e39 Mitochondrial DNA and Heritable Traits and Diseases

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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 electron transport chain (ETC) multiprotein enzyme complexes I–V, and the two electron carriers, coenzyme Q (CoQ) and cytochrome-c. Other cellular processes to which mitochondria make a major contribution include apoptosis (programmed cell death), as well as additional cell-type specific functions (Table e39-1). The efficiency of the mitochondrial ETC in ATP production is a major determinant of overall body energy balance and thermogenesis. In addition, mitochondria serve as the predominant source for the generation of reactive oxygen species (ROS), whose rate of production also relates to the coupling of ATP production to oxygen consumption. 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. e39-1). Thus, physicians in many specialties might encounter patients with mitochondrial diseases and should be aware of their existence and characteristics.

TABLE e39-1 FUNCTIONS OF MITOCHONDRIA

All cells and tissues
Oxidative phosphorylation
Apoptosis (programmed cell death)
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

The integrated activity of several hundred proteins is required for normal mitochondrial biogenesis, function, and integrity. Most of these are encoded by nuclear genes and thus follow the rules and patterns of nuclear genomic inheritance (see Part 3, Genetics and Disease). These nuclear-encoded proteins are synthesized in the cell cytoplasm and imported to their location of activity in mitochondria through a complex biochemical process. In addition, the mitochondria themselves have their own genome consisting of numerous copies (polyploidy) per mitochondrion of a circular, double-strand mitochondrial DNA (mtDNA) molecule consisting of a 16,569-nucleotide sequence. This mtDNA sequence contains a total of 37 genes, of which 13 encode mitochondrial protein components of the ETC (Fig. e39-2). The remaining 22 tRNA- and 2 rRNA-encoding genes are dedicated to the process of translation of the 13 mtDNA-encoded proteins. This dual genetic control of mitochondrial function can result in fascinating patterns of inheritance, which may be challenging to unravel. The current chapter focuses on diseases and heritable traits related to the mtDNA component of the dual genetic control of mitochondrial function. The reader is referred elsewhere for consideration of mitochondrial disease originating from mutations in the nuclear genome. The latter include (1) nuclear genomic mutations that disrupt the integrity of the mitochondrial genome itself (mtDNA deletion and depletion states), (2) disorders due to mutations in nuclear genes encoding structural components or assembly factors of the oxidative phosphorylation complexes, and (3) mitochondrial disorders due to mutations in nuclear genes encoding proteins indirectly related to oxidative phosphorylation.

MITOCHONDRIAL DNA (mtDNA) STRUCTURE AND FUNCTION

As a result of its circular structure and extranuclear location, the replication and transcription mechanisms of mtDNA differ from the corresponding mechanisms in the nuclear genome, whose nucleosomal packaging and structure is more complex. In terms of mtDNA replication, at least two major models have been proposed, which differ principally in whether the two strands of the mtDNA double helix replicate simultaneously or consecutively. Since each mitochondrion contains many copies of mtDNA, and because the number of mitochondrion per cell can vary during the lifetime of a cell through the processes of fission, fusion, and mitochondrial biogenesis, mtDNA copy number per mitochondrion and per cell can also vary within the lifetime of a cell, and it is not directly coordinated with the cell cycle. Thus, it is not surprising that 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 greatly reduced stringency of proofreading and replication error correction, leading to a much greater degree of sequence variation compared to the nuclear genome. Some of these sequence variants are silent polymorphisms that do not

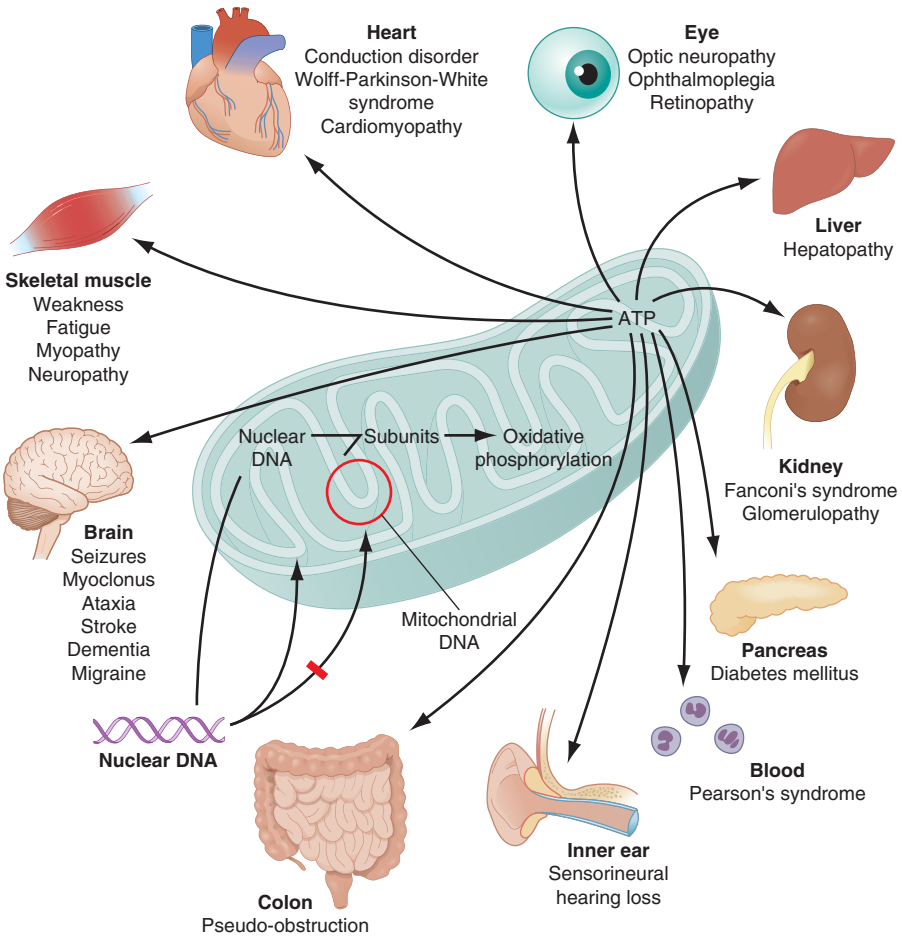

FIGURE e39-1 Dual genetic control and multiple organ system manifestations of mitochondrial disease. *(Reproduced with permission from DR Johns: Mitochondrial DNA and disease. N Engl J Med 333:638, 1995.)*

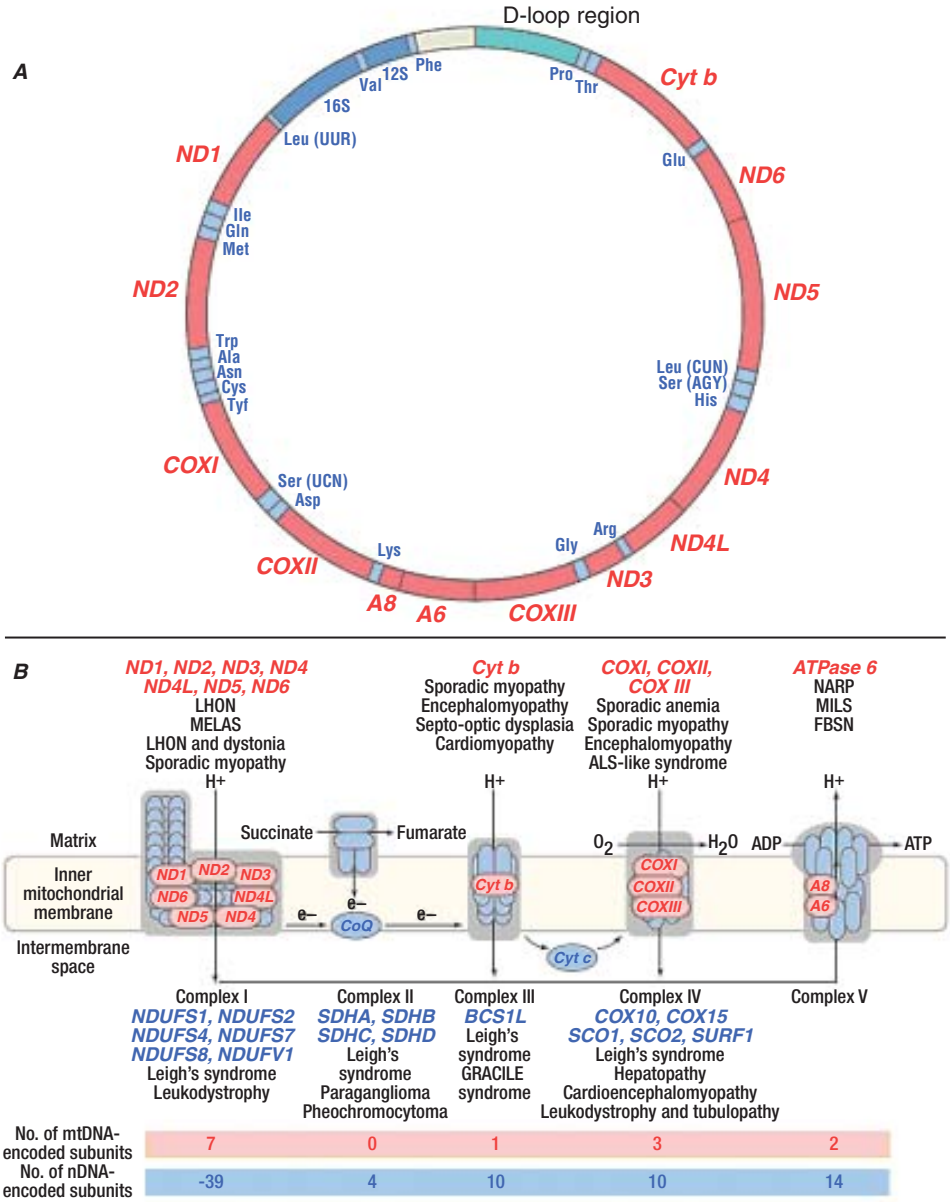

FIGURE e39-2 Mitochondrial DNA (mtDNA) and the mitochondrial respiratory chain. ***A.*** The map of the human mitochondrial genome. The protein-coding genes—seven subunits of complex I (ND), three subunits of cytochrome-c oxidase (COX), the cytochrome-b subunit of complex III (Cyt b), and two subunits of adenosine triphosphate (ATP) synthase (A6 and A8)—are shown in red. The protein-synthesis genes—the 12S and 16S ribosomal RNAs and the 22 transfer RNAs (three-letter amino acid symbols)—are shown in blue. The D-loop region controls the initiation of replication and transcription of mtDNA. ***B.*** The subunits of the respiratory chain encoded by nuclear DNA (nDNA) are shown in blue and the subunits encoded by mtDNA are shown in red. As electrons (e^-) flow along the electron-transport chain, protons (H^+) are pumped from the matrix to the intermembrane space through complexes I, III, and IV and then back into the matrix through complex V, to produce ATP. Coenzyme Q (CoQ) and cytochrome-c (Cyt c) are electron-transfer carriers. Genes responsible for the indicated respiratory-chain disorders are also shown. ATPase 6 denotes ATP synthase 6; BCS1L, cytochrome b–c complex assembly protein (complex III); NDUF, NADH dehydrogenase–ubiquinone oxidoreductase; SCO, synthesis of cytochrome oxidase; SDHA, SDHB, SDHC, and SDHD, succinate dehydrogenase subunits; SURF1, surfeit gene 1; FBSN, familial bilateral striatal necrosis; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MILS, maternally inherited Leigh's syndrome; NARP, neuropathy, ataxia, and retinitis pigmentosa; GRACILE, growth retardation, aminoaciduria, lactic acidosis, and early death; and ALS, amyotrophic lateral sclerosis. *(Reproduced with permission from DiMauro and Schon.)*

have the potential for a phenotypic or pathogenic effect, whereas others may be considered pathogenic mutations.

With respect to transcription, initiation can occur on both strands and proceeds through the production of an intronless polycistronic precursor RNA that 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* consists of ~1.1 kilobases (kb) of noncoding DNA, thought to have a major role in replication and transcription initiation. The mutation rate is considerably higher in the control region, which contains a displacement, or D, loop, in turn containing two adjacent hypervariable regions (HVR-I and HVR-II) that give rise to large interindividual variability within the human population. Indeed mtDNA sequence variants at both the coding and control regions are more highly partitioned across geographically defined populations than sequence variants in other parts of the ge-

nome, and combinations of these sequence variants define phylogeographic mtDNA haplogroups and haplotypes. A major active research question is whether or not differences in these haplotypes are of medical significance in terms of disease predisposition. The foregoing structural and functional features of mtDNA lead to the expectation that phenotypic inheritance and disease patterns for disorders related to mtDNA sequence variation and mutation should be quite different from the more familiar inheritance and disease patterns attributed to variation and mutation of nuclear DNA. Intensive research during the past two decades has confirmed that this is, indeed, the case.

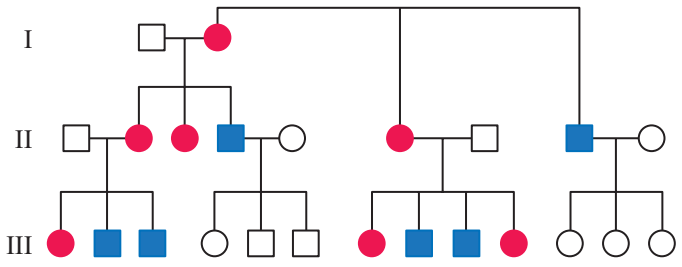

FIGURE e39-3 Maternal inheritance of mtDNA disorders and heritable traits. Affected women (filled circles) transmit the trait to their children. Affected men (filled square) do not transmit the trait to any of their offspring.

MATERNAL INHERITANCE AND LACK OF RECOMBINATION

The nuclear genome is characterized by homologous pairs of chromosomes of biparental origin. With the exception of the nonrecombining region of the Y chromosome in males, these homologous pairs undergo meiotic recombination during gametogenesis, which, together with mutation, serves as the source of universal genetic diversity. In contrast, mtDNA molecules do not undergo recombination, such that mutational events represent the only source of mtDNA genetic diversification. Moreover, with very rare exceptions, it is only the maternal DNA that is transmitted to the offspring. The fertilized oocyte degrades mtDNA carried from the sperm in a complex process involving the ubiquitin proteasome system. Thus, while mothers transmit their mtDNA to both their sons and daughters, only the daughters are able to transmit the inherited mtDNA to future generations. Accordingly, mtDNA sequence variation and associated phenotypic traits and diseases are inherited exclusively along maternal lines.

As will be noted below, because of the complex relationship between mtDNA mutations and disease expression, sometimes this maternal inheritance is difficult to recognize at the clinical or pedigree level. However, evidence of paternal transmission can almost certainly rule out 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. e39-3). One interesting consequence of uniparental inheritance and lack of recombination is the utility of mtDNA marker and sequence analysis in tracing matrilineal ancestry in phylogenetic research.

MULTIPLE COPY NUMBER (POLYPLOIDY), MITOTIC SEGREGATION, AND HIGH MUTATION RATE

Each aerobic cell in the body has multiple mitochondria, often numbering many hundreds or more in cells with extensive energy production 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 each individual, newly acquired somatic mutation 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 the 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. According to this formulation, the high somatic mtDNA mutation rate and the global effect on mitochondrial function counterbalance the reduced impact of the multiple copy number of each individual mtDNA mutation. The potential contribution of such acquired somatic mtDNA mutations to aging and to common age-related disturbances, such as metabolic syndrome and diabetes, cancer, neurodegenerative, and cardiovascular disease, will be considered in greater detail below.

It is evident that just as in the case of acquired somatic mutations in the nuclear genome, so, too, the somatic mutations in mtDNA are not carried forward to the next generation. Therefore, in terms of heritable traits and disease, it is important to focus on the consequence of mtDNA polyploidy within the germ cells of the female reproductive system. The multiple mtDNA copy number within the maternal germ cells result in the phenomenon of heteroplasmy of inherited mtDNA mutations. Heteroplasmy for a given mtDNA sequence variant or mutation arises as a result of the coexistence within the oocyte of mtDNA molecules bearing both versions of the sequence variant (Fig. e39-4). In the case of pathogenic mutations, this means coexistence within the oocyte of both the wild-type and mutant versions. For each oocyte, the percentage of mtDNA molecules bearing each version of the polymorphic sequence variant 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 carried forward to the zygote and then, to varying degrees, depending on mitotic segregation of mtDNA molecules, during organ system development and maintenance.

Mitotic segregation refers to the unequal distribution of wild-type and mutant versions of the mtDNA molecules during all cell divisions that occur during prenatal development and subsequently throughout the lifetime of an individual. The particular mtDNA sequence variant may be entirely silent in terms of phenotype or disease predisposition

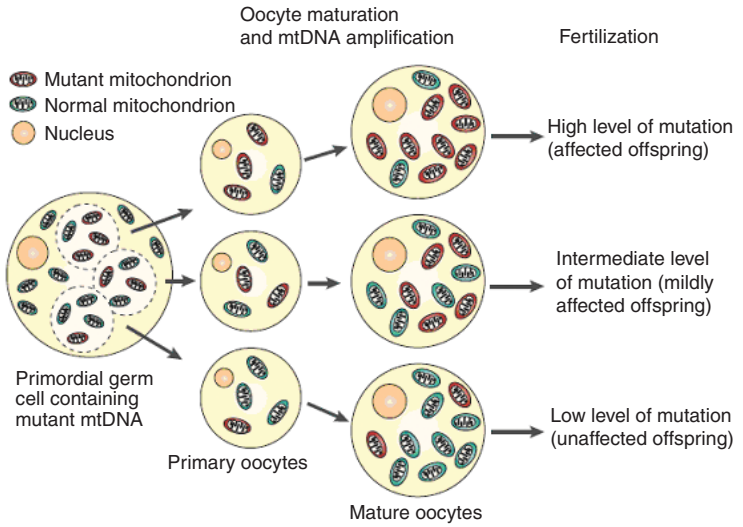

FIGURE e39-4 Heteroplasmy and the mitochondrial genetic bottleneck. During the production of primary oocytes, a selected number of mitochondrial DNA (mtDNA) molecules are transferred into each oocyte. Oocyte maturation is associated with the rapid replication of this mtDNA population. This restriction-amplification event can lead to a random shift of mtDNA mutational load between generations and is responsible for the variable levels of mutated mtDNA observed in affected offspring from mothers with pathogenic mtDNA mutations. Mitochondria that contain mutated mtDNA are shown in red, and those with normal mtDNA are shown in green. *(Reproduced with permission from Taylor and Turnbull.)*

and only detectable upon mtDNA sequencing. By contrast, the mtDNA sequence variant may affect one or more aspects of mitochondrial function in a manner that gives rise to a phenotypic effect or predisposes to a disease. The phenotypic effect or disease impact of a given mtDNA sequence variant will be a function not only of its inherent disruptive effect (pathogenicity) on the mtDNA-encoded gene (coding region mutations) or integrity of the mtDNA molecule (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. This leads to the notion of a "threshold" effect, wherein the actual expression of disease depends upon the relative percentage of mitochondria whose function is disrupted by mtDNA mutations. Consequently, there is tremendous heterogeneity in disease penetrance and severity, as well as complexity of organ system involvement among the offspring of women with pathogenic heteroplasmic mutations. This heterogeneity arises from differences in the degree of heteroplasmy among oocytes and with subsequent mitotic segregation of the pathogenic mutation during tissue and organ development, and throughout the lifetime of the individual. This may create difficulty in recognizing a maternal pattern of inheritance and making the diagnosis of an mtDNA genetic cause of disease.

During the course of human evolution, certain heteroplasmic mtDNA sequence variants may drift 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. e39-4). In other words, during certain stages of oogenesis, the mtDNA copy number becomes substantially reduced, such that the particular mtDNA species bearing the novel or derived sequence variant may become the increasingly predominant, and eventually exclusive, version of the mtDNA for that particular nucleotide site. The offspring of a woman bearing an mtDNA sequence variant or mutation that has become homoplasmic will also be homoplasmic for that variant, and the female offspring will transmit it forward in subsequent generations; this process establishes a new mtDNA haplotype in the human population.

Considerations of reproductive fitness limit the evolutionary or population emergence of homoplasmic mutations that are lethal or cause severe disease in infancy or childhood. Thus, with a number of notable exceptions (e.g., mtDNA mutations causing Leber hereditary optic neuropathy; see below), most homoplasmic mutations were considered to be neutral markers of human evolution—useful and interesting in the population genetics analysis of shared maternal ancestry but with little significance in human phenotypic variation or disease predisposition. However, recent research and clinical attention have focused on the potential for certain of these homoplasmic mtDNA sequence variants to contribute to the evolutionary adaptation of populations to their climatic environment or to predispose to heritable late postreproductive and age-associated disease predisposition.

MITOCHONDRIAL DNA DISEASE

The true prevalence of mtDNA disease is difficult to estimate because of the phenotypic 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 function and inheritance described above. Very rough estimates suggest that heteroplasmic germ-line pathogenic mtDNA mutations may affect up to approximately 1 in 5000 individuals. The true overall impact of mtDNA mutation in human health and disease is certainly much greater, if the potential contribution of homoplasmic mtDNA sequence variation to common complex diseases appearing in the postreproductive age is also considered. Only when the ability to distinguish a completely neutral sequence variant from a true phenotype-modifying or pathogenic mutation is achieved, and when an accurate assessment of heteroplasmy can be determined with fidelity, will the true extent and contribution of mtDNA sequence variation to human traits and health be determined. In addition, the combination of interactions of mtDNA sequence variation with mutations in the nuclear genome also complicates our ability to ascertain the extent of contribution of heritable mtDNA mutations to human illness. Finally, assessment of the impact of the accumulation of acquired somatic mtDNA mutations on late-onset common disease predisposition, or on diseases arising from exposure to metabolic stress, also needs to be considered in order to appreciate the full impact of mtDNA in human health and disease.

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 mtDNA mutations have been described to date, all affecting ETC function. **Figure e39-5** provides an mtDNA map of some of the better characterized disorders. A number of clues can increase the index of suspicion for 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 neurodegenerative 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 reflecting a heteroplasmic state.

Heteroplasmy can sometimes be elegantly demonstrated at the tissue level using histochemical staining for enzymes in the oxidative phosphorylation pathway, with a mosaic pattern indicating heterogeneity of the genotype for the coding region for the mtDNA-encoded enzyme. Complex II, CoQ, and cytochrome-c are exclusively encoded by nuclear DNA. In contrast, complexes I, III, IV, and V contain at least some subunits encoded by mtDNA. Just 3 of the 13 subunits of the ETC complex IV enzyme, cytochrome-c oxidase, are encoded by mtDNA, and, therefore, this enzyme has the lowest threshold for dysfunction when a threshold of mutated mtDNA is reached. Histochemical staining for cytochrome-c oxidase 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 **(Fig. e39-6)**. Heteroplasmy can also be detected at the genetic level through direct mtDNA genotyping under special conditions. It is not always possible to detect heteroplasmy readily in genomic samples extracted from whole blood. Only when a substantial proportion of mtDNA molecules carry the mutant genotype within a sampled tissue does heteroplasmy become detectable by more conventional sequencing or genotyping approaches.

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, while fairly consistent and well-defined "classic" syndromes have been attributed to specific mutations, frequently "nonclassic" combinations of disease phenotypes ranging from isolated myopathy to extensive multisystem disease are often encountered, rendering genotype-phenotype correlation challenging. In both classic and nonclassic mtDNA disorders, there is often a clustering of some combination of abnormalities affecting the neurologic system (including optic nerve atrophy, pigment retinopathy, 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, though 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

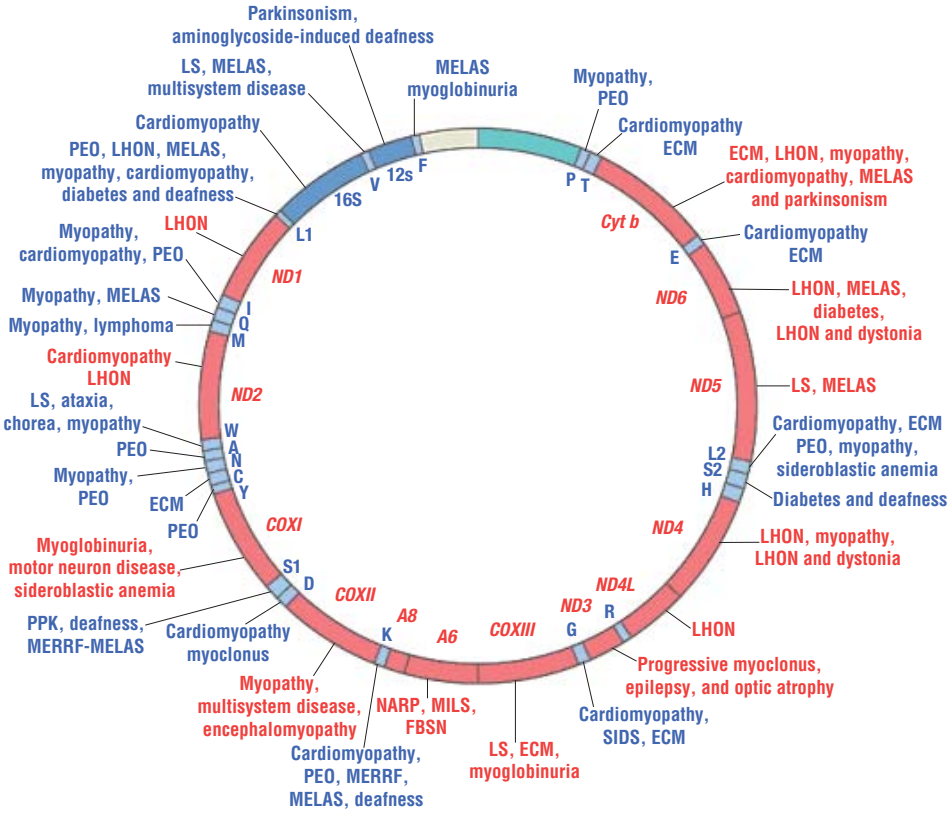

FIGURE e39-5 Mutations in the human mitochondrial genome known to cause disease. Disorders that are frequently or prominently associated with mutations in a particular gene are shown in boldface. Diseases due to mutations that impair mitochondrial protein synthesis are shown in blue. Diseases due to mutations in protein-coding genes are shown in red. ECM denotes encephalomyopathy; FBSN, familial bilateral striatal necrosis; LHON, Leber 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; and SIDS, sudden infant death syndrome. *(Reproduced with permission from DiMauro and Schon.)*

genes. Clinical manifestations do not readily distinguish these two categories, though lactic acidosis and muscle pathologic findings 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 reactive oxygen species has been invoked as the mediating 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 e39-2), 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).

Table e39-3 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 molecular 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 germ line.

Leber hereditary optic neuropathy (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 after the initial onset. In some instances, cerebellar ataxia, peripheral neuropathy, and cardiac conduction defects are observed. In >95% of cases, LHON is due to one of three homoplasmic point mutations of mtDNA that affect genes encoding different subunits of complex I of the mitochondrial ETC; however, not all individuals who inherit a primary LHON mtDNA mutation develop optic neuropathy, indicating that additional environmental (e.g., tobacco exposure) or genetic factors are important in the etiology of the disorder. Both the nuclear as well as mitochondrial genomic background modify disease penetrance. Thus, for example, LHON has a greater penetrance and severity in men than in women, pointing to an epistatic interaction with the nuclear genome. Moreover, disease susceptibility for a given mutation is modulated by mtDNA haplotype background, with certain haplotypes being protective. Of interest, patients with this syndrome are often homoplasmic for the disease-causing mutation. The somewhat later onset in young adulthood and modifying effect of genetic background may have enabled homoplasmic pathogenic mutations to have escaped evolutionary censoring.

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is probably the most common mtDNA disease, consisting of a progressive encephalomyopathy characterized by repeated stroke-like events involving mainly posterior cerebral areas. Of note, brain lesions do not respect the distribution of vascular territories. Recurrent migraine-like headache and vomiting, exercise intolerance, seizures, short stature, and lactic acidosis are other frequent clinical features. The most commonly described pathogenic point mutations are A3243G and T3271C in the gene encoding the leucine tRNA.

Myoclonic epilepsy with ragged red fibers (MERRF) is a multisystem disorder characterized by myoclonus, seizures, ataxia, and myopathy with ragged red fibers. Hearing loss, exercise intolerance, neuropathy, and short stature are often present. Almost all MERRF patients have

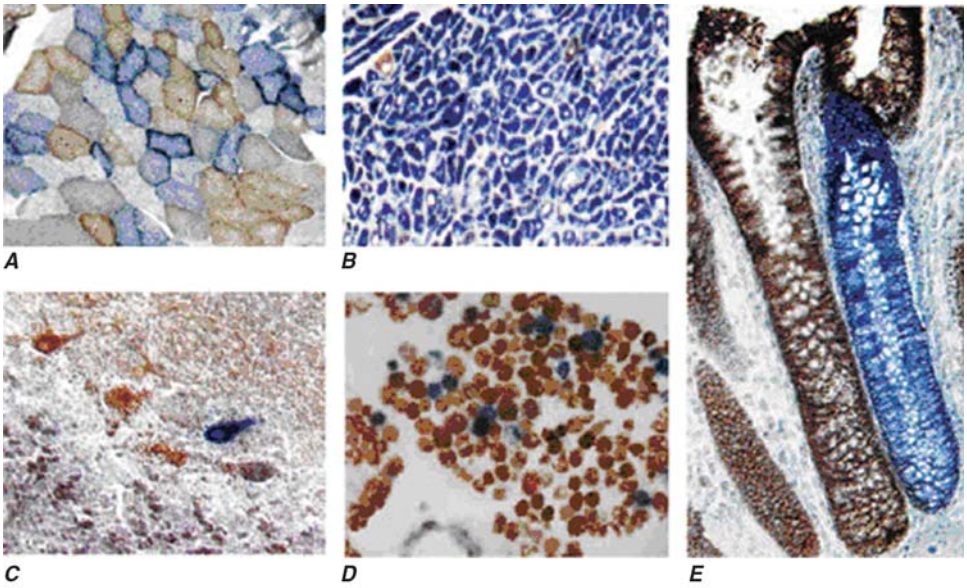

FIGURE e39-6 Cytochrome-c oxidase deficiency in mitochondrial DNA–associated disease. Transverse tissue sections that are reacted for both cytochrome-c oxidase (COX) and succinate dehydrogenase (SDH) activities sequentially, with COX-positive cells shown in brown and COX-deficient cells shown in blue. ***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, whichdemonstrates 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 Taylor and Turnbull.)*

mutation in the mtDNA $tRNA^{lys}$ gene and the A8344G mutation in the mtDNA gene encoding the lysine amino acid tRNA is responsible for 80–90% of MERRF cases.

Neurogenic weakness, ataxia, and retinitis pigmentosa (NARP) is characterized by moderate diffuse cerebral and cerebellar atrophy and symmetric lesions of the basal ganglia on MRI. A heteroplasmic T8993G mutation in the gene ATPase 6 subunit gene has been identified as causative. Ragged red fibers are not observed in muscle biopsy. When >95% of mtDNA molecules are mutant, a more severe clinical, neuroradiologic and neuropathologic picture (Leigh's syndrome) emerges. Point mutations in the mtDNA gene encoding the 12S rRNA result in heritable nonsyndromic hearing loss. One such mutation causes heritable ototoxic susceptibility to aminoglycoside antibiotics, which opens a pathway for a simple pharmacogenetic test in the appropriate clinical settings.

Kearns-Sayre syndrome (KSS), sporadic progressive external ophthalmoplegia (PEO), and Pearson syndrome are three disease phenotypes caused by large-scale mtDNA rearrangements including partial deletions or partial duplication. The majority of single large-scale rearrangements of mtDNA are thought to result from clonal amplification of a single sporadic mutational event, occurring in the maternal oocyte or during early embryonic development. Since germ line involvement is rare, most cases are sporadic rather than inherited.

KSS is characterized by the triad of onset before age 20, chronic progressive external ophthalmoplegia, and pigmentary retinopathy. Cerebellar syndrome, heart block, increased cerebrospinal fluid protein content, diabetes, 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 progressive external ophthalmoplegia, proximal myopathy, and exercise intolerance. In both KSS and PEO, diabetes mellitus and hearing loss are frequent accompaniments. Pearson syndrome is also characterized by diabetes mellitus from pancreatic insufficiency, together with pancytopenia and lactic acidosis, caused by the large-scale sporadic deletion of several mtDNA genes.

TABLE e39-2 COMMON FEATURES OF mtDNA-ASSOCIATED DISEASES IN ADULTS

Neurologic: stroke, epilepsy, migraine headache, peripheral neuropathy, cranial neuropathy (optic atrophy, sensorineural deafness, dysphagia, dysphasia)
Skeletal myopathy: ophthalmoplegia, exercise intolerance, myalgia
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)

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. e39-7. 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 in adulthood have normal blood lactate. A raised cerebrospinal fluid lactate is a more specific test for mitochondrial disease if there is central neurologic involvement. The serum creatine kinase may be elevated but is often normal, even in the presence of a proximal myopathy. Urine organic and amino acids may also be abnormal. Every patient with seizures or cognitive decline should have an electroencephalogram. A brain CT scan may show calcified basal ganglia or bilateral hypodense regions with cortical atrophy. MRI is indicated in patients with brain stem signs or stroke-like episodes.

For some 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 three mtDNA point mutations (A11778G, A3460G, and T14484C). These patients have very high levels of mutated mtDNA in peripheral blood cells, and it is, therefore, appropriate to send a blood sample for molecular genetic analysis by polymerase chain reaction (PCR) or restriction fragment length polymorphism. 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 A3243G 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 be investigated further by performing a skeletal muscle biopsy.

Muscle biopsy histochemical analysis is the cornerstone for investigation of patients with suspected mitochondrial disease. Histochemical analysis may show subsarcolemmal accumulation of mitochondria with the appearance of ragged red fibers. Electron microscopy might show abnormal mitochondria with paracrystalline inclusions. Muscle histochemistry may show cytochrome-c oxidase (COX)–deficient fi-

TABLE e39-3 MITOCHONDRIAL DISEASES DUE TO mtDNA POINT MUTATIONS AND LARGE-SCALE REARRANGEMENTS

Disease	Phenotype	Most Frequent mtDNA Mutations	Homoplasmy or Heteroplasmy	Inheritance
Leber hereditary optic neuropathy (LHON)	Loss of central vision leading to blindness in young adult life	G1778A, T14484C, G3460A	Homoplasmic (usually)	Maternal
NARP, Leigh's disease	*N*europathy, *a*taxia, *r*etinitis *p*igmentosa, developmental delay, mental retardation, lactic acidemia	Point mutation in ATPase subunit 6 gene	Heteroplasmic	Maternal
MELAS	*M*itochondrial *e*ncephalomyopathy, *l*actic *a*cidosis, and *s*troke-like episodes; may manifest only as diabetes	Point mutation in $tRNA^{leu}$	Heteroplasmic	Maternal
MERRF	*M*yoclonic *e*pilepsy, *r*agged *r*ed *f*ibers in muscle, ataxia, increased CSF protein, sensorineural deafness, dementia	Point mutation in $tRNA^{lys}$	Heteroplasmic	Maternal
Deafness	Progressive sensorineural deafness, often induced by aminoglycoside antibiotics.	A1555G mutation in 12S rRNA	Homoplasmic	Maternal
	Nonsyndromic sensorineural deafness	A7445G mutation in 12S rRNA	Homoplasmic	Maternal
Chronic *p*rogressive *e*xternal ophthalmoplegia (PEO)	Late-onset bilateral ptosis and ophthalmoplegia, proximal muscle weakness, and exercise intolerance	Single deletions or duplications	Heteroplasmic	Mostly sporadic, somatic mutations
Pearson syndrome	Pancreatic insufficiency, pancytopenia, lactic acidosis	Large deletion	Heteroplasmic	Sporadic, somatic mutations
Kearn-Sayre syndrome (KSS)	External ophthalmoplegia, heart-block, retinal pigmentation, ataxia	The 5-kb "common deletion"	Heteroplasmic	Sporadic, somatic mutations

Note: CSF, cerebrospinal fluid.

bers, which indicate mitochondrial dysfunction (Fig. e39-6). Respiratory chain complex assays may also show a deficiency. Either of these two abnormalities confirm that a patient has mitochondrial disease, and this should lead to in-depth molecular genetic analysis.

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 phosphorylation and the consequent reduction in reproductive fitness. Therefore, it has been previously thought that sequence variants that have reached homoplasmy should be neutral in terms of human evolution and useful only for tracing human evolution, demography, and migration; however, recent studies have suggested that some homoplasmic mtDNA sequence variants may affect heritable traits or health through one or more mechanisms.

The first such mechanism relates to locally adaptive evolutionary forces. As noted above, homoplasmic mtDNA sequence variants that partition population groups are designated as defining maternal "haplogroups." Striking discontinuities have been observed in mtDNA haplogroup distribution among climatic zones across the globe. For example, of the extensive mtDNA sequence diversity in Africa, only a limited number of haplogroups and their derivative lineages successfully colonized all of Eurasia and then the Americas. Furthermore, it was shown that ancient missense mutations that define these haplogroups alter amino acids that are as highly conserved in evolution as are those known to result from pathogenic mutations. Retention of mutations altering such highly conserved amino acids, over many tens of thousands of years, suggests that they must be adaptive since they could not have been maintained if they were pathogenic and destructive to reproductive fitness. This phenomenon has been attributed to adaptive differences in the efficiency of oxidative phosphorylation and consequent thermogenesis, according to differences in prevailing climates in different global geographic regions during much of human evolution. A potential health implication of this finding is the possibility that these same mutations might result in deleterious effects on energy metabolism and caloric balance in the current era of human transglobal migration or climate control.

A much broader extrapolation of the foregoing mechanism states that many homoplasmic mtDNA mutations affect human health in the postreproductive age only and therefore escaped evolutionary censoring altogether. In the modern era of increased median life span, such mutations are thought to account for a considerable burden of age-associated common complex disease. Mean life expectancy has risen from ~47 years to ~77 years during the past century alone; there-

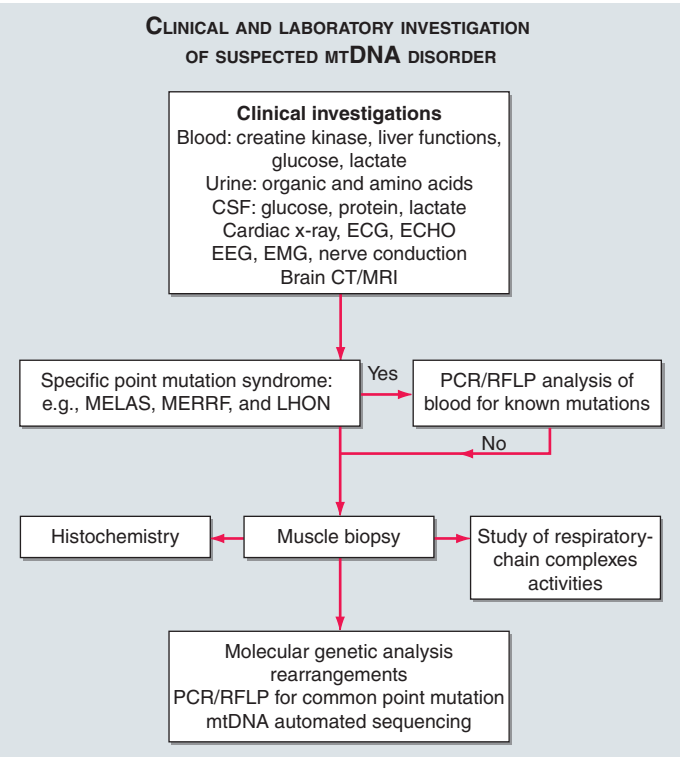

FIGURE e39-7 Clinical and laboratory investigation of suspected mtDNA disorder. CSF, cerebrospinal fluid; ECG, electrocardiogram; EEG, electroencephalogram; EMG, electromyogram; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stoke-like episodes; MERFF, myoclonic epilepsy with ragged red fibers; LHON, Leber hereditary optic neuropathy; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

fore, late-onset effects of a subset of homoplasmic mtDNA mutations may contribute significantly to the burden of human illness only in the current era when a relatively higher percentage of the population is surviving beyond reproductive age. Many homoplasmic mtDNA variants have been and will continue to be identified by whole mtDNA sequencing in various global populations. The challenge is to identify the subsets that modify mtDNA function and contribute to late-onset, common complex disease. Indeed, given the finding that global populations are more differentiated at the level of mtDNA than they are at the level of the nuclear genome, it also attractive to postulate that population differences in the predisposition to certain late-onset common complex metabolic diseases may be attributed in part to population-based mtDNA sequence variation. The diseases that have been of particular interest are those that affect the very organ systems familiar from the known classic mtDNA syndromes described above.

METABOLIC SYNDROME AND TYPE 2 DIABETES MELLITUS (T2DM)

Insulin release by pancreatic beta cells is modulated in response to ATP metabolism, and insulin action is perturbed by metabolites of mitochondrial fatty acid oxidation. This has led investigators to consider mtDNA itself as a potential genomic locus for susceptibility to T2DM. A rather clear-cut case is that of a mutation in mtDNA nucleotide 3243 encoding the mitochondrial tRNA for the amino acid leucine. Even a low level of heteroplasmy for a particular point mutation in the mtDNA tRNA gene encoding the leucine tRNA is thought to contribute to the pathogenesis of up to 1% of all cases of T2DM. This and other findings at the biochemical and population genetics levels have motivated the search for more definitive evidence of the role of homoplasmic variants in the predisposition to metabolic syndrome and T2DM. Such evidence has been obtained with the finding of significant segregation of a homoplasmic mtDNA tRNA mutation (T→ C transition in the nucleotide immediately 5′ to the isoleucine tRNA anticodon) with metabolic syndrome phenotypes in a large Caucasian kindred.

Since the metabolic syndrome is so common, and can result from numerous different genetic susceptibility loci and environmental causes, and since its pathogenesis involves a large number of recently identified genetic susceptibility loci coupled with environmental factors—additional features in this particular reported kindred—researchers were able to tease out affected from unaffected individuals for purposes of the association study. The affected individuals had signs of hypomagnesemia, hypertension, and hypercholesterolemia. This finding highlights the problem of underdiagnosing homoplasmic mtDNA sequence variants as pathogenic causes of common symptoms and syndromes. This particular mutation in a tRNA-encoding mtDNA gene also highlights the expected difference in the phenotypic impact of mutations in genomic regions encoding tRNAs in the mitochondrial versus the nuclear genome.

In the case of the mitochondrial genome, mutations affecting mtDNA-encoded tRNAs perturb the translation of only up to 13 protein products and may be compatible with life. In contrast, severe loss of function of nuclear genome–encoded tRNA might be expected to perturb the function of a much greater array of nuclear-encoded proteins. It is also of interest to contrast the anticipated effects of point mutations in one of the 13 protein-coding genes of mtDNA with those of the 24 genes encoding tRNAs and rRNAs. Whereas the former would disrupt the function of a single protein product, the latter group would perturb translation of up to all 13 of the mtDNA-encoded proteins. Under these circumstances, a tolerable loss of function of all 13 proteins might be compatible with life, although it would be expected to cause a pleiotropic multiorgan heritable syndrome, as was indeed observed for the mtDNA isoleucine tRNA mutation causing an extended metabolic syndrome phenotype. At the population level, the finding of an apparent excess of maternal inheritance in T2DM suggests the potential involvement of mtDNA. A common variant mtDNA sequence variant (T16189C) has been related to both low birth weight, impaired glucose tolerance, and metabolic syndrome in specific populations. However, rigorous population-based association studies using case-control designs have not yet provided definitive evidence for a relationship between mtDNA haplogroups and susceptibility to T2DM or its complications.

NEURODEGENERATIVE DISEASE

The prominence of neurologic injury in classic mtDNA diseases, together with the presumed role of reactive oxygen species in neuronal injury and late age of onset of neurodegenerative diseases, have led investigators to consider the possibility that homoplasmic variants in mtDNA sequence that define population haplogroups might also modify the susceptibility to neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Thus, for example, particular configurations of mtDNA sequence polymorphisms that define population haplogroups designated in phylogenetics by the labels J, T, U, and K have been reported to be potentially protective against Parkinson's disease in different populations. In the case of Alzheimer's disease, some studies have shown haplogroup J to increase risk, with haplogroup D decreasing risk. Mutations located in the mtDNA control region do not produce defective polypeptide products but affect both the light and heavy strand promoters, as well as the heavy strand origin of replication, and thus may modulate mtDNA replication and transcription. Mitochondrial DNA control region sequence variants (e.g., T414G) have been identified in Alzheimer's disease brains in association with a significant reduction in mtDNA copy number and reduction in specific transcripts. A number of studies have focused on the interaction of mtDNA haplogroup–designating mutations with the well-established Alzheimer's disease risk alleles at the nuclear APOE4 locus. From these studies it was postulated that ETC-uncoupling mutations that minimize ROS production are those that confer protection against neuronal injury, but definitive proof of this postulate awaits further studies.

OTHER DISEASES AND NONDISEASE HERITABLE TRAITS

Consideration of the potential contribution of mtDNA mutations to numerous heritable traits and common complex diseases requires consideration of the common variant–common phenotype model (including disease phenotype) versus the rare variant–common phenotype model, which are also applicable to the nuclear genome. According to the common variant–common phenotype model, DNA sequence variants inherited identically by descent and present in large numbers of individuals within one or more populations, may predispose to common phenotypes. In the rare variant–common phenotype model, different mutations within one or more genetic loci involved in a particular molecular pathway may predispose to a common phenotype or disease. In this regard, the entire mtDNA can be considered as a single genomic locus. Genome-wide association studies have been utilized to try to map common variants responsible for common diseases, using case-control or multiplex family approaches. These approaches have been applied to common variants in mtDNA sequence as well, as noted above for metabolic syndrome and neurodegenerative disease. Additional examples include the variable length of an mtDNA control region polycytosine stretch (16189 variant) as a contributing genomic influence in the onset of age-related cardiomyopathy with T2DM. An association of mtDNA haplogroup T, and a polymorphism at position 13368 with hypertrophic cardiomyopathy has been reported in a European population, and a number of studies have suggested an association between mtDNA mutations and mitochondrial dysfunction in heart failure predisposition. In the case of age-related cancers as well, the association of a number of heritable homoplasmic mtDNA mutations with certain cancers has been reported, including prostate, kidney, and breast cancer.

The association of mtDNA haplogroups with at least two nondisease heritable traits has also been studied. These are life expectancy and exercise endurance. Several mtDNA control region mutations, including the C150T mutation that shifts the heavy chain origin of replication, have been reported to accumulate with age in specific tissues, including lymphocytes of centenarians and their twins. The relationship between the C150T mutation and longevity has been replicated in Italian, Finnish, and Japanese populations—suggesting a common ancient origin.

The alternative of evolutionary convergence of this mutation for longevity seems less likely, as trait does not confer reproductive advantage. The association of haplogroup J and its sub-haplogroups with longevity has been demonstrated in north Italian, north Irish, and Finnish sample sets. At least in the Italian study, this association was shown to be population specific, since it was not reproduced in sample sets of southern Italian communities. Furthermore, in the case of the north Italian communities, an additional interaction of the mtDNA haplogroup designated as J2 with several different mutations adjacent to replication origins, including the aforementioned C150T, has been noted.

The functional importance of one or more of the mutations designated as haplogroup J is further strengthened by the finding of the interesting interaction with the mtDNA mutations causing LHON, as noted previously. Reduced disease predilection suggests that one or more of the ancient sequence variants designated as haplogroup J appear to attenuate predisposition to degenerative disease, in the face of other risk factors. It has been proposed that the mtDNA haplogroups associated with exceptional longevity favor a relatively uncoupled state of the ETC, with reduced efficiency of production of ATP and ROS and increased thermogenesis. While this has not been demonstrated biochemically, the notion is strengthened by the finding of a relative paucity of these mtDNA haplogroups among successful endurance athletes, for whom maximum efficiency of oxidative phosphorylation confers an athletic competitive advantage.

It should be noted that not all studies have replicated associations of mtDNA haplogroups with longevity, athletic performance, or other heritable phenotypes. Most of these studies are limited by small sample sizes and the possibility of population stratification or ethnic ancestry bias. Since mtDNA haplogroups are so prominently partitioned along phylogeographic lines, it is difficult to rule out the possibility that a haplogroup for which an association has been found is simply a marker for differences in populations with a societal or environmental difference or with different allele frequencies at other genomic loci, which are actually causally related to the heritable trait or disease of interest. The 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 different mtDNA haplogroup–defining homoplasmic mutations serve to provide different "risk backgrounds" for common age-related diseases whose major molecular pathogenesis emanates from a combination of mutations in the nuclear genome, together with environmental influences. Progress in minimizing potentially misleading associations in mtDNA heritable trait and disease studies should include ensuring adequate sample size taken from a large sample recruitment base, together with the use of carefully matched controls, and analysis that takes into account the interaction 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 those 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 A3344G and A3243G mutations responsible for the MERRF and MELAS syndromes, respectively. However, the cumulative 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 mtDNA point mutations. Along the same lines, an age-associated and tissue-specific accumulation 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 cytochrome-c oxidase activity upon histochemical staining, especially in skeletal and cardiac muscle and brain. A particularly well studied and potentially important example is the accumulation of mtDNA deletions and cytochrome-c oxidase 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 disease pathogenesis (Fig. e39-8). As noted above, ROS are a byproduct of oxidative phosphorylation and are removed by detoxifying antioxidants into less harmful moieties; however, exaggerated production of ROS or impaired removal results in their accumulation. One of the main targets for ROS-mediated injury is DNA, and mtDNA is particularly vulnerable because of its lack of protective histones and less efficient injury repair systems compared with nuclear DNA. In turn, accumulation of mtDNA mutations results in inefficient oxidative phosphorylation, 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 mutation can potentially occur in postmitotic cells as well, since 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 function 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.

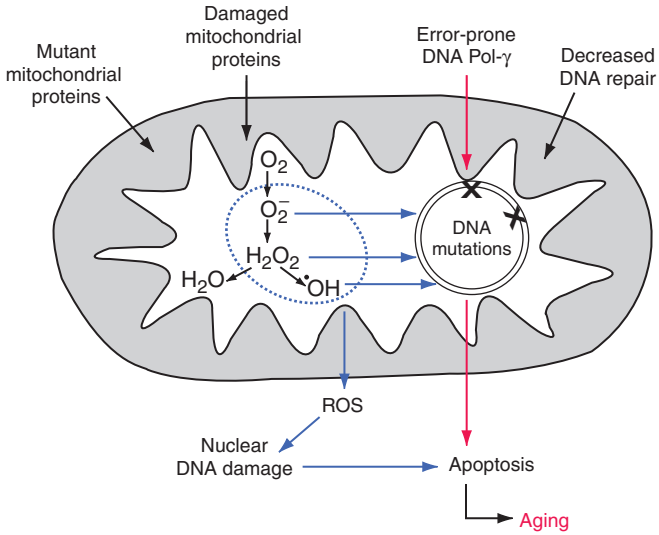

FIGURE e39-8 Multiple pathways of DNA damage and aging. Mitochondrial DNA damage and aging. Multiple factors may impinge on the integrity of mitochondria that lead to loss of cell function, apoptosis, and aging. The classic pathway is indicated with blue arrows; the generation of ROS (superoxide anion, hydrogen peroxide, and hydroxyl radicals), as a by-product of mitochondrial oxidative phosphorylation, results in damage to mitochondrial macromolecules including the mtDNA, 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 Loeb L et al.)*

Replication of the mitochondrial genome is mediated by the activity of the nuclear-encoded polymerase gamma gene. A transgenic homozygous mouse knock-in mutation of this gene renders the polymerase enzyme deficient in proofreading and results in a three- 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. However, not all animal models of enhanced mtDNA mutation rate have shown a similar accelerated aging phenotype. Furthermore, it has been difficult to demonstrate a consistent relationship between rates of mtDNA mutation and ROS production. While the finding of increased in mtDNA mutation and mitochondrial dysfunction with age has been solidly established, the causative role and specific contribution of mitochondrial ROS to aging and age-related disease has yet to be proved. Similarly, while 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 mutation 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 tissue, usually skeletal muscle. The presentation consists of decreased exercise tolerance and myalgias, sometimes progressing to rhabdomyolysis. As in the case of the sporadic heteroplasmic large-scale deletion classic syndromes of chronic PEO, Pearson syndrome, and KSS, the absence of a maternal inheritance pattern, together with the finding of limited tissue distribution, suggests a molecular pathogenic mechanism emanating from mutations arising de novo in muscle stem cells after germ-line 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 particular tissue within a given individual, without evidence of heritability.

PROSPECTS FOR PREVENTION AND TREATMENT OF mtDNA DISEASE

GENETIC COUNSELING IN mtDNA DISORDERS

The provision of accurate genetic counseling and reproductive options to families with mtDNA mutations is complicated by the unique genetic features of mtDNA that distinguish it from Mendelian genetics. While there is no risk of disease transmission from an affected male, the risk of maternal transmission of disease phenotypes associated with heteroplasmic mutations is a function of differential segregation and copy number of mutant mtDNA during oogenesis and subsequently, following tissue and organ development in the offspring. This is rarely predictable with any degree of accuracy. In addition, 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. Environmental interactions are also of importance, as in the case of ototoxic susceptibility to aminoglycosides in the case of the A1555G mutation of the 12SrRNA encoding gene.

The value of prenatal genetic testing is also questionable, partly owing to the absence of data on the rules that govern the segregation of wild-type and mutant mtDNA species (heteroplasmy) among tissue in the developing embryo. Three factors are required to ensure the reliability of prenatal testing: (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 T8993G 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 provide clinically useful information regarding the postnatal and adult state.

TREATMENT OF mtDNA DISORDERS

The polyploid nature of the mitochondrial genome, the inability to deliver therapeutic nucleic acids to the organelle through mitochondrial transfection, and the phenomenon of heteroplasmy coupled with the relative unavailability of useful preclinical experimental models have hampered progress in the development of curative treatments for mtDNA disease. One possible approach to "diluting" or even entirely eliminating the mutant mtDNA is applicable only in the earliest embryonic state and in effect represents a form of germ-line preventive therapy. This would involve cytoplasmic transfer of normal mitochondria into the oocyte of a woman affected with the heteroplasmic mtDNA mutation or, more radically, the use of a normal donor oocyte that has been enucleated and into which the nucleus of the affected recipient mother has been introduced. These approaches have not yet been met with widely reported clinical success. On the hopeful side, the threshold effect suggests that even a small increase in nonmutant mtDNA copies or limited amelioration of metabolic dysfunction might greatly improve the disease phenotype and benefit patient health. Therefore counteracting the most damaging metabolic changes currently represents the mainstay of treatment. Combined treatment strategies include dietary intervention and removal of toxic metabolites. Cofactors and vitamin supplements are widely used in the treatment of diseases of mitochondrial oxidative phosphorylation, although there is little evidence, apart from anecdotal reports, to support their use. This includes administration of artificial electron acceptors, including vitamin K_3, vitamin C, and ubiquinone (coenzyme Q_{10}); administration of cofactors (coenzymes) including riboflavin, carnitine, and creatine; and use of oxygen radical scavengers, such as vitamin E, copper, selenium, ubiquinone, and idebenone. Drugs that could interfere with mitochondrial defects, such as the anesthetic agent propofol, barbiturates, and high doses of valproate, should be avoided. Supplementation with the nitric oxide synthase substrate, L-arginine, has been advocated as a vasodilator treatment during stroke-like episodes.

In the case of homoplasmic mtDNA variants that predispose to late-onset common complex disease, it is more realistic to think of using their identification in a given patient as a nonmodifiable risk factor, which guides the aggressiveness of medical intervention for the associated modifiable risk factors for the same disorder. For example, the identification of a haplogroup-defining homoplasmic mtDNA mutation that confers added risk for metabolic syndrome should trigger intensive dietary, lifestyle, and medical intervention to reduce other factors that promote the metabolic syndrome and its complications. In the case of acquired somatic mutations—to the extent that a vicious cycle of ROS production with mtDNA mutation plays a role—effective antioxidant and ROS scavenging therapeutic strategies may prove to be of benefit.

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