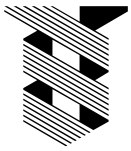
REVIEW ARTICLE

SEMINARS IN MEDICINE OF THE BETH ISRAEL HOSPITAL, BOSTON



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MITOCHONDRIAL DNA AND DISEASE

DONALD R. JOHNS, M.D.

THE mitochondrial encephalomyopathies are a diverse group of disorders that result from the structural, biochemical, or genetic derangement of mitochondria.[1] Since mitochondrial dysfunction can affect virtually all organ systems (Fig. 1), physicians in many specialties see patients with mitochondrial diseases. Despite a bewildering array of clinical manifestations (Tables 1 and 2) and variations in the mode of onset, course, and progression of disease, many mitochondrial disorders share prominent systemic effects (Table 2) that contribute to their morbidity.

Our understanding of the role of mitochondrial DNA in certain diseases has evolved rapidly since 1988, when the first mutations in mitochondrial DNA were discovered.[2,3] Such mutations have subsequently been identified in a variety of diseases,[4-7] and the pathogenic role of cumulative mitochondrial-DNA damage is being explored in many common diseases that develop late in life, and even in the aging process itself.

STRUCTURE AND FUNCTION OF MITOCHONDRIAL DNA

To understand mitochondrial disease, one must first examine the unique features of mitochondrial DNA. Mitochondria generate energy for cellular processes by producing ATP through oxidative phosphorylation. These organelles contain their own, extrachromosomal DNA, which is distinct from DNA in the nucleus.[8] Human mitochondrial DNA (known as "the other human genome") is a double-stranded, circular molecule that encodes 13 protein subunits of 4 biochemical complexes and the 24 structural RNAs (2 ribosomal RNAs [rRNAs] and 22 transfer RNAs [tRNAs]) that are required for the intramitochondrial translation of the protein-coding units.[9] Mitochondrial-DNA mutations have been found in each type of mitochondrial gene (Fig. 2).

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Mitochondria, which probably evolved from independent organisms that became part of the cell, are able to replicate, transcribe, and translate their DNA independently of nuclear DNA. However, cellular function and mitochondrial function are interdependent.[10] Nuclear DNA encodes protein subunits of oxidative phosphorylation and the myriad macromolecular compounds required for mitochondrial structure and function (e.g., replication, transcription, and translation). Proteins encoded by nuclear DNA must be imported from the cytoplasm into the correct position within the mitochondria.[8] During oxidative phosphorylation, energy is derived from intermediary metabolites to produce ATP by means of an electrochemical gradient. This biochemical process depends on the mitochondrial-DNA–encoded tRNAs that cleave multigene transcripts into individual messenger RNAs and tRNAs.[10]

Several features of mitochondrial DNA may be related to its frequent association with disease. Mitochondrial DNA mutates more than 10 times as frequently as nuclear DNA and has no introns, so that a random mutation will usually strike a coding DNA sequence. In addition, mitochondrial DNA has neither protective histones nor an effective repair system, and it is exposed to oxygen free radicals generated by oxidative phosphorylation.

Mitochondrial DNA is inherited maternally and does not recombine; mutations thus accumulate sequentially through maternal lineages. Each mitochondrion contains 2 to 10 DNA molecules, and each cell contains multiple mitochondria. Thus, normal and mutant mitochondrial DNA can coexist within the same cell. This condition, known as heteroplasmy, allows an otherwise lethal mutation to persist. Homoplasmy is the presence of either completely normal or completely mutant mitochondrial DNA. Through the process of replicative segregation, the proportions of mutant and normal molecules can shift as mitochondrial DNA is partitioned into daughter cells. Thus, the principles of population genetics, rather than those of mendelian genetics, govern mitochondrial DNA. Selection pressures occur at the molecular and cellular levels, as well as at the level of the organism itself. The proportion of mutant mitochondrial DNA required for the occurrence of a deleterious phenotype, known as the threshold effect, varies among persons, among organ systems, and within a given tissue. The threshold effect depends on the delicate balance between oxidative supply and demand.

The classic mitochondrial phenotypes described below are caused by gross structural rearrangements (single deletions, multiple deletions, or duplications) or by point mutations in mitochondrial DNA. Mutations with the potential to cause a lethal impairment of oxidative phosphorylation (gross structural defects or point mutations in critical regions) are viable only if they are heteroplasmic. The majority of the milder, missense mutations in protein-coding genes are homoplasmic. Although mutations have been found in each type of

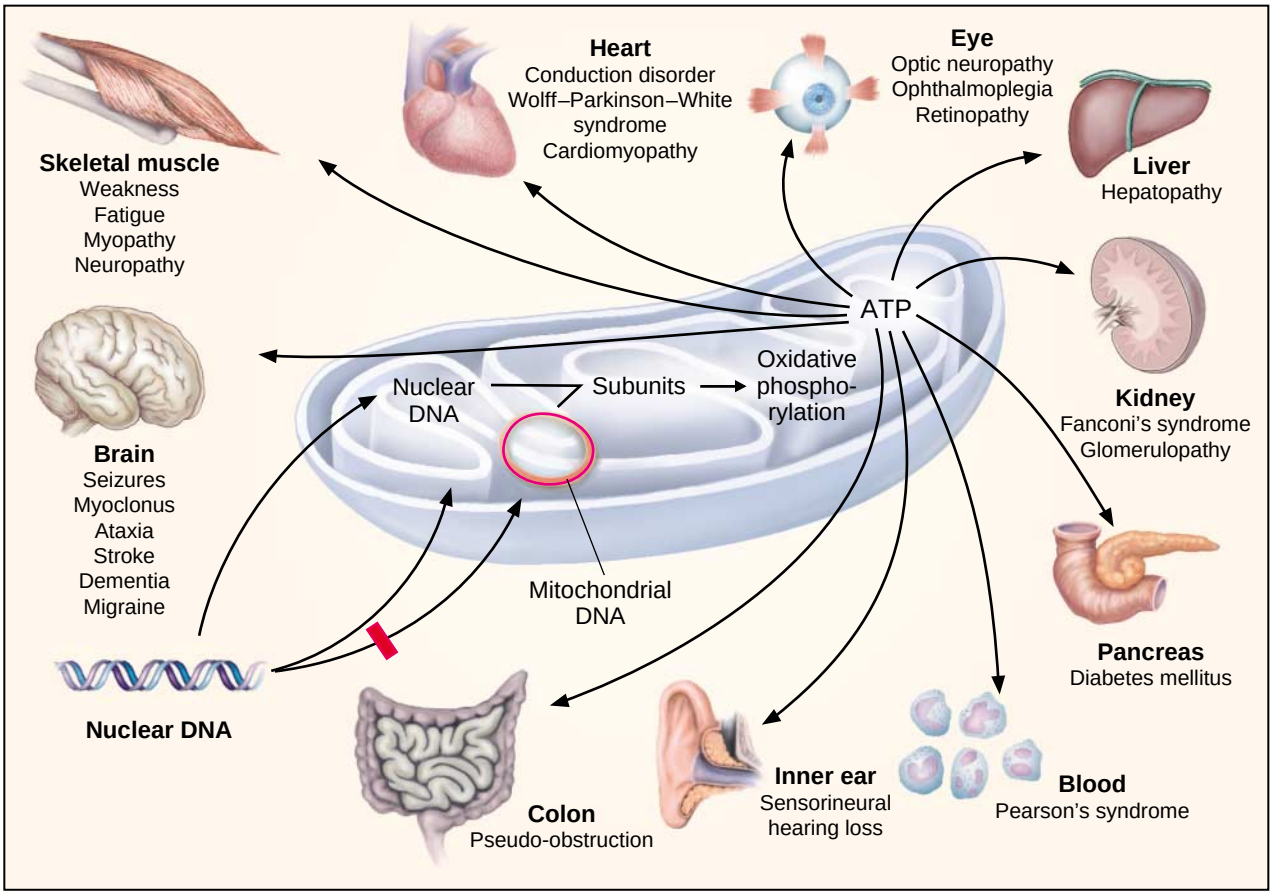

Figure 1. Interaction between Genes Encoded by Nuclear DNA and Those Encoded by Mitochondrial DNA in Oxidative Phosphorylation.

The intricate function of the oxidative-phosphorylation complexes can be disrupted by defects in the subunits encoded by nuclear DNA and mitochondrial DNA or by defects in intergenomic communication between the two types of DNA. The resulting deficits in the production of ATP have deleterious effects on a number of organ systems, causing the disorders shown. The red bar indicates the site of defects in intergenomic communication (depletion and multiple deletions of mitochondrial DNA).

mitochondrial-DNA gene, tRNA mutations predominate in the phenotypes of mitochondrial encephalomyopathy, and protein-coding gene mutations predominate in Leber's hereditary optic neuropathy. A recent report suggests that a point mutation in the 12S rRNA gene is associated with both spontaneous and antibiotic-associated sensorineural deafness[11] (Fig. 2).

Classic Phenotypes of Mitochondrial Encephalomyopathy

Before 1988, when the first abnormal mitochondrial DNA was identified, many diseases were provisionally classified as mitochondrial disorders because of abnormal morphologic or biochemical features of mitochondria or a pattern of maternal inheritance. The first direct evidence that mitochondrial DNA was involved in a disease came from two observations: the finding of large deletions in mitochondrial DNA from patients with mitochondrial myopathies[2] and the detection of a missense mutation in mitochondrial DNA from patients with Leber's hereditary optic neuropathy.[3] Over the next several years, the molecular genetic basis of the classic mitochondrial encephalomyopathies was elucidated.[4-7] These disorders are relatively uncommon, but they were the first molecularly defined examples of many cardinal neurologic diseases, including stroke (the syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS]), seizures (the MELAS syndrome and myoclonic epilepsy

Table 1. Neurologic Manifestations of Mitochondrial Diseases.

Ophthalmoplegia
Stroke in a young person
Seizures
Myoclonus
Optic neuropathy
Myopathy
Fatigability and exercise intolerance
Elevated levels of cerebrospinal fluid protein
Sensorineural hearing loss
Ataxia
Dementia
Peripheral neuropathy
Vascular headache
Myelopathy
Dystonia
Basal-ganglia calcification

Table 2. Systemic Manifestations of Mitochondrial Diseases.

Cardiac conduction defects
Cardiomyopathy
Diabetes mellitus
Short stature
Hypoparathyroidism
Pigmentary retinopathy
Cataracts
Lactic acidosis
Hearing loss
Proximal-nephron dysfunction
Glomerulopathy
Hepatopathy
Intestinal pseudo-obstruction
Episodic nausea and vomiting
Pancytopenia
Exocrine pancreatic dysfunction
Psychiatric disorder (especially depression)

with ragged-red fibers), and optic neuropathy (Leber's hereditary optic neuropathy).

Chronic Progressive External Ophthalmoplegia

The main features of chronic progressive external ophthalmoplegia are ptosis, ophthalmoplegia, and limb myopathy, but additional clinical features (Tables 1 and 2) and the laboratory abnormalities characteristic of mitochondrial disorders (Table 3) may also be present. Skeletal-muscle biopsies in patients with chronic progressive external ophthalmoplegia reveal abnormal proliferating mitochondria that cause ragged-red fibers, a hallmark of the severe biochemical defects in oxidative phosphorylation in many mitochondrial encephalomyopathies. The Kearns–Sayre syndrome, a form of chronic progressive external ophthalmoplegia that begins before the age of 20 years, is characterized by atypical pigmentary retinopathy, as well as elevated levels of cerebrospinal fluid protein, ataxia, and heart block.[1]

Most patients with chronic progressive external ophthalmoplegia have large, single deletions in mitochondrial DNA.[2,12-15] Almost all these deletions occur sporadically by an unknown mechanism.[15] In most cases, the junction contains directly repeated sequences, including a molecular "hot spot" that accounts for approximately 25 percent of all deletions.[13-15] A few patients have partially duplicated mitochondrial-DNA molecules.[16] Many patients without gross structural abnormalities of mitochondrial DNA have a point mutation at nucleotide position 3243.[17]

Autosomally Transmitted Multiple Deletions in Mitochondrial DNA

The syndrome of autosomally transmitted multiple deletions in mitochondrial DNA has diverse phenotypic manifestations, most of which are variants of chronic progressive external ophthalmoplegia.[18,19] Multiple deletions differ from single deletions in their inheritance pattern, location within the mitochondrial genome, and molecular structure. Unlike single deletions, which are almost always sporadic, multiple deletions can be transmitted in autosomal dominant and autosomal recessive patterns.[18] The pattern of autosomal inheritance suggests the existence of a nuclear-DNA–encoded gene that influences the structure of normal mitochondrial DNA. Tissue-specific, autosomally transmitted depletion of mitochondrial DNA has also been reported.[18] Molecular genetic methods can reliably detect mitochondrial-DNA deletions,[12-15] but these methods require DNA from skeletal muscle.

The MELAS Syndrome

In the MELAS syndrome, seizures and stroke-like events cause subacute brain dysfunction, cerebral structural changes, and several other clinical and laboratory abnormalities (Tables 1, 2, and 3). The disease is inherited maternally, but it may not be evident in relatives who do not have all the overt symptoms. In 80 percent of cases, there is a point mutation at nucleotide position 3243 in the $tRNA^{Leu(UUR)}$ gene. Other mutations have also been found in this gene, which appears to be a common target for pathogenetic mutations. The mutation at nucleotide position 3243 apparently has multiple phenotypic effects, because it is also associated with nondeletion chronic progressive external ophthalmoplegia, myopathy, deafness, diabetes, and dystonia.[17,20,21]

Myoclonic Epilepsy with Ragged-Red Fibers

The syndrome of myoclonic epilepsy with ragged-red fibers consists of myoclonus, seizures, cerebellar ataxia, and mitochondrial myopathy, as well as neurologic (Table 1) and laboratory (Table 3) abnormalities that are common in other mitochondrial encephalomyopathies. Maternal relatives may be asymptomatic or have partial clinical syndromes, including lipomas in a characteristic "horse collar" distribution and cardiovascular disease.[22,23] Pathogenetic mutations have been demonstrated at nucleotide positions 8344 and 8356 in the $tRNA^{Lys}$ gene.[22]

Neuropathy, Ataxia, and Retinitis Pigmentosa and Maternally Inherited Leigh Disease

The syndrome of neuropathy, ataxia, and retinitis pigmentosa is characterized by proximal-muscle weakness, sensory neuropathy, developmental delay, ataxia, seizures, dementia, and retinal pigmentary degeneration.[24] This maternally inherited disorder is associated with heteroplasmic missense mutations at nucleotide position 8993 in the ATPase 6 gene.[24] Large proportions of the same mutations are also present in patients with maternally inherited Leigh disease.[25] Molecular studies readily detect these point mutations in mitochondrial DNA extracted from muscle, blood, or urine.

Leber's Hereditary Optic Neuropathy

Leber's hereditary optic neuropathy, the first disease in humans that was linked to heritable point mutations in mitochondrial DNA,[3] is the exemplar of mitochondrial disease caused by homoplasmic missense mutations. The main clinical phenotype is painless, subacute, bilateral visual loss, with central scotomas and abnormal color vision.[26] The mean age at the onset is 23 years, and three to four times as many males are affected as females.[27]

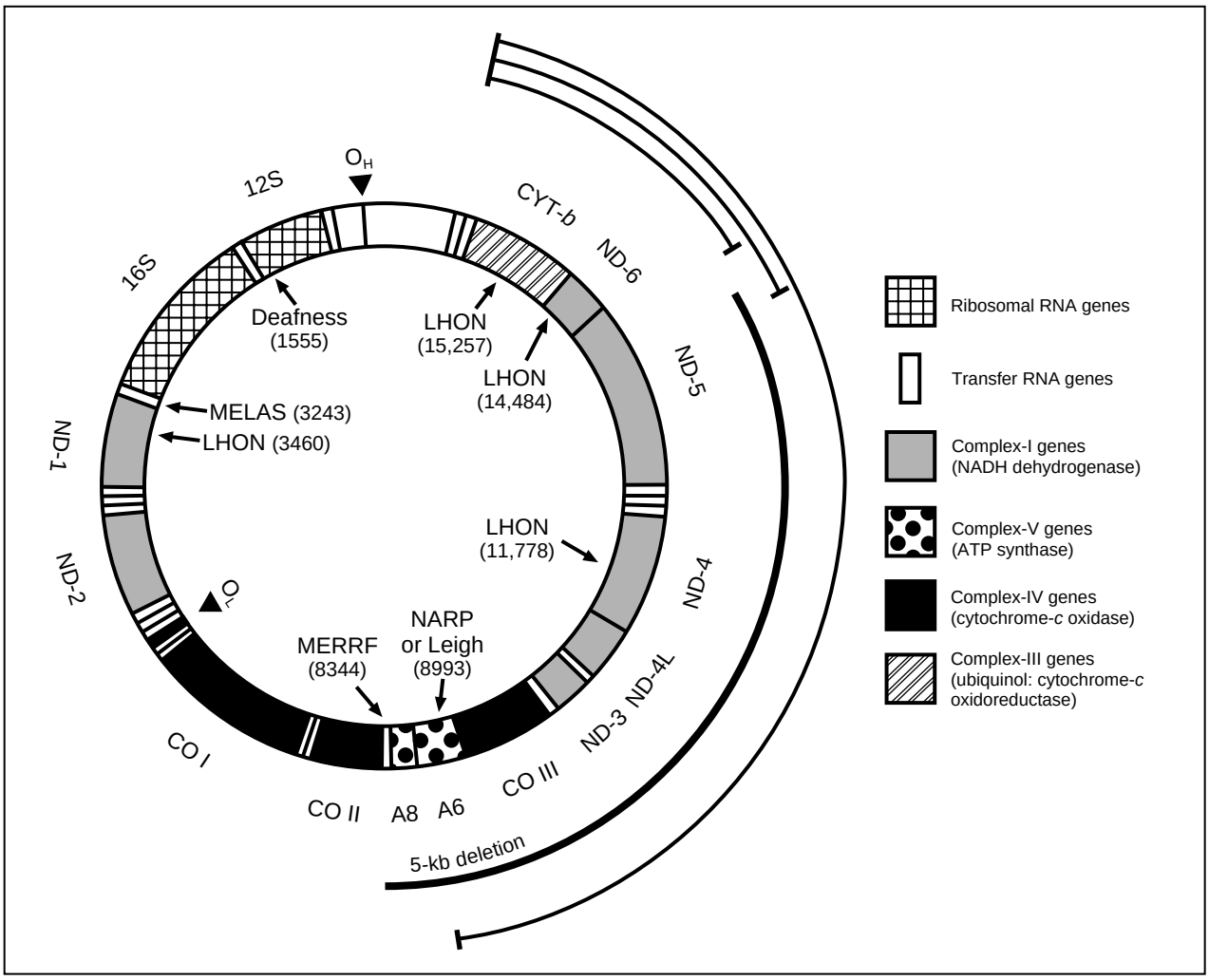

Figure 2. Diagram of Human Mitochondrial DNA and the Most Common Associated Pathogenetic Mutations.

Point mutations in structural and protein-coding genes are shown inside the circle, with the clinical phenotype indicated and the nucleotide position of the mutation shown in parentheses. The position of the most common single deletion, which is 5 kilobases (kb) long, and the multiple deletions are indicated by the arcs outside the circle. MERRF denotes myoclonic epilepsy with ragged-red fibers; NARP, neuropathy, ataxia, and retinitis pigmentosa; Leigh, maternally inherited Leigh disease; LHON, Leber's hereditary optic neuropathy; and MELAS, the syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes. O_H denotes the origin of heavy-stranded DNA replication, and O_L the origin of light-stranded DNA replication. CYT-*b* denotes the apocytochrome-*b* subunit; ND-1, ND-2, ND-3, ND-4L, ND-5, and ND-6, NADH dehydrogenase subunits; CO I, CO II, and CO III, cytochrome-*c* oxidase subunits; 12S and 16S, ribosomal RNA subunits; and A6 and A8, ATPase subunits. The large open space at the top (which includes O_H) is the noncoding D (displacement) loop.

Leber's hereditary optic neuropathy, which bears little clinical resemblance to the other mitochondrial diseases, was first classified as such a disease because of its pattern of maternal inheritance.[26] The visual loss appears to involve both genetic and epigenetic factors.[28] The mitochondrial-DNA mutations are heterogeneous, with mutations in at least eight genes that encode subunits of three biochemical complexes. Four mutations, at nucleotide position 11,778, position 3460, position 15,257, and position 14,484, may have primary pathogenetic importance[29] (Fig. 2). Two other mitochondrial-DNA mutations were recently found in cytochrome-*c* oxidase subunit III in patients with Leber's hereditary optic neuropathy.[30]

Several other mitochondrial-DNA mutations may have secondary roles in Leber's hereditary optic neuropathy.[29] The prevalence of a secondary mutation at nucleotide position 13,708 in subunit 5 of the NADH dehydrogenase gene increases significantly in association with four different primary mutations.[31] An X-linked nuclear-DNA factor may explain the marked predominance of symptomatic Leber's hereditary optic neuropathy in males, but the evidence is conflicting.[29] The most important epigenetic factors are consumption of alcohol and use of tobacco.[32]

The diversity of the primary mitochondrial-DNA mutations in Leber's hereditary optic neuropathy has clinical relevance. For example, the probability of visual recovery varies widely, depending on the mutation.[27] Some variants of the disorder, such as Leber's hereditary optic neuropathy plus dystonia[33] and subacute optic neuropathy and myelopathy,[34] are also associated with specific mitochondrial-DNA mutations.

Leber's hereditary optic neuropathy illustrates the

Table 3. Possible Laboratory Findings in Patients with Mitochondrial Diseases.

Ragged-red fibers in skeletal-muscle–biopsy specimens
Elevated lactate concentrations in serum and cerebrospinal fluid
Myopathic potentials on electromyography
Axonal and demyelinating peripheral neuropathy on nerve-conduction studies
Sensorineural hearing loss on audiography
Cardiac conduction defects
Basal-ganglia calcification or focal signal abnormalities on magnetic resonance imaging
Abnormalities on phosphorus-31 nuclear-magnetic-resonance spectroscopy
Defective oxidative phosphorylation on biochemical studies
Molecular genetic evidence of mitochondrial-DNA mutation

problems involved in determining the pathogenicity of any mitochondrial-DNA mutation.[35] It can be difficult to link a mutation to a clinical disease for several reasons: mitochondrial DNA is highly polymorphic, there is a dissociation between the genotype and the phenotype, different mutations can be associated with the same phenotype,[29] the same mutation can be associated with different phenotypes,[17] and epigenetic factors can affect clinical manifestations.

Systemic Manifestations of Mitochondrial-DNA Mutations

Virtually all tissues in the body depend to some extent on oxidative metabolism and can therefore be affected by mitochondrial-DNA mutations. Patients often first seek care because of the systemic manifestations listed in Table 2, which may thus provide the first opportunity to consider the diagnosis of a mitochondrial disease. Since these manifestations may be important comorbid features of the disease, they should be diagnosed and vigorously treated.

The neurologic manifestations of mitochondrial disorders, involving both the central and the peripheral nervous systems, have been reported extensively and reviewed elsewhere.[1,4,6] Less well known are the nonneurologic manifestations of mitochondrial-DNA mutations. Ophthalmologic manifestations are very common, involving virtually the entire visual axis from the lids, cornea, and extraocular muscles to the occipital cortex.[36] The cardinal findings include ptosis, ophthalmoplegia, optic neuropathy, pigmentary retinopathy, and cortical visual-field defects.[36] Cardiac findings, which are also common and can be life-threatening, include cardiomyopathy, conduction disease and heart block, the Wolff–Parkinson–White syndrome, and hypertension.[23]

Endocrine manifestations are frequent, and the incidence of diabetes mellitus is relatively high. Pancreatic islet cells are extremely active metabolically and are thus susceptible to the disruption of oxidative phosphorylation. Diabetes mellitus associated with mitochondrial-DNA mutations is mainly due to a defect in insulin secretion. The disorder has been linked with a heteroplasmic point mutation in the $tRNA^{Leu(UUR)}$ gene at nucleotide position 3243, usually in association with sensorineural hearing loss.[20] Other neurologic findings associated with this mutation, such as the MELAS syndrome or nondeletion chronic progressive external ophthalmoplegia, were not found. Diabetes mellitus, which is a treatable feature of a number of mitochondrial diseases, can thus be the predominant manifestation of a mutation in mitochondrial DNA.

Gastrointestinal manifestations of mitochondrial-DNA mutations include colonic pseudo-obstruction,[19] hepatopathy, and weight loss. The most prominent renal manifestation is a type of nonselective proximal-nephron dysfunction, with aminoaciduria, phosphaturia, and glycosuria, that resembles Fanconi's syndrome. Lactic acidosis and an acid–base disturbance or glomerulopathy may bring patients to the attention of nephrologists. Pearson's syndrome of exocrine pancreatic dysfunction, a sideroblastic hypoproliferative anemia, and pancytopenia occurs in association with large-scale, single deletions in mitochondrial DNA. Other forms of sideroblastic anemia or aplastic anemia may also be associated with acquired or inherited mitochondrial-DNA mutations. Multiple symmetric lipomas, with their characteristic horse-collar distribution on the thorax, occur in association with the mitochondrial-DNA mutation at nucleotide position 8344.[22,23]

The most prominent pulmonary manifestations of mitochondrial-DNA mutations are the central hypoventilatory abnormalities of Leigh disease and severe cases of myoclonic epilepsy with ragged-red fibers. Mild elevations of creatine kinase levels, along with muscle fatigability, poor stamina, and poor endurance, may bring patients with mitochondrial disease to the attention of rheumatologists, perhaps resulting in an evaluation for an inflammatory myopathy. Psychiatric manifestations, especially depression, have been noted in association with multiple mitochondrial-DNA deletions.[37]

Mitochondrial-DNA Mutations in Common Diseases

One of the next challenges for researchers is to define the role of mitochondrial-DNA mutations in common diseases. The genetic basis of many prevalent diseases is complex and does not follow a simple, single-gene mendelian inheritance. Leber's hereditary optic neuropathy illustrates the complex interactions between genetic and epigenetic factors.[28,32] These interactions may involve mitochondrial-DNA mutations in subgroups of common diseases, such as diabetes mellitus, in which the pattern of maternal inheritance is not prominent.

Thus far, I have focused on inherited mutations or gross structural derangements at the gametic level. Another category of mitochondrial-DNA mutations that may be relevant to late-onset degenerative disorders is the tissue-specific accumulation of somatic (noninherited) mutations.[38] Because of the high rate of mutations in mitochondrial DNA, postmitotic tissues or those with a slow turnover of DNA accumulate the largest number of somatic mitochondrial-DNA mutations. External factors may also affect mitochondrial DNA; for example, the antiretroviral drug zidovudine depletes muscle mitochondrial DNA and causes an acquired mitochondrial myopathy.[39]

The accumulation of mitochondrial-DNA mutations

above a threshold level in certain critical neuronal subpopulations and the consequent deficit in the production of ATP may contribute to the pathogenesis of neurodegenerative diseases,[40] particularly those that are more common with advanced age, such as Alzheimer's disease and Parkinson's disease. Mitochondrial energy deficits may contribute to neuronal injury through excitotoxic mechanisms.[41-43]

Such mechanisms may also contribute to aging itself. Shigenaga and colleagues have outlined an elegant concept of aging that incorporates the role of mitochondria.[38] These researchers argue that cumulative, age-dependent mitochondrial dysfunction, mediated to a substantial degree by oxidative damage to mitochondrial DNA and other mitochondrial macromolecules, plays an important part in the aging of cells, tissues, organ systems, and even the whole organism.

Summary

Data on the role of mitochondrial-DNA mutations in the pathogenesis of several diseases have accumulated at a breathtaking pace in the seven years since the first pathogenetic mutations in mitochondrial DNA were discovered. Elucidation of the complete mitochondrial-DNA sequence in humans and knowledge about variations in that sequence[44] have contributed to a nascent understanding of the clinical implications of mitochondrial-DNA mutations. The rapid accumulation of disease-specific knowledge about "the other human genome" may well foreshadow advances in our understanding of diseases involving the nuclear genome, which will emerge from the Human Genome Project. Detailed studies of mitochondrial disease have also provided insight into fundamental biologic processes, such as oxidative phosphorylation and aging.

Mitochondrial-DNA mutations appear to cause an extensive array of disorders. As the number and types of mitochondrial diseases increase, internists and subspecialists will be in a pivotal position to recognize and treat these diseases. The advances in our understanding of the molecular genetic basis of mitochondrial diseases have already had a profound effect on the detection and evaluation of mitochondrial diseases. A set of sensitive and specific molecular genetic tests has been developed for a number of the mitochondrial encephalomyopathies.[45] The unequivocal diagnosis of a mitochondrial disease by such methods is obviously the first step in appropriate genetic counseling and treatment. Although the advances in treatment have not paralleled the advances in diagnosis,[46] at least we are now aware of the biochemical targets. Mitochondrial gene therapy for these disorders must overcome obstacles that are different from those encountered in the search for effective nuclear gene therapy, but such therapy is not beyond the realm of possibility.

Discussion

DR. JEFFREY FLIER: Undernutrition is known to protect against aging and nuclear-DNA mutations. Does overnutrition damage mitochondrial DNA, and if so, could this process be related to any of the pathogenic consequences of obesity?

DR. JOHNS: I am unaware of any data on this topic, but I can speculate that overnutrition may increase oxidative damage to mitochondrial DNA that results in dysfunctional oxidative phosphorylation within mitochondria. These mitochondria may then become the source of excess free radicals that further damage mitochondrial DNA and other macromolecules in a feed-forward fashion.

DR. DAVID MOLLER: Because the ratio of mutant mitochondrial DNA to the wild type varies so much among tissues, how do you go about ruling out a mitochondrial mutation, especially one that may be inherited, when you cannot obtain biopsy specimens of the relevant tissue (e.g., beta cells of the pancreas or the optic nerve)? If you do not have a candidate mutation, how can you screen the whole genome?

DR. JOHNS: With respect to the availability of DNA from the relevant tissue, virtually all the point mutations can be detected readily in available tissues, such as leukocytes. Homoplasmic mutations are the same in all tissues. The proportions of heteroplasmic point mutations may vary according to the type of tissue, but all that is needed to demonstrate the presence of the mutation is a detectable proportion. Major structural mutations, such as deletions, do require the analysis of skeletal muscle. There is no easy, overall screening test that is sensitive and specific for the presence of mitochondrial-DNA mutations. Sequencing the entire mitochondrial-DNA region is impractical, and it is easy to miss heteroplasmic mutations. My colleagues and I use very specific molecular genetic assays for particular mutations, and we are interested in developing a method of analyzing larger mitochondrial-DNA regions.

DR. JOSEPH MAZJOUB: Do particular tissues have characteristic ratios of mutant mitochondrial DNA to the wild type, or do the ratios vary? If they vary, what factors control the variation? Is the half-life of a defective mitochondrion any different from that of a normal mitochondrion?

DR. JOHNS: For heteroplasmic mutations, the ratio of normal-to-mutant mitochondrial DNA varies within a tissue — for example, from fiber to fiber in skeletal muscle. The initial differences within and between tissues are determined by mitotic segregation during embryogenesis. These ratios may change over time, depending on a complex interplay of selection pressures at the molecular, organelle, cellular, tissue, and organismal levels. Mitochondria are not static structures within a cell; they undergo dynamic morphologic changes. I am unaware of any specific data on a difference in the half-lives of defective and normal mitochondria.

DR. FLIER: What is known about the rate of mutations in the mitochondria of brown fat?

DR. JOHNS: I am not aware of studies of mitochondrial-DNA mutations in normal brown fat. However, the lipomas that occur in association with the mitochondrial-DNA mutation at position 8344 are found in the areas where brown fat is more abundant.

DR. CHAIM MAYMAN: What is the role of mitochondrial-DNA mutations in the optic neuropathies associated with the use of tobacco or alcohol?

DR. JOHNS: The presence of mitochondrial-DNA mutations in patients with tobacco–alcohol amblyopia illustrates the interplay between genetic and epigenetic factors.[32] The mutations appear to confer a genetic susceptibility to the deleterious effects of tobacco, alcohol, or both on the optic nerve. However, not all patients with tobacco–alcohol amblyopia have detectable mitochondrial-DNA mutations, and my colleagues and I postulate that other mutations are present elsewhere in mitochondrial or nuclear DNA.

DR. LAKSHMI KANTHAM: Are there animal models of mitochondrial dysfunction?

DR. JOHNS: No. There is a large gap in our knowledge of these diseases. We have extensive knowledge at the molecular and in vitro cellular levels (rho^0 cell lines), and we also have extensive knowledge of the clinical phenotypes in humans. However, we have few data on organ systems and no data obtained under experimental conditions. The rho^0 human cell lines, which are depleted of endogenous mitochondrial DNA and repopulated with mutant mitochondria, have been indispensable systems for pathophysiologic studies at the cellular level.[47]

DR. COLIN M. COSSI: What do you think is the current level of public and medical awareness of the mitochondrial disorders?

DR. JOHNS: I believe that we are at a crucial juncture in the perception of these disorders. The recent announcement by the American cyclist Greg Le Mond that he is retiring from competitive cycling because of a mitochondrial myopathy[48] has brought these "mysterious" disorders to the attention of the public.

REFERENCES

1. DiMauro S, Moraes CT. Mitochondrial encephalomyopathies. Arch Neurol 1993;50:1197-208.
2. Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature 1988;331:717-9.
3. Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 1988;242:1427-30.
4. Wallace DC. Diseases of the mitochondrial DNA. Annu Rev Biochem 1992; 61:1175-212.
5. Brown MD, Wallace DC. Molecular basis of mitochondrial DNA disease. J Bioenerg Biomembr 1994;26:273-89.
6. Schon EA, Hirano M, DiMauro S. Mitochondrial encephalomyopathies: clinical and molecular analysis. J Bioenerg Biomembr 1994;26:291-9.
7. Howell N. Primary LHON mutations: trying to separate "fruyt" from "chaf." Clin Neurosci 1994;2:130-7.
8. Attardi G, Schatz G. Biogenesis of mitochondria. Annu Rev Cell Biol 1988; 4:289-333.
9. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. Nature 1981;290:457-65.
10. Clayton DA. Structure and function of the mitochondrial genome. J Inherit Metab Dis 1992;15:439-47.
11. Prezant TR, Agapian JV, Bohlman MC, et al. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet 1993;4:289-94.
12. Johns DR, Hurko O. Preferential amplification and molecular characterization of junction sequences of a pathogenetic deletion in human mitochondrial DNA. Genomics 1989;5:623-8.
13. Johns DR, Rutledge SL, Stine OC, Hurko O. Directly repeated sequences associated with pathogenic mitochondrial DNA deletions. Proc Natl Acad Sci U S A 1989;86:8059-62.
14. Schon EA, Rizzuto R, Moraes CT, Nakase H, Zeviani M, DiMauro S. A direct repeat is a hotspot for large-scale deletions of human mitochondrial DNA. Science 1989;244:346-9.
15. Shoffner JM, Lott MT, Voljavec AS, Soueidan SA, Costigan DA, Wallace DC. Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. Proc Natl Acad Sci U S A 1989;86:7952-6.
16. Poulton J. Duplications of mitochondrial DNA: implications for pathogenesis. J Inherit Metab Dis 1992;15:487-98.
17. Moraes CT, Ciacci F, Silvestri G, et al. Atypical clinical presentations associated with the MELAS mutation at position 3243 of human mitochondrial DNA. Neuromuscul Disord 1993;3:43-50.
18. Zeviani M. Nucleus-driven mutations of human mitochondrial DNA. J Inherit Metab Dis 1992;15:456-71.
19. Johns DR, Threlkeld AB, Miller NR, Hurko O. Multiple mitochondrial DNA deletions in myo-neuro-gastrointestinal encephalopathy syndrome. Am J Ophthalmol 1993;115:108-9.
20. van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, et al. Mutation in mitochondrial $tRNA^{(Leu)(UUR)}$ gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet 1992;1:368-71.
21. Johns DR, Plotkin GM, Logigian EL, Sudarsky LR. Dystonia as a manifestation of the 3243 mtDNA mutation. Ann Neurol 1994;36:315. abstract.
22. Silvestri G, Ciafaloni E, Santorelli FM, et al. Clinical features associated with the A→G transition at nucleotide 8344 of mtDNA ("MERRF mutation"). Neurology 1993;43:1200-6.
23. Austin SG, Thandroyen FT, Hecht JT, Vriesendorp FJ, Jones OW, Johns DR. Expanding the phenotype of the 8344 $tRNA^{lys}$ mitochondrial DNA mutation. Neurology 1994;44:Suppl 2:A403. abstract.
24. Holt IJ, Harding AE, Petty RKH, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 1990;46:428-33.
25. Santorelli FM, Shanske S, Macaya A, DeVivo DC, DiMauro S. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh's syndrome. Ann Neurol 1993;34:827-34.
26. Nikoskelainen EK, Savontaus M-L, Wanne OP, Katila MJ, Nummelin KU. Leber's hereditary optic neuroretinopathy, a maternally inherited disease: a genealogic study in four pedigrees. Arch Ophthalmol 1987;105:665-71.
27. Johns DR. Genotype-specific phenotypes in Leber hereditary optic neuropathy. Clin Neurosci 1994;2:146-50.
28. Johns DR, Smith KH, Miller NR, Sulewski ME, Bias WB. Identical twins who are discordant for Leber's hereditary optic neuropathy. Arch Ophthalmol 1993;111:1491-4.
29. Newman NJ. Leber's hereditary optic neuropathy: new genetic considerations. Arch Neurol 1993;50:540-8.
30. Johns DR, Neufeld MJ. Cytochrome *c* oxidase mutations in Leber hereditary optic neuropathy. Biochem Biophys Res Commun 1993;196:810-5.
31. Johns DR, Neufeld MJ, Park RD. An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Commun 1992;187:1551-7.
32. Cullom ME, Heher KL, Miller NR, Savino PJ, Johns DR. Leber's hereditary optic neuropathy masquerading as tobacco-alcohol amblyopia. Arch Ophthalmol 1993;111:1482-5.
33. Jun AS, Brown MD, Wallace DC. A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. Proc Natl Acad Sci U S A 1994;91:6206-10.
34. Johns DR, Hurko O, Attardi G, Griffin JW. Molecular basis of a new mitochondrial disease: acute optic neuropathy and myelopathy. Ann Neurol 1991;30:234. abstract.
35. Howell N. Mitochondrial gene mutations and human diseases: a prolegomenon. Am J Hum Genet 1994;55:219-24.
36. Johns DR. Mitochondrial DNA mutations and eye disease. In: Wiggs JL, ed. Molecular genetics of ocular disease. New York: Wiley–Liss, 1995:201-18.
37. Suomalainen A, Majander A, Haltia M, et al. Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia. J Clin Invest 1992;90:61-6.
38. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci U S A 1994;91:10771-8.
39. Dalakas MC, Illa I, Pezeshkpour GH, Laukaitis JP, Cohen B, Griffin JL. Mitochondrial myopathy caused by long-term zidovudine therapy. N Engl J Med 1990;322:1098-105.
40. Shoffner JM, Brown MD, Torroni A, et al. Mitochondrial DNA variants observed in Alzheimer disease and Parkinson disease patients. Genomics 1993; 17:171-84.
41. Beal MF. Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann Neurol 1992;31:119-30.
42. Beal MF, Hyman BT, Koroshetz W. Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases? Trends Neurosci 1993;16:125-31.
43. Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol 1994;36:747-51.
44. Cann RL, Stoneking M, Wilson AC. Mitochondrial DNA and human evolution. Nature 1987;325:31-6.
45. Johns DR, Neufeld MJ. Pitfalls in the molecular genetic diagnosis of Leber hereditary optic neuropathy (LHON). Am J Hum Genet 1993;53:916-20.
46. Johns DR. Mitochondrial encephalomyopathy. In: Johnson RT, Griffin JW, eds. Current therapy in neurologic disease. St. Louis: Mosby–Year Book, 1993:329-32.
47. King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. Science 1989;246:500-3.
48. Almond E. Le Mond announces retirement. Los Angeles Times. December 4, 1994:C5.