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The Neuro-Ophthalmology of Mitochondrial Disease

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Abstract

Mitochondrial diseases frequently manifest neuro-ophthalmologic symptoms and signs. Because of the predilection of mitochondrial disorders to involve the optic nerves, extraocular muscles, retina, and even the retrochiasmatal visual pathways, the ophthalmologist is often the first physician to be consulted. Disorders caused by mitochondrial dysfunction can result from abnormalities in either the mitochondrial DNA or in nuclear genes which encode mitochondrial proteins. Inheritance of these mutations will follow patterns specific to their somatic or mitochondrial genetics. Genotype-phenotype correlations are inconstant, and considerable overlap may occur among these syndromes. The diagnostic approach to the patient with suspected mitochondrial disease entails a detailed personal and family history, careful ophthalmic, neurologic, and systemic examination, directed investigations, and attention to potentially life-threatening sequelae. Although curative treatments for mitochondrial disorders are currently lacking, exciting research advances are being made, particularly in the area of gene therapy. Leber hereditary optic neuropathy, with its window of opportunity for timely intervention and its accessibility to directed therapy, offers a unique model to study future therapeutic interventions. Most patients and their relatives benefit from informed genetic counseling.

Keywords

Mitochondria; neuro-ophthalmology; Leber hereditary optic neuropathy; dominant optic atrophy; chronic progressive external ophthalmoplegia; Kearns-Sayre syndrome; pigmentary retinopathy; NARP; MELAS

1. Introduction

Mitochondrial diseases, once believed to be esoteric and rare, are now recognized as a common cause of metabolic disease. Over the past thirty years, the sequencing of the mitochondrial genome, the subsequent identification of pathogenic mutations, and the elucidation of the major principles of mitochondrial genetics has led to the recognition of the protean manifestations of mitochondrial disease. Prevalence estimates have been reappraised, and combined adult and pediatric mitochondrial disease is now estimated to have a prevalence of at least 1:5000.²⁰⁶ Because ophthalmic manifestations figure prominently in mitochondrial disease, general and

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LITERATURE SEARCH

Literature search was based on a Medline search with Pubmed, initially including the search words: *mitochondria, mitochondrial, LHON, Leber, optic atrophy, optic neuropathy, dominant optic atrophy, DOA, Friedreich ataxia, hereditary motor sensory neuropathy, Charcot-Marie-Tooth, hereditary spastic paraplegia, Wolfram, DIDMOAD, ophthalmoplegia, CPEO, PEO, Kearns-Sayre syndrome, KSS, MNGIE, pigmentary retinopathy, retinitis pigmentosa, maculopathy, NARP, MELAS, retrochiasmatal vision loss, treatment, gene therapy, brimonidine, l-arginine, and coenzyme Q10*. The search covered the years 1966 to June 2009 and included English articles and other-language publications with English abstracts. References within relevant articles were also obtained for review.

subspecialty ophthalmologists are likely to encounter patients with mitochondrial disease in their everyday practice, often as the first physician the patient consults. Recognition of the four most common neuro-ophthalmic manifestations of mitochondrial disease (optic neuropathy, chronic progressive external ophthalmoplegia (CPEO), pigmentary retinopathy, and retrochiasmal visual loss), combined with a basic knowledge of mitochondrial genetic principles, allows for effective diagnosis, treatment, and counseling of patients and their relatives.

2. Physiology and genetics of mitochondria

A basic understanding of the role of mitochondria in cellular function and the genetics and modes of inheritance of mitochondrial disorders is essential in appreciating the richness of clinical expression of mitochondrial disease.

2.1. The functions of mitochondria in the cell

Mitochondria are double-membrane cytoplasmic organelles, whose principal role is the generation of energy needed for cellular growth, function, and maintenance. Each cell may have hundreds of mitochondria, and depends on them for the production of ATP, the universal “currency” of energy in the cell. Cells in highly metabolically active tissues, such as the central nervous system (including the eye and the optic nerve), cardiac conduction system, oxidative muscle, endocrine pancreas, kidneys, and liver, rely heavily on ATP and have increased numbers of mitochondria. Hence, these tissues are the most susceptible to the defective mitochondrial energy output seen in mitochondrial disease.^{29,152-259}

Although the production of ATP by oxidative phosphorylation (OXPHOS) is an essential and well-known task of mitochondria in the cell, other mitochondrial processes include the detoxification of reactive oxygen species, regulation of cellular apoptosis, the fission and fusion of organelle membranes among mitochondrial networks, and aspects of iron metabolism, fatty acid oxidation, and amino acid biosynthesis.^{29,217} Dysfunction of OXPHOS seems to be the most important factor in the pathogenesis of mitochondrial disorders, but dysfunctions in other mitochondrial processes have been increasingly implicated in human disease.

The morphology and genetics of mitochondria reflect their probable evolutionary origins as autonomous prokaryotic organisms. Like prokaryotes, mitochondria have their own genome, encoded in a 16569-base-pair circle of mitochondrial DNA (mtDNA) (Figure 1), and their own ribosomal apparatus for gene transcription. Moreover, like prokaryotes, mitochondria replicate by fission independent of their host cell’s replicative cycle, and may fuse with other mitochondria inside a cell to exchange genetic material.¹⁴⁵ According to the widely-accepted “endosymbiotic theory”, popularized by Lynn Margulis, ancient autonomous prokaryotic “proto-mitochondria” were ingested and incorporated into the cytoplasm of larger eukaryotic cells.^{146,217} The relationship that developed between eukaryotic cells and proto-mitochondrial prokaryotes was mutually beneficial, with the eukaryotic cell providing cytosolic proteins and creating a protected environment for these proto-mitochondria, and proto-mitochondria supplying ATP and detoxifying oxygen species for the eukaryotic cell.¹⁴⁶ From these beginnings, eukaryotic cells and mitochondria evolved an extraordinary interdependence, which now informs our understanding of mitochondrial disease pathogenesis and inheritance.

2.2. Physiology of the OXPHOS system

The generation of ATP by mitochondrial OXPHOS is accomplished by the mitochondrial respiratory chain – a five-complex chain of polypeptides embedded in the inner mitochondrial membrane. The first four complexes of the mitochondrial respiratory chain (complexes I–IV) oxidize NADH and FADH₂ through a controlled series of redox reactions, while complex V

harnesses the resultant electrochemical gradient to phosphorylate ADP to ATP. Cofactors, including ubiquinone (also called coenzyme Q₁₀, or CoQ₁₀) and cytochrome c (cyt c), act as electron shuttles between respiratory complexes and are essential for mitochondrial respiratory chain function.^{29,57,118,247,259} Defects of these complexes, cofactors, or the machinery that transcribes, assembles, and maintains them may result in interruptions of mitochondrial ATP supply to the host cell or increases in reactive oxygen species, with potentially grave implications for cellular function.

2.3. Genetics of mitochondria

The mitochondrial genome consists of 37 genes, which encode for 13 structural proteins (all of which are subunits of the five mitochondrial respiratory complexes) and the two 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) required to synthesize these 13 structural proteins (Figure 1).^{29,152} However, the majority of the protein subunits of the OXPHOS system (more than 80) are encoded by nuclear DNA and are imported into the mitochondria from the cytosol (Figure 2). Although complex II is assembled from nuclear DNA-encoded proteins alone, the remaining respiratory complexes are fusions of nuclear DNA- and mtDNA-encoded proteins, illustrative of the intricate symbiosis between host cell and mitochondria. The nuclear genome therefore plays an essential role in both the structure of the mitochondrial respiratory chain and the function of mitochondrial OXPHOS. Furthermore, the processes governing most mitochondrial activity, including the expression, replication, and repair of the mitochondrial DNA itself, are governed by nuclear genes (Figure 2).

2.4. Inheritance of mitochondrial disease

Because OXPHOS is mediated by two genomes, nuclear and mitochondrial, “mitochondrial disease” may result from genetic defects in either mtDNA or nuclear DNA (Figure 2).^{57,169} Common mitochondrial syndromes and their possible modes of inheritance are summarized in Table 1. The nuclear genome obeys the usual rules of mendelian inheritance (*e.g.*, autosomal dominant, autosomal recessive, or sex-linked transmission), the mitochondrial genome follows a different set of rules of inheritance, characterized by four unique properties: maternal inheritance, heteroplasmy, replicative segregation, and the threshold effect.

“**Maternal inheritance**” refers to the transmission of the mitochondrial genome from a mother to all her children, with no paternal mtDNA contribution. During fertilization, a human sperm cell, with its few mitochondria, does not contribute significantly to the zygote. Although approximately 100 paternal mitochondria may enter the ovum at fertilization, these organelles are tagged with ubiquitin for prompt proteolytic destruction by the zygote,²²³ and virtually all the zygote’s mitochondria come from its mother (Figure 3).

Each mitochondrion contains several copies of mtDNA in its matrix at any given time.²⁵⁹ Usually, all copies of wild-type mtDNA are identical, a condition known as “homoplasmy”. After a mutation has arisen in one copy of mtDNA, however, wild-type and mutant species of mtDNA may coexist within the same mitochondrion – a condition known as “**heteroplasmy**”. Heteroplasmy becomes important during division of the host cell as mitochondria (and the mtDNA they contain) are distributed between the two daughter cells in a process called “**replicative segregation**”. Replicative segregation is a *stochastic* process, meaning it is governed by both predictable and random elements: although all mtDNA copies are predictably distributed between two daughter cells during cell division, the proportions of mutant mtDNA the two daughter cells receive are subject to random influences, and may be unequal. When heteroplasmic mtDNA mutations are segregated unequally during cell mitosis, mutation loads may increase in certain cells and tissues, particularly when reiterative mitotic events occur, as in embryogenesis. Heteroplasmy and replicative segregation, therefore,

contribute strongly to the heterogeneity of disease phenotype, even among individuals of the same pedigree.

In a heteroplasmic state, a certain proportion of mtDNA remains normal and may compensate for the deleterious effects of the mutant mtDNA population in a cell or tissue. When the proportion of mutant mtDNA exceeds a certain tissue-specific threshold, however, the wild-type mtDNA is no longer able to compensate to sustain normal cellular function, and the disease phenotype is expressed. This “**threshold effect**” is tissue-specific, and tissues with high metabolic needs, such as the central nervous system, including the optic nerve, retinal pigment epithelium, and extraocular muscles, may have lower thresholds for mutant mtDNA loads than less metabolically active tissues.¹⁰⁰ Post-mitotic cells, such as neurons, are believed to accumulate mutant mtDNA copies over time, which eventually overwhelm the wild-type mtDNA and result in age-dependent expression of disease.¹⁵²

Point mutations account for over 200 disease-causing primary mutations in mtDNA (www.mitomap.org).^{217,253} Most disease-causing point mutations are heteroplasmic, but several mutations, notably the common Leber hereditary optic neuropathy (LHON) mutations, are most often homoplasmic. Point mutations in mtDNA are a common cause of mitochondrial disease and are maternally inherited.²⁵⁹

Single large-scale rearrangements (deletions or duplications) of mtDNA comprise another class of primary mtDNA mutations, and are mainly sporadic in origin. reasons that are incompletely understood, only one in 25 females with sporadic single large-scale rearrangements of mtDNA transmits the mutation to her progeny.^{42,57,259}

2.5. Nuclear influences on mitochondrial genetics

Mutations in the nuclear genome may also cause mitochondrial dysfunction, most often by damaging nuclear genes that control the stability of the mtDNA molecule. Such nuclear DNA mutations lead to secondary mutations in mtDNA, usually the accumulation of multiple large-scale deletions in the mitochondrial genome.^{57,58,259} Although these secondary mutations are localized to the mtDNA and result in defective OXPHOS, the underlying tendency to develop such secondary mutations results from a **nuclear** DNA mutation, and the resulting disease is therefore inherited in mendelian fashion.

Other nuclear genes encode subunits and assembly components for complexes I to V of the mitochondrial respiratory chain, and mutations in these genes may cause dysfunctional OXPHOS directly. Many such diseases have been described, most often resulting in a Leigh disease phenotype without prominent ophthalmological features.^{57,259} Finally, nuclear DNA mutations may cause disease by interfering with non-OXPHOS aspects of mitochondrial function, such as membrane stabilization and mitochondrial fusion (as in autosomal dominant optic atrophy) and iron metabolism (as in Friedreich ataxia). These diseases will also be inherited in mendelian fashion, reflecting their mutational origin in the nuclear genome.

2.6. Classification of mitochondrial diseases

Mitochondrial diseases are difficult to classify. Within a pedigree, a single mtDNA mutation may have protean clinical and biochemical manifestations, varying in expression and penetrance among individuals and even within a single individual over time. Conversely, a single common phenotype may be due to one of several mtDNA or nuclear DNA mutations (Table 1 and Table 2). The factors influencing disease expression in an individual include heteroplasmy, replicative segregation, and the tissue-specific threshold effect, as described above, but may also include environmental, toxic, and other non-genetic factors. Despite the variable and overlapping correspondence between genotype and phenotype, a clinical

classification of mitochondrial diseases may be made based on four distinct neuro-ophthalmic syndromes: optic neuropathy, CPEO, pigmentary retinopathy, and retrochiasmal visual loss.

3. Optic neuropathy

3.1. Leber hereditary optic neuropathy (LHON)

3.1.1. History and epidemiology—First defined as a clinical entity by the German ophthalmologist Theodore Leber in 1871,¹²⁹ LHON was the first human disease to be etiologically linked to a point mutation in mtDNA.²⁴⁴ It is the most common primary disease of mtDNA associated with bilateral optic neuropathies,^{169,196,257} with a minimum point prevalence of 1 in 31,000 in the northeast of England,¹⁴³ and similar prevalences elsewhere in Europe.^{186,218} LHON typically affects males of the lineage, with a male predominance of 80–90% in most pedigrees.¹⁷¹ The onset of symptoms typically occurs between the ages of 15 and 35 years, but ages of onset between 2 and 87 years have been reported.^{71,171,195}

3.1.2. Clinical presentation—The usual presentation of LHON is that of rapid, painless loss of central vision in one eye, followed by similar loss of vision in the fellow eye within days to months.^{29,169,257} More than 97% of patients develop second eye involvement within one year,^{57,171,195} with a median delay between involvement of the two eyes of 6 to 8 weeks.⁸⁰ Bilateral simultaneous onset of vision loss may occur in up to 50% of cases, but may include cases of bilateral sequential vision loss in which vision loss in the first eye was unnoticed by the patient.^{80,169,195,257}

Most patients progress to visual acuities of 20/200 or worse, with accompanying dense central or cecentral scotomas on visual field testing (Figure 4).^{167,169,172,195} Compared to other causes of optic neuropathy, LHON may maintain relative preservation of pupillary light reflexes,²⁹ although this remains debated.⁹⁸

Fundus findings in the acute and subacute stages of LHON often include circumpapillary telangiectatic microangiopathy and elevation of the retinal nerve fiber layer (RNFL) around the disc (pseudoeedema) (Figure 5). There is no true disc edema, as demonstrated by the absence of disc leakage on fluorescein angiography. Tortuosity of the retinal vasculature has also been described.¹⁷¹ These funduscopic findings, although helpful when present, do not rule out LHON when absent; in up to 50% of acute LHON cases, the optic disc appears normal.^{171,194}

Interestingly, these fundus abnormalities may also be seen in the eyes of “pre-symptomatic” carriers of a LHON mutation and in the eyes of asymptomatic maternal relatives, some of whom never lose vision in their lifetime.¹⁷² Although “asymptomatic”, these LHON carriers may also have subtle abnormalities in optic nerve structure and function on more subtle testing. These abnormalities, which may wax and wane with time, include relative paracentral or arcuate scotomas on automated perimetry, impairments in color vision and contrast sensitivity, and depressed or asymmetric electrophysiological responses in the retina and optic nerve.^{167,201}

As the disease progresses in patients with symptomatic LHON, disc hyperemia, pseudoeedema, and telangiectasias resolve, and rapid axonal loss in the papillomacular bundle leads to temporal atrophy of the optic nerve head. This pattern of atrophy may progress further to cupping of the disc or to diffuse optic pallor.^{148,169}

Optical coherence tomography (OCT) has allowed objective confirmation and quantification of the characteristic fundus changes in LHON. The RNFL, as measured by OCT, is thickened during the acute phase (less than 6 months from symptom onset) of LHON, and markedly thinned, especially temporally, during the chronic phase (more than 6 months from symptom

onset).¹² Asymptomatic carriers of LHON mutations may show thickening of temporal fibers on OCT, more often in males than females, suggestive of early involvement of the papillomacular bundle in preclinical or subclinical LHON.²⁰²

In most patients, vision loss is devastating and permanent; however, in a minority of patients, visual recovery may occur, with the development of “fenestrations” within a visual field defect or with more diffuse return of central visual acuity and color vision, usually bilaterally.^{171·172·222} Visual recovery, when it occurs, generally happens slowly between 6 and 12 months after the onset of visual loss; however, sudden dramatic improvement in vision may occur many years after symptom onset.¹⁷¹ Positive prognostic features for visual recovery include, most importantly, a favorable mutation status (the 14484 mutation has the best prognosis, as described below), and an age of onset less than 20 years.^{11·169·172} It has also been suggested that thicker RNFL and larger optic disc vertical diameter on OCT may be associated with a better visual prognosis.^{11·12·191}

Most LHON patients have visual loss as the only manifestation of their disease. In some pedigrees, however, cardiac conduction defects such as Wolff-Parkinson-White syndrome, Lown-Ganong-Levine syndrome, and long QT syndromes may occur, and EKG should be performed.¹⁶⁹ Minor neurological and skeletal abnormalities have also been reported.¹⁶⁹ Uncommonly, major neurological abnormalities may occur with LHON, and the disorders in these patients or pedigrees have been termed “Leber Plus”.¹⁷¹ Examples of “Leber Plus” manifestations include basal ganglionic degeneration with early-onset dystonia, spasticity, psychiatric disturbances, and encephalopathy.^{75,126,171} A large Australian family has been described in which maternal family members developed optic neuropathies, spasticity, movement disorders, psychiatric disturbances, and acute encephalopathic episodes.^{88,171,245} Some patients with LHON have been observed to develop a syndrome clinically and radiologically indistinguishable from multiple sclerosis, but whether the concurrence of these events exceeds the incidence expected by chance alone remains unresolved, particularly as an underlying LHON mutation may worsen the prognosis of optic neuritis in a patient with multiple sclerosis.^{19,171,257}

3.1.3. The selective vulnerability of the optic nerve in LHON—The selective vulnerability of the optic nerve (and the retinal ganglion cells (RGCs) which comprise it) to mitochondrial dysfunction in LHON may relate to uneven energy demands along each RGC axon. Histochemical studies of RGC axons have shown mitochondrial clustering in areas with a high density of repolarizing sodium-potassium membrane pumps, and an abrupt decrease in mitochondrial numbers is seen posterior to the lamina cribrosa where myelination begins and energy-efficient saltatory conduction occurs.^{8·24·29·256} This uneven distribution of mitochondria suggests an increased energy requirement and special vulnerability of the unmyelinated retinal and prelaminar portions of the RGC axons to bioenergetic failure in LHON. The smaller-diameter P-type RGCs that comprise the majority of fibers in the papillomacular bundle may be particularly affected by this bioenergetic “chokepoint”, potentially explaining the susceptibility to loss of central visual function in LHON.^{30,89}

3.1.4. Mitochondrial DNA LHON mutations—Three point mutations in mtDNA, known as the “primary LHON mutations”, account for about 90% of cases of LHON worldwide.^{118·171} These mutations are located at mtDNA nucleotide positions 11778 (about 69% of cases), 14484 (about 14% of cases), and 3460 (about 13% of cases)¹⁷¹ and all involve genes encoding subunits of complex I of the mitochondrial respiratory chain (Figure 6). The true prevalence of each mutation varies with the population being studied. For example, in China and Japan, more than 90% of LHON cases are due to the 11778 mutation,^{149,255} while among French Canadians, 87% of cases are attributable to the 14484 mutation, as a result of a founder effect.^{140,141} Many other primary LHON mtDNA point mutations have been described, but

each accounts for only one or a few pedigrees worldwide.^{171,257} Most often, these other primary mutations also involve genes encoding the ND1 and ND6 subunits of complex I – so-called mutational “hot spots”.^{41,239}

Although the 11778, 14484, and 3460 mutations usually cause isolated “nonsyndromic” optic neuropathy, these mutations may, in some pedigrees, be expressed as a “Leber Plus” syndrome. For example, the syndrome of LHON plus spastic dystonia and basal ganglionic lesions has been reported in at least two 11778 families and in one 3460 family.^{126,154} Other mtDNA pathogenic point mutations have been more often associated with “Leber Plus” syndromes: for example, the syndrome of LHON plus spastic dystonia occurs with mutations at the 14596, and 14459 locations;¹⁷¹ the large Australian pedigree with optic neuropathies, movement disorders, and encephalopathy was found to have both a LHON primary 14484 mutation *and* a point mutation at the 4160 position in the *ND1* gene.⁸⁸

Some mtDNA variants are found with higher frequency in populations of LHON patients than in control populations and are referred to as “secondary” mutations, because their causal significance remains unclear, despite their association with LHON.^{169,171} The association between these secondary mutations and LHON may be explained, in part, by the concept of the mtDNA “haplogroup”: a cluster of stable background mtDNA polymorphisms found among individuals descended from a common female ancestor.²⁵⁷ These polymorphisms may have epigenetic effects on the expression of LHON mutations within a haplogroup, and may increase disease penetrance.⁹¹ Alternatively, certain haplogroups may have increased susceptibility to *de novo* primary LHON mutations. Many secondary mutations may therefore be markers of an underlying LHON-prone haplogroup rather than pathogenic mutations in themselves.

Because the three primary LHON mutations account for the vast majority of cases of LHON, they should be the first to be screened in a patient with vision loss suspicious for LHON. If none of these three mutations are detected, and clinical suspicion remains high, then testing for other mtDNA mutations associated with LHON is indicated, particularly when a family history of LHON-like disease is elicited. Alternatively, sequencing of the entire mitochondrial genome is possible; however, the difference between polymorphisms and pathogenic mutations may be subtle, and consultation with an expert versed in the intricacies of mitochondrial genetics is recommended.⁴³

3.1.5. Prognosis—Among the three primary LHON mutations, clinical phenotype is virtually indistinguishable, and the only consistent mutation-dependent clinical feature is the prognosis for spontaneous recovery of visual acuity. The 14484 mutation has a 37–71% chance of some degree of visual improvement, while the 11778 mutation has only a 4% chance.^{169, 172,195,222,257} The 3460 mutation appears to have the same chance of recovery as the 11778 mutation, but numbers are too small for meaningful comparison.^{12,195}

3.1.6. Factors affecting disease expression—Although a pathogenic mtDNA mutation is necessary for the development of LHON, phenotypic expression is not determined by genotype alone, as is apparent from the incomplete and variable penetrance of the disease among pedigrees harboring one of the three primary mtDNA mutations. Although most individuals with LHON are homoplasmic for their mtDNA mutation, 10–15% are heteroplasmic,^{80,143,257} and tissue-specific segregation in the setting of such heteroplasmy may account for some phenotypic variability among pedigrees. In the more usual setting of homoplasmic primary mtDNA mutations, however, additional nuclear genetic factors, mitochondrial genetic factors, and/or environmental factors may partially determine disease expression.

Nuclear genes regulate many normal mitochondrial functions, including the expression of mitochondrial genes, and nuclear genetic factors have been implicated in the phenotypic expression of mitochondrial disease. Because LHON mutations are phenotypically expressed more commonly among men than women, an X-linked vision loss susceptibility gene has been hypothesized. Early linkage analysis studies were non-revealing, but more recent studies have identified a region of interest on the X chromosome for the putative susceptibility gene and have defined a high-risk haplotype at Xp21 associated with a 35-fold increase in vision loss among patients with the 11778 and 14484 mutations.^{92,208} The presence of an X-linked susceptibility locus does not exclude the possibility of autosomal genetic factors also having influence over disease expression, although no such factors have yet been identified.

An influence of mitochondrial genetic factors on disease expression in LHON has been shown through the analysis of mitochondrial haplogroups. Haplogroup J, one of several mitochondrial haplogroups found in central Europeans, has been associated with the 11778 and 14484 mutations in LHON,^{86,123} and a meta-analysis of 159 European pedigrees has suggested the penetrance of these two primary mutations is increased on a haplogroup J background.⁹¹ The 3460 mutation, on the other hand, although distributed equally among haplogroups, was more often expressed phenotypically on a haplogroup K background. Mitochondrial haplogroups may also confer protection from disease expression, as evidenced by the lower risk of visual loss from the 11778 mutation on a haplogroup H background.⁹¹

A role for environmental factors in the expression of the LHON phenotype is suggested by reports of discordant expression of a primary LHON mutation by monozygotic twins.²⁰ Tobacco smoking and alcohol have been proposed as precipitants for vision loss in individuals carrying a primary LHON mutation.^{113,200,237} One large case-control study found no association,¹¹² but a large, multicenter, retrospective telephone survey of members of 125 LHON pedigrees in three European countries found an association between smoking and vision loss, and a significant increase in lifetime clinical penetrance among male smokers (93% penetrance) than male non-smokers (66% penetrance).¹¹³ The extent to which LHON may “unveil” an underlying male susceptibility to tobacco-related toxic optic neuropathy is not known.¹⁷⁰

Theoretically, nutritional deficiencies might also play a role in disease expression through an insufficiency of important metabolic cofactors,¹⁸⁵ but widespread malnutrition in Cuba during the early 1990s did not appear to change the penetrance of LHON in a large 11778-positive pedigree.¹⁶⁸ Various other systemic illnesses, medications, and toxins have been proposed as triggers for vision loss in the setting of LHON mutations, none with robust epidemiological confirmation.^{136,195,200} No specific environmental precipitant for vision loss in LHON mutation carriers has been clearly identified, and the true extent to which environmental factors influence LHON expression remains unknown.

In clinical practice, physicians are not infrequently queried by carriers of LHON primary mutations as to their risk of developing vision loss, as well as their risk of transmitting the disease to their children. The lifetime risk of vision loss in a homoplasmic carrier varies with sex and mutation type, and the magnitude of this risk varies in the literature depending on ascertainment method. The risk of vision loss across all mutations is about 46% for men and 11% for women.^{80,141,143} Familial LHON may have a lower risk of vision loss – as low as 20% in men and 4% in women in one Australian study.¹³⁹ The risk of vision loss in familial LHON has been observed to decline with successive generations, and this effect may relate to changes in environmental factors over decades of observation.^{139,200}

Because LHON is caused by a mtDNA point mutation, it is inherited maternally, and therefore affected or carrier *men* cannot transmit their mutation to their children. Affected or carrier

women, on the other hand, transmit their LHON mutation to all of their children. Children of homoplasmic women are at the highest risk for LHON disease expression, as they are homoplasmic themselves. Children of heteroplasmic women, however, receive a variable and unpredictable amount of mutant mtDNA from their mothers, not determined by the extent of maternal heteroplasmy, and may have insufficient mutant mtDNA to reach the threshold for disease expression.^{40,93}

3.2. Dominant optic atrophy (DOA)

3.2.1. History and epidemiology—Autosomal dominant optic atrophy (DOA) was first described in detail by the Danish ophthalmologist Poul Kjer in 1959,¹¹⁶ and is the most common autosomal hereditary optic neuropathy, with a reported prevalence of 1:50,000 overall¹³⁷ and up to 1:10,000 in Denmark.^{114,233} DOA, unlike LHON, shows no gender predilection.

3.2.2. Clinical presentation—DOA typically presents as slowly progressive, painless, bilateral, symmetric visual loss, beginning insidiously, often imperceptibly, in the first two decades of life. are usually unable to recall a precise onset of visual symptoms,¹¹⁷ and not infrequently, DOA is discovered incidentally in asymptomatic individuals during routine vision testing or as part of screening of family members of a proband.^{29,117}

Visual loss in DOA is detected between ages 4 and 6 in the majority of patients, and 58–84% of patients with DOA report visual impairment by age 11.^{46,171} Compared to LHON, vision loss is typically mild in DOA, with a mean visual acuity of 20/80 to 20/120.^{47,114,242} More than 80% of patients retain vision of 20/200 or better,²⁴² although visual acuities can range from 20/20 to light perception.^{116,171} Although not as rapid or as devastating as LHON, DOA may nevertheless preclude safe motor vehicle operation and significantly impair quality of life in some patients.

Progressive decline in visual acuity occurs in 19–50% of patients.^{46,116,187,242} The rate of progression varies considerably among and within families;²⁴² however, in general, disease progression in DOA follows a relatively indolent course, and is independent of visual acuity at diagnosis.⁶⁰ In one long-term follow-up study of 69 patients with a confirmed DOA-causing mutation, of whom 58 (84%) were symptomatic, 43 (62%) had stable visual acuity in at least one eye at 10 year follow-up.⁴⁶

Defects in color vision occur in DOA, and, although tritanopic (blue-green) defects were initially felt to be characteristic of the disorder, subsequent studies have shown that a generalized dyschromatopsia (involving red-green and blue-yellow axes) accounts for the majority of color perceptual abnormalities in DOA.^{5,242} Central, cecocentral, and paracentral scotomas are the most frequent visual field abnormalities in DOA, consistent with early involvement of the papillomacular bundle, and may show a predilection for the superotemporal quadrant.^{169,242} Pupillary light reflexes may be relatively spared in DOA, similar to reports in LHON.²³

Optic disc atrophy seen in DOA may be subtle (12%), may present as a suggestive “temporal wedge” of pallor (classically associated with temporal excavation and absence of fine superficial capillaries) (44%) (Figure 7), or may be diffuse (44%).^{47,116,117,242,243} In one series of 29 patients with 58 eyes, pallor of the temporal neuroretinal rim was a universal finding (100%), often accompanied by other features such as shallow shelving (or “saucerization”) of the disc (79%), peripapillary atrophy (69%), cup-to-disc ratio of more than 0.5 (48%), grey temporal pigmentary crescent (31%), or deep excavation (21%).²⁴³

The RGCs appear to be the primary target in DOA. Histopathological reports in DOA are scarce, but two published postmortem studies described atrophy of RGCs, with preferential loss of neurons in the papillomacular bundle.^{102,115} Similarly, OCT has shown thinning of the RGC layer and RNFL, with preservation of the photoreceptor layer in patients with DOA.^{96,156} Visual evoked potentials are generally unrevealing and are either absent or show nonspecific decreased amplitudes and prolonged latencies.²⁴² Pattern electroretinography often shows selective depression of the N95 component, consistent with RGC dysfunction.²⁴²

3.2.3. Mutations causing DOA—Mutations in the *OPA1* gene, located on chromosome 3q28-29, account for the majority of cases of DOA (60–70%).⁴⁷ The *OPA1* gene is ubiquitously expressed in mitochondria and encodes for the OPA1 protein, a dynamin-related GTPase anchored to the inner membrane of mitochondrial cristae.^{5,52,177} More than 200 pathogenic mutations in the *OPA1* gene have been identified to date, including missense, nonsense, deletion/insertion, and splicing mutations.⁶⁵ The majority of mutations lead to a truncated OPA1 protein and haploinsufficiency, and the most frequent mutation in DOA patients of European descent is a frameshift-inducing microdeletion.⁵

The OPA1 protein has been implicated in several subcellular functions including mitochondrial fusion, membrane stabilization, apoptosis, and OXPHOS.^{37,38,70,176,178,258} Each normal mitochondrion undergoes repeated fission and fusion events with other neighboring mitochondria, with an associated transfer of genetic information and organelle contents. These events are believed to serve a protective purpose in the cell, allowing mitochondria with damaged or deficient mtDNA to share in the genetic material of other mitochondria, thereby preserving OXPHOS and maintaining mitochondrial homeostasis.^{35,37,38} Mitochondrial fusion is promoted by the dynamin-related family of mitofusin proteins, which includes OPA1, mitofusin-1, and mitofusin-2. Through a stabilizing effect on the inner mitochondrial membrane, OPA1 sequesters cytochrome c, a pro-apoptotic molecule, within the mitochondrion, thereby preventing its intracellular release and subsequent organelle fragmentation and apoptotic cell death.^{47,70,176} OPA1 may also participate in OXPHOS through a role in the assembly and stabilization of mitochondrial respiratory chain complexes I, II, and III.²⁵⁸ Mitochondria with decreased *OPA1* expression were observed to lack fusion events, to have disrupted membrane organization, and to have impaired OXPHOS.^{157,176,178,258}

Because the majority of *OPA1* mutations causing DOA result in nonsense or frameshift mutations, the resultant mutant mRNA is unstable or truncated and is degraded by intracellular mechanisms, resulting in failure of successful expression of one *OPA1* allele. This *OPA1* haploinsufficiency is believed to be the major pathophysiologic mechanism of disease in DOA.^{64,257} Less commonly, missense mutations occur in the *OPA1* gene and result in DOA via a loss of function mechanism rather than a protein truncation mechanism.²⁹ One particular missense mutation, the R445H mutation, has been associated in several families with a classic phenotype of DOA *plus* sensorineural deafness – so-called “ADOAD” for “autosomal dominant optic atrophy and deafness”.^{6,211}

Recently, missense mutations in *OPA1* have been implicated in severe multi-systemic syndromes, called the “OPA1 Plus” (or “DOA Plus”) syndromes.⁷ These mutations result in a dysfunctional rather than nonfunctional (haploinsufficient) OPA1 protein, with toxic consequences for the mitochondrial genome. A dysfunctional OPA1 protein permits aberrant replication of mtDNA through several putative mechanisms (discussed in further detail later), including excessive GTPase activity, impaired mitochondrial fusion, and exposure of mtDNA molecules to oxidative damage. These culminate in multiple large-scale deletions of mtDNA, resulting in optic atrophy as a component of a larger CPEO-like syndrome (see section on CPEO).^{7,90}

Although *OPA1* mutations account for the majority of cases of DOA, other loci have also been implicated in the DOA phenotype. In a single family of German descent with DOA, the causative *OPA4* locus has been mapped to chromosome 18q12.2-12.3.¹¹⁰ In two unrelated French families, an *OPA5* locus has been mapped to chromosome 22q12.1-13.1.¹⁰ The genes at these two loci have not been characterized further and the function of the proteins they encode is unknown. The *OPA3* gene on chromosome 19q13.2-13.3, has been associated with DOA and premature cataracts (ADOAC) in two French families.^{193,241} This gene, whose mutations are usually associated with an autosomal recessive syndrome of optic atrophy, extrapyramidal signs, and cognitive impairment (Costeff syndrome, or type III 3-methylglutaconic aciduria), encodes a protein that, like OPA1, localizes to the inner mitochondrial membrane.⁵⁰ The function of the OPA3 protein is currently unknown.

3.2.4. Selective vulnerability of the optic nerve in classic DOA—*OPA1* mRNA is widely distributed in the body and is not confined to RGCs, although it is abundantly expressed in these cells.^{4,177} In mammalian models, the OPA1 protein has been detected in the inner and outer plexiform layers, the inner nuclear layer, and the photoreceptor layer of the retina.^{103, 104,183} Extraocularly, OPA1 has been found in high levels in the hair cells and ganglion cells of the cochlea and in the brain,^{16,17} and may therefore be implicated in the ADOAD phenotype seen with certain OPA1 mutations. The reason why RGCs are preferentially affected, while other tissues are not, with *OPA1* mutations in classic DOA (*i.e.*, not “OPA1 Plus” syndromes) is incompletely understood; however, RGCs in DOA may exhibit the same susceptibility to bioenergetic failure at the level of the lamina cribrosa that is believed to occur in LHON. Mutations in *OPA1* may therefore be most apparent at this “chokepoint”, where impairment of mitochondrial fusion, derangement of mitochondrial networks, and disruption of OXPHOS are most stressful to mitochondrial homeostasis resulting, ultimately, in the release of cytochrome c and apoptotic RGC death.

3.2.5. Factors affecting disease expression—Although DOA was believed to be nearly completely (98%) penetrant in the pre-molecular era,¹¹⁶ this figure has been revised since the discovery of the *OPA1* gene and the development of molecular genetic testing. More recent studies of OPA1-positive families have modified the estimated penetrance of DOA to 66–88%.^{47,235} Penetrance rates between families may be mutation-specific,^{234,235} but considerable variability in visual dysfunction and disease course may also be seen among family members, all of whom share the same mutation. Therefore, as in LHON, DOA likely has genetic, epigenetic, and environmental factors which modify its phenotypic expression. Background mtDNA haplogroup may have an influence on the expression of disease in at least one subset of DOA patients: OPA1-negative DOA patients were found to have a three-fold overrepresentation of mitochondrial haplogroup J versus controls in one study.⁷⁸ The other genetic, epigenetic, and environmental factors affecting DOA expression remain to be elucidated.

3.2.6. Comparisons with LHON—Since the identification of DOA as a mitochondrial disease, many parallels have been found between LHON and DOA. Although their clinical progressions differ dramatically, both diseases share clinical features of central scotomas, dyschromatopsia, and possibly relative preservation of pupillary reflexes. In the late-stage of both conditions, the funduscopic appearance may be strikingly similar, with disc excavation (“cupping” or “saucerization”) and optic atrophy as common findings.^{148,243} Both DOA and LHON cause predominant damage to RGCs, particularly those in the papillomacular bundle, with cell death likely mediated in each disease by a mitochondrially-initiated apoptotic pathway.^{29,52} Although arising from different genomes, DOA and LHON are diseases related through their degenerative effects on RGCs, and the two conditions highlight the dependence of vital mitochondrial processes on both nuclear and mitochondrial DNA integrity.

3.3. Other “mitochondrial” optic neuropathies

Although LHON and DOA are sometimes considered the primary “nonsyndromic” mitochondrial optic neuropathies, as they classically have no accompanying neurologic or systemic symptoms, optic neuropathy is sometimes encountered as a secondary feature in other “syndromic” mitochondrial diseases. The scope of what constitutes a “mitochondrial” optic neuropathy has been broadened in recent years with the discovery that certain proteins important for mitochondrial function, even if only indirectly related to the OXPHOS system, are implicated in genetic syndromes with optic nerve dysfunction as part of a larger clinical phenotype. For example, genetic disorders such as Friedreich ataxia (FRDA), variants of Charcot-Marie-Tooth (CMT) disease, and hereditary spastic paraplegia (HSP) have been shown to have mitochondrial dysfunction at their core, resulting in “syndromic” mitochondrial optic neuropathies. Furthermore, as we better elucidate the pathogenesis of a variety of other syndromic disorders, we may find that mitochondrial dysfunction provides a final common pathogenesis underlying their optic neuropathies.

3.3.1. Friedreich ataxia (FRDA)—FRDA is the most common autosomal recessive hereditary ataxia, affecting 1 in 50,000 people in the United States. Age of onset is typically before 25 years. The disease is caused by a GAA trinucleotide repeat expansion in the *frataxin* gene on chromosome 9q13-q21.1,⁵⁷ which encodes for frataxin, a nuclear-encoded mitochondrial protein targeted to the inner mitochondrial membrane, whose main role involves iron-sulfur protein homeostasis within mitochondria.^{207,227} Mitochondrial respiratory chain complexes I, II, and III contain iron-sulfur and depend on frataxin for proper assembly and maintenance,¹³³ with abnormalities in frataxin resulting in impaired OXPHOS, decreased bioenergetic output, increased reactive oxygen species, iron accumulation in mitochondria, and apoptosis.^{133,227,252}

FRDA is a neurodegenerative disorder characterized by progressive limb and gait ataxia, dysarthria, loss of deep tendon reflexes, loss of joint position and vibration sense, pes cavus, cardiomyopathy, and scoliosis.⁶⁹ Although optic neuropathy is also a common feature of FRDA, most patients are visually asymptomatic, and severe vision loss occurs only rarely.⁶⁹⁻⁷² In a recent study of 26 patients with FRDA, 21 were completely visually asymptomatic, 3 were symptomatic with decreased visual acuity, and 2 presented with sudden onset of bilateral central scotomas, resembling LHON.⁶⁹ On evaluation with automated perimetry, OCT, and pattern visual evoked potentials, however, all 26 patients had evidence of underlying optic neuropathy. Unlike LHON and DOA, FRDA showed no preferential involvement of the papillomacular bundle on visual fields or OCT, suggesting that its pathophysiological mechanisms differ from those of LHON and DOA.⁶⁹ The involvement of complexes I, II, and III in FRDA (compared to complex I in LHON, for example) may limit alternative mitochondrial respiratory pathways, thereby causing less selective damage to RGC populations.⁶⁹

3.3.2. Charcot-Marie-Tooth (CMT) disease—CMT disease is one of the most common inherited peripheral neuropathies. A subtype of CMT, hereditary motor and sensory neuropathy type VI (HMSN VI), is defined by the combination of axonal peripheral neuropathy and optic atrophy, and has both autosomal dominant and autosomal recessive forms of inheritance.³³⁻³⁴ HMSN VI-related optic atrophy typically develops in late adolescence, a decade or more after the onset of the peripheral neuropathy, with a subacute visual decline, resulting in bilateral central scotomas, severe decrease in visual acuity (down to 20/400), and impairment of color vision.²⁶² Like LHON, but unlike DOA, a subset of patients with HMSN VI recover vision, often to near-normal levels, years after the onset of optic neuropathy.²⁶²

The autosomal dominant form of HMSN VI is caused by a mutation in the nuclear *mitofusin-2* gene,²⁶² and constitutes a subclass of CMT2A, the most common autosomal dominant form of axonal CMT. The mitofusin-2 protein is a GTPase localized to the mitochondrial outer membrane, with similar structure to the inner-membrane OPA1 protein in DOA.²⁶² Like OPA1, mitofusin-2 is involved in the fusion of mitochondria and creation of mitochondrial networks, as well as in the maintenance of mitochondrial OXPHOS and regulation of mtDNA gene expression.^{57,134}

3.3.3. Hereditary spastic paraplegia (HSP)—Hereditary spastic paraplegia is characterized by progressive and often severe spasticity of the lower extremities.¹³⁸ HSP may be “pure”, if spasticity is the only manifestation of disease, or “complicated” if other features, such as optic atrophy, are present. Complicated HSP with optic atrophy may result from several nuclear DNA mutations,^{138,158} at least one of which (in the *SPG7* gene) impairs mitochondrial function.^{32,57} The *SPG7* gene, found on chromosome 16q24.3, encodes for a mitochondrial metalloproteinase, paraplegin, which is believed to have proteolytic and chaperone-like activity at the inner mitochondrial membrane.^{32,51} In a consanguineous family with autosomal recessive complicated HSP with optic atrophy, an absence of paraplegin resulted in defective mitochondrial OXPHOS, although further elements of its role in the development of optic atrophy remain to be elucidated.³²

3.3.4. Wolfram syndrome—Wolfram syndrome is a rare neurodegenerative disorder characterized by juvenile-onset nonautoimmune type 2 diabetes mellitus and progressive bilateral optic neuropathy.^{13:251} Because other common associated features include diabetes insipidus and sensorineural hearing loss, Wolfram syndrome is also called DIDMOAD (for diabetes insipidus, diabetes mellitus, optic atrophy, and deafness). The disease may also be associated with multiple psychiatric, neurological, and urological features, as well as central respiratory failure, which is the usual cause of death at a median age of 30 years.^{48:59} Diabetes mellitus is typically the first feature of the syndrome to occur, developing in the first or second decade of life, shortly before the onset of optic neuropathy. Optic atrophy begins insidiously with symptoms of dyschromatopsia, but progresses to a state of severely decreased visual acuity (commonly worse than 20/200), loss of central and peripheral visual fields, and excavation of the optic disc.^{13:171} Sensorineural hearing loss and diabetes insipidus may also be severe in Wolfram syndrome, the latter potentially life-threatening.

Many of the features of Wolfram syndrome are seen in diseases commonly accepted as mitochondrial, such as chronic progressive external ophthalmoplegia (CPEO).¹⁶⁹ Wolfram syndrome may arise sporadically or be inherited autosomally recessively, although maternal transmission of the disease in some pedigrees has not been ruled out.¹⁷¹ In pedigrees showing autosomal recessive inheritance, one causative gene has been localized to the *WFS1* gene on chromosome 4p16.1. The *WFS1* gene, although originally presumed to encode a mitochondrial protein, was recently shown to encode an endoplasmic reticulum protein, wolframin, which plays a role in the regulation of intracellular calcium.^{226,238} Although *WFS1* mRNA and protein have been found in multiple cell types in the mouse visual system, the mechanism by which wolframin-mediated endoplasmic reticulum dysfunction results in optic atrophy and other features of Wolfram syndrome is currently unexplained. Not all cases of Wolfram syndrome are autosomal recessive, however, and not all autosomal recessive cases localize to the 4p16.1 locus. The appearance of multiple mtDNA deletions in some pedigrees with Wolfram syndrome and the tendency of Wolfram syndrome to occur in the same mitochondrial haplogroups associated with LHON remain unexplained and may yet suggest an underlying mitochondrial pathophysiology for some variants of Wolfram syndrome.^{14,85}

3.3.5. Optic neuropathy as part of other mitochondrial syndromes—A “syndromic” mitochondrial optic neuropathy may also occur as a secondary feature in the

setting of other known mitochondrial diseases. Examples include myoclonic epilepsy and ragged-red fibers (MERRF), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), maternally inherited Leigh syndrome (MILS), and mitochondrial neurogastrointestinal encephalomyopathy (MNGIE).¹⁷¹ The other, more constant, phenotypic features of these diseases help differentiate them from LHON and DOA, in which optic neuropathy causing vision loss is the primary feature.

4. Chronic progressive external ophthalmoplegia (CPEO)

Chronic progressive ophthalmoplegia is a frequent manifestation of mitochondrial myopathies and is characterized by insidious painless progressive bilateral ptosis and ophthalmoparesis.^{58,84,97}

Ptosis typically precedes ophthalmoparesis by months to years and may progress to become complete. As the disease progresses, the ptotic eyelids occlude the pupils, interfering with vision, and patients may adopt a backward head tilt, accompanied by elevation of the frontalis muscles, to compensate. Orbicularis oculi muscles may also become weak in CPEO, resulting in lagophthalmos and ectropion and predisposing patients to exposure keratopathy. This risk is compounded by the loss of a protective Bell's phenomenon and natural spontaneous eye movements as a result of extraocular muscle dysfunction.^{49,214}

The ophthalmoparetic process affects all extraocular muscles symmetrically, and patients do not commonly complain of diplopia. In fact, many are unaware of their limitation of ocular motility until it has become severe (Figure 8). The first clinical sign of difficulty usually occurs with reading, when bimedial rectus weakness causes convergence insufficiency and diplopia. As the disease progresses, extraocular muscles may become fibrotic, exhibiting restriction on forced duction testing.⁸⁴

Visual acuity is usually not affected in CPEO, except as a possible secondary effect from exposure keratopathy. Pupillary function is always spared in CPEO, and pain and proptosis are not features of the disorder.¹³⁰ Skeletal muscle weakness is present in most patients with CPEO and may involve the neck, limb, or bulbar musculature, with bifacial weakness the rule. Chronic progressive external ophthalmoplegia also occurs as a disease-defining finding in oculopharyngeal muscular dystrophy and as a non-specific finding in the setting of other multisystem diseases, including the spinocerebellar ataxias, Refsum disease, myotonic dystrophy, and abetalipoproteinemia.

4.1. Mitochondrial CPEO

When seen as the primary manifestation of a mitochondrial myopathy, CPEO may occur at any age; however, the condition starts in childhood or early adulthood in up to 90% of patients.⁹⁷ Ptosis and ophthalmoplegia may be isolated in CPEO, or may occur with other ophthalmologic, neurologic and systemic manifestations, the most common of which include pigmentary retinopathy (see below), optic neuropathy, corneal opacities, cataracts, myopathy, sensorineural hearing loss, ataxia, spasticity, peripheral neuropathy, encephalopathy, calcification of the basal ganglia, and gastrointestinal dysmotility. Life-threatening systemic abnormalities may occur, and include cardiac conduction defects, respiratory insufficiency, and hormonal and electrolyte imbalances. Short stature, and skin and skeletal abnormalities are also seen.^{152,184}

Skeletal muscle biopsy is the diagnostic test of choice in CPEO patients. The presence of ragged red fibers (RRFs) on Gomori trichrome stain is the hallmark of mitochondrial disease, representing a massive accumulation of subsarcolemmal mitochondria in OXPHOS-impaired

muscle cells (Figure 9). Ragged red fibers are seen in approximately 50% of CPEO muscle biopsies,²² and are frequently accompanied by paracrystalline (“parking lot”) inclusions on transmission electron microscopy (Figure 9).

The most frequent finding on brain imaging in CPEO is that of cerebral and cerebellar cortical atrophy, although periventricular white matter abnormalities and calcifications or altered signal in the basal ganglia may also be seen.⁸¹ Extraocular muscles may appear atrophied on MRI, consistent with longstanding myogenic weakness in CPEO, and may help exclude other causes of ophthalmoplegia, such as Graves ophthalmopathy.³¹

Kearns-Sayre syndrome is a subtype of CPEO, defined by the following criteria: 1) onset before age 20 years; 2) CPEO; 3) pigmentary retinopathy; 4) one or more of the following: cardiac conduction abnormality, cerebrospinal fluid protein greater than 100 mg/dL, or cerebellar dysfunction.^{66,108} The additional neurological and systemic features seen in CPEO are also seen in KSS, usually with a more severe phenotype, and life expectancy may be significantly shortened. Indeed, KSS is not a single clinical entity, but rather a more severe phenotype along the spectrum of CPEO syndromes. Ragged red fibers are always seen on muscle biopsy in KSS, and neuroradiologic and histopathologic studies reveal abnormalities affecting the subcortical white matter and deep gray structures of the brain.^{45,81,175} Because of their risk of sudden death from heart block, all patients with CPEO, particularly those with KSS, should have regular cardiac evaluations and electrocardiograms performed.^{84,246}

Pearson syndrome is a rare hematologic disorder of early infancy, characterized by refractory sideroblastic pancytopenia, severe lactic acidosis, and gastrointestinal malabsorption from exocrine pancreatic insufficiency.¹⁹⁹ Although Pearson syndrome frequently results in death during infancy, those patients who survive through childhood may have improvement of their hematologic abnormalities, only to develop a KSS phenotype in later life.^{161,210}

4.2. Genetics of CPEO syndromes

The CPEO syndromes may develop sporadically or may be inherited in either a maternal or nuclear fashion. Although maternally inherited CPEO arises from a mtDNA point mutation, sporadic and nuclearly inherited CPEO arise from one or more *large-scale rearrangements* of the mitochondrial genetic code. Large-scale rearrangements are mutations resulting from either a partial deletion or partial duplication of mtDNA. Mitochondrial DNA that lacks a portion of its genome is called a *partial deletion*. When such a species fuses to a wild-type mtDNA molecule, the new species is called a *partial duplication*.

In sporadic CPEO, a single mutational event causes a partial deletion or duplication in one mtDNA molecule of an oocyte or embryo, and this mutation is then propagated to all future mutant mtDNA during embryogenesis. Mutant mtDNA molecules in sporadic CPEO are therefore characterized by a **single** large-scale rearrangement, identical across all mitochondria, cells, and tissues. In contrast, in nuclearly inherited CPEO, a mutational event in the nuclear genome damages the machinery underlying accurate mtDNA replication and repair, and ongoing secondary large-scale rearrangements of mtDNA ensue. Mutant mtDNA molecules in nuclearly inherited CPEO are therefore characterized by **multiple** large-scale rearrangements, varying from mtDNA molecule to mtDNA molecule in location, size, and number. Large-scale rearrangements are typically heteroplasmic, and deletions, duplications, and wild-type mtDNA may all coexist in the same cell or tissue.²⁵⁹

4.2.1. Sporadic CPEO—Most cases of mitochondrial disease associated with CPEO are sporadic, arising from a **single** large-scale rearrangement of mtDNA. The single large-scale rearrangements responsible for CPEO syndromes are likely due to single mutational events in the maternal oocyte or early in development,^{39,204} and are distributed to the tissues of the body,

in varying proportions, by replicative segregation during embryogenesis. The onset and severity of disease resulting from a mtDNA rearrangement is determined, in part, by the proportion of mutant mtDNA in each tissue, and CPEO syndromes fall on a spectrum, with isolated CPEO having the smallest tissue distribution of mutant mtDNA and KSS and Pearson syndrome having the largest. Within an individual with a sporadic CPEO syndrome, all mutated copies of mtDNA are identical, and a certain 4,997-base-pair deletion, called the “common deletion”, accounts for more than one third of cases (Figure 1).^{84,204}

Large-scale mutations in mtDNA result in defective OXPHOS due to the loss of genetic material from the mitochondrial genome. Although genes encoding important respiratory chain proteins may be deleted, the deletion of mitochondrial tRNA genes prevents even undeleted genes from being translated.⁵³ An earlier disease onset has been observed in mutations involving more tRNA genes.²⁵⁴ Mitochondrial DNA carrying large-scale deletions have shorter length and may replicate faster than wild-type mtDNA, thereby accumulating over time, and explaining the progressive nature of CPEO.⁸⁴ The extraocular muscles, tonically metabolically active, have high oxidative demands and may be especially prone to bioenergetic dysfunction in the setting of large-scale rearrangements of mtDNA.⁸⁴

4.2.2. Maternally inherited CPEO—Although most cases of CPEO are sporadic in origin, maternal transmission of CPEO has been observed in several pedigrees.^{27,79} The responsible point mutations typically affect highly-conserved regions of mitochondrial tRNA genes (thereby preventing translation of other mitochondrial genes.) Seventeen point mutations in six of the 22 mitochondrial tRNA genes have been implicated in CPEO so far,²⁷ the most frequent of which, the A3243G point mutation, is more often associated with MELAS than with CPEO (see below).⁷⁹

4.2.3. Nuclearily inherited CPEO—Autosomal dominant and autosomal recessive patterns of CPEO inheritance are associated with **multiple** different large-scale rearrangements of mtDNA. Unlike sporadic CPEO, in which all mutated mtDNA molecules within an individual have the same rearrangement, nuclearily inherited CPEO is associated with mtDNA rearrangements that vary in length and location among family members and within individuals.^{57,162} Six nuclear genes have been implicated in autosomally inherited CPEO – *TP*, *ANTI*, *Twinkle*, *POLG1*, *POLG2*, and *OPA1* – their common feature being their normal function in stabilization of mtDNA replication and maintenance.

TP (*ECGF1*) encodes thymidine phosphorylase, an important enzyme in pyrimidine catabolism. Deficiency in this enzyme results in the autosomal recessive syndrome of **MNGIE**, a devastating multisystem disorder characterized by CPEO, gastrointestinal dysmotility, cachexia, peripheral neuropathy, and leukoencephalopathy.^{57,173} The accumulation of dTTP that results from the enzyme deficiency in MNGIE upsets the balance of nucleosides available for mtDNA replication, resulting in multiple deletions and partial depletion of mtDNA and subsequent impairment of OXPHOS.¹⁷³

The other five mutations that result in CPEO and multiple mtDNA rearrangements are inherited in an autosomal dominant fashion. *ANTI* (*SLC25A4*) encodes the heart/muscle-specific adenine nuclear translocator protein, the most abundant protein in the inner mitochondrial membrane, which regulates the transport of ATP and ADP across the inner mitochondrial membrane.¹⁰⁷ *Twinkle* (*PEO1*) encodes a mtDNA helicase, involved in the uncoiling of mtDNA during replication.^{118,215} *POLG1* and *POLG2* encode the two subunits of polymerase gamma (POLG), the mtDNA-specific DNA polymerase.^{118,135,240}

Recently, and unexpectedly, the *OPA1* gene was also implicated in a disorder of multiple large-scale rearrangements of mtDNA, resulting in a syndrome of DOA, CPEO, sensorineural

hearing loss, ataxia, axonal sensorimotor (CMT2A-like) peripheral neuropathy, and myopathy with RRFs, and was dubbed the “OPA1 Plus” (or “DOA Plus”) syndrome.^{7,90} Unlike most *OPA1* mutations in “pure” DOA which truncate the *OPA1* protein via nonsense or frameshift mutations and lead to haploinsufficiency, the *OPA1* mutations in “OPA1 Plus” are missense mutations, and encode for a dysfunctional (rather than nonfunctional) protein. Interestingly, the *OPA1* protein is a dynamin-related GTPase, which, in contrast to the proteins encoded by *ANT1*, *Twinkle*, *POLG1*, and *POLG2*, is not directly involved in mtDNA replication.²⁶¹ The mechanisms by which multiple large-scale rearrangements of mtDNA arise from a dysfunctional *OPA1* protein are currently under scrutiny. Mutant *OPA1* proteins (themselves GTPases) may pathologically increase GTP hydrolysis, resulting in a disruption of nucleoside supply for mtDNA replication, similar to the pathogenesis of MNGIE. Alternatively, mutant *OPA1* protein may inadequately shelter mtDNA molecules from oxidative damage within the mitochondrial matrix. Finally, mutant *OPA1* proteins may promote aberrant and autonomous replication of mutated mtDNA via impaired mitochondrial fusion and the disintegration of mitochondrial networks.^{7,261} *OPA1* missense mutations as a cause of autosomal dominant CPEO are second only to *POLG1* mutations in frequency, and are more common than *ANT1*, *Twinkle*, and *POLG2*.²²¹ Therefore, in *POLG1*-negative autosomal dominant CPEO, *OPA1* gene sequencing is recommended, particularly when optic atrophy is present.²²¹

5. Pigmentary retinopathy

Pigmentary retinopathy is a variable and nonspecific finding in many mitochondrial disorders.^{95,181} The retinal pigment epithelium (RPE) is susceptible to oxidative damage in the setting of compromised mitochondrial function;⁹⁹ and mtDNA is prone to damage in RPE cells, presumably due to a high degree of oxidant stress during the phagocytosis of photoreceptor outer segments.¹⁰¹

5.1. Clinical presentation

The most common fundusoscopic manifestation of mitochondrial pigmentary retinopathy is a pattern of mottled hypopigmented and hyperpigmented patches of RPE – *i.e.*, salt-and-pepper retinopathy (Figure 10). Though initially mild in degree and peripheral in location, salt-and-pepper retinopathy may become more prominent and widespread with age, and may even progress to bone spicule formation – a pattern usually seen in retinitis pigmentosa.¹¹¹ Other patterns of retinopathy include a stippled pigmentary disruption, patchy atrophy of the RPE or choriocapillaris, and bull’s eye maculopathy.^{95,108,111,122} Vision loss occurs in approximately 50% of patients with pigmentary retinopathy, and is generally mild; however, macular involvement may occur in mitochondrial disease and may cause severe loss of central vision.² Vascular attenuation is a frequent finding accompanying pigmentary retinopathy.

Patients with subclinical pigmentary retinopathy may have very mild retinal disease, detectable only on detailed dilated fundusoscopic examination or with ancillary tests such as electroretinography, fluorescein angiography, or fundus autofluorescence.^{95,192} Electroretinography may be normal or may reveal subtle dysfunction of photoreceptors (rods, cones, or both) and fluorescein angiogram may detect subtle subclinical RPE changes.¹⁸⁴ Fundus autofluorescence may reveal subtle RPE changes and may also be useful to distinguish mitochondrial pigmentary retinopathies from other etiologies.¹⁹² The maculopathy described with the A3243G mtDNA mutation, for example, is associated with a characteristic autofluorescence appearance of a discontinuous ring of perifoveal atrophy associated with widespread RPE speckling, which helps distinguish it from other maculopathies such as Stargardt’s macular dystrophy (characterized by a pattern of flecks of increased autofluorescence, discrete areas of decreased autofluorescence, and normal intervening RPE) and geographic atrophy due to age-related macular degeneration (characterized by a patch of

decreased autofluorescence involving the fovea, surrounded by a ring of increased autofluorescence without widespread RPE speckling.)¹⁵⁵

5.2. Pathology

Histopathological examination of mitochondrial pigmentary retinopathy suggests primary degeneration of the RPE with secondary disturbance of rods, cones, and choriocapillaris.¹⁵³ Electron microscopy shows numerous enlarged and abnormal mitochondria within RPE cells.^{36,153} The upregulation of mitochondria may represent a compensatory mechanism on the part of RPE cells to stimulate the increased ATP synthesis required for the regeneration of photoreceptor cell rhodopsin.

5.3. Neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP) and maternally inherited Leigh syndrome (MILS)

Pigmentary retinopathy is a prominent feature in the syndrome of neurogenic muscle weakness, ataxia, and retinitis pigmentosa – termed “NARP”. The term is somewhat of a misnomer, as the pigmentary retinopathy seen in NARP is not invariably that of retinitis pigmentosa; bone spicules are usually absent on presentation, although they may appear several years after diagnosis.¹¹¹ Furthermore, neurogenic weakness and ataxia may occur in the absence of retinopathy.²³⁶

NARP most often results from a point mutation at mtDNA position 8993, located in the gene encoding the ATPase-6 subunit of mitochondrial respiratory chain complex V (Figure 1).^{87,205} This mutation is also responsible for a phenotype of maternally inherited Leigh syndrome (MILS) – a syndrome of severe and progressive childhood encephalopathy related to necrotic degeneration of the basal ganglia, diencephalon, and brainstem and associated with dystonia, optic atrophy, ataxia, nystagmus, seizures, lactic acidosis, central respiratory hypoventilation, and early death.^{57,152}

The 8993 NARP/MILS mutation is a heteroplasmic mtDNA mutation, and its phenotypic expression varies across a spectrum, increasing in severity with increasing mutation load. A mild NARP phenotype becomes apparent at mutation loads of 60–70%, while the MILS phenotype occurs at mutation loads above 90%.^{87,250} Overlap phenotypes occur with intermediate mutation loads, while mutation loads below 60% are generally asymptomatic.¹⁴² Because of stochastic replicative segregation during oogenesis or early embryogenesis, variable degrees of tissue heteroplasmy and correspondingly variable clinical phenotypes may coexist among family members of the same pedigree.⁶² Analogously, in cell cultures and in tissue-derived mitochondria, increased mtDNA mutation loads correspond with decreased mitochondrial ATP synthesis, suggesting impaired cellular energy production as a probable underlying etiology.²⁸

5.4. Other mitochondrial disorders with pigmentary retinopathy

Pigmentary retinopathy is one of the defining features of KSS.^{95,108,160} Patients with CPEO usually have a milder retinal degeneration than patients with KSS; however, in some cases of CPEO, a pigmentary retinopathy may be the only additional manifestation. Asymptomatic relatives of patients with typical CPEO may have isolated pigmentary retinopathy as well.⁹⁵ Mild pigmentary mottling of the posterior pole may also be seen in MERRF.⁹⁵

Patients with MELAS are prone to develop pigmentary retinopathy. In one Finnish population-based study, pigmentary retinopathy was found in 38% of MELAS patients carrying the A3243G mtDNA point mutation, with the risk of RPE degeneration proportional to overall disease severity and to the mtDNA mutation load.¹²⁸ Retinal degeneration in MELAS most often conforms to a classic salt-and-pepper retinopathy pattern; however, degeneration may

be confined to the macula in MELAS and in the related mitochondrial syndrome of maternally inherited diabetes and deafness (MIDD).^{2,127,151,213} The macular lesions in such cases tend to be bilateral, symmetric, pigmented and mostly asymptomatic, but may result in annular perifoveal RPE atrophy or, less commonly, a pattern dystrophy appearance of RPE degeneration.^{2,150,151,155,213} Fundus autofluorescence often reveals subtle, yet widespread, abnormalities not apparent on funduscopy.^{155,192}

Because subtle or overt pigmentary retinopathy is a feature of many mitochondrial diseases, and may be seen in *formes frustes* of otherwise well-known mitochondrial syndromes, its presence may be a helpful diagnostic sign in a patient with an unexplained neurological or multisystem disorder, raising suspicion of an underlying mitochondrial etiology.

6. Retrochiasmal visual loss

Some patients with mitochondrial disease suffer visual loss, not from optic neuropathy or pigmentary retinopathy, but from damage to the retrochiasmal visual pathways, resulting in homonymous hemianopic visual field defects or cortical blindness. In the absence of other superimposed anterior visual pathway disease, retrochiasmal visual pathway lesions should always be associated with identical visual acuities in both eyes, normal pupillary reflexes, and normal fundoscopic appearance.

6.1. Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)

6.1.1. Clinical features—The most common mitochondrial disease associated with retrochiasmal visual loss is MELAS,¹⁸² defined by a core clinical triad of: 1) stroke-like episodes before age 40 years; 2) encephalopathy characterized by seizures, dementia, or both; and 3) lactic acidosis, RRFs, or both.^{83,231} Other abnormalities are also frequently seen in MELAS, including muscle weakness and early fatiguability,⁸² sensorineural hearing loss,⁸² mixed axonal-demyelinating peripheral neuropathy,¹⁰⁶ diabetes mellitus,^{83,182} growth failure,¹⁷⁴ cardiomyopathy,⁸² cardiac conduction defects,^{82,216} and renal¹⁵⁹ and gastrointestinal dysfunction.⁶⁷

Stroke-like episodes are seen in virtually all patients with MELAS and often have a stuttering onset, accompanied by a migraine-like prodrome lasting hours.^{82,216} The episodes have a predilection for the occipital and parietal lobes and result in homonymous hemianopia in up to 79% of patients.⁸² Loss of consciousness is common, and other focal neurological deficits may also occur, including aphasia, alexia without agraphia, and hemiplegia.¹⁸² Stroke-like events occur paroxysmally, with no clear precipitant. They tend to resolve partially or completely over hours to weeks, a prognosis for recovery better than that of true cerebral ischemia.²³² Focal seizures are seen in relation to stroke-like episodes and may perpetuate neurological dysfunction, but whether they are the cause or effect of stroke-like episodes is unclear.^{82,152,231,232}

Stroke-like episodes tend to punctuate a chronic progressive deterioration in mental and neurologic function in MELAS. This dementing encephalopathy may affect language, perception, memory, and executive function, with MELAS patients scoring poorly on tests of memory, orientation, nonverbal intelligence, working memory, verbal fluency, visuomotor skills, processing speed, and attention.^{124,217} Acute confusional states may occur independently of stroke-like episodes and focal seizures, and are associated with transient elevations in cerebrospinal fluid (CSF) and serum lactate.

Among all the mitochondrial syndromes, MELAS appears to have the most variable phenotype, and frequently presents as an “overlap” syndrome with other mitochondrial diseases. Although

the primary visual morbidity in MELAS is retrochiasmal visual loss, pigmentary retinopathies, especially maculopathies, are quite common among MELAS patients (see above). Less commonly, optic neuropathies may be found.²¹ Patients with MELAS can even manifest the typical features of CPEO/KSS and MERRF.²⁶⁰

6.1.2. Ancillary testing—Elevated serum lactate is detected in over 90% of patients, and is one of the most constant features of MELAS; high CSF lactate is found in 50–100% of MELAS patients.⁸² In early disease, lactate levels may be within normal limits; however, repeat CSF examinations almost invariably reveal increased levels,⁸² with the highest levels detected during and shortly after a stroke-like episode.²³¹

Magnetic resonance imaging (MRI) is the most sensitive means of detecting cerebral lesions in MELAS (Figure 11), and can be used to help differentiate the stroke-like events in MELAS from cerebrovascular ischemia. Stroke-like episodes are associated with asymmetric cortical lesions on neuroimaging predominantly located in the occipital and parietal lobes. These lesions are typically confined to the cortex, with sparing of the deep white matter, and, unlike true ischemic strokes, do not respect arterial vascular territories.⁸² Lesions on neuroimaging may be transient, in which case the resolution of lesions parallels the resolution of clinical symptoms.²³¹ Diffusion-weighted imaging (DWI) sequences are very sensitive for the detection of both stroke-like lesions and true ischemia, but apparent diffusion coefficient (ADC) maps help differentiate these lesion-types: within the first 48 hours, a decreased lesional signal on the ADC map is expected with cerebrovascular ischemia, whereas an increased lesional signal on the ADC map is classic for MELAS.^{180,203} Magnetic resonance spectroscopy (MRS) is useful for demonstrating a lactate peak (Figure 11) within stroke-like lesions,¹ and ventricular MRS can be used to confirm a CSF lactate level greater than 4.0 mmol/L.¹³² Other neuroimaging features of MELAS include global cerebral atrophy, consistent with the relentless cognitive decline in MELAS, and basal ganglia calcification, best seen on CT.⁸²

Muscle biopsy can be used for biochemical assays of mitochondrial respiratory enzyme function. The most frequent MELAS-associated defects are deficiencies in complex I and complex IV.⁸² On histopathologic examination, RRFs, the hallmark of mitochondrial disease, are seen in 80–100% of MELAS skeletal muscle biopsies using the Gomori trichrome stain (Figure 9).^{74,82} These RRFs stain strongly for succinate dehydrogenase (SDH), *i.e.*, the nuclearly-encoded complex II, indicating compensatory mitochondrial proliferation.²¹⁷ Unlike other mitochondrial myopathies, MELAS has only partial reduction in cytochrome c oxidase (COX), or complex IV, staining, and this may represent a characteristic histopathological feature.^{74,232} Electron microscopy reveals enlarged and dystrophic mitochondria in subsarcolemmal regions in the RRFs.²¹⁷

Pathological changes are not confined to muscle cells in MELAS. Enlarged and dystrophic mitochondria have been found in capillary pericytes, endothelial cells, and smooth muscle cells of pial arterioles and intracerebral end-arterioles. These vessels stain strongly positive for SDH, suggesting mitochondrial angiopathy (Figure 9).^{18,119} The precise role of this angiopathy in the stroke-like episodes characterizing MELAS remains unclear.

6.1.3. MELAS mutations—The first reported MELAS mutation was the A3243G mutation in the *MTTL1* gene encoding for mitochondrial tRNA^{Leu(UUR)}.⁷³ Although many other MELAS point mutations have since been identified, the A3243G point mutation is the most frequent cause of MELAS, accounting for over 80% of cases.²⁵³

These mtDNA point mutations, as expected, are inherited maternally. However, because of the exceptionally poor correlation between genotype and phenotype in MELAS, maternal family

members of a MELAS patient may be normal, have asymptomatic elevation of serum lactate or positive muscle biopsy for RRFs, have an oligosymptomatic *forme fruste* of MELAS (e.g., isolated migraines, sensorineural hearing loss, or diabetes mellitus), or have frank MELAS themselves.⁸² In large studies, 20–86% of MELAS patients have a positive family history, a wide range indicative of the large spectrum of disease phenotype in MELAS and the difficulty in recognizing oligosymptomatic and subclinical expression.⁸²

Heteroplasmic point mutations in mtDNA account for the bulk of cases of MELAS; however, large-scale mtDNA deletions causing MELAS have also been reported, usually as part of a more general “overlap” syndrome. MELAS/KSS and MELAS/Fanconi syndrome are two such overlap syndromes attributable to large-scale deletions in mtDNA,^{26,263} highlighting the importance of searching for mtDNA deletions when testing for point mutations is negative.

6.1.4. Pathogenesis—MELAS mutations result in deficient expression of mitochondrial respiratory chain proteins and impaired OXPHOS. As with other mitochondrial diseases, a state of chronic bioenergetic failure results, with subsequent dysfunction of cells and tissue. In MELAS, more than other mitochondrial disorders, pyruvate is shunted through anaerobic pathways of metabolism, bypassing OXPHOS to create ATP and generating lactic acid in the process.^{82,217}

Aside from these general considerations, the pathogenesis of MELAS is unclear. The mechanisms for stroke-like episodes and the predilection of these lesions to occur in the posterior cerebrum, causing retrochiasmal visual loss, are not fully understood, although several theories have been proposed. According to one model, cortical mitochondrial neuropathy may be a primary defect, with secondary neuronal hyperexcitability resulting in migraines and seizures. In the context of OXPHOS deficiency, such hyperexcitability may result in membrane pump incompetence, with leakage of ions and glutamate, causing excitotoxic damage and further neuronal hyperexcitability, spreading to other neurons and culminating in a stroke-like episode.^{94,217} A second model incorporates the observed decrease in L-citrulline levels in MELAS patients to posit a derangement in nitric oxide (NO)-citrulline metabolism that impairs cerebral vasodilation, precipitating stroke-like episodes.¹⁶⁴ The role of L-arginine in inhibiting this effect forms the basis of its theoretical therapeutic utility in MELAS.¹¹⁹

7. Diagnostic approach to mitochondrial disorders

7.1. Clinical approach

An underlying mitochondrial disorder should be suspected in any patient with unexplained optic neuropathy, external ophthalmoplegia, pigmentary retinopathy, or retrochiasmal visual loss, especially when accompanied by other “mitochondrial” features such as ptosis, muscle weakness, sensorineural hearing loss, seizures, stroke-like episodes, ataxia, cardiac abnormalities with palpitations or syncope, diabetes, malabsorption, growth failure, or tetany. A detailed personal and family history specifically addressing these potential manifestations is essential, although their absence does not exclude the possibility of mitochondrial disease. Family history should include questions pertaining to neonatal or childhood deaths. A detailed neurologic and systemic examination should be performed, with attention to the cardiac and endocrine systems. Examination of relatives may also aid in the diagnosis.

7.2. Investigations

Routine blood tests may provide support for a clinical diagnosis or may help eliminate alternatives, and may include complete blood count, electrolytes, urea, creatinine, random glucose, hemoglobin A_{1C}, thyroid stimulating hormone, thyroxine, creatine kinase, and resting

lactate.¹⁵² CSF examination is often a valuable investigation, and may show abnormally high protein and lactate levels in mitochondrial disease.⁹⁷ Serum and CSF lactate may increase after exercise in patients with MELAS, CPEO, and Leigh syndrome, although this finding is variable.

An electrocardiogram is essential in any suspected case of mitochondrial disease, as cardiac conduction defects and cardiac hypertrophy are common, and potentially life-threatening. Echocardiography may be indicated in some cases.

Neuroimaging is recommended whenever signs localizable to the central nervous system are present, or when an abnormal electroencephalogram (EEG) is recorded. Imaging of the brain and orbits is useful to exclude alternate causes of optic neuropathy or neurological impairment, and to detect features consistent with mitochondrial disease, such as basal ganglia calcification, spongiform changes, and the stroke-like lesions of MELAS. The modality of choice is MRI in most circumstances, although CT may be useful to detect basal ganglia calcification when poorly visualized on MRI.

Skeletal muscle biopsy can provide a wealth of diagnostic information. Histological and histochemical analysis may be used to confirm the presence of mitochondriopathy and to exclude alternate myopathies. As such, it is often considered the “gold standard” for the diagnosis of mitochondrial disease; however, it is not 100% sensitive for mitochondrial disease, as muscle biopsies from LHON and NARP patients are typically normal. The Gomori trichrome stain is the most helpful in the diagnosis of mitochondrial disease, revealing RRFs, the “signature” of mitochondrial disease (Figure 9). RRFs are commonly seen in MELAS, MERFF, KSS, and CPEO in about 50% of cases.²² Other helpful histochemical stains include NADH, SDH, and COX, which stain for different complexes of the mitochondrial respiratory chain (complexes I, II, and IV, respectively), allowing better localization of an OXPHOS defect. Transmission electron microscopy (TEM) on muscle specimens may reveal characteristic paracrystalline (“parking lot”) inclusions (Figure 9), which represent compensatory accumulations of mitochondrial creatine kinase in the setting of OXPHOS impairment.²¹⁹ Enzymatic assays on muscle tissue can be performed in specialized laboratories, and may reveal biochemical deficiencies in complexes I–V of the mitochondrial respiratory chain.

Genetic analysis for point mutations in mtDNA can be performed on any tissue containing mitochondria, including whole blood, skin fibroblasts, buccal epithelial cells, hair follicles, and urine sediment;²⁰⁹ however, muscle tissue has the highest mtDNA mutational load and is the tissue of choice when large-scale rearrangements of mtDNA are suspected, as in CPEO. Blood is usually sufficient for analysis of mtDNA point mutations, as in LHON. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques are routinely used to detect common point mutations, while Southern blot techniques are used to detect large-scale rearrangements of mtDNA.¹⁵² The full sequencing of a patient’s mtDNA may allow the identification of novel gene mutations; however, when abnormalities are found, they must be judged as benign polymorphisms or pathogenic mutations. This distinction is often difficult and dependent on clinical context, family history, and important principles of mitochondrial genetics, and is therefore best performed by an experienced geneticist well-versed in the nuances of mitochondrial disease.

Nuclear mutations responsible for mitochondrial disease may be detected in whole blood or other tissue through conventional genetic techniques, including sequence analysis, deletion/duplication analysis, and targeted mutation analysis. Sequence analysis of a gene allows rapid nucleotide sequencing of fragments of frequently mutated DNA and identification of common point mutations, and is the molecular diagnostic technique of choice for most mitochondrial

diseases with mendelian inheritance, including DOA, CMT2A/HMSN VI, HSP, *WFS1*-related Wolfram syndrome, autosomal dominant CPEO, and MNGIE. Sequence analysis of the *OPA1* gene, for example, has a detection rate of about 50% in patients with sporadic DOA, and a detection rate of 70–90% in patients with familial DOA.¹⁶⁵ Deletion/duplication analysis, via quantitative PCR, real-time PCR or multiplex ligation-dependent probe amplification, may uncover mutations not detected through sequence analysis alone. This analysis is generally performed as second-line testing to increase diagnostic yield when sequence analysis is unrevealing. Targeted mutation analysis identifies specifically-sought mutations and is best reserved for diseases in which a single known mutation causes the bulk of disease within a population or within a pedigree. For example, targeted mutation analysis is the technique of choice for the diagnosis of FRDA, in which more than 95% of cases are attributable to a GAA trinucleotide repeat in intron 1 of the *frataxin* gene.⁵⁷ Finally, linkage analysis is still a useful molecular technique to localize a genetic mutation when a mitochondrial disease is transmitted in a mendelian fashion, but the underlying gene has yet to be identified.

8. Treatment of mitochondrial disease

8.1. Symptomatic treatments

Symptomatic treatments should be considered in all patients with mitochondrial disease to prevent secondary morbidity and to improve quality of life.

Strabismus or ptosis surgery may be helpful for patients with CPEO who have diplopia or vision-limiting ptosis.^{3,248} Lid surgery must be undertaken with care, as weakness of the orbicularis oculi in CPEO commonly results in lagophthalmos and ectropion, predisposing the patient to exposure keratopathy.^{49,214} The limitation of extraocular motility and impairment of Bell's phenomenon may further increase the risk of corneal damage. If ocular motility is not examined and conventional ptosis surgery is performed in CPEO, complications related to corneal exposure are likely to result.²¹⁴ A frontalis suspension surgery is the procedure of choice in CPEO when levator function is less than 8mm.^{15,214} Ptosis crutches, affixed to a patient's glasses, are a nonsurgical alternative for patients with CPEO.

Low vision aids benefit patients with severe vision loss from optic atrophy, pigmentary retinopathy, and maculopathy.²²⁰ In particular, patients with LHON and DOA are often young adults with preserved peripheral vision, and make excellent candidates for low vision rehabilitation.

Hyperglycemia from diabetes may be treated through a combination of diet modification and antihyperglycemic agents, including insulin. Seizures typically respond well to common antiepileptic medications, although valproic acid should be used with caution due to its tendency to aggravate epilepsy in MELAS.¹³¹ Cardiac pacemakers may prevent life-threatening events in patients with KSS and cardiac conduction defects.²⁴⁹ Cochlear implants may help some patients with sensorineural hearing loss.²¹² Aerobic exercise training appears to ameliorate biochemical and oxidative parameters of muscle dysfunction and also improves quality of life.^{118,163,225} Antidepressants and antipsychotics may benefit patients with psychiatric symptoms of mitochondrial disease.⁵⁶

Although avoiding agents that may act as mitochondrial "stressors" is a non-specific recommendation that has not been scientifically tested or confirmed, it may nevertheless be prudent to caution patients to avoid tobacco use, excessive alcohol intake, cyanide-containing products, medications which may have mitochondrial toxicity, and environmental toxins.^{113, 170}

8.2. Disease-modifying treatments

8.2.1. General treatments—Therapies for mitochondrial disorders are very limited, and, despite numerous anecdotal reports of success with various therapies, a recent Cochrane review of 678 abstracts and articles found no evidence supporting any intervention in the management of mitochondrial disease.⁴⁴ Nevertheless, on a practical level, there may still be a rationale for recommending certain agents to patients with mitochondrial disease.

General therapies that have been studied in mitochondrial disease fall into four main categories:⁵⁶ 1) vitamins and cofactors (ubiquinone (CoQ₁₀), folic acid, vitamin B₁₂, thiamine, riboflavin, L-carnitine, and creatine); 2) electron acceptors (vitamin C, menadiol); 3) free radical scavengers (ubiquinone, idebenone, alpha-lipoic acid, and vitamin E); and 4) inhibitors of toxic metabolites (dichloroacetate(DCA)). Most of these general therapies are harmless at their usual doses, although some may be expensive. In the absence of any other proven therapy in mitochondrial disease, many clinicians resort, on theoretical or anecdotal grounds alone, to “mitochondrial cocktails” – various combinations of these agents – to treat their patients.

Ubiquinone (Coenzyme Q₁₀) is a lipophilic molecule found in the mitochondrial membrane that shuttles electrons from complex I and II to complex III. In patients with primary ubiquinone deficiency, OXPHOS is interrupted and ATP synthesis is impaired with consequent mitochondrial encephalomyopathy. In some of these patients, supplementation with exogenous ubiquinone has led to clear improvement in function, and doses of up to 3000mg/d of ubiquinone were tolerated without side-effects in other neurological populations.^{54,63,198} Because of its therapeutic usefulness in treating primary ubiquinone deficiency, exogenous ubiquinone therapy is frequently used to treat other diseases of the OXPHOS system, including those causing neuro-ophthalmological manifestations. Doses of greater than 400mg per day are typical.⁵⁶, exogenous riboflavin (100mg daily) and L-carnitine (3g daily) supplementation, useful in the treatment of multiple acyl-CoA dehydrogenase deficiency and primary carnitine deficiency, respectively, have had their use extended to mitochondrial disorders,^{179,230} despite the absence of deficiency of these cofactors in primary mitochondrial diseases.

Vitamin C (4g daily) and menadiol diphosphate (40mg daily) were used as electron acceptors in patients with severe exercise intolerance and mitochondrial myopathy related to complex III deficiency to facilitate electron transfer from complexes I and II to complex IV.¹⁰⁹ One patient had dramatic improvement initially on 31 P MRS of muscle, but this effect was not sustained and was not seen in other patients with complex III deficiency.¹⁰⁹

Because oxidative stress in mitochondrial disorders causes release of free radicals and can lead to apoptosis, free radical scavengers including ubiquinone (400mg daily), idebenone (up to 75mg/kg daily), alpha-lipoic acid (600mg daily), and vitamin E (400 IU daily), are often used in the treatment of mitochondrial disease.^{197,229} The combination of creatine (3g bid), ubiquinone (120mg bid), and alpha-lipoic acid (300mg bid) was shown to reduce serum lactate and markers of oxidative stress in patients with mitochondrial cytopathies in one randomized double-blind controlled trial, probably through a free-radical-scavenging mechanism.¹⁹⁷

8.2.2. Disease-specific treatments—Despite the lack of progress in developing effective general treatments for mitochondrial disease, advances have been made in specific diseases. Allogenic stem cell transplantation has shown initial success in two MNGIE patients in partially replacing the deficient enzyme, thymidine phosphorylase, although further clinical followup is necessary.¹²⁵ L-arginine has been shown in a prospective, unblinded, and unrandomized trial of 24 MELAS patients to reduce the frequency and severity of stroke-like episodes.¹²⁰ Alpha-ketoglutarate and aspartate have been used to increase mitochondrial substrate-level phosphorylation and improve cell survival in human fibroblast and cybrid line models of NARP, although trials in human subjects have not yet been undertaken.²⁰⁵

Dichloroacetate, which reduces lactate levels by inhibiting pyruvate dehydrogenase, was recently studied in patients with MELAS in a randomized, placebo-controlled trial. This trial was terminated prematurely, however, because of an excessively high incidence of peripheral nerve toxicity, overshadowing any potential benefit in MELAS.¹⁰⁵

Idebenone, a short-chain benzoquinone structurally related to ubiquinone, readily enters the brain and localizes to the mitochondria. It both stimulates net ATP formation and acts as a potent free radical scavenger protecting the mitochondrial membrane against lipid peroxidation. Compared to other analogs of ubiquinone, idebenone is particularly suited for bypassing the functional impairment of mitochondrial complex I. Idebenone has been successfully used in Friedreich ataxia^{55,147} to improve both cardiac and neurological symptoms, and is currently being tested in LHON patients.

Brimonidine purite is an α -2 agonist used in the treatment of glaucoma. Because brimonidine may have antiapoptotic properties, its efficacy as a prophylactic agent for second eye visual loss in LHON was evaluated in an open-labeled, non-randomized, multicenter study of nine patients with acute vision loss in one eye from LHON.¹⁶⁶ Despite the use of the drug, all patients had deterioration of visual acuity, and seven of eight patients followed for longer than two months had visual acuity in the second eye of 20/200 or worse at the end of the study.

As illustrated by the brimonidine study, LHON offers a unique “laboratory” for the investigation of new interventions in mitochondrial disease. In patients with LHON, vision loss often occurs in a bilateral sequential fashion, the second eye becoming involved after the first with a median delay of two months in 50–75% of patients.^{77,80,195} Therefore, a window of opportunity exists for possible therapeutic intervention after vision loss in the first eye but before second eye involvement.^{77,166} LHON has the useful characteristic that drugs, adenovirus gene vectors, and other agents may be easily and directly delivered to the tissue at risk, the RGCs and optic nerve, by intravitreal injection. Although LHON alone presents this opportunity for experimentation, intervention studies in this “laboratory” have enormous potential for generalization to other mitochondrial diseases, and perhaps to apoptosis-mediated diseases as a whole.

8.2.3. Gene therapy—Gene therapy shows significant promise in the treatment of mitochondrial diseases. Many ingenious strategies have been devised using transfected nuclear and mitochondrial genes to reduce the overall proportion of heteroplasmic mutant mtDNA *in vitro*, in yeast models, and in animal models – a strategy called “gene shifting”.^{56,118}

Although it is possible to introduce DNA into the cell nucleus using a variety of vectors, the techniques required to introduce genes directly into mitochondria have not yet been developed. Targeted repair or replacement of mutated mitochondrial genes is therefore not currently possible; however, “allotopic rescue” is one means of circumventing this barrier.^{56,77,144} Allotopic rescue refers to the technique of using the nuclear genome, transfected by a genetically engineered adenovirus-associated virus (AAV) or other vector, to express a protein usually expressed by the mitochondrial genome. The transfected gene is engineered to attach a mitochondrial targeting polypeptide to the end of the transcribed protein, ensuring the nuclear protein is transported into the mitochondria. The nuclear protein, once in the mitochondria, may replace or complement a protein expressed by mutated mtDNA. This technique of allotopic rescue has been used to replace a mutated ND4 protein in a cybrid cell line homoplasmic for the 11778 LHON mutation, with consequent improvement in biochemical function and ATP synthesis.⁷⁷

Allotopic rescue has also been shown to be effective in two animal models of LHON, one a mouse model^{76,190} and one a rat model.⁶¹ In both studies, an animal model of LHON-like optic

neuropathy was induced by intravitreal injection of the human *ND4* gene harboring the LHON 11778 mutation. Subsequent intravitreal injection of the wild-type *ND4* gene prevented both retinal ganglion cell loss and impairment of visual function.⁶¹

Another gene therapy strategy involves the *in vitro* transfection of homoplasmic 11778 LHON cells with an AAV vector containing the human mitochondrial superoxide dismutase (*SOD2*) gene.^{118,189} Superoxide dismutase, an antioxidant, is encoded by the nuclear *SOD2* gene and detoxifies free radical species within the mitochondrial matrix, thereby acting as an anti-apoptotic agent. Although the *SOD2* gene is expressed in LHON cells, superoxide dismutase activity is attenuated in cells homoplasmic for the LHON mutation.⁶⁸ When LHON cells were transfected with the *SOD2*-AAV vector, superoxide dismutase was overexpressed, and three-day survival was increased by 89% in transfected LHON cells compared to non-transfected controls.¹⁸⁹ This strategy of bolstering antioxidant mechanisms to prolong cell survival was also observed to protect against optic neuropathy in complex I-deficient mice, animals with similar histopathology to human LHON patients, and may represent another avenue for treatment with gene therapy.¹⁸⁸

In heteroplasmic diseases, such as NARP/MILS related to the 8993 mtDNA mutation, another gene therapy strategy exists: selective destruction of mutant mtDNA with a mutation-specific restriction endonuclease, shifting heteroplasmy toward the wild-type state and allowing repopulation of mitochondria with wild-type mtDNA. This strategy has been shown to be effective both *in vitro*^{118,228} and in a murine model of NARP.⁹

One form of gene therapy “treatment” for children of mothers with known mtDNA mutations would be the *in vitro* replacement of the entire mitochondrial genome of an oocyte which could then be fertilized *in vitro* and implanted for normal embryo development. This technique has been successfully demonstrated in primates, in which the nuclear contents from the mother’s egg is transferred by a technique known as “spindle replacement” to an enucleated, mitochondrial-replete donor cytoplasm.²²⁴

Gene therapy is a nascent technology. While it holds significant promise in human mitochondrial disease, its clinical use currently faces several challenges.⁵⁶ As with gene therapy in mendelian diseases, appropriate transfection vectors must be selected, and their delivery to affected tissues must be optimized. The duration of gene therapy effect must be improved, as current transfection methods have not resulted in prolonged and autonomous maintenance of transfected genetic material.¹²¹ The presence of multiple species of mtDNA within a mitochondrion or cell may complicate mutation-specific mtDNA gene therapy in some disorders such as autosomal dominant CPEO. Finally, patient safety from immunological and oncological side effects and from mtDNA depletion must be guaranteed,^{9,56} and efficacy must be shown in appropriate animal models before human trials can begin.

9. Genetic counseling

Genetic counseling of patients and their relatives is an important part of the management of mitochondrial disease, and knowledge of the basic principles of mitochondrial genetics is essential.

9.1. Genetic counseling with nuclear DNA mutations

Men and women with nuclear DNA mutations causing mitochondrial disease must be counseled about the risk of transmission to their offspring according to the mode of inheritance of their disease. For example, a patient with an *OPA1* mutation has a 50% probability of transmitting the pathogenic allele to each of his or her children. Children with the mutant allele then have a 66–88% chance of developing DOA, in keeping with the known penetrance of the

disease,^{47,235} although penetrance may be nearly complete when the parent manifests DOA themselves.¹¹⁶ By contrast, patients with FRDA, an autosomal recessive condition, will not transmit the disease to any of their children (unless their mate also has at least one mutated *frataxin* gene), but all of their children will be carriers for FRDA. In this situation, the patient's *siblings* each have a 25% probability of having the disease, 50% chance of being a carrier, and 25% chance of having two normal alleles. Indeed, when a patient is diagnosed at a young age with FRDA, genetic counseling is primarily directed towards the patient's *parents* to assist with *their* future childbearing decisions.

9.2. Genetic counseling with mtDNA mutations

Men with mitochondrial DNA mutations must be counseled differently from women. Because mtDNA is inherited entirely from the maternal oocyte, with negligible paternal contribution, men with mtDNA mutations should be uniformly reassured that they have no chance of transmitting their mtDNA mutation to their children.²⁵ Women with mtDNA mutations, on the other hand, always have a high probability of transmitting mitochondrial disease to their children. The risk of transmission depends on several factors, including the type of mtDNA mutation responsible for disease (large-scale deletion or point mutation), and the presence or absence of heteroplasmy. Replicative segregation in the setting of heteroplasmy introduces a large element of unpredictability into the genetic counseling process.^{25,56}

Single large-scale mtDNA deletions causing CPEO/KSS usually arise sporadically, with only a 4% risk of transmission of sporadic CPEO/KSS from mother to child.⁴² Additionally, an unaffected mother of a child with a single large-scale mtDNA deletion disorder is unlikely to have more than one affected child.⁴² On the other hand, multiple large-scale mtDNA deletions resulting from gene mutations in the nuclear genome will be inherited following the rules of mendelian genetics (*e.g.*, autosomal dominantly or autosomal recessively), and patients must be counseled accordingly.

Homoplasmic mtDNA point mutations are inherited maternally, with the mutation transmitted from each affected woman to all of her children. A mother who is homoplasmic for a mtDNA point mutation has no wild-type mtDNA to transmit to her children, and therefore children of homoplasmic women are homoplasmic themselves. LHON is the prototypical example of a homoplasmic mtDNA point mutation, with more than 85% of patients homoplasmic for their mutation. A woman with homoplasmy for a LHON mutation should be advised that all her children will inherit her LHON mutation and be at some degree of risk for bilateral visual loss, depending on the penetrance of the mutation and on the sex of the child.

Heteroplasmic mtDNA mutations are also inherited maternally, and both mutant and wild-type mtDNA molecules are transmitted, in some proportion, from each woman to all of her children; however, the presence of heteroplasmy leads to unpredictability in disease inheritance, penetrance, and phenotype. Because of replicative segregation – a consequence of heteroplasmy – maternal tissues can have varying proportions of mutant and wild-type mtDNA. The mutation load measured in a woman's blood cells, for example, does not accurately reflect the mutation load in her other cells, such as oocytes.⁹³ Zygotes may therefore begin embryogenesis with a mutation load quite different from the total mutation load in the mother. Replicative segregation during embryogenesis complicates matters further, as mutation loads may become magnified or diminished in various tissues of the developing fetus in a stochastic, unpredictable way. Even asymptomatic heteroplasmic mothers with very low mutation loads in blood may have children with severe disease from very high mutation loads.⁹³ The risk of transmission of disease with heteroplasmic mtDNA point mutations is therefore impossible to predict accurately.

Prenatal testing with amniocentesis or chorionic villus sampling is confounded by heteroplasmy as well: amniocytes and chorionic villi may have mutation loads different from other fetal tissues and are unlikely to reflect the child's ultimate phenotypic outcome, as large shifts in the proportion of mutant mtDNA may occur in developing tissue *in utero* or after birth as a result of replicative segregation.⁵⁶ NARP is an example of a disease related to a mtDNA point mutation, in which accurate genetic counseling is complicated by heteroplasmy. NARP pedigrees may show extreme variability in clinical phenotypes among closely-related family members, and children of women with NARP may have NARP themselves, may be normal, or may develop a lethal MILS phenotype.⁶²

10. Conclusions

The past thirty years have seen an explosion of interest and research in the field of mitochondrial medicine. Once viewed as esoteric and irrelevant to daily clinical practice, mitochondrial diseases are now understood to be much more common than previously believed, and have assumed a new importance in all fields of medicine, and ophthalmology in particular. Early eye, muscle, and visual pathway involvement in mitochondrial disease is the rule rather than the exception, and ophthalmologists are among the first physicians consulted by patients with undiagnosed mitochondrial disorders. Early recognition of the four major neuro-ophthalmic syndromes – optic neuropathy, CPEO, pigmentary retinopathy, and retrochiasmal visual loss – expedites correct diagnosis and allows the detection and treatment of potentially life-threatening systemic abnormalities.

Rapid advances are being made in therapeutics for mitochondrial diseases. In particular, exciting and innovative gene therapy techniques are being developed, and hold promise for future disease intervention studies. Leber hereditary optic neuropathy may be the ideal mitochondrial disease for experimental intervention, as its natural history of bilateral sequential vision loss lends itself to timely intervention, and its monosymptomatic localization to the optic nerve allows for directed delivery of the intervention. The results of future LHON treatment trials may be generalizable to other mitochondrial diseases (and perhaps to apoptosis-mediated disease as a whole) and offer exciting new avenues for research. Until effective and enduring treatments for patients with mitochondrial disease are readily available, however, informed genetic counseling remains the cornerstone of disease management.

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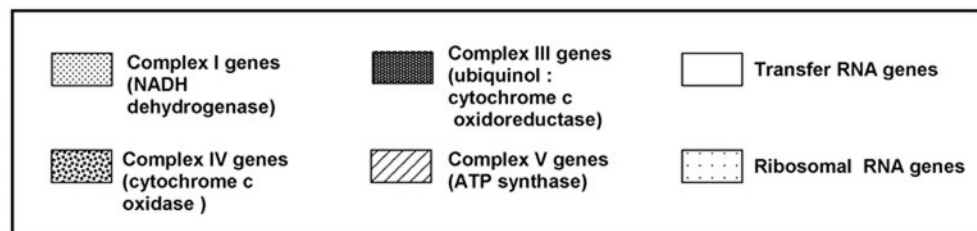
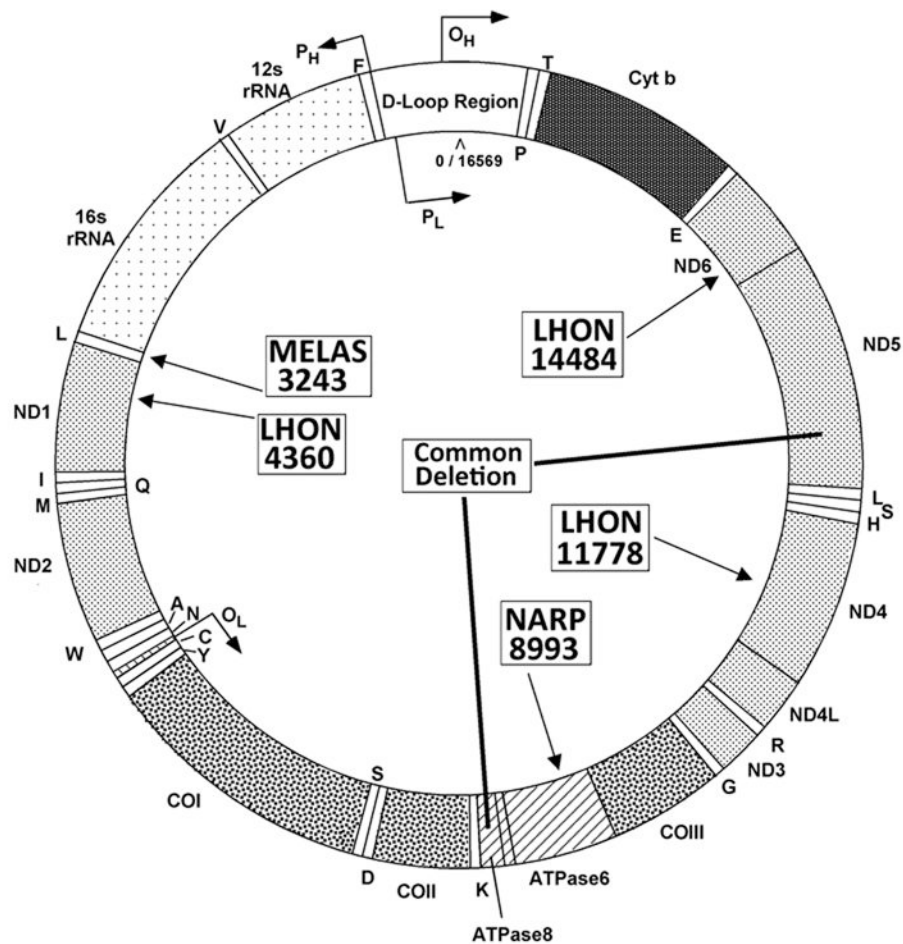


Figure 1. Map of the human mitochondrial genome

The human mitochondrial genome is comprised of 16,569 base pairs of nucleotides encoding 37 genes. Shown are the most frequent mtDNA point mutations responsible for mitochondrial disorders (Leber hereditary optic neuropathy (LHON); neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP); and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)), and the 5 kilobase “common deletion” seen in chronic progressive external ophthalmoplegia (CPEO). (Adapted from: www.mitomap.org).

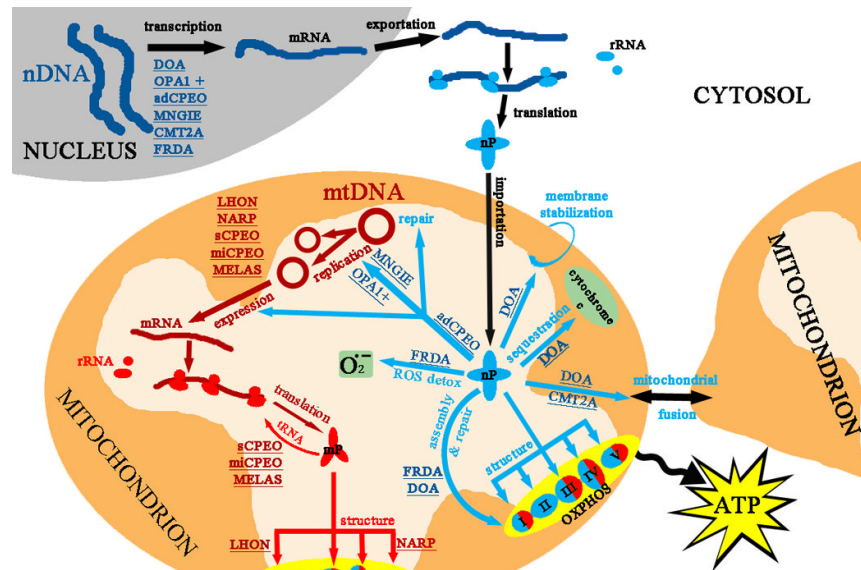


Figure 2. Nuclear and mitochondrial DNA influences on mitochondrial function

Normal mitochondrial function depends on the symbiotic relationship of nuclear (*blue*) and mitochondrial (*red*) DNA influences. Normal cellular homeostatic processes (*arrows with plain text*) are under the dual control of these two genomes (*red arrows: mitochondrial-DNA-related processes; black arrows: nuclear-DNA-mediated extramitochondrial processes; blue arrows: nuclear-DNA-mediated intramitochondrial processes*). Mitochondrial diseases (*underlined text*) may therefore arise from dysfunctional homeostatic processes mediated by either genome (*red underlined text: diseases of mitochondrial DNA origin; blue underlined text: diseases of nuclear DNA origin*).

The mitochondrial genome (*dark red circle*) encodes 13 structural subunits of the OXPHOS machinery (complexes I, III, IV, and V) (*light red semicircles*) and 24 molecules required for mitochondrial gene translation (2 rRNAs (*light red ovals*) and 12 tRNAs (*light red cloverleaf*)).

The nuclear genome (*paired dark blue lines*) encodes all other proteins used by mitochondria. Nuclear proteins (*light blue cloverleaf*) imported into the mitochondrial matrix comprise the remaining structural subunits of the OXPHOS machinery (*dark blue semicircles and circles*) and are involved in: the assembly and repair of the OXPHOS complexes; the fusion of mitochondria within the cytosol; the detoxification of reactive oxygen species; the stabilization of mitochondrial membranes; the sequestration of the pro-apoptotic molecule cytochrome c; and the repair, replication, and expression of mitochondrial genes (*blue arrows pointing to red arrows*).

Abbreviations:

OXPHOS: oxidative phosphorylation system (comprised of complexes I to V)

ATP: adenosine triphosphate

ROS: reactive oxygen species

nP: nuclear product (proteins)

mP: mitochondrial product (proteins, tRNAs, and rRNAs)

nDNA: nuclear DNA

mtDNA: mitochondrial DNA

mRNA: messenger RNA

tRNA: transfer RNA

rRNA: ribosomal RNA

DOA: dominant optic atrophy

OPA1+: “OPA1-Plus” syndrome
adCPEO: autosomal dominant chronic progressive external ophthalmoplegia
MNGIE: mitochondrial neurogastrointestinal encephalomyopathy
CMT2A: Charcot-Marie-Tooth disease, type 2A
FRDA: Friedreich ataxia
sCPEO: sporadic chronic progressive external ophthalmoplegia
miCPEO: maternally inherited chronic progressive external ophthalmoplegia
LHON: Leber hereditary optic neuropathy
MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
NARP: neurogenic muscle weakness, ataxia, and retinitis pigmentosa

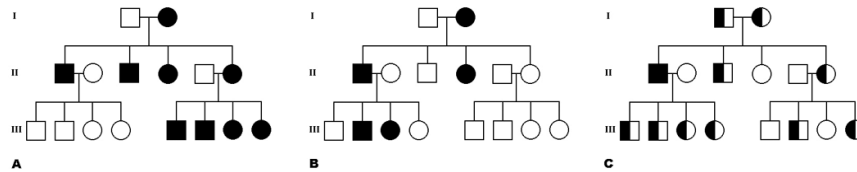


Figure 3. Modes of genetic inheritance

(A) Maternal inheritance (*e.g.*, Leber hereditary optic neuropathy (LHON)): women transmit the mutation to all children, while men do not transmit the mutation to any children; (B) autosomal dominant inheritance (*e.g.*, dominant optic atrophy (DOA)): men and women alike have a 50% chance of transmitting the mutation to each child; (C) autosomal recessive inheritance (*e.g.*, Friedreich ataxia (FRDA)): each parent's mutant allele has a 50% chance of being transmitted to the next generation, but disease is only expressed when a child receives an allele from both parents.

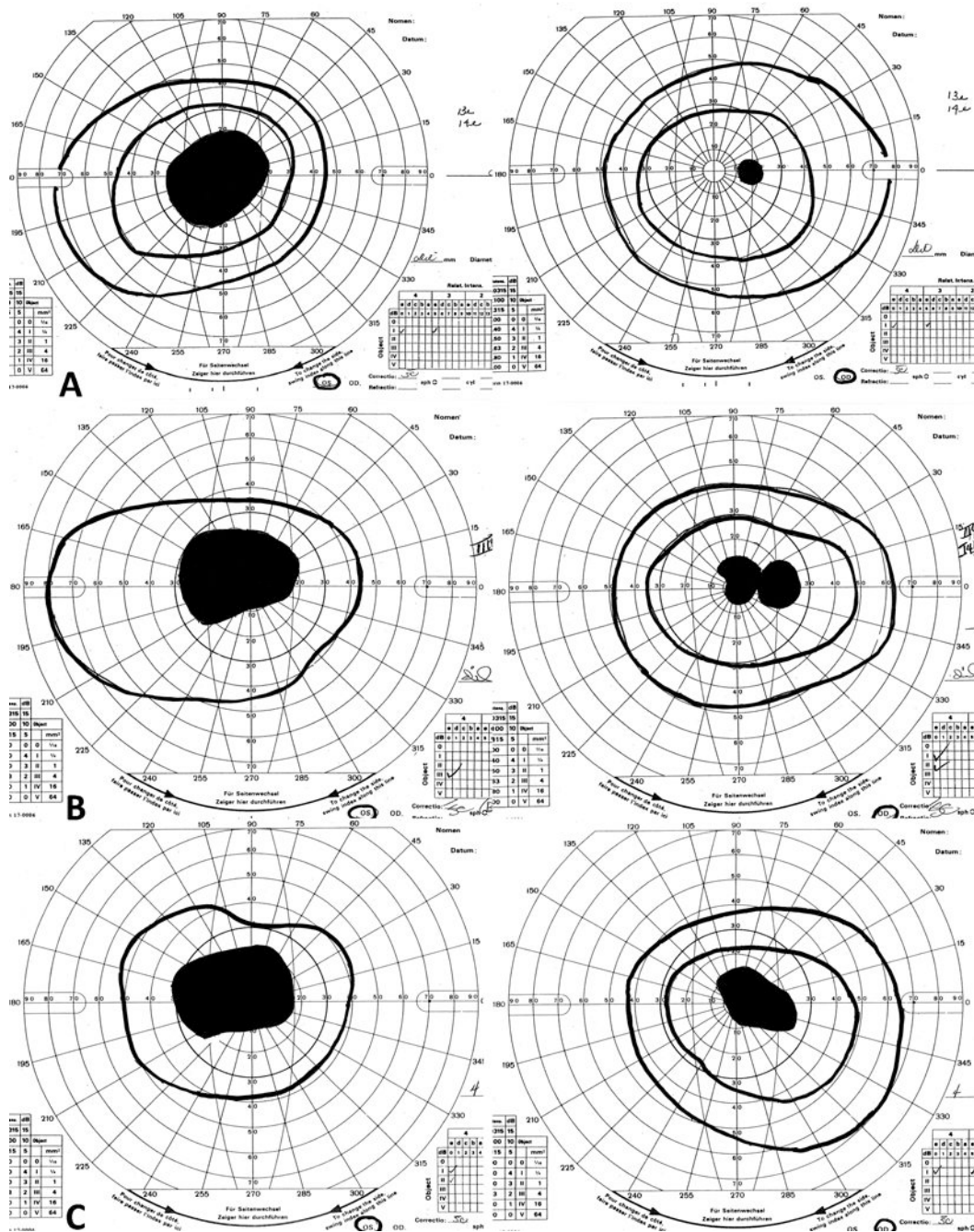


Figure 4. Goldmann visual fields (GVF) in a patient with Leber hereditary optic neuropathy (LHON)

Progressive central scotoma in the right eye of a 21 year old man with the 11778 LHON mutation. He had suffered a painless central scotoma in the left eye three months prior. (A) GVF from March 5, 2009; (B) GVF from March 19, 2009; (C) GVF from April 16, 2009.

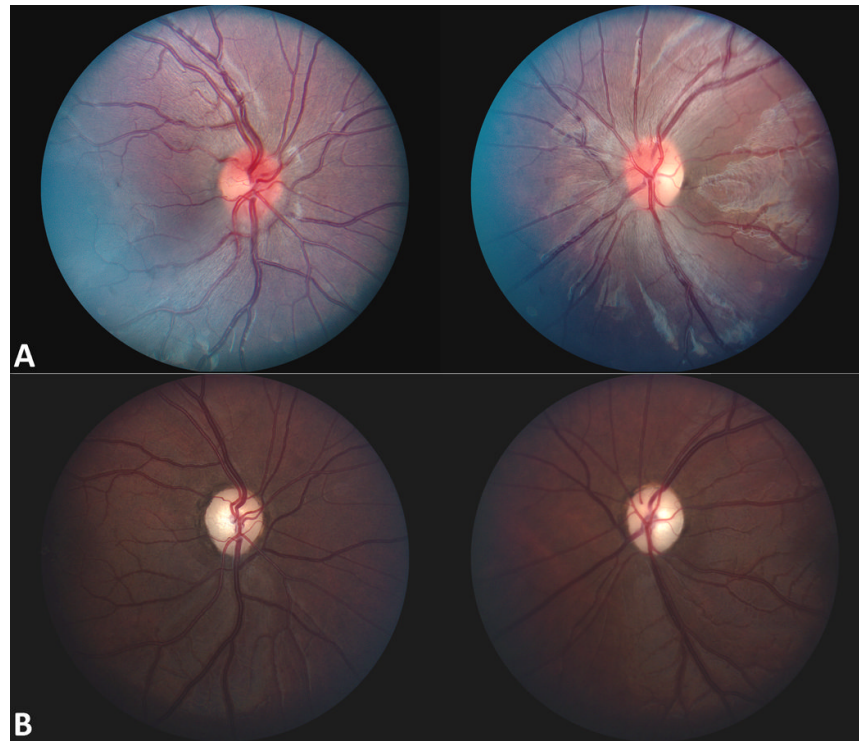


Figure 5. Ocular fundus appearance in a patient with Leber hereditary optic neuropathy (LHON)
(A) Acute pattern: disc hyperemia, pseudoedema, and telangiectasias; the left eye was affected one month prior to the right eye, and early temporal optic disc pallor is evident in the left eye.
(B) Chronic pattern: diffuse optic atrophy, most apparent temporally, three years later.

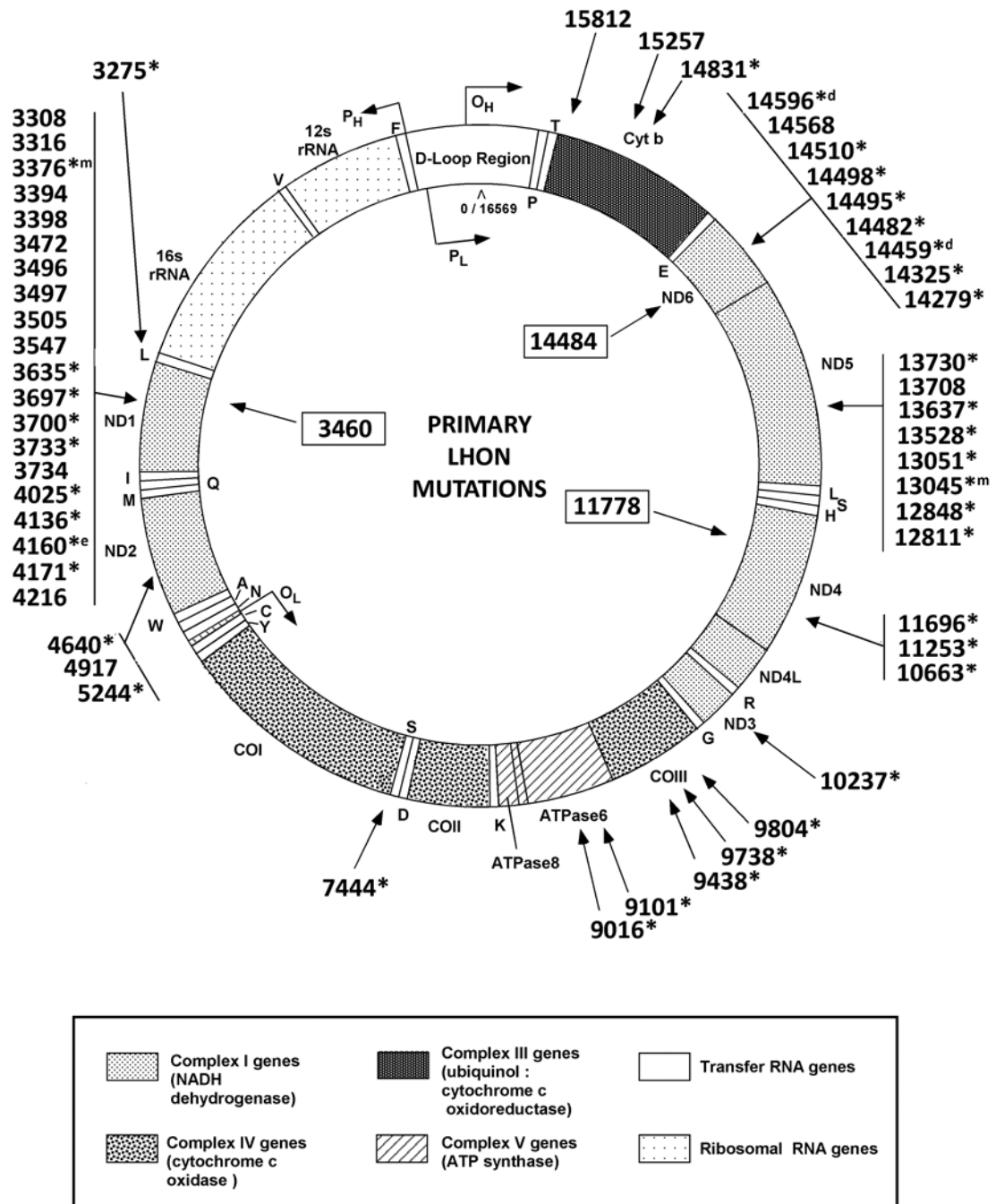


Figure 6. Primary and secondary LHON mutations

Primary LHON point mutations (inside circular mitochondrial genome) and secondary LHON point mutations (outside circular mitochondrial genome) are shown. Mutations marked * may be primary, but they account for only one or a few pedigrees worldwide. Mutations marked *d are primary mutations associated with LHON and dystonia; mutations marked *m are primary mutations associated with LHON and MELAS; mutation marked *e is a primary mutation associated with LHON and encephalopathy. (Adapted from: www.mitomap.org).

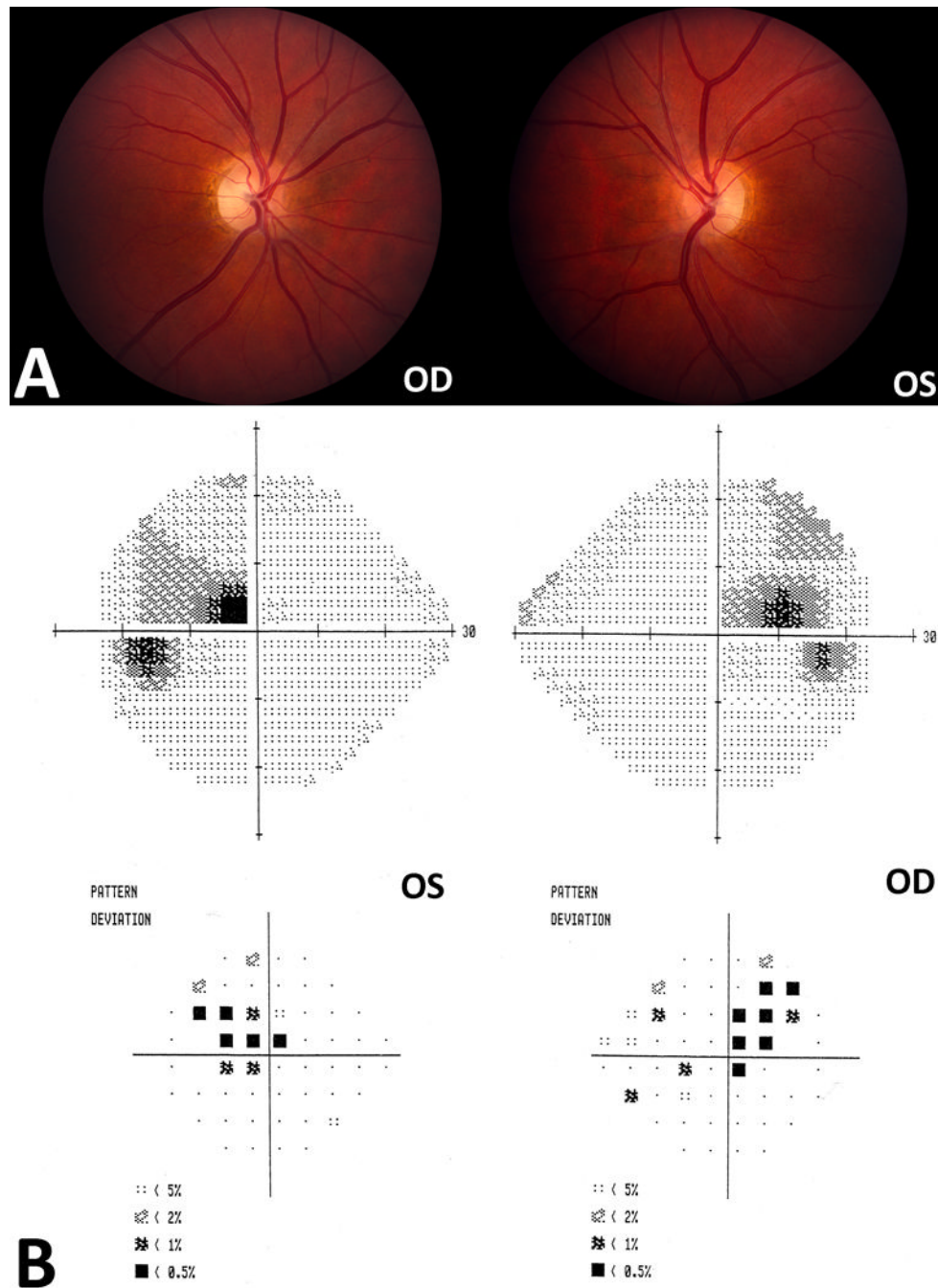


Figure 7. Ocular fundus appearance and visual fields in a patient with dominant optic atrophy (DOA)

(A) Classic “temporal wedge” of optic disc pallor, symmetric between eyes; (B) Humphrey visual fields showing bilateral cecocentral scotomas in grayscale (top) and pattern deviation (bottom), reflecting involvement of the papillomacular bundles OU. Note the superobitemporal predilection of the visual field defect.

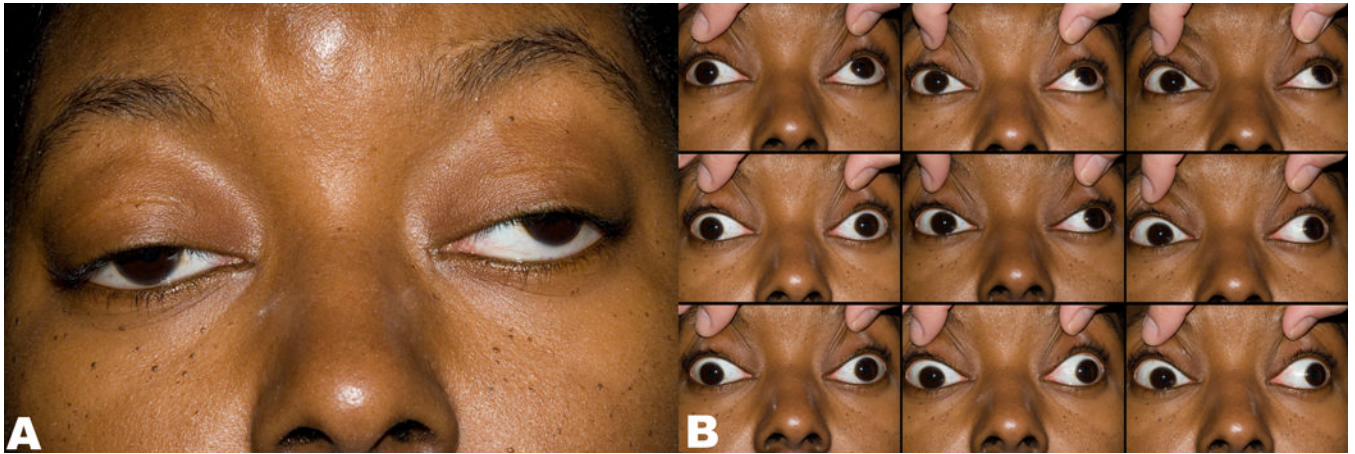


Figure 8. External motility photographs in chronic progressive external ophthalmoplegia
(A) Bilateral ptosis and bifacial weakness; (B) Limitation of ocular motility in all directions in both eyes.

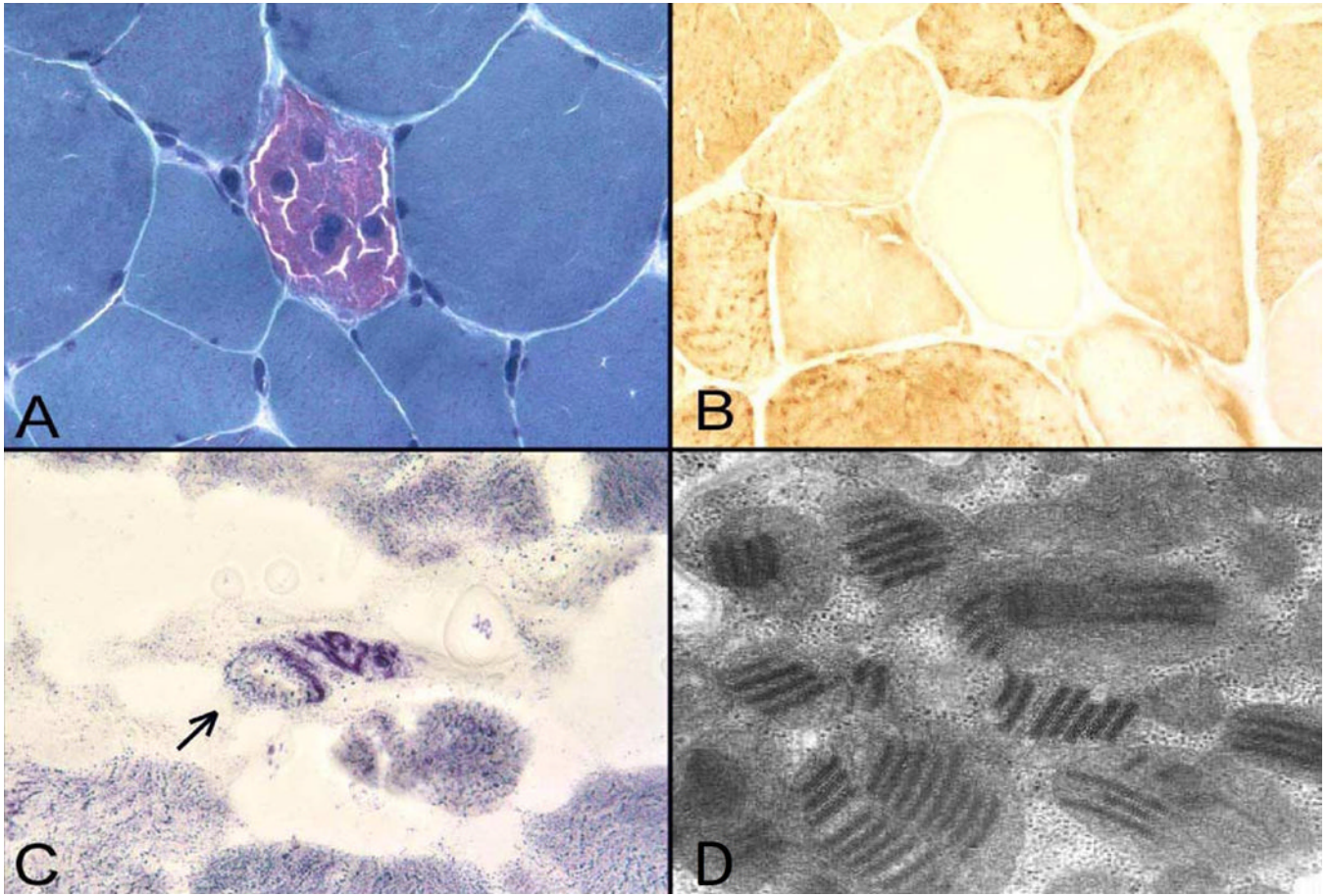


Figure 9. Classic histopathological findings in mitochondrial myopathy

(A) Gomori trichrome stain: ragged red fibers (RRFs); (B) Cytochrome oxidase (COX) stain: isolated COX-negative fiber (center); (C) Succinate dehydrogenase (SDH) stain: increased staining of a blood vessel (arrow) in a patient with MELAS; (D) Electron microscopy: paracrystalline “parking lot” inclusions. (Images reproduced with permission from: Bourgeois JM, Tarnopolsky MA. Pathology of skeletal muscle in mitochondrial disorders. *Mitochondrion*. 2004;4(5–6):441–42).

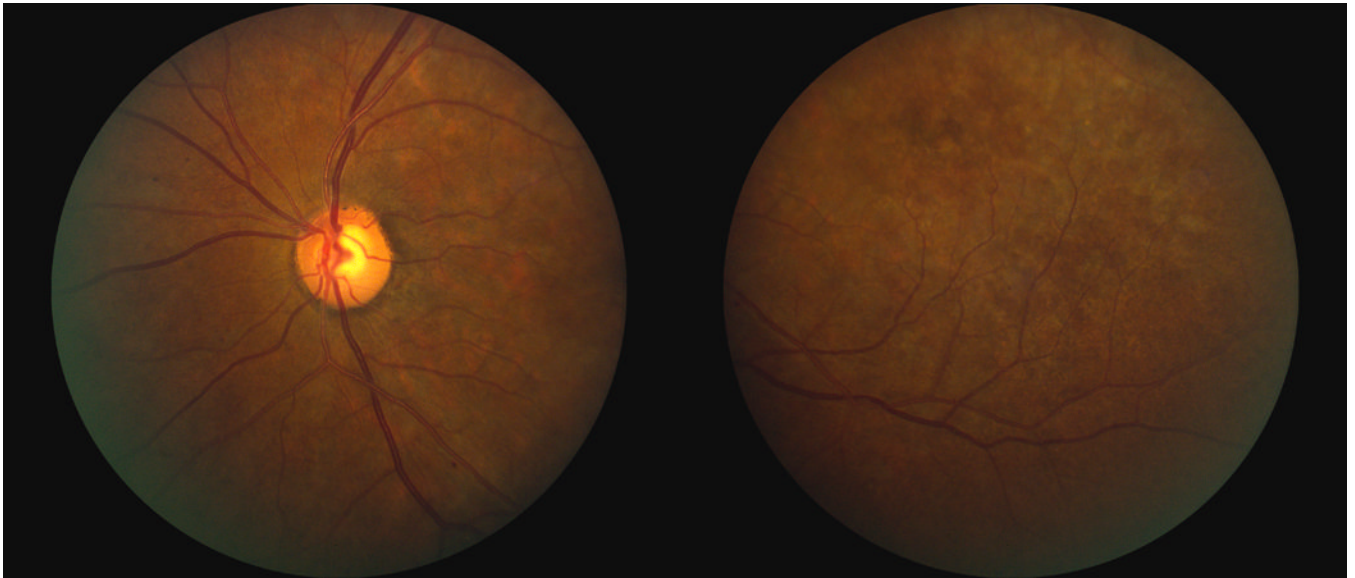


Figure 10. Ocular fundus appearance in a patient with pigmentary retinopathy associated with Kearns-Sayre syndrome (KSS)
(A) Diffuse retinal arteriolar attenuation and mild waxy pallor of the optic disc; (B) salt and pepper retinopathy.

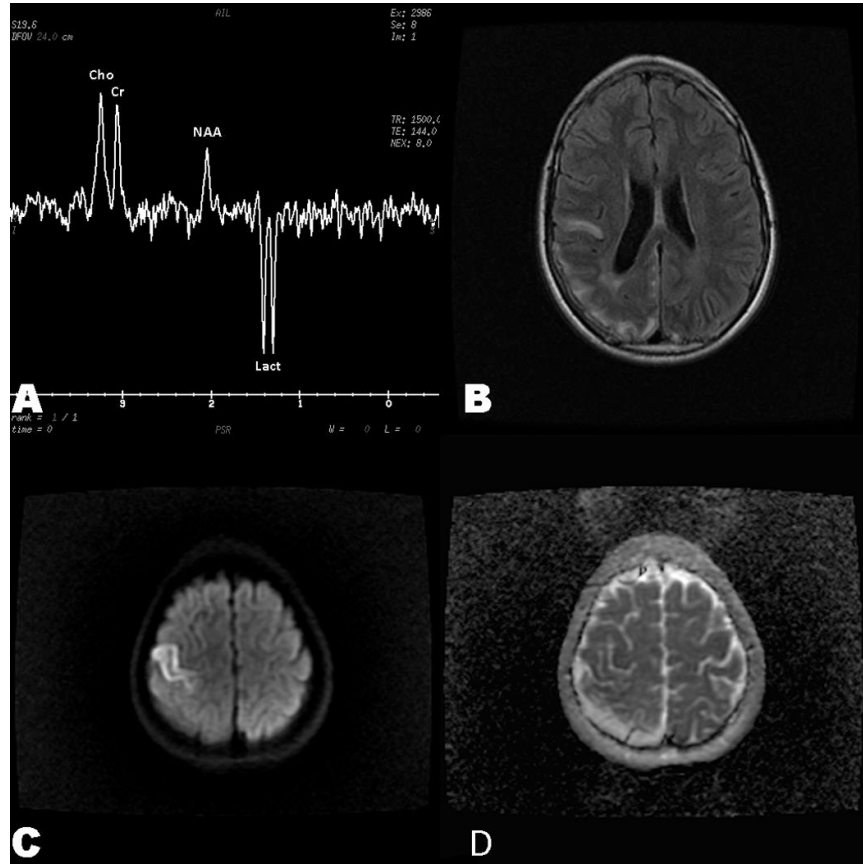


Figure 11. Neuroimaging features of MELAS

(A) Magnetic resonance spectroscopy (MRS) showing a decreased N-acetyl aspartate (NAA) peak relative to choline (Cho) and creatine (Cr) and a large lactate doublet (Lact); (B) Fluid-attenuated inversion recovery (FLAIR) imaging sequence showing high T2 signal in a cortical laminar distribution of the right brain, predominantly posteriorly, sparing deep white matter, and not respecting cerebral arterial territories; (C) Diffusion weighted imaging (DWI) sequence, showing a right cortical focus of restricted diffusion during a stroke-like episode; (D) Apparent diffusion coefficient (ADC) map in the same patient as (C), showing no decreased signal in the involved area of cortex. (Images courtesy of Dr. Chad Holder, Emory University).

Table 1

Mutation types in each mitochondrial syndrome

Syndrome	Nuclear DNA		Mitochondrial DNA			
	AD	AR	Single large scale deletion	Homoplasmic point mutation	Heteroplasmic point mutation	
LHON	-	-	-	+++	+	
DOA	+++ ^o	-	-	-	-	
FRDA	-	+++	-	-	-	
HMSN VI	+++	+	-	-	-	
HSP	-	+	-	-	-	
Wolfram syndrome	-	++	+?	-	+?	
CPEO/KSS	++ ^{oo}	+ ^{oo}	+++	-	+	
MNGIE	-	+++ ^{oo}	-	-	-	
NARP	-	-	-	-	+++	
MELAS	-	-	-	+	+++	

+++ = Common

++ = Less common

+ = Rare

- = Does not occur

^{oo} = Secondary multiple large-scale mtDNA deletions always occur

^o = Secondary multiple large-scale mtDNA deletions may rarely occur

AD: autosomal dominant; AR: autosomal recessive; LHON: Leber hereditary optic neuropathy; DOA: dominant optic atrophy; FRDA: Friedreich ataxia; HMSN VI: hereditary motor sensory neuropathy type VI; HSP: hereditary spastic paraplegia; CPEO: chronic progressive external ophthalmoplegia; KSS: Kearns-Sayre syndrome; MNGIE: mitochondrial neurogastrointestinal encephalomyopathy; NARP: neurogenic muscle weakness, ataxia, and retinitis pigmentosa; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.

Table 2

Mitochondrial diseases with neuro-ophthalmological manifestations

Disease	Age of onset	First major sign	Other signs	Classic pattern of inheritance	Genetic defect	Product encoded by gene
LHON	15 – 35 y	Optic neuropathy	Early pseudo disc edema; late optic atrophy	Maternal	11778G>A 14484T>C 3460G>A	ND4 ND6 ND1
DOA	< 10 y	Optic atrophy	Sensorineural hearing loss	Autosomal dominant	<i>OPA1</i>	OPA1
FRDA	10 – 15 y	Ataxia and dysarthria	Optic atrophy, peripheral neuropathy, spasticity, scoliosis, pes cavus, diabetes mellitus, hypertrophic obstructive cardiomyopathy	Autosomal recessive	<i>Frataxin</i>	Frataxin
HMSN VI	< 10 y	Peripheral neuropathy	Optic atrophy, hoarse voice	Autosomal dominant	<i>Mitofusin-2</i>	Mitofusin-2
HSP	20 – 42 y	Paraplegia	Optic atrophy, dysarthria, dysphagia, ataxia, nystagmus, peripheral neuropathy	Autosomal recessive	<i>SPG7</i>	Paraplegin
Wolfram syndrome	< 10 y	Diabetes mellitus, optic atrophy, diabetes insipidus, sensorineural hearing loss	Growth retardation, ataxia, dementia, central apnea	Autosomal recessive	<i>WFS1</i> , <i>WFS2</i> , others?	Wolframin
CPEO/KSS	> 10 y	Ptosis and ophthalmoplegia	Pigmentary retinopathy, cardiac conduction block, ataxia, growth retardation, endocrinopathy, myopathy	Sporadic	Large-scale deletion	Multiple products
MNGIE	< 20 y	Gastrointestinal dysmotility and cachexia	Ptosis, ophthalmoplegia, pigmentary retinopathy, peripheral neuropathy, myopathy, leukoencephalopathy	Autosomal recessive	<i>EGF1</i>	Thymidine phosphorylase
NARP	< 20 y	Pigmentary retinopathy, peripheral neuropathy, ataxia	Optic atrophy, nystagmus, dystonia, seizures	Maternal	8993T>G	ATPase-6
MELAS	2 – 10 y	Stroke-like episodes (including retrochiasmal visual loss)	Pigmentary retinopathy, Seizures, dementia, headaches, gastrointestinal dysmotility, growth retardation, diabetes mellitus, ophthalmoplegia, myopathy, cardiac conduction block	Maternal	3243A>G	tRNA ^{Leu} (UUR)

LHON: Leber hereditary optic neuropathy; DOA: dominant optic atrophy; FRDA: Friedreich ataxia; HMSN VI: hereditary motor sensory neuropathy type VI; HSP: hereditary spastic paraplegia; CPEO: chronic progressive external ophthalmoplegia; KSS: Kearns-Sayre syndrome; MNGIE: mitochondrial neurogastrointestinal encephalomyopathy; NARP: neurogenic muscle weakness, ataxia, and retinitis pigmentosa; MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.