDNA mutations have been described affecting ETC function. Figure 481-4 provides a partial mtDNA map of some of the better characterized of these disorders. A number of clinical clues can increase the index of suspicion for a heteroplasmic mtDNA mutation as an etiology of a heritable trait or disease, including (1) familial clustering with absence of paternal transmission; (2) adherence to one of the classic syndromes (see below) or paradigmatic combinations of disease phenotypes involving several organ systems that normally do not fit together within a single nuclear genomic mutation category; (3) a complex of laboratory and pathologic abnormalities that reflect disruption in cellular energetics (e.g., lactic acidosis and neurodegen erative and myodegenerative symptoms with the finding of ragged red fibers, reflecting the accumulation of abnormal mitochondria under the muscle sarcolemmal membrane); or (4) a mosaic pattern reflect ing a heteroplasmic state. There are no truly sensitive and specific biomarkers of disease, and the presence of a historically quintessential finding of ragged red fibers can be seen in numerous

muscle disorders, so laboratory tests must always be interpreted in the context of their limitations and should not be used to define the disease. Because of the improved availability and decreasing cost for mtDNA sequencing, the presence or absence of a pathogenic mtDNA mutation can be diag nostic when the clinical phenotype and family history are suggestive.

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Heteroplasmy can sometimes be elegantly demonstrated at the tissue level using histochemical staining for enzymes in the oxidative phos phorylation pathway, with a mosaic pattern indicating heterogeneity of the genotype for the coding region for the mtDNA-encoded enzyme. MELAS myoglobinuria Myopathy, PEO Cardiomyopathy ECM ECM, LHON, myopathy, cardiomyopathy, MELAS and parkinsonism 16S V 12sF PT Cardiomyopathy ECM L1 E ND1 LHON, MELAS, diabetes, LHON and dystonia ND6 M Q I ND2 ND5 LS, MELAS Cardiomyopathy, ECM PEO, myopathy, sideroblastic anemia Y C N A W L2 S2 H Diabetes and deafness COXI ND4 LHON, myopathy, LHON and dystonia D S1 ND4L ND3 COXIII COXII R G K A6 A8 LHON Progressive myoclonus, epilepsy, and optic atrophy Myopathy, multisystem disease, encephalomyopathy NARP, MILS, FBSN Cardiomyopathy, SIDS, ECM Cardiomyopathy, PEO, MERRF, MELAS, deafness LS, ECM, myoglobinuria

Complex II, CoQ, and cytochrome c are exclusively encoded by nuclear DNA. In contrast, complexes I, III, IV, and V contain at least some sub units encoded by mtDNA. Just 3 of the 13 subunits of the ETC complex IV enzyme, cytochrome c oxidase (COX), are encoded by mtDNA, and therefore, this enzyme has the lowest threshold for dysfunction when a threshold level of mutated mtDNA is reached. Histochemical stain ing for COX activity in tissues of patients affected with heteroplasmic inherited mtDNA mutations (or with the somatic accumulation of mtDNA mutations, see below) can show a mosaic pattern of reduced histochemical staining in comparison with histochemical staining for the complex II enzyme succinate dehydrogenase (SDH) (Fig. 481-5).

PART 16 Genes, the Environment, and Disease Next-generation sequencing (NGS) has dramatically improved the clinical genetic diagnostic evaluation of mitochondrial diseases at the level of both the nuclear genome and mtDNA. Low sequencing costs, high throughput, and short turnaround time expedite whole exome (WES) or whole genome sequencing (WGS) to identity genes and mutations with known pathogenicity or based on bioinformat ics assessment of likely pathogenicity. In the context of the mtDNA, the deep coverage enabled by NGS compared to Sanger sequencing now provides rapid and reliable detection of heteroplasmy in differ ent affected tissues. NGS yields accurate information about a patient’s predominant mtDNA sequence as well as lower frequency heteroplas mic variants and can reliably reach detection of even single mutant nucleotide heteroplasmy down to levels of <10%. Lower levels are often only clinically relevant if in the setting of a striking difference in heteroplasmy in different tissues. Clinically, the most striking overall characteristic of mitochondrial genetic disease is the phenotypic heterogeneity associated with mtDNA mutations. This extends to intrafamilial phenotypic heterogeneity for the same mtDNA pathogenic mutation and, conversely, to the overlap of phenotypic disease manifestations with distinct mutations. Thus, although fairly consistent and well-defined “classic” syndromes have been attributed to specific mutations, frequently “nonclassical” com binations of disease phenotypes ranging from isolated myopathy to extensive multisystem disease are often encountered, rendering geno type-phenotype correlation challenging. In both classical and nonclas sical mtDNA disorders, there is often a clustering of some combination A B C D E FIGURE 481-5 Cytochrome c

oxidase (COX) deficiency in mitochondrial DNA (mtDNA)–associated disease. Transverse tissue sections that have been stained for COX and succinate dehydrogenase (SDH) activities sequentially, with COX-positive cells shown in brown and COX-deficient cells shown in blue. A. Skeletal muscle from a patient with a heteroplasmic mitochondrial tRNA point mutation. The section shows a typical “mosaic” pattern of COX activity, with many muscle fibers harboring levels of mutated mtDNA that are above the crucial threshold to produce a functional enzyme complex. B. Cardiac tissue (left ventricle) from a patient with a homoplasmic tRNA mutation that causes hypertrophic cardiomyopathy, which demonstrates an absence of COX in most cells. C. A section of cerebellum from a patient with mtDNA rearrangement that highlights the presence of COX-deficient neurons. D, E. Tissues that show COX deficiency due to clonal expansion of somatic mtDNA mutations within single cells—a phenomenon that is seen in both postmitotic cells (D; extraocular muscles) and rapidly dividing cells (E; colonic crypt) in aging humans. (Reproduced with permission from R Taylor, D Turnbull: Mitochondrial DNA mutations in human disease. Nat Rev Genetics 6:389, 2005.)

of abnormalities affecting the neurologic system (including optic nerve atrophy, pigment retinopathy, and sensorineural hearing loss), cardiac and skeletal muscle (including extraocular muscles), and endocrine and metabolic systems (including diabetes mellitus). Additional organ systems that may be affected include the hematopoietic, renal, hepatic, and gastrointestinal systems, although these are more frequently involved in infants and children. Disease-causing mtDNA coding region mutations can affect either one of the 13 protein-encoding genes or one of the 24 protein synthetic genes. Clinical manifesta tions do not readily distinguish these two categories, although lactic acidosis and specific muscle pathologic findings (e.g., ragged red and ragged blue fibers, immunohistochemical staining, paracrystalline inclusions on ultrastructure) tend to be more prominent in the latter. In all cases, either defective ATP production due to disturbances in the ETC or enhanced generation of ROS has been invoked as the mediat ing biochemical mechanism between mtDNA mutation and disease manifestation. ■ ■mtDNA DISEASE PRESENTATIONS The clinical presentation of adult patients with mtDNA disease can be divided into three categories: (1) clinical features suggestive of mitochondrial disease (Table 481-2) but not a well-defined classic syndrome; (2) classic mtDNA syndromes; and (3) clinical presentation confined to one organ system (e.g., isolated sensorineural deafness, cardiomyopathy, or diabetes mellitus). It is important to note, espe cially when young adults come to medical attention, that symptoms of an mtDNA disorder may have begun during childhood. Table 481-3 provides a summary of eight illustrative classic mtDNA syndromes or disorders that affect adult patients and highlights some of the most interesting features of mtDNA disease in terms of molecu lar pathogenesis, inheritance, and clinical presentation. The first five of these syndromes result from heritable point mutations in either protein-encoding or protein synthetic mtDNA genes; the other three result from rearrangements or deletions that usually do not involve the germline. LHON is a common cause of maternally inherited visual failure. LHON typically presents during young adulthood with subacute painless loss of vision in one eye, with symptoms developing in the other eye 6–12 weeks later. In some instances, cerebellar ataxia, peripheral neuropa thy, and cardiac conduction defects are observed. In >95% of cases, LHON is due to one of the three homoplas mic point mutations of mtDNA that affect genes encoding different sub units of complex I of the mitochon drial ETC; however, not all individuals who inherit a primary LHON mtDNA mutation develop optic neuropathy, and the male-to-female ratio is 8.2, indi cating that additional environmental (e.g., tobacco exposure) or independent genetic factors are important in the eti ology of the disorder.

Estrogen may also play a role in the decreased clinical pen etrance in women. Both the nuclear and mitochondrial genomic backgrounds modify disease penetrance. Indeed, a region of the X chromosome containing a high-risk haplotype for LHON has been identified, supporting the formu lation that nuclear genes act as modifi ers and affording an explanation for the male prevalence of LHON. This haplo type can be used in predictive genomic testing and prenatal screening for this disease. In contrast to the other classic mtDNA disorders, it is of interest that

TABLE 481-2 Common Features of Mitochondrial DNA–Associated Diseases in Adults Neurologic: stroke, epilepsy, migraine headache, peripheral neuropathy, ataxia, dystonia, myoclonus, cranial neuropathy (optic atrophy, sensorineural deafness, dysphagia, dysphasia) Skeletal myopathy: ophthalmoplegia, exercise intolerance, myalgia, weakness Cardiac: conduction block, cardiomyopathy Respiratory: hypoventilation, aspiration pneumonitis Endocrine: diabetes mellitus, premature ovarian failure, hypothyroidism, hypoparathyroidism Ophthalmologic: cataracts, pigment retinopathy, neurologic and myopathic (optic atrophy, ophthalmoplegia) patients with this syndrome are often homoplasmic for the diseasecausing mutation. The somewhat later onset in young adulthood and modifying effect of protective background nuclear genomic haplotypes may have enabled homoplasmic pathogenic mutations to have escaped evolutionary censoring. Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is a multisystem disorder with a typical onset between 2 and 10 years of age, although adult presentations also occur. Following normal early psychomotor development, the most com mon initial symptoms are seizures, recurrent headaches, anorexia, and recurrent vomiting. Exercise intolerance or proximal limb weakness can be the initial manifestation, followed by generalized tonic-clonic seizures. Short stature is common. Seizures are often associated with stroke-like episodes of transient hemiparesis or cortical blindness that may produce recurrent encephalopathy with impaired consciousness. It is often not possible to determine if the encephalopathy is due to refractory clinical or subclinical seizures or should be attributed to an independent effect. The cumulative residual effects of the stroke-like episodes gradually impair motor abilities, vision, and cognition, often by adolescence or young adulthood. Sensorineural hearing loss adds to the progressive decline of these individuals. A plethora of less common symptoms have been described including myoclonus, ataxia, episodic coma, optic atrophy, cardiomyopathy, pigmentary retinopathy, ophthal moplegia, diabetes mellitus, hirsutism, gastrointestinal dysmotility, and nephropathy. The typical age of death ranges from 10 to 35 years, but some individuals live into their sixth decade. Intercurrent infections or intestinal obstructions are often the terminal events. It is proposed that the clinical diagnosis of MELAS can only be applied if the following three criteria are met: (1) stroke-like episode before age 40 years, (2) encephalopathy due to seizures and/or dementia, and (3) lactic acidosis and/or ragged red fibers. It is not atypical for some family members to have much less severe or later onset illness, presumably because of a TABLE 481-3 Mitochondrial Diseases Due to Mitochondrial DNA (mtDNA) Point Mutations and Large-Scale Rearrangements DISEASE PHENOTYPE NARP, Leigh’s syndrome Loss of central vision leading to blindness in young adult life MELAS Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; may manifest only as diabetes mellitus MERRF Myoclonic epilepsy, ragged red fibers in muscle, ataxia, increased CSF protein, sensorineural deafness, dementia Deafness Progressive sensorineural deafness, often induced by aminoglycoside antibiotics Nonsyndromic sensorineural deafness m.7445A>G mutation in 12S rRNA Chronic progressive external ophthalmoplegia (PEO) Late-onset bilateral ptosis and ophthalmoplegia, proximal muscle weakness, and exercise intolerance Pearson’s syndrome Pancreatic insufficiency, pancytopenia, lactic acidosis Large

deletion Heteroplasmic Sporadic, somatic mutations Kearns-Sayre syndrome (KSS) External ophthalmoplegia, heart block, retinal pigmentation, ataxia Abbreviations: CSF, cerebrospinal fluid; NARP, neuropathy, ataxia, and retinitis pigmentosa.

lessor mutation load, and “MELAS” is not used as a diagnosis for these restricted phenotypes. This creates somewhat of a disconnect between the genotype for MELAS (most commonly the m.3243A>G mutation) and a diverse phenotype, which includes the syndrome MELAS, a syndrome of high-frequency hearing loss and diabetes with onset later in life, as well as many other phenotypes between these two extreme syndromes. Certain other mtDNA mutations can also cause such patterns of diverse phenotypic expression. Laboratory investigation commonly demonstrates elevated blood lactate concentrations at rest with excessive increase after moderate exercise. Magnetic resonance imaging (MRI) of the brain shows areas of involvement on T2- or fluid-attenuated inversion recovery (FLAIR) sequences, with decreased signal on perfusion-weighted sequences, which typically involve the posterior cerebrum and do not conform to the distribution of major arteries. These abnormalities may be temporary or evolve to subse quent atrophy (Fig. 481-6). Electrocardiography (ECG) may show evidence of cardiomyopathy, preexcitation, or incomplete heart block. Electromyography and nerve conduction studies are consistent with a myopathic process, without or with coexisting axonal and sensory neuropathic findings. Muscle biopsy typically shows ragged red fibers with the modified Gomori trichrome stain or “ragged blue fibers” with the SDH histochemical stain, resulting from the hyperintense reaction. The diagnosis of MELAS is based on a combination of clinical findings and molecular genetic testing. Mutations in the mtDNA gene MT-TL1 encoding tRNAleu are causative. The most common mutation, pres ent in ~80% of individuals with typical clinical findings, is an A-to-G transition at nucleotide 3243 (m.3243A>G). Mutations can usually be detected in mtDNA from leukocytes in individuals with typical MELAS; however, the occurrence of heteroplasmy can result in vary ing tissue distribution of mutated mtDNA. In the absence of specific treatment, various manifestations of MELAS are treated according to standard modalities for prevention, surveillance, and treatment. Recent developments in therapy are described below.

Myoclonus epilepsy with ragged red fiber (MERRF) is a multisystem disorder characterized by myoclonus, seizures, ataxia, and myopathy with ragged red fibers. Hearing loss, exercise intolerance, neuropathy, ataxia, cervical lipomas, and short stature are often present. Ataxia and lipomas can be a feature in adults or adult-onset MERRF. Cerebrospi nal fluid (CSF) analysis reveals an elevated protein content. Almost all MERRF patients have a mutation in the mtDNA tRNAlys gene, and the m.8344A>G mutation in the mtDNA gene encoding the lysine amino acid tRNA is responsible for 80–90% of MERRF cases. Neuropathy, ataxia, and retinitis pigmentosa (NARP) is character ized by moderate diffuse cerebral and cerebellar atrophy and sym metric lesions of the basal ganglia on MRI (Figs. 481-7 and 481-8). A heteroplasmic m.8993T>G mutation in the ATPase 6 subunit gene MOST FREQUENT mtDNA MUTATIONS HETEROPLASMIC/ HOMOPLASMIC MATERNAL m.1778G>A, m.14484T>C, m.3460G>A Heteroplasmic Maternal Point mutation in tRNAleu Heteroplasmic Maternal Point mutation in tRNAlys Heteroplasmic Maternal m.1555A>G mutation in 12S rRNA Homoplasmic Maternal Homoplasmic Maternal Single deletions or duplications Heteroplasmic Mostly sporadic, somatic mutations The 5-kb “common deletion” Heteroplasmic Sporadic, somatic mutations

PART 16 Genes, the Environment, and Disease FIGURE 481-6 A 15-year-old girl with MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) due to m.A3243G (tRNALeu(UUR)), 85% mutant heteroplasmy, presenting at age 5 with focal motor seizures, ataxia, and short stature, with episodes of acute language and motor dysfunction and progress cognitive impairment. The fluid-attenuated inversion recovery (FLAIR) magnetic resonance image (MRI) shows increased signal intensity (white arrows) in the left temporal-parietal region in addition to global mild volume loss (increased extraaxial cerebrospinal fluid spaces). has been identified as causative, which underscores the lack of defini tive genotype-phenotype correlation in mtDNA diseases. Ragged red fibers are not observed in muscle biopsy. When >95% of mtDNA molecules are mutant, a more severe clinical, neuroradiologic, and FIGURE 481-7 A 9-year-old girl with Leigh's syndrome due to m.T8993G (ATPase subunit 6), 99% heteroplasmy, presenting at age 14 months with a motor delay and who underwent magnetic resonance imaging (MRI) at 24 months, at which time she had just begun to walk. She has moderate cognitive impairment, arm chorea, and distal leg dystonia. The fluid-attenuated inversion recover (FLAIR) MRI shows symmetric bilateral increased signal in the caudate nuclei (thin arrow) and putamen (thick arrow); only left-sided lesions indicated with arrows.

FIGURE 481-8 A 12-year-old boy with Leigh's syndrome due to m.T10191C (ND3 gene, complex I), heteroplasmy percentage not determined, presenting with infantile spasms at 8 months of life. He responded well to adrenocorticotropic hormone (ACTH), and his magnetic resonance imaging (MRI) and development were normal until 30 months when he developed dystonia and progressive medically intractable epilepsy. The fluid-attenuated inversion recover (FLAIR) MRI at 6 years of life shows global atrophy with large extra-axial cerebrospinal fluid spaces, increased signal intensity in the cortex (thin arrows), necrotic bilaterally symmetric lesions in the putamina, and enlarged lateral ventricles due to loss of bilateral caudate nuclei volume (stars). neuropathologic picture (Leigh's syndrome) emerges. Not uncom monly, an infant is diagnosed with Leigh's syndrome due to the m.8993T>G mutation and not until several years later will the mother present with symptoms of NARP, a situation that highlights the concept of a higher threshold for lower levels of tissue heteroplasmy. Point mutations in the mtDNA gene encoding the 12S rRNA (m.A1555G) result in heritable nonsyndromic hearing loss. One such mutation causes heritable ototoxic susceptibility to standard dosing of aminoglycoside antibiotics, which opens a pathway for a simple phar macogenetic test in the appropriate clinical settings. This is an example of an ecogenetic disorder in that most people with this mutation do not develop any symptoms until exposed to an external agent. KSS, sporadic progressive external ophthalmoplegia (PEO), and Pearson's syndrome are three disease phenotypes caused by large-scale mtDNA rearrangements including partial deletions or partial duplica tion. The majority of single large-scale rearrangements of mtDNA are thought to result from clonal amplification of a single sporadic muta tional event, occurring in the maternal oocyte during early embryonic development. The typical mtDNA deletion specifically involves 4977 nucleotides, lost at identical breakpoints, and accounting for most KSS and PEO of mtDNA deletion origin. Because germline involvement is rare, most cases are sporadic rather than inherited. KSS is characterized by the triad of onset before age 20, chronic PEO, and pigmentary reti nopathy. Cerebellar syndrome, heart block, increased CSF protein con tent, diabetes mellitus, and short stature are also part of the syndrome. Single deletions/duplication can also result in milder phenotypes such as PEO, characterized by late-onset PEO, proximal myopathy, and exer cise intolerance. In both KSS and PEO, diabetes mellitus and hearing loss are frequent accompaniments. Pearson's syndrome is characterized by infantile onset of a sideroblastic anemia accompanied by

lactic aci dosis and failure to thrive caused in part by exocrine pancreatic insuf ficiency. If the child survives, the manifestations appear phenotypically similar to those of severe KSS with myopathy, PEO, encephalopathy, and cardiomyopathy. Pearson's syndrome is generally caused by

large-scale sporadic deletion of several mtDNA genes that differ from the common deletion seen in KSS. Typically, the deletion size is larger in Pearson's syndrome, and located with different breakpoints, than in KSS or PEO, but this is not always the case. Two important dilemmas in classic mtDNA disease have benefited from recent important research insights. The first relates to the greater involvement of neuronal, muscular, renal, hepatic, and pancreatic manifestations in mtDNA disease in these syndromes. This observa tion has appropriately been mostly attributed to the high energy uti lization of the involved tissues and organ systems and, hence, greater dependency on mitochondrial ETC integrity and health. However, because mutations are stochastic events, mitochondrial mutations should occur in any organ during embryogenesis and development. Recently, additional explanations have been suggested based on studies of the common m.3243A>G transition. The proportion of this muta tion in peripheral blood cells was shown to decrease exponentially with age. A selective process acting at the stem cell level with a strong bias against the mutated form would have its greatest effect to reduce the mutant mtDNA only in highly proliferating cells, such as those derived from the hematopoietic system. Tissues and organs carrying pathogenic mtDNA mutations and having a lower cell turnover, such as brain, nerve, or retina, would not benefit from this effect and, thus, would be expected to accumulate mutational load and be the most affected. However, age-related clonal hematopoiesis might mitigate some of this disparity. The other dilemma arises from the observation that only a sub set of mtDNA mutations accounts for the majority of the familial mtDNA diseases. The random occurrence of mutations in the mtDNA sequence should yield a more uniform distribution of disease-causing mutations. However, recent studies using the introduction of one severe and one mild point mutation into the female germline of experi mental animals demonstrated selective elimination during oogenesis of the severe mutation and selective retention of the milder mutation, with the emergence of mitochondrial disease in offspring after multiple Clinical Investigations – Initial Biochemical Screening Blood: CK, liver functions, glucose, lactate, carnitine/acylcarnitines, amino acids, GDF-15 Urine: organic acids, amino acids CSF: glucose, protein, lactate, amino acids Cardiac: ECG, ECHO Brain: MRI with MRS (or CT) Nerve/Muscle: EMG, nerve conduction Depending on Available Technology, Select

- Specific mtDNA point mutations with LR-PCR (mtDNA); or
- Whole mtDNA genome (NextGen) with LR-PCR (mtDNA); or
- WES or WGS (including mtDNA genome) with LR-PCR (mtDNA) Muscle Biopsy Immunohistochemistry Respiratory Chain Enzymology Special Testing on Muscle
- LR-PCR (mtDNA)
- mtDNA whole genome sequencing
- mtDNA depletion quantification FIGURE 481-9 Clinical and laboratory investigation of a suspected mitochondrial DNA (mtDNA) disorder. Following history including the family history and examination, a screening biochemical evaluation and other testing is selectively warranted, and if the evaluation suggests a mitochondrial disease, further genetic evaluation is warranted. The specific molecular genetic testing depends on available technology and costs, with clinical acumen essential for determining the extent of testing. The use of muscle investigation can support the genetic testing. Of note,

mtDNA deletion disorders at times require LR-PCR on skeletal muscle tissue to find the deletion. CSF, cerebrospinal fluid; CT, computed tomography; ECG, electrocardiogram; ECHO, echocardiography; EEG, electroencephalogram; EMG, electromyogram; LHON, Leber's hereditary optic neuropathy; LR-PCR, long-range polymerase chain reaction; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stoke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy, PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; WES, whole exome sequencing; WGS, whole genome sequencing.

generations. Thus, oogenesis itself can act as an "evolutionary" filter for the most harmful mtDNA disease.

■ ■THE INVESTIGATION OF SUSPECTED

mtDNA DISEASE The clinical presentations of classic syndromes, groupings of disease manifestations in multiple organ systems, or unexplained isolated presentations of one of the disease features of a classic mtDNA syndrome should prompt a systematic clinical investigation as outlined in Fig. 481-9. However, some tests are not universally available or are costly, and WGS is not only more readily available but also less costly than the cost of obtaining tissue such as muscle for biochemical and pathologic evaluation. In many medical centers, a history and exam suggestive of an mtDNA disorder will result in a molecular genetic evaluation before tissue evaluation. Indeed, mitochondrial disease should be considered in the differential diagnosis of any progressive multisystem disorder. Despite the centrality of disruptive oxidative phosphorylation, an elevated blood lactate level is neither specific nor sensitive, because there are many causes of blood lactic acidosis and many patients with mtDNA defects presenting at any age may have normal blood lactate levels. An elevated CSF lactate is a more spe cific test for mitochondrial disease if there is central nervous system involvement but is still not diagnostic. The serum creatine kinase may be elevated but is often normal, even in the presence of a proximal myopathy. Recently, testing for elevated levels of growth differentiat ing factor 15 (GDF15) has shown a high degree of sensitivity and specificity in those with a mitochondrial myopathy, but the degree of elevation for an individual patient reflects the severity of the illness and does not seem to be a sensitive marker of disease activity. Urinary organic acids (specifically TCA cycle intermediates) and amino acids (alanine, proline) may also be abnormal, reflecting metabolic as well as kidney proximal tubule dysfunction. Every patient with seizures, epi sodes of confusion or atypical behavioral changes, or cognitive decline should have an electroencephalogram. A brain computed tomography

(CT) scan may show calcified basal ganglia or bilateral hypodense regions with cortical atrophy. MRI is indicated in patients with brainstem signs or stroke-like episodes.

For an increasing number of mitochondrial diseases, it is possible to obtain an accurate diagnosis with a simple molecular genetic screen. For examples, 95% of patients with LHON harbor one of the three mtDNA point muta tions (m.11778A>G, m.A3460A>G, or m.14484T>C). These patients have very high levels of mutated mtDNA in peripheral blood cells, and therefore, it is appropriate to send a blood sample for molecular genetic analysis by polymerase chain reaction (PCR) or

restriction fragment length polymorphism (RFLP). The same is true for most MERRF patients who harbor a point mutation in the lysine tRNA gene at position 8344. In contrast, patients with the m.3243A>G MELAS mutation often have low levels of mutated mtDNA in blood. If clinical suspicion is strong enough to warrant peripheral blood testing, then patients with a negative result should have testing repeated using a saliva sample or be investigated further by performing a skeletal muscle biopsy to obtain mtDNA from a relatively nonreplicative tissue. ANT1 adPEO PART 16 Genes, the Environment, and Disease Deoxyguanosine kinase MPV17 Thymidine kinase (TK2) RRMB2 (p53-R2) Succinyl-CoA synthase (SUCLA2, SUCLG1) TP Thymidine phosphorylase FIGURE 481-10 Disorders associated with perturbations in nuclear-mitochondrial genomic crosstalk. Clinical features and genes associated with multiple mitochondrial DNA (mtDNA) deletions, mtDNA depletion, and mitochondrial neurogastrointestinal encephalomyopathy syndromes. adPEO, autosomal dominant progressive external ophthalmoplegia; ANT, adenine nucleotide translocators; arPEO, autosomal recessive progressive external ophthalmoplegia; IOSCA, infantile-onset spinocerebellar ataxia; SCAE, spinocerebellar ataxia and epilepsy. (Reproduced with permission from A Spinazzola, M Zeviani: Disorders from perturbations of nuclear-mitochondrial intergenomic cross-talk. J Intern Med 265:174, 2009.) Muscle biopsy histochemical analysis had been the historical cornerstone for investigation of patients with suspected mitochondrial disease. Histochemical analysis may show subsarcolemmal accumulation of mitochon dria with the appearance of ragged red fibers, especially in those with mtDNA mutations affecting the tRNA and rRNA genes. Electron microscopy might show abnormal mitochondria with paracrystalline inclusions. Muscle histochemistry may show COX-deficient fibers, which indi cate mitochondrial dysfunction (Fig. 481-5). Respiratory chain complex assays may also show reduced enzyme function. If enzy matic or polarographic data are used to aid in the confirmation of diagnosis, a standard method of analysis should be employed. Either of these two abnormalities, within the exact context of established peer-reviewed criteria, may confirm the presence of a mitochondrial disease, to be followed by an in-depth molecular genetic analysis. In most major centers, genetic testing has become the primary means of obtaining a definitive diagnosis, using muscle pathology and bio chemistry to assist with interpretation of inconclusive genetic results. It is proposed to use of the term primary mitochondrial disease only when a pathogenic mutation is identified that matches the clinical phenotype. Recent evidence has provided important insights into the impor tance of nuclear-mtDNA genomic cross-talk and has provided a descriptive framework for classifying and understanding disorders that emanate from perturbations in this cross-talk. Although not strictly considered as mtDNA genetic disorders, manifestations do overlap those highlighted above (Fig. 481-10). IMPACT OF HOMOPLASMIC SEQUENCE VARIATION ON HERITABLE TRAITS AND DISEASE The relationship among the degree of heteroplasmy, tissue distribution of the mutant mtDNA, and disease phenotype simplifies inference of a clear causative relationship between heteroplasmic mutation and disease. With the exception of certain mutations (e.g., those causing most cases of LHON), drift to homoplasmy of such mutations would be precluded normally by the severity of impaired oxidative phosphoryla tion and the consequent reduction in reproductive fitness. Therefore, sequence variants that have reached homoplasmy should be neutral in terms of human evolution and, hence, useful for tracing phylogeny, demography, and migration, as described above. Thus, novel homo plasmic variants are seldom pathogenic. One important exception is in the case of one or more of the homoplasmic population-level variants, which designate the mtDNA haplogroup J, and the interaction with the mtDNA mutations causing LHON. Reduced disease predilection

Multiple ΔmtDNA adPEO arPEO Pol γ adPEO A B Twinkle mtDNA depletion Pol γA Twinkle Patient Control Alpers' like IOSCA Alpers's. SCAE dNTP pool Pyrimidine salvage suggests that one or more of the ancient sequence variants designating mtDNA haplogroup J appear to attenuate predisposition to degen erative disease, in the presence of other risk factors. Whether or not additional epistatic interactions between population-level mtDNA haplotypes and common health conditions will be found remains to be determined. If such influences do exist, then they are more likely to be relevant to health conditions in the postreproductive age groups, wherein evolutionary filters would not have had the opportunity to censor deleterious effects and interactions and wherein the effects of oxidative stress during aging or with poor diet or lack of exercise may play a role. Although much has been written about the possible associations between population-level common mtDNA variants and human health and disease phenotypes or adaptation to different envi ronmental influences (e.g., climate), clinical implications have not been forthcoming. Many studies that purport to show such associations with phe notypes such as longevity, athletic performance, and metabolic and neurodegenerative disease are limited by small sample sizes, possible genotyping inaccuracies, and the possibility of population stratifica tion or ethnic ancestry bias. Because mtDNA haplogroups are so prominently partitioned along phylogeographic lines, it is difficult to exclude the possibility that a haplogroup for which an association has been reported is simply a marker for otherwise unappreciated popula tion heterogeneity, wherein a nongenetic (societal or environmental) difference among the populations marked by the mtDNA haplogroup differences is actually causally related to the disease of interest. The experimental difficulty in generating cellular or animal models to test the functional influence of homoplasmic sequence variants (as a result of mtDNA polyploidy) further compounds the challenge. The most likely formulation is that the risk conferred by different mtDNA haplogroup-defining homoplasmic mutations for common diseases depends on the concomitant nuclear genomic background, together with environmental influences. Progress in minimizing potentially misleading associations in mtDNA heritable trait and disease stud ies should include ensuring adequate sample size taken from a large sample recruitment base, using carefully matched controls and population structure determination, and performing analysis that takes into account epistatic interactions with other genomic loci and environmental factors.

IMPACT OF ACQUIRED SOMATIC

mtDNA MUTATION ON HUMAN

HEALTH AND DISEASE Studies on aging humans and animals have shown a potentially important correlation of age with the accumulation of heterogeneous mtDNA mutations, especially in organ systems that undergo the most prominent age-related degenerative tissue phenotype. Sequencing of PCR-amplified single mtDNA molecules has demonstrated an average of two-to-three-point mutations per molecule in elderly subjects when compared with younger ones. Point mutations observed include those responsible for known heritable heteroplasmic mtDNA disorders, such as the m.3344A>G and m.3243A>G mutations responsible for the MERRF and MELAS syndromes, respectively. However, the cumula tive burden of these acquired somatic point mutations with age was observed to remain well below the threshold expected for phenotypic expression (<2%). Point mutations at other sites not normally involved in inherited mtDNA disorders have also been shown to accumulate to much higher levels in some tissues of elderly individuals, with the description of tissue-specific "hot spots" for acquired somatic mtDNA point mutations. Likewise, an age-

associated and tissue-specific accu mulation of mtDNA deletions has been observed, including deletions involved in known heritable mtDNA disorders, as well as others. The accumulation of functional mtDNA deletions in a given tissue is expected to be associated with mitochondrial dysfunction, as reflected in an age-associated patchy and reduced COX activity on histochemi cal staining, especially in skeletal and cardiac muscle and brain. A particularly well-studied and potentially important example is the accumulation of mtDNA deletions and COX deficiency observed in neurons of the substantia nigra in Parkinson's disease patients. The progressive accumulation of ROS has been proposed as the key factor connecting mtDNA mutations with aging and age-related dis ease pathogenesis (Fig. 481-11). As noted above, ROS are a by-product of normal oxidative phosphorylation and are removed by detoxifying antioxidants into less harmful moieties; however, environmental factors or mutations that result in exaggerated production of ROS or impaired Damaged mitochondrial proteins Error-prone DNA Pol-γ Mutant mitochondrial proteins Decreased DNA repair O2 X − O2 X DNA mutations H2O2 H2O OH ROS Nuclear DNA damage Apoptosis Aging FIGURE 481-11 Multiple pathways of mitochondrial DNA (mtDNA) damage and aging. Multiple factors may impinge on the integrity of mitochondria that lead to loss of cell function, apoptosis, and aging. The classic pathway is indicated with blue arrows; the generation of reactive oxygen species (ROS; superoxide anion, hydrogen peroxide, and hydroxyl radicals), as a by-product of mitochondrial oxidative phosphorylation, results in damage to mitochondrial macromolecules, including the mtDNA, with the latter leading to deleterious mutations. When these factors damage the mitochondrial energy-generating apparatus beyond a functional threshold, proteins are released from the mitochondria that activate the caspase pathway, leading to apoptosis, cell death, and aging. (Reproduced with permission from L Loeb et al: The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mt DNA mutations. Proc Natl Acad Sci USA 102 (52):18769-18770, 2005.)

removal result in ROS accumulation and subsequent cellular injury. One of the main targets for ROS-mediated injury is DNA, and mtDNA is particularly vulnerable because of its proximity to the origin of free radical production, the lack of protective histones, and less efficient injury repair systems compared with nuclear DNA. In turn, accumula tion of mtDNA mutations results in inefficient oxidative phosphoryla tion, with the potential for excessive production of ROS, generating a "vicious cycle" of cumulative mtDNA damage. Indeed, measurement of the oxidative stress biomarker 8-hydroxy-2-deoxyguanosine has been used to measure age-dependent increases in mtDNA oxidative damage at a rate exceeding that of nuclear DNA. It should be noted that mtDNA mutations can potentially occur in postmitotic cells as well, because mtDNA replication is not synchronized with the cell cycle. Two other proposed links between mtDNA mutation and aging, besides ROS-mediated tissue injury, are the perturbations in efficiency of oxidative phosphorylation with disturbed cellular aerobic func tion and perturbations in apoptotic pathways, whose execution steps involve mitochondrial activity.

Genetic intervention studies in animal models have sought to clarify the potential causative relationship between acquired somatic mtDNA mutation and the aging phenotype and the role of ROS in particular. Replication of the mitochondrial genome is mediated by the activity of the nuclear-encoded POLG. A transgenic homozygous mouse knockin mutation of this gene renders the polymerase enzyme deficient in proofreading and results in a threefold to fivefold increase in mtDNA mutation rate. Such mice develop a premature aging phenotype, which includes

subcutaneous lipoatrophy, alopecia, kyphonia, and weight loss with premature death. Although the finding of increased mtDNA muta tion and mitochondrial dysfunction with age has been solidly established, the causative role and specific contribution of mitochondrial ROS to aging and age-related disease in humans have yet to be proved. Similarly, although many tumors display higher levels of heterogeneous mtDNA mutations, a causal relationship to tumorigenesis has not been proved. Besides the age-dependent acquired accumulation in somatic cells of heterogeneous point mutations and deletions, a quite different effect of nonheritable and acquired mtDNA mutations has been described affecting tissue stem cells. In particular, disease phenotypes attributed to acquired mtDNA mutation have been observed in sporadic and apparently nonfamilial cases involving a single individual or even tis sue, usually skeletal muscle. The presentation consists of decreased exercise tolerance and myalgias, sometimes progressing to rhabdo myolysis. As in the case of the sporadic, heteroplasmic, large-scale deletion, classic syndromes of chronic PEO, Pearson’s syndrome, and KSS, the absence of a maternal inheritance pattern and the finding of limited tissue distribution suggest a molecular pathogenic mechanism emanating from mutations arising de novo in muscle stem cells after germline differentiation (somatic mutations that are not sporadic and occur in tissue-specific stem cells during fetal development or in the postnatal maintenance or postinjury repair stage). Such mutations would be expected to be propagated only within the progeny of that stem cell and affect a singular tissue within a given individual, without evidence of heritability. PROSPECTS FOR CLINICAL MANAGEMENT OF mtDNA DISEASE ■ ■TREATMENT OF mtDNA DISORDERS No specific curative treatment for mtDNA disorders is currently available; therefore, the management of mitochondrial disease is largely supportive. Management issues may include early diagnosis and medical management of epilepsy, gastrointestinal dysfunction, weakness, diabetes mellitus, cardiac dysrhythmia, hearing loss, endo crinopathy, ptosis, and cataracts. Rapid identification of subclinical seizures, which may present with focal neurologic signs or even mild mental status changes, is critical in the management of MELAS and other mtDNA disorders associated with epilepsy. The value of aggres sive symptom management cannot be understated. Less specific interventions in the case of other disorders involve combined treat ment strategies including dietary intervention and removal of toxic

metabolites. Cofactors and vitamin supplements are widely used in the treatment of diseases of mitochondrial oxidative phosphoryla tion, although there is little evidence, apart from anecdotal reports, to support their use. This includes administration of artificial electron acceptors, including vitamin K3, vitamin C, and ubiquinone (CoQ10); administration of cofactors (coenzymes) including riboflavin, car nitine, and creatine; and use of oxygen radical scavengers, such as vitamin E, copper, selenium, ubiquinone, and idebenone. Drugs that could interfere with mitochondrial function, such as the anesthetic agent propofol, barbiturates, and high doses of valproate, can gen erally be avoided if possible. The use of valproate in patients with pathogenic mutations in POLG and possibly other mutations affect ing mtDNA stability and replication is especially contraindicated. Supplementation with the nitric oxide synthase substrate l-arginine and, more recently, l-citrulline has been advocated as a vasodilator treatment during stroke-like episodes as well as for chronic manage ment in patients with MELAS. Open-label studies demonstrate that levoarginine and levocitrulline may be helpful in reducing the strokelike symptoms in MELAS but may have serious side effects. As CSF folate deficiency has been reported in some cases of mitochondrial disease, this can be treated with oral folinic acid.

PART 16 Genes, the Environment, and Disease The physician should also be familiar with environmental interac tions, such as the strong and consistent association between visual loss in LHON and smoking or ethanol consumption. A clinical penetrance of 93% was found in men who smoked. Asymptomatic carriers of an LHON mtDNA mutation should, therefore, be strongly advised not to smoke and to moderate their alcohol intake. Although not a cure, these interventions might stave off the devastating clinical manifesta tions of the LHON mutation. Another example is strict avoidance of aminoglycosides in the familial syndrome of ototoxic susceptibility to aminoglycosides in the presence of the mtDNA m.1555A>G mutation of the 12SrRNA encoding gene. Clinical trials using novel agents have been initiated and launched. These agents include elamipretide (Stealth Biotherapeutics) and KL-1333 (Abliva). In an open-label study of α-tocotrienol used to treat 10 children with Leigh's syndrome, there were improvements in the primary endpoints, including the Newcastle Pediatric Mitochondrial Diseases Scale, the Gross Motor Function Measure, and the PedsQL Neuromuscular Module. GENETIC COUNSELING, PRENATAL DIAGNOSIS, AND PGD IN

mtDNA DISORDERS The provision of accurate genetic counseling and reproductive options to families with mtDNA mutations is challenging due to the unique genetic features of mtDNA inheritance that distinguish it from Mendelian genetics. mtDNA defects are transmitted by mater nal inheritance. mtDNA de novo mutations are often large deletions, affect one family member, and usually represent no significant risk to other members of the family. In contrast, mtDNA point mutations or duplications can be transmitted maternally. Accordingly, the father of an affected individual has no risk of harboring the disease-causing mutation, and a male cannot transmit the mtDNA mutation to his offspring. In contrast, the mother of an affected individual usually harbors the same mutation but may be completely asymptomatic. This wide phenotypic variability is primarily related to the phenomena of heteroplasmy and the mutation load carried by different members of the same family. Consequently, a symptomatic or asymptomatic female harboring a disease-causing mutation in a heteroplasmic state will transmit to her offspring variable amounts of the mutant mtDNA molecules. The offspring will be symptomatic or asymptomatic pri marily according to the mutant load transmitted via the oocyte and, to some extent, subsequent mitotic segregation during development. Interactions with the mtDNA haplotype background or nuclear human genome (as in the case of LHON) serve as an additional important determinant of disease penetrance. Because the severity of the disease phenotype associated with the heteroplasmic mutation load is a function of the stochastic differential segregation and copy

number of mutant mtDNA during the oogenesis bottleneck and, sub sequently, following tissue and organ development in the offspring, it is rarely predictable with any degree of accuracy. For this reason, prenatal diagnosis (PND) and PGD techniques that have evolved into integral and well-accepted standards of practice are severely ham pered in the case of mtDNA-related diseases. The value of PND and PGD is limited, partly due to the absence of data on the rules that govern the segregation of wild-type and mutant mtDNA species (heteroplasmy) among tissue in the devel oping embryo. Three factors are required to ensure the reliability of PND and PGD: (1) a close correlation between the mutant load and the disease severity, (2) a uniform distribution of mutant load among tissues, and (3) no major change in mutant load with time. These criteria are suggested to be fulfilled for the NARP m.8993T>G mutation but do not seem to apply to other mtDNA disorders. In fact, the level of mutant mtDNA in a chorionic villous or amniotic fluid sample may be very different from the level in the fetus, and it would be difficult to deduce whether the mutational load in the prenatal samples provides clinically useful information regarding the

postnatal and adult state. ■ ■PREVENTION OF MITOCHONDRIAL

DISEASE INHERITANCE BY ASSISTED REPRODUCTIVE TECHNOLOGIES Because the treatment options for patients with mitochondrial dis ease are rather limited, with no current U.S. Food and Drug Admin istration (FDA)–approved therapies for established mitochondrial DNA disease, preventive interventions that eliminate the likelihood of transmission of affected mtDNA into offspring are desirable. The poor reliability of prenatal and preimplantation approaches in pre dicting mitochondrial DNA disease has resulted in the search for alternative preventive approaches. The common purpose underly ing various emerging approaches is to reduce mutant heteroplasmy levels to a level below a pathogenic threshold. This is based on the observed relationship between heteroplasmy and disease inheritance patterns, which indicates that even a small increase in copy number of nonmutant mtDNA molecules in the fertilized egg can exceed the threshold required to ameliorate serious clinical disease. Use of gene editing, with clustered regularly interspaced short palindromic repeats (CRISPR) or mitochondrial-targeted TALEN (transcrip tion activator-like effector nucleases) technology, and others, for example, to shift the heteroplasmy load in affected tissues will require future development of corrective gene delivery techniques. Likewise, induced pluripotent cell technology has not yet met with widespread success in the preclinical research setting. This has prompted the application of mitochondrial replacement therapy (MRT) approaches (Fig. 481-12). These approaches substitute in vitro the entire oocyte or zygote complement of mitochondria, together with their mtDNA from the carrier mother, with the unaffected complement of mito chondria and their unaffected mtDNA from a donor woman. This can be accomplished either by removing and transferring the car rier mother’s spindle with her nuclear DNA into the unfertilized oocyte of the donor or, alternatively, by transferring the pronucleus from the fertilized oocyte of the carrier mother to the unfertilized donor oocyte from which the pronucleus has been removed. These approaches provide a “bulk” substitution and hence do not target the specific mtDNA mutation, and they are potentially applicable to a wide variety of mtDNA disorders. This is a form of germline genetic therapy, and therefore, it projects onto future generations in the case of a female offspring. Accordingly, ethical and regulatory bodies have appropriately weighed in on the societal implications of such approaches and have been tentatively supportive of human clinical investigation for situations that would prevent great suffering and when the clinical need is clear and unambiguous, subject to specified conditions and principles and subject to ethical scrutiny. Several such studies have been initiated, and careful examination and follow-up are needed to determine developmental and longer-term health and fertility of children who have undergone genetic manipulation at the earliest stages of human development and whose genomes comprise

FIGURE 481-12 Mitochondrial replacement techniques—maternal spindle transfer and pronuclear transfer. In both procedures, some mutant mtDNA, estimated at 1–2%, might be carried over together with the spindle or pronucleus, but the levels are low enough to avoid disease risk. IVF, in vitro fertilization. (From MJ Falk et al: Mitochondrial replacement techniques—Implications for the clinical community. N Engl J Med 374:1103, 2016. Copyright © 2016, Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.) separate maternal origins of nuclear and mtDNA genomes. It has been recommended that such studies be limited to male offspring, who cannot then transmit the donor mtDNA to future generations, until such time as the health, ethical, and societal issues are well understood and live up to the exciting promise of reducing the bur den of clinical mtDNA disease in the future. ■ ■FURTHER READING Alston CL et

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