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OXIDATIVE PHOSPHORYLATION DISEASES AND MITOCHONDRIAL DNA MUTATIONS: Diagnosis and Treatment

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INTRODUCTION

Oxidative phosphorylation (OXPHOS) diseases were first recognized in 1962 when a young woman was found to have a hypermetabolic state, structurally abnormal mitochondria, and abnormalities of OXPHOS function, all signs of a rare disorder now known as Luft's disease (47, 107). For almost three

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decades, the diagnosis of OXPHOS diseases was defined by many of the pathological and biochemical criteria identified in this patient. During this period, OXPHOS diseases were considered complex neuromuscular disorders characterized by a unique type of degeneration of the OXPHOS-dependent type I skeletal muscle fibers (ragged-red fibers); by ultrastructural abnormalities of mitochondria, including paracrystalline inclusions and abnormal membrane structures (179, 180); and by various abnormalities in OXPHOS enzyme activity. These diagnostic limitations changed rapidly when, in 1988, gene mutations in the mtDNA were found to cause OXPHOS diseases that had markedly different clinical presentations. The first two types of pathogenic mtDNA mutations discovered were large deletions in the mtDNA and a point mutation that produced a missense mutation in a polypeptide subunit of OXPHOS. The mtDNA deletions removed several thousand nucleotide pairs (np) and were found in patients who had Kearns-Sayre syndrome or chronic progressive external ophthalmoplegia (CPEO) syndromes (68). The mtDNA point mutation was shown to be a major cause of a type of maternally inherited blindness called Leber's hereditary optic neuropathy (LHON) (213).

Within two years after the discovery of these mutations, the major classes of mtDNA mutations were defined: insertion-deletion mutations (68, 147, 148), missense base substitutions (213), and base substitutions that impair mitochondrial protein synthesis (174, 215). The term OXPHOS disease now encompasses a wide array of clinical disorders whose onset can occur at any time from birth to old age. These diseases no longer are confined to rare neuromuscular disorders with ragged-red fibers and structurally abnormal mitochondria. In fact, as the complex relationships among mtDNA mutations, nuclear DNA mutations, and OXPHOS disease expression are investigated, the catalog of patient phenotypes encompassed by this class of diseases will continue to expand rapidly. It already has grown to include various types of cardiac and ophthalmological diseases as well as endocrinopathies and may also include genetically complex disorders such as Alzheimer's disease and Parkinson's disease. Diagnosis of these disorders is complex and requires specialized techniques performed at centers experienced in evaluating the clinical, biochemical, and genetic intricacies of these disorders. Hence, OXPHOS diseases may be one of the most commonly encountered classes of degenerative diseases, thus making the development of novel therapeutic interventions essential to patient management. Treatment protocols for OXPHOS diseases are still in their infancy. High doses of certain coenzymes and vitamins can produce mild degrees of symptomatic improvement in some patients. However, significant advances in OXPHOS disease therapy are needed to ameliorate or cure the vast array of clinical manifestations exhibited by these patients. In this regard, novel gene therapy approaches may hold significant promise for the treatment of OXPHOS diseases.

mtDNA GENETICS AND OXIDATIVE PHOSPHORYLATION

Oxidative Phosphorylation (OXPHOS)

OXPHOS consists of five protein-lipid enzyme complexes located in the mitochondrial inner membrane that contain flavins (FMN, FAD), quinoid compounds (coenzyme Q10), and transition metal compounds (iron-sulfur clusters, hemes, protein-bound copper) (Figure 1) (177, 178). These enzymes

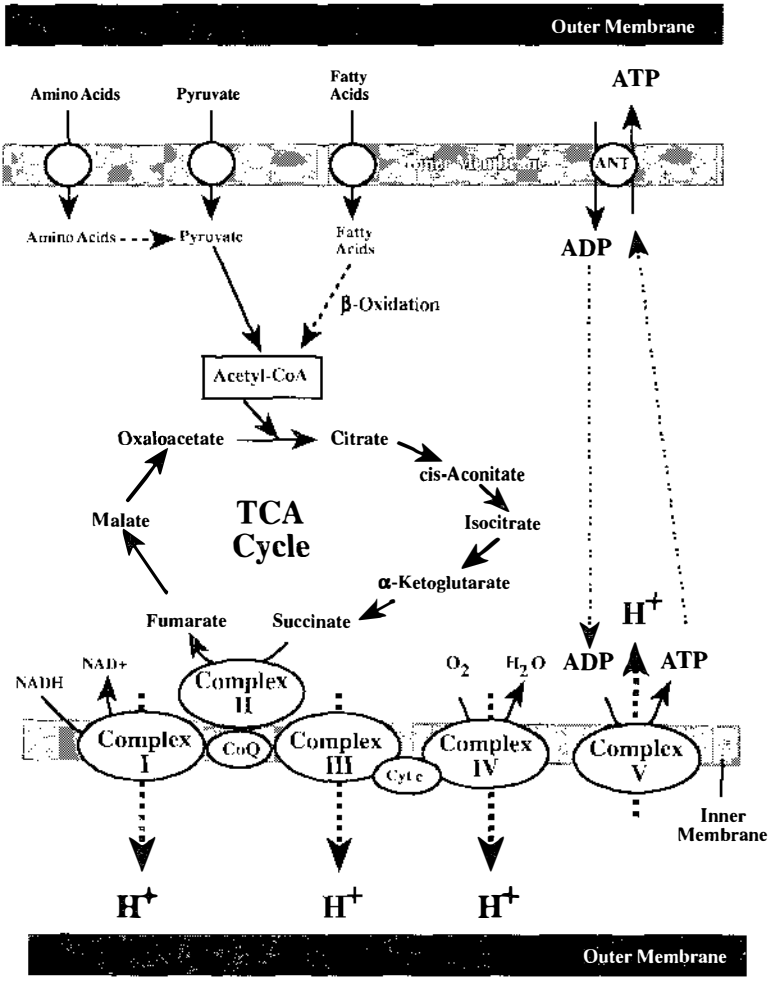

Figure 1 Oxidative phoshorylation and the tricarboxylic acid cycle (TAC). Dashed arrows indicate proton (H^+) translocation into the space between the inner and outer mitochondrial membrane (intermembrane space). CoQ, ubiquinone (coenzyme Q); Cyt c, cytochrome c. Reprinted with permission of the American Heart Association, Inc. from *Heart Disease and Stroke*. 1993. 2:439–45.

are designated Complex I (NADH: ubiquinone oxidoreductase, EC 1.6.5.3), Complex II (succinate: ubiquinone oxidoreductase, EC 1.3.5.1), Complex III (ubiquinol: ferrocytochrome c oxidoreductase, EC 1.10.2.2), Complex IV (ferrocytochrome c: oxygen oxidoreductase or cytochrome C oxidase, EC 1.9.3.1), and Complex V (ATP synthase, EC 3.6.1.34). Complexes I and II collect electrons from the catabolism of fats, proteins, and carbohydrates and transfer them to ubiquinone (coenzyme Q10). The electrons then move sequentially through Complex III, cytochrome c, and Complex IV and finally react with oxygen, the terminal electron acceptor. Complexes I, III, and IV use the energy in electron transfer to pump protons across the inner mitochondrial membrane, thereby producing a proton gradient. Complex V uses the potential energy stored in the proton gradient to condense ADP and inorganic phosphate (Pi) into ATP. The resulting ATP is exchanged across the inner membrane with ADP by the adenine nucleotide translocase (ANT) (95).

mtDNA and Nuclear DNA OXPHOS Genes

The human mtDNA is a double-stranded, circular molecule of 16,569 bp that codes for the ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) of mitochondrial protein synthesis as well as for 13 polypeptides of OXPHOS (Figure 2) (4, 211). The guanine-rich strand is called the heavy (H) strand, and the cytosine-rich strand is called the light (L) strand. Twelve OXPHOS subunit genes, the 12S and 16S rRNA genes, and 14 tRNA genes are present on the H strand. One OXPHOS subunit gene (ND6 of Complex I) and eight tRNA genes are located on the L strand. A 1122-bp stretch of mtDNA designated the displacement loop (D-loop) contains genetic information necessary for replication and transcription.

Over the past several years, significant progress has been made in identifying nuclear genes that encode OXPHOS polypeptides. The precise function of many of these subunits is obscure, and nuclear gene mutations that cause OXPHOS diseases have not been identified. The human gene sequences and the chromosomal locations of known nuclear OXPHOS genes are reviewed in Ref. 178.

Mitochondrial Genetics

The high mtDNA copy number and cytoplasmic location of mitochondria produce genetic principles unique to the mtDNA. Current concepts in mitochondrial genetics embody five main features: maternal inheritance, replicative segregation, threshold expression of phenotype, an accumulation of somatic mtDNA mutations with aging and in degenerative diseases, and a high mtDNA mutation rate.

Maternal transmission of mtDNA defines the inheritance pattern of this genome in all vertebrates (178). Although low levels of paternally transmitted

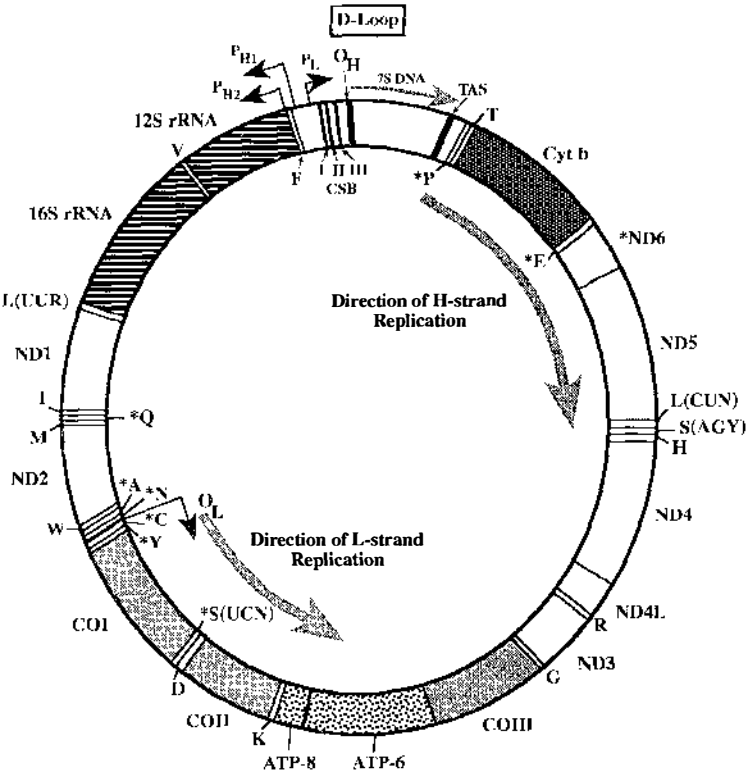

Figure 2 Mitochondrial DNA gene arrangement. * = mtDNA genes coded on the L strand. The mtDNA map positions are derived from the human gene mapping (HGM) conventions. The mitochondrial genome is composed of 16,569 bp and has the following features: (*a*) D-loop, a 1122-bp stretch of mtDNA extending from bp 16,204 to 576 responsible for directing replication and transcription. It consists of the termination-associated sequence (TAS, bp 16,157–16,172), the origin of H-strand replication (O_H, bp 110–441), the 7S-like DNA region (OL-16,104–16,106), conserved sequence block I (CSBI, bp 213–235), CSBII (bp 299–315), and CSBIII (bp 346–363), mtTF-binding sites (bp 233–260, 276–303, 418–445, and 523–550), the mitochondrial replication primer (bp 317–321), the L-strand promotor (P_L, bp 392–445), the major H-strand promotor (P_{H1}, bp 545–567), and the minor H-strand promotor (P_{H2}, bp ~ 645). The membrane attachment site is located at ~ bp 15,925–499. Other regulatory sequences are located outside the D-loop and include the origin of L-strand replication (OL, bp 5721–5798) and a transcription terminator that regulates the rRNA and mRNA ratio (bp 3237–3249). (*b*) rRNAs: 12S rRNA (bp 648–1601), 16S rRNA (bp 1671–3229). (*c*) tRNAs: alanine (A, Ala, bp 5587–5655), arginine (R, Arg, bp 10,405–10,469), asparagine (N, Asn, bp 5657–5729), asparate (D, Asp, bp 7518–7585), cysteine (C, Cys, bp 5761–5826), glutamate (E, Glu, bp 14,674–14,742), glutamine (Q, Gln, bp 4329–4400), glycine (G, Gly, bp 9991–10,058), histadine (H, His, bp 12,138–12,206), isoleucine (I, Ile, bp 4263–4331), leucine [L(UUR), Leu, bp 3230–3304 and L(CUN), bp 12,266–12,336], lysine (K, Lys, bp 8295–8364), methionine (M, Met, bp 4402–4469), phenylalanine (F, Phe, bp 577–647), proline (P, Pro, bp 15,955–16,023), serine [S(UCN), Ser, bp 7445–7516 and S(AGY), bp 12,207–12,265], threonine (T, Thr, bp 15,888–15,953), tryptophan (W, Trp, bp 5512–5576), tyrosine (Y, Tyr, bp 5826–5891), valine (V, Val, bp 1602–1670). (*d*) OXPHOS subunits. Complex I: NADH dehydrogenase 1 (ND1, bp 3307–4262), ND2 (bp 4470–5511), ND3 (bp 10,059–10,404), ND4 (bp 14,149–14,673); Complex III: cytochrome b (Cytb, bp 14,747–15,887); Complex IV: cytochrome c oxidase I (COI, bp 5904–7444), COII (bp 7586–8262), COIII (bp 9207–9990); Complex V: ATPase 6 (ATP-6, bp 8527–9207), ATP-8 (bp 8366–8572).

mtDNA representing ~ 10^{-4} of the total mtDNA have been identified in mice (59), paternal transmission has not been found to be a significant feature of mtDNA inheritance in human pedigrees.

The mtDNA found in normal individuals is homoplasmic, i.e. all cells have the same mtDNA sequence. However, pathogenic mtDNA mutations are frequently heteroplasmic, i.e. mtDNAs with both normal and mutant sequences coexist. An important factor in determining the relative proportions of normal and mutant mtDNAs is the replicative potential of the cell line. For example, skeletal muscle, cardiac muscle, and neurons have a low replicative potential, which explains their ability to maintain high proportions of mutant mtDNAs. In contrast, hematopoietic cells replicate throughout an individual's life, resulting in highly variable concentrations of mutant and normal mtDNAs. Hence, replicative segregation accounts for a significant proportion of the variation in cellular genotypes observed in patients with OXPHOS diseases caused by heteroplasmic mtDNA mutations (122, 210).

When organs are rank ordered according to oxygen consumption per gram of tissue, the heart has the highest consumption, followed by kidney, brain, liver, and skeletal muscle (168). However, OXPHOS diseases generally produce central nervous system (CNS) dysfunction early during the course of the disease. Clinically significant manifestations commonly are identified in the highly OXPHOS-dependent type I skeletal muscle fibers and in cardiac tissue. In contrast, liver and kidney generally have minor clinical manifestations. Hence, the differential sensitivity of these organs to OXPHOS dysfunction is due to the combined influences of replicative segregation of mutant and normal mtDNAs, the pathogenicity of the mtDNA mutation, the different energetic requirements of human organs and tissues, and the different abilities of human organs and tissues to compensate for cellular injury. Of these variables, tissue-specific energy requirements are particularly important and result in a phenomenon known as threshold expression. Each tissue requires a different minimum level (threshold) of mitochondrial ATP production to sustain normal cellular functions. Throughout a broad concentration range of mutant mtDNAs, the cellular phenotype remains normal. Once the mutant mtDNAs surpass a certain level or threshold, cellular phenotype changes rapidly from normal to abnormal. For example, in hybrid cell lines containing both drug-resistant and drug-sensitive mtDNAs, the cell expresses resistance only after the drug-resistant mtDNAs reach a level above the cellular threshold (72, 210). Likewise, patients (174) and their cell lines (30, 122) that harbor mutations in a mitochondrial tRNA do not show severe inhibition of protein synthesis until >85% of the mtDNAs are mutant.

The relationship between tissues and their energetic thresholds changes throughout an individual's life. ATP production by OXPHOS declines with age (2, 8, 14, 15, 18, 29, 94, 204, 207, 224). This decline in respiratory

function is associated with an accumulation of Complex IV–deficient fibers in various muscle groups (124–126). An important mechanism for this process may be the accumulation of mtDNA mutations in somatic cells as a result of increased free radical production. This hypothesis of free radical–mediated mtDNA damage has three essential elements (115). First, free radicals are continuously produced in the mitochondria by a variety of reactions. Second, oxidative degradation of mitochondrial lipids, proteins, and mtDNAs impairs OXPHOS efficiency and stimulates more free radical accumulation. Third, accumulation of mtDNA damage blocks mitochondrial biogenesis, resulting in permanent organelle dysfunction that ultimately leads to cell death. The mtDNA is particularly susceptible to oxygen radical damage because of its proximity to oxygen radical production by OXPHOS, a lack of protective histones, and minimal mtDNA repair mechanisms. Consistent with these elements, the mtDNA accumulates 16 times more oxidized bases than does the nuclear DNA (64, 75, 113, 154, 155) and is more susceptible to alkylating agents and polycyclic aromatic hydrocarbons (10, 221).

Significant progress has been made in evaluating the age-related accumulation of somatic mtDNA mutations in human tissues. Heterogeneous classes of mtDNA deletions increase with age in brain (35, 81, 189), heart (6, 36–38, 63, 141, 197), skeletal muscle (93), liver (39, 224), kidney (39), and various other tissues (39). Base modifications also increase in aging tissues, with an age-related increased in 8-hydroxydeoxyguanosine in human diaphragm (64) and brain (113). These observations support the hypothesis that OXPHOS declines with age in certain tissues in accordance with the accumulation of mtDNA damage. Impairment of OXPHOS by inherited OXPHOS gene mutations, environmental toxins, or processes such as chronic tissue ischemia and reperfusion would stimulate oxygen radical generation and thus promote somatic mtDNA mutations. Tissues such as the basal ganglia that produce high levels of free radicals or that are subjected to disease processes that impair OXPHOS, e.g. ischemia and reperfusion of coronary artery heart disease, accumulate significant amounts of damaged mtDNAs (35–37). Thus the accumulation of somatic mtDNA mutations could contribute to the progression of mitochondrial disease, the occurrence of various classes of degenerative diseases, and the age-related decline in OXPHOS.

The final principle of mitochondrial genetics that relates to OXPHOS diseases is that the mtDNA is more likely to harbor heritable, pathogenic mutations than the nuclear DNA. Nucleotide substitutions in genes that encode polypeptides fall into two classes: those that change the amino acid sequence (replacement mutations) and those that do not (synonymous mutations). The potential for deleterious effects of replacement mutations on a particular pro-

tein can be estimated by determining the selective constraint for a protein and the rate of point mutation fixation for its gene (129, 214). In a highly constrained protein, replacement mutations are more likely to be deleterious. In this regard, nucleotide substitution mutations appear to be fixed ~ 6–17 times more rapidly in mtDNA than in nDNA genes (129, 214). The nuclear-encoded ANT gene fixes 1.6 times the synonymous mutations and 3.9 times the replacement mutations of the nuclear-encoded β subunit of the ATP synthase (129). In contrast, the average mtDNA OXPHOS genes fix synonymous and replacement mutations approximately 10 times faster than these nuclear OXPHOS genes (129, 214). Because the mtDNA OXPHOS genes are comparably evolutionarily constrained to the nuclear OXPHOS genes, the high mtDNA mutation rate means that gene for gene, deleterious OXPHOS mutations are much more likely for the mtDNA than for the nuclear DNA.

OXIDATIVE PHOSPHORYLATION DISEASES

Diagnostic Overview

Identification of patients with OXPHOS diseases requires application of the principles of both mitochondrial and Mendelian genetics. To formulate an appropriate diagnostic, management, and genetic counseling plan, patients must be referred to centers experienced in these diseases for clinical, metabolic, pathological, biochemical, and genetic evaluations. Phenotype recognition and pedigree analysis are important steps in the evaluation of patients suspected of having an OXPHOS disease. OXPHOS defects can result from mutations in any of the hundreds of nuclear and mitochondrial OXPHOS genes. Hence, all forms of Mendelian inheritance, including autosomal dominant, recessive, and X-linked, are possible. Maternal inheritance of homoplasmic or heteroplasmic mutations, abnormalities of the interaction between nuclear DNA and mtDNA genes, and spontaneous mutations also must be considered.

The quantitation of organic and amino acids in blood and urine as well as in the cerebrospinal fluid of some patients can provide useful information. Elevations of lactate, pyruvate, and alanine and/or a generalized amino aciduria can be important diagnostic clues to the presence of an OXPHOS disease. However, normal values for these tests do not exclude the diagnosis. Most patients suspected of having an OXPHOS disease will require a muscle biopsy. Pathological analysis of the muscle biopsy by histochemistry and electron microscopy can be helpful in supporting the diagnosis of an OXPHOS disease. Mitochondrial myopathies are distinguished pathologically by the proliferation of subsarcolemmal mitochondria and by varying degrees of degeneration of the muscle fibers as detected by the modified Gomori trichrome stain (the red-staining mitochondria of a ragged-red fiber) or by the more sensitive

succinate dehydrogenase stain (blue-staining mitochondria). Ultrastructural analysis of the muscle often reveals structurally abnormal mitochondria with paracrystalline inclusions, which are intermembranous condensations of mitochondrial creatine kinase (194). These pathological findings are most commonly present in OXPHOS disorders caused by defects in mitochondrial protein synthesis. Diseases associated with mtDNA missense mutations rarely show this clinical feature. Therefore, detection of muscle mitochondrial abnormalities can provide useful clues as to the type of mtDNA mutation involved. However, a normal study does not exclude OXPHOS defects as the cause of the disease.

Biochemical and/or genetic testing can confirm the presence of an OXPHOS disease. When specific mutations are not implicated, OXPHOS enzyme analysis in skeletal muscle mitochondria provides a more general diagnostic approach for OXPHOS diseases. To perform accurate assessments of this delicate enzyme system, mitochondria should be isolated immediately from fresh muscle biopsies. This approach avoids the artifacts in OXPHOS enzyme analysis that can be associated with freezing the biopsy prior to mitochondrial isolation (229). A small portion of the biopsy is frozen in liquid nitrogen for DNA or RNA extraction. Finally, lymphoblast and/or myoblast cell lines can be established for cellular respiration studies, for somatic cell genetic studies, and as a source of total genomic DNA for future analyses. This integrated clinical-genetic, metabolic, and biochemical-genetic protocol, utilized routinely in our clinic, increases the probability of a correct biochemical and genetic diagnosis, which is necessary for accurate genetic counseling and effective patient management. Although one can now achieve a precise diagnosis of certain OXPHOS diseases by DNA analysis alone, OXPHOS enzymology is the only means of diagnosing many cases. Because most experience with OXPHOS disease treatment has been in patients who harbor mtDNA mutations, this review focuses on the features of OXPHOS diseases caused by these mutations.

mtDNA Mutations

Three classes of mtDNA mutations have been identified: insertion-deletion mutations, missense base substitutions, and base substitutions that impair mitochondrial protein synthesis. Many of these mutations are distinguished by their clinical manifestations, which permit diagnosis and genetic counseling of affected individuals and their family members. Examples of diseases caused by each class of mutation are discussed in the subsequent sections.

mtDNA INSERTION-DELETION MUTATIONS

Kearns-Sayre syndrome and CPEO syndromes Kearns-Sayre syndrome is a clinical term that has been applied to patients with the onset of disease symptoms before 20 years of age, ophthalmoplegia, atypical retinitis pigmentosa, mitochondrial myopathy, and one

of the following: cardiac conduction defects, cerebellar syndrome, or a cerebrospinal fluid (CSF) protein above 100 mg/dl (161). When these symptoms first appear after 20 years of age, the disorder is referred to as a CPEO syndrome. Although a large variety of clinical manifestations can be observed in patients with Kearns-Sayre syndrome or CPEO syndromes, ophthalmoplegia is the most characteristic clinical feature of these disorders (177, 178). Kearns-Sayre syndrome and CPEO syndromes usually are caused by spontaneously occurring mtDNA insertion-deletions, which explains the sporadic occurrence of the phenotype within the pedigree (120, 167). However, some families harbor mtDNA insertion-deletion mutations transmitted along the maternal lineage of the pedigree (156).

A large array of mtDNA deletions that remove ~ 9–50% of the mtDNA genome can cause Kearns-Sayre syndrome and CPEO syndromes. The mtDNA from these patients is heteroplasmic for mutant and normal mtDNAs, and the severity of the clinical manifestations is determined by the distributions of these molecules within the individual. Hence, the clinical distinction between these two diseases is somewhat arbitrary. Approximately 80% of Kearns-Sayre patients, 70% of patients with CPEO plus manifestations in other organs, and 40% of CPEO patients have mtDNA deletions (67, 120). Of the 100 different deletions that have been reported in the larger O_H to O_L arc, the most common is a 4.9-kb deletion, which accounts for ~ 50% of cases.

The original descriptions of mtDNA deletion and insertion mutations suggested that these genetic phenomena may have occurred by different mechanisms (68, 147, 148, 167). However, recent investigations suggest that complex rearrangements in the mtDNA are more common than previously suspected and that the genesis of both types of mutations may be related (146). mtDNA deletions were originally recognized by digesting a patient's mtDNAs with a restriction endonuclease such as BamHI or PvuII that produces a single cut in the mtDNA. Because of the sequence organization of the mtDNA rearrangements, this approach often cannot distinguish between mtDNAs, which harbor only mtDNA deletions, and complex mtDNA molecules, which contain both duplicated and deleted regions. Modifications of these initial analyses indicate that maternally transmitted as well as spontaneously occurring Kearns-Sayre syndrome and CPEO syndromes may be produced by the accumulation of mtDNA deletions, which occur when large, unstable mtDNA molecules with both duplicated and deleted regions undergo rearrangements into smaller, more stable molecules (146).

Pearson's syndrome Pearson's syndrome is a systemic disorder of oxidative phosphorylation in infants that predominantly affects the bone marrow. Hematopoietic dysfunction is manifested as a severe, transfusion-dependent, macrocytic anemia with varying degrees of neutropenia and thrombocytopenia

(144). Bone marrow examination reveals normal cellularity but extensive vacuolization of erythroid and myeloid precursors, hemosiderosis, and ringed sideroblasts. This disease occurs sporadically within pedigrees with no evidence of hematologic dysfunction in other family members and is caused by spontaneously occurring deletions in the mtDNA (20, 34, 157–160). Patients with this disorder may die early in the course of the disease owing to complications of bone marrow failure and repeated transfusions. Some individuals experience spontaneous improvement of the pancytopenia, presumably as a result of repopulation of the bone marrow with stem cells, which have predominantly normal mtDNAs. However, individuals who show improvement of the pancytopenia may later develop symptoms in other organs from OXPHOS defects caused by the deleted mtDNA. Lactic acidosis, growth restriction, pancreas dysfunction, mitochondrial myopathy, and progressive neurological dysfunction frequently occur. This syndrome further supports the concept that the phenotypes produced by mtDNA deletions are highly variable and dependent on the chance distribution of the deleted molecules among the tissues through replicative segregation.

Maternally inherited diabetes mellitus and deafness Diabetes mellitus is the primary clinical manifestation of a genetically heterogeneous group of diseases that cause dysfunction or degeneration of the insulin-secreting β-cells of the pancreas. Although the precise genetic abnormality in most cases of diabetes mellitus is unknown, mtDNA mutations are among the recognizable causes of this disease (11, 208). In a large African-American family, both diabetes and deafness segregated with a complex mtDNA rearrangement (11). The mtDNA from these patients exhibited a trimolecular heteroplasmy. mtDNAs of normal size (16.5 kb), deleted mtDNAs of smaller size (6.1 kb), and monocircular dimers of the normal and deleted mtDNAs of very large size (22.5 kb) were present in leukocytes and/or skeletal muscle from maternal lineage family members. One of the functional effects of this complex set of rearrangements was the inhibition of mitochondrial protein synthesis.

The physiological characteristics of the insulin-producing β-cells and of the glucagon-producing α-cells of the pancreas were investigated in two severely affected, insulin-dependent individuals who had experienced episodes of diabetic ketoacidosis. During periods of euglycemia, these individuals produced both insulin and glucagon. As their blood glucose increased from normal to hyperglycemic levels, the blood levels of insulin and glucagon remained relatively constant, which suggests that the pancreas could not sense and respond to changes in blood glucose. This interpretation is consistent with current concepts of insulin regulation. In normal pancreatic cells, the ability of the β-cells to increase energy production in relation to the blood glucose level distinguishes them from other tissues in which glucose-dependent energy

production remains stable (170). This glucose-dependent regulation of cellular energetics is directly related to insulin release. Glucokinase catalyzes the first step in β-cell glycolysis through an ATP-dependent reaction that converts glucose to glucose-6-phosphate. Recent investigations have indicated that even minor decrements in glucose phosphorylation by glucokinase decrease the ability of β-cells to release insulin in response to hyperglycemia (50). Hence, the glucokinase (49, 195, 209) and mtDNA mutations (11, 208) may share similar mechanisms for producing diabetes mellitus. The glucokinase mutations directly impair glucose sensing by decreasing the activity of this enzyme (51), whereas mtDNA mutations could impair glucose sensing by reducing intracellular concentrations of ATP, thus limiting the rate of glucose phosphorylation.

MISSENSE BASE SUBSTITUTIONS *Leber's hereditary optic neuropathy (LHON)*
LHON was the first human disease to be associated with a mtDNA point mutation (213). It presents with acute or subacute painless loss of central visual acuity that usually occurs between 12 and 30 years of age (130–132). Typical ophthalmoscopic features of acute LHON include circumpapillary telangiectatic microangiopathy and swelling of the nerve fiber layer around the optic disc (184, 185). However, since the advent of molecular approaches for LHON detection, it has become clear that these characteristic ophthalmological features are not present in all patients. Once visual loss has occurred, spontaneous recovery is uncommon but has been reported in a few patients (103, 196). As many as 80–90% of males within Caucasian pedigrees experience visual loss, whereas only 8–32% of females are affected (131).

The most common cause of LHON is a mutation at bp 11,778 (MTND4* LHON11778A), which accounts for ~ 50–70% of cases in Europe and for more than 90% of cases in Japan (128). This G-to-A transition changes a highly conserved arginine to a histidine at the 340th amino acid of the ND4 gene (213), which encodes a component of the large hydrophobic protein domain of Complex I (151). The second most common cause of LHON is a G-to-A transition mutation in the ND1 gene at bp 3,460 (MTND1*LHON3460A). This mutation changes an alanine to a threonine at amino acid 52 of the ND1 polypeptide (Table 1) and accounts for ~ 15–25% of cases (70, 78). MTND1* LHON3460A and MTND4*LHON11778A have several features in common. Both typically present as classical LHON with no or only minor neurological manifestations. Moreover, they can be heteroplasmic or homoplasmic; they both preferentially affect males; both are found in maternal pedigrees as well as in singleton cases; and both are sufficiently pathogenic to produce LHON (70, 78, 105, 131, 213). Although most individuals with the MTND4* LHON11778A mutation generally have an uncomplicated optic atrophy, atypical presentations do occur. Some cases are associated with a painful loss of

vision, central scotomas, and features of multiple sclerosis (61). A Swedish LHON family that was homoplasmic for the MTND4*LHON11778A mutation had complex neurological manifestations in addition to the classic ophthalmological findings (99). To determine whether these atypical phenotypes are part of the normal spectrum of MTND4*LHON11778A expression, the mtDNA sequences from these individuals must be evaluated for the presence of additional deleterious mtDNA mutations.

In the ~ 20% of patients who do not harbor the MTND4*LHON11778A and MTND1*LHON3460A mutations, several novel mutations have been discovered by investigating the evolutionary relationship between controls and patients with LHON. Certain mtDNA lineages harbor various combinations of mildly deleterious mutations. These mtDNA mutations appear to be less pathogenic than the MTND4*LHON11778A and MTND1*LHON3460A mutations and have a lower probability of causing blindness. By themselves, these mutations would not be sufficiently pathogenic to produce LHON. As a group, however, their pathogenicity is enhanced, which increases an individual's risk of developing blindness. These mtDNA mutations are listed in Table 1.

Table 1 MtDNA mutations associated with LHON. Human gene map (HGM) designations are given for each mutation (212). LDYT, Leber's hereditary optic neuropathy plus dystonia. A, adenine; G, guanine, C, cytosine; T, thymine

HGM designation	NP	AA[a]	Homo-plasmy	Hetero-plasmy	References
MTND1*LHON3394C	3394 (T→C)	Y→H	YES	NO	26,88,136
MTND1*LHON3460A	3460(G→A)	A→T	YES	YES	26,70,74,78,84, 90,109,143
MTND1*LHON4136G	4316(A→G)	Y→C	YES	NO	73
MTND1*LHON4160C	4160(T→C)	L→P	YES	NO	73
MTND1*LHON4216C	4216(T→C)	Y→H	YES	NO	27,85
MTND2*LHON4917G	4917(A→G)	D→N	YES	NO	85
MTND2*LHON5244A	5244(G→A)	G→S	NO	YES	27
MTCOI*LHON7444A	7444(G→A)	TERM→K	YES	NO	26,28,89
MTCOI*LHON9438A	9438(G→A)	G→S	YES	NO	89
MTCOI*LHON9804A	9804(G→A)	A→T	YES	NO	89
MTND4*LHON11778A	11778(G→A)	R→H	YES	YES	2,3
MTND5*LHON13708A	13708(G→A)	A→T	YES	NO	26,27,85,87,88
MTND5*LHON13730A	13730(G→A)	G→E	NO	YES	71
MTND6*LDYT14459A	14459(G→A)	A→V	NO	YES	92
MTND6*LHON14484C	14484(T→C)	M→V	YES	YES	26,86,88,108
MTCYB*LHON15257A	15257(G→A)	D→N	YES	NO	27,66,91
MTCYB*LHON15812A	15812(G→A)	V→M	YES	NO	27,87

[a] Amino acids (AA): A, alanine; C, cysteine; D, asparate; E, glutamate; F, phenylalanine; G, glycine; H, histidine; I, isoleucine, K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

The molecular basis of a complex neurological disorder in which LHON is maternally inherited in conjunction with variable degrees of neurodegeneration has been identified. This disease, referred to as LHON + dystonia, exhibits early onset dementia with dystonia, bulbar dysfunction, corticospinal tract abnormalities, and short stature (135). Brain imaging revealed bilateral and symmetrical basal ganglia abnormalities similar to those observed in Leigh's disease. Severity of disease expression varied significantly among affected pedigree members. Individuals with the mildest manifestations had adolescent onset visual loss with optic atrophy similar to that observed in LHON. The most severely affected individual had infantile bilateral striatal necrosis and later developed optic atrophy. Sequencing 90% of the mtDNA from a severely affected individual revealed an heteroplasmic G-to-A mutation at np 14,459 of the mtDNA (MTND6*LDYT14459A) (92). A moderately conserved alanine was changed to a valine at amino acid 72 of the ND6 polypeptide, which is thought to be located in the iron-protein fragment of Complex I (152, 153).

Neurodegeneration + retinitis pigmentosa and Leigh's disease. Retinitis pigmentosa associated with variable degrees of neurological dysfunction has been associated with a T-to-G transversion at bp 8993 in the ATP-6 gene (MTATP6*NARP8993G) (69). This point mutation changes the highly conserved, hydrophobic leucine at position 156 to an arginine. A T-to-C transition at this position, which changes the leucine to a proline in the ATPase 6 polypeptide, has also been observed and has been associated with Leigh's syndrome (MTATP6*NARP8993C) (165). The MTATP6*NARP8993G mutation was originally described in a large family in which individuals manifested primarily neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP) (69). Patients may experience dementia, corticospinal tract degeneration, generalized seizures, and an axonal sensory neuropathy. Metabolic investigations in patients with this mutation are generally unremarkable and reveal lactate accumulation only in severely affected individuals. Muscle histology can be normal, demonstrate neurogenic atrophy, or show subtle abnormalities, such as minor variations in fiber diameter and minor accumulations of lipid in the type I fibers.

The most frequently encountered manifestation in patients who harbor the MTATP6*NARP8993G mutation is a pigmentary retinopathy. Detailed ophthalmological examination frequently reveals some degree of retinal degeneration, even in asymptomatic individuals. As in all patients who harbor pathogenic mtDNA mutations, long-term clinical follow-up is important in order to screen for varying degrees of systemic expression. Like many of the other pathogenic mtDNA mutations, the MTATP6*NARP8993G mutation is

functionally recessive, with the entire range of clinical manifestations occurring over ~ 70–100% mutant mtDNAs.

The most severe clinical phenotype known to be caused by this mutation is Leigh's disease, a severe neurodegenerative disorder often characterized by symptoms of brainstem and basal ganglia dysfunction (176, 203). Pathologically, patients with this disease exhibit demyelination, gliosis, necrosis, relative neuronal sparing, and capillary proliferation involving primarily the basal ganglia, brainstem, and cerebellum (118, 145). Patients with Leigh's disease who harbor the MTATP6*NARP8993G mutation have >95% mutant mtDNAs in their tissues. This high concentration of mutant mtDNAs correlates with the clinical severity of this disease.

BASE SUBSTITUTIONS THAT IMPAIR MITOCHONDRIAL PROTEIN SYNTHESIS

$tRNA^{Lysine}$ mutations In 1990, the first mtDNA point mutation that caused a defect in mitochondrial protein synthesis was identified in the TψC loop of the $tRNA^{Lysine}$ gene at position 8344 (MTTK*MERRF8344G) (Figure 3) (174). Since then, numerous additional mutations have been found in mitochondrial tRNAs. These represent a large class of mtDNA mutations with an extraordinary range of clinical presentations (Table 2). Severely affected patients with the MTTK*MERRF8344G mutation generally present with myoclonic epilepsy and ragged-red fiber disease (MERRF). This disease is characterized by progressive myoclonic epilepsy, mitochondrial myopathy with ragged-red fibers, and slowly progressive dementia. The MTTK*-MERRF8344G mutation causes ~ 80–90% of MERRF cases (60, 174). MERRF cases in two additional families have been attributed to a heteroplasmic T-to-C transition mutation at bp 8356 in the $tRNA^{Lysine}$ gene (MTTK*-MERRF8356C) (Figure 3) (181, 228). The MTTK*MERRF8356C mutation

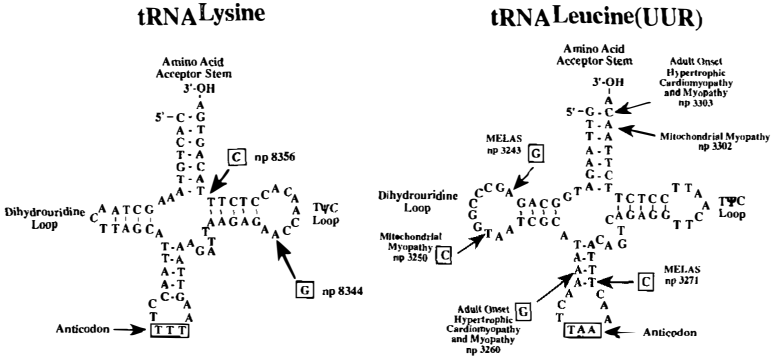

Figure 3 The morbid anatomy of mitochondrial $tRNA^{Lysine}$ and $tRNA^{Leucine(UUR)}$. The location of pathogenic mtDNA mutations with the secondary structures of these tRNAs is shown.

alters a moderately conserved nucleotide predicted to disrupt base pairing in the stem of the TψC loop. Individuals in one of these families exhibited features of two OXPHOS diseases: MERRF and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS; see section on $tRNA^{Leucine}$ mutations for a description of this disease). Both of these mutations are heteroplasmic and can produce significant phenotypic heterogeneity in families owing to replicative segregation.

As predicted from the location of the mutation within the $tRNA^{Lysine}$ gene, the MTTK*MERRF8344G mutation impairs mitochondrial protein synthesis when present at high concentrations within the cell (31). Heteroplasmic myotubes with varying percentages of the MTTK*MERRF8344G mutation experienced a sharp decline in mitochondrial protein synthesis levels when the intracellular percentage of mutant mtDNAs exceeded 85%. This result indicates that the MTTK*MERRF8434G mutation is functionally recessive (21). Hence, essentially all of the phenotypic variation observed in patients with this mutation is confined to a narrow range of mutant mtDNA concentrations.

$tRNA^{Leucine}$ mutations To date, six pathogenic mtDNA mutations have been identified in the mitochondrial $tRNA^{Leucine(UUR)}$ gene (Figure 3). The first $tRNA^{Leucine(UUR)}$ gene mutation that was discovered produced MELAS (56). This disorder is first suspected when patients present with focal or generalized seizures, recurrent migraine headaches, vomiting, and stroke. Not all patients who harbor the MTTL1*MELAS3243G mutation have MELAS. Some may present with Kearns-Sayre syndrome or CPEO syndromes (32, 56, 60), mitochondrial encephalomyopathies with or without a ragged-red fiber myopathy (32, 60), mitochondrial myopathy (32, 60), or diabetes mellitus and deafness (208).

Approximately 70–80% of individuals with the clinical features of MELAS have an heteroplasmic A-to-G point mutation in the dihydrouridine loop of the $tRNA^{Leucine(UUR)}$ gene at bp 3243 (MTTL1*MELAS3243G) (Figure 3) (46, 55, 56, 82, 97, 121, 137). An additional 7.5% have a heteroplasmic T-to-C point mutation at bp 3271 in the terminal nucleotide pair of the anticodon stem of the $tRNA^{Leucine(UUR)}$ gene (MTTL1*MELAS3271C) (Figure 3) (57, 163a, 206). An A-to-G point mutation at bp 11,084 of ND4 that substitutes a threonine for an alanine within a stretch of ~ 20 hydrophobic amino acids was proposed as the cause of MELAS in a single family based on heteroplasmy of the mutation, absence of the mutation in 109 normal and disease controls, and evolutionary conservation of this nucleotide pair in 8 of 10 species (102). Further investigation of this site has revealed that this nucleotide change does not cause MELAS and is more likely to be a neutral polymorphism. The A-to-G mutation at bp 11,084 was present in 15 of 105 (14%) Japanese controls and was not observed in African Americans or Caucasians (163b). Hence, this mutation appears to be an ethnic-specific polymorphism. The MTND4*-

MELAS11084G mutation had been observed previously in a Japanese patient with hypertrophic cardiomyopathy and various neurological manifestations (140). The remaining cases appear to be caused either by nuclear DNA mutations (42) or by other as yet unidentified mtDNA mutations.

Four other mtDNA mutations with distinct clinical presentations have been described. A hypertrophic cardiomyopathy and a mitochondrial myopathy are the primary clinical manifestations produced by a C-to-T mutation at bp 3303 (MTTL1*MMC3303T) (182) and by an A-to-G mutation at bp 3260 (MTTL1*MMC3260G) (227) (Figure 3). Severely affected maternal lineage relatives with the MTTL1*MMC3260G mutation began to manifest symptoms of congestive heart failure in their twenties. The heart of a severely affected individual who died at 30 years of age exhibited myocardial fibrosis, hypertrophy, and structural disarray of cardiomyocytes. Other clinical manifestations included insulin-dependent diabetes mellitus, bilateral cataracts, and the Wolf-Parkinson-White preexcitation syndrome. Maternal lineage relatives showed evidence of a mild proximal myopathy with ragged-red fibers. In a pedigree with the MTTL1*MMC3303T mutation, the clinical manifestations began in infancy and in two individuals resulted in death at 10 weeks and 9 months of age (182). Although hypertrophic cardiomyopathy was a prominent clinical manifestation in these families, it may not be entirely specific to these two mutations. Hypertrophic cardiomyopathies can also occur in conjunction with the MTTK*MERRF8344G and MTTL1*MELAS3243G mutations.

The other two mutations in the $tRNA^{Leucine(UUR)}$ gene are an A-to-G mutation at bp 3302 (MTTL1*MM3302G) (17, 173) and a T-to-C mutation at bp 3250 (MTTL1*MM3250C) (Figure 3) (58). These mutations are heteroplasmic and are associated with a mitochondrial myopathy. In contrast to the other $tRNA^{Leucine(UUR)}$ gene mutations, significant involvement of organs other than skeletal muscle was not observed in patients who harbored these mutations. An interesting feature of the MTTL1*MM3302G mutation is its proposed effect on mRNA processing. Accumulations of a polycistronic mRNA precursor containing 16S rRNA, $tRNA^{Leucine(UUR)}$, and ND1 sequences have been observed in skeletal muscle and skin fibroblasts (17). Further analysis of this mutation will be needed to determine whether its pathological effects are due to an impairment of mitochondrial protein synthesis or to abnormal mRNA processing.

rRNA and other tRNA mutations The first rRNA mutation to be definitively associated with a maternally inherited disease was a homoplasmic A-to-G mutation in the 12S rRNA gene at bp 1555 of the mtDNA (MTRNR1*-DEAF1555G) (150). This mutation was observed originally in a large Israeli-Arab kindred in which 55 individuals across 5 generations were identified with sensorineural hearing loss (83) and in 3 Chinese families who had multiple

maternal lineage family members with aminoglycoside-induced deafness (76). The MTRNR1*DEAF1555G mutation occurs in a highly conserved region of the 12S rRNA, which has been proposed as the aminoacyl site. Because this region is homologous to the aminoglycoside-binding region of *Escherichia coli,* the location of this mutation within the 12S rRNA is consistent with the enhanced sensitivity of these patients to this class of drugs (116). In bacteria, aminoglycosides preferentially bind to this region and stabilize mismatched aminoacyl tRNAs, thus impairing bacterial protein synthesis. Hence, patients who harbor the MTRNR1*DEAF1555G mutation may exhibit a similar toxic response when aminoglycosides are administered. Because sensorineural hearing loss is the only identifiable clinical manifestation in ~ 60% of cases of hereditary deafness, investigations into why some families spontaneously develop deafness and why others must be exposed to a mitochondrial toxin such as an aminoglycoside for deafness to occur will be important for understanding the role of mtDNA mutations in nonsyndromic deafness.

A number of other mitochondrial tRNA mutations are associated with a wide array of phenotypes. Table 2 contains a comprehensive list of these point mutations, which include mutations that have been independently confirmed by several research groups as well as those observed by a single research group. Of these point mutations, the G-to-A mutation at position 15,990 of the mtDNA (MTTP*MM15990A) produces an interesting change in the anticodon structure of the $tRNA^{Proline}$ (119). The anticodon sequence in the $tRNA^{Proline}$ gene is changed from the normal UGG to UGA, the anticodon sequence in the $tRNA^{Serine(UCN)}$ gene. As has been observed in other mitochondrial tRNA point mutations (21), the MTTP*MM15990A mutation is functionally recessive and affects cellular phenotype only when present at levels > 90% of the total mtDNA. The MTTP*MM15990A mutation was associated with childhood onset mitochondrial myopathy. Interestingly, no other family members had clinical manifestations at the time the proband was ascertained, which underscores the ability of mtDNA mutations to confound clinical assessments of inheritance by presenting as singleton cases within a pedigree. Unless the patient's phenotype is clearly associated with a nuclear DNA mutation with autosomal recessive inheritance, genetic counseling of families with a single affected individual should include a discussion of transmission risks associated with both mtDNA and nuclear DNA mutations.

OXPHOS DISEASE THERAPY

Over the past several years, rapid advancements have been made in the diagnosis and genetic counseling of OXPHOS diseases. However, therapeutic advancements for these diseases have been more difficult. Various factors complicate the treatment of OXPHOS diseases. These include limitations in

Table 2 MtDNA mutations associated with OXPHOS diseases other than LHON. Provisonal (PROV) status indicates that only one research group has reported the mutation as pathological. Confirmed (CFRM) status indicates that more than one group has reported the mutation as pathological

HGM Designation[a]	NP	AA or RNA[b]	Homo-plasmy	Hetero-plasmy	References
Missense Mutations					
MTATP6*NARP8993G	8993(T→G)	L→R	NO	YES	(69) CFRM
MTATP6*NARP8993C	8993(T→C)	L→P	NO	YES	(41,165) CFRM
MTND4*MELAS11084G	11084(A→G)	R→H	YES	YES	(102) PROV
tRNA Mutations					
MTTL1*MELAS3243G	3243(A→G)	$tRNA^{Leu(UUR)}$	NO	YES	(56) CFRM
MTTL1*DMDF3243G	3243(A→G)	$tRNA^{Leu(UUR)}$	NO	YES	(208) PROV
MTTL1*MM3250C	3250(T→C)	$tRNA^{Leu(UUR)}$	NO	YES	(58) PROV
MTTL1*MMC3260G	3260(A→G)	$tRNA^{Leu(UUR)}$	NO	YES	(227) PROV
MTTLI*MELAS3271C	3271(T→C)	$tRNA^{Leu(UUR)}$	NO	YES	(57,163a,206) CFRM
MTTL1*MM3302G	3302(A→G)	$tRNA^{Leu(UUR)}$	NO	YES	(17,173) CFRM
MTTL1*MMC3303T	3303(C→T)	$tRNA^{Leu(UUR)}$	NO	YES	(182) PROV
MTTI*FICP4269G	4269(A→G)	$tRNA^{Ile}$	NO	YES	(202) PROV
MTTI*FICP4317G	4317(A→G)	$tRNA^{Ile}$	?	?	(201) PROV
MTTQ*ADPD4336G	4336(A→G)	$tRNA^{Gln}$	YES	NO	(172) PROV
MTTK*MERRF8344G	8344(A→G)	$tRNA^{Lys}$	NO	YES	(174) CFRM
MTTK*MERRF8356C	8356(T→C)	$tRNA^{Lys}$	NO	YES	(181) CFRM
MTTK*MERRF/MELAS 8356C	8356(T→C)	$tRNA^{Lys}$	NO	YES	(228) CFRM
MTTG*MHCM9997C	9997(T→C)	$tRNA^{Gly}$	?	YES	(114) PROV
MTTG*CIPO10006G	10006(A→G)	$tRNA^{Gly}$	?	?	(101) PROV
MTTS1ICIPO12246G	12246(C→G)	$tRNA^{Ser(AGY)}$	?	?	(101) PROV
MTTL2*CIPO12308G	12308(A→G)	$tRNA^{Leu(CUN)}$	?	?	(101) PROV
MTTL2*CPEO12308G	12308(A→G)	$tRNA^{Leu(CUN)}$	?	?	(101) PROV
MTTT*LIMM15923G	15923(A→G)	$tRNA^{Thr}$	YES	NO	(25,225,226) PROV
MTTP*MM15990A	15990(G→A)	$tRNA^{Pro}$	NO	YES	(119) PROV
rRNA Mutations					
MTRNR1*DEAF1555G	1555(A→G)	12SrRNA	YES	NO	(150) PROV
MTRNR2*ADPD3196A	3196(G→A)	16SrRNA	YES	YES	(172) PROV

[a] Phenotype designations: ADPD, Alzheimer's disease and Parkinson's disease; CIPO, chronic intestinal pseudoobstruction with myopathy and cardiomyopathy; CPEO, chronic progressive external opthalmoplegia; CEAF, maternally inherited deafness or aminoglycoside-induced deafness; DMDF, diabetes mellitus and deafness; LIMM, lethal infantile mitochondrial myopathy; MHCM, maternally inherited hypertrophic cardiomyopathy; MM, mitochondrial myopathy; MMC, maternally inherited mitochondrial myopathy and cardiomyopathy; NARP, neurogenic muscle weakness, ataxia, and retinitis pigmentosa.

[b] tRNAs: Gln, glutamine; Gly, glycine; Ile, isoleucine; Leu, leucine; Lys, lysine; Pro, proline; Ser, serine; Thr, threonine; ?, unknown.

our understanding of the extent of the pathophysiological derangements produced by pathogenic mutations, the effects of genetic heterogeneity on therapeutic outcome, and the nuclear and mitochondrial mechanisms for OXPHOS control. Furthermore, the formation of homogeneous treatment groups that can be used for the interpretation of therapeutic trials is made more difficult by the large number of pathogenic mtDNA mutations, the replicative segregation of normal and mutant mtDNAs, tissue-specific thresholds for phenotype expression, and the accumulation of somatic mtDNA mutations.

The fact that mtDNA mutations can be functionally recessive may be important for the treatment of certain OXPHOS diseases, particularly in patients with relatively mild disease manifestations. Relatively small increases in ATP production may change the threshold for disease expression and allow clinical improvements to occur. To date, most attempts to improve OXPHOS function have used naturally occurring vitamins, coenzymes, and metabolic intermediates. Metabolic therapies reported to produce a positive therapeutic effect include coenzyme Q10, phylloquinone, menadione, succinate, ascorbate, and riboflavin. Little is known about the role of pharmaceutical therapies in OXPHOS diseases. Four pharmaceutical therapies that have been tried in OXPHOS diseases are idebenone, corticosteroids, dichloroacetate, and chloramphenicol. The nutritional and pharmaceutical therapies reviewed here generally produce only mild to moderate degrees of symptomatic improvement in patients with OXPHOS diseases. As we learn more about the relationship between OXPHOS function and disease expression, more effective therapeutic alternatives, such as improved pharmaceutical therapies and gene therapy protocols, are likely to emerge.

Coenzymes

COENZYME Q10 (CoQ10) (2,3-DIMETHOXY-5-METHYL-6-DECAPRENYL-1,4-BENZOQUINONE) Of all the agents used to treat OXPHOS diseases, CoQ10 has undergone the most investigations and is the agent of choice for the treatment of these diseases. CoQ10 is a fat-soluble quinone that contains a side chain of 10 isoprenoid units. Various functions have been proposed for CoQ10. These include the transfer of electrons from Complex I to III and from Complex II to III (Figure 1) (62), the stabilization of the OXPHOS subunits within the mitochondrial inner membrane, and the detoxification of oxygen free radicals (98), even at physiological concentrations (48). In a MERRF pedigree (215) and a patient with CPEO (175), we found that the lability of Complex IV was increased over that of controls, which suggests that one mechanism by which CoQ may improve OXPHOS function may be the stabilization of the OXPHOS complexes within the mitochondrial inner membrane. In humans, the highest concentrations of coenzyme Q have been found in heart, liver, kidney, and

pancreas (104). Studies using rat hepatocytes demonstrated that 25–30% of coenzyme Q was localized in the cell nucleus, 40–50% in the mitochondrion, 15–20% in microsomes, and 5–10% in the cytosol (199).

When administered in high doses, CoQ10 has been reported to have a beneficial effect on the clinical manifestations of a number of OXPHOS diseases. Numerous clinical and metabolic improvements with CoQ10 administration have been noted in various classes of OXPHOS disease (1, 13, 16, 22, 23, 43, 52, 79, 133, 138, 139, 175, 222). Patients with MELAS, Kearns-Sayre syndrome, or CPEO syndromes have exhibited some of the best-documented responses to CoQ10 administration. For example, recurrent stroke-like episodes are among the most debilitating manifestations of MELAS. The frequency of these transient neurological deficits can be reduced when doses of 300 mg/day or ~ 4.3 mg/kg per day are administered (52, 222). A positive therapeutic response is less likely with lower doses, possibly owing to difficulties in maintaining adequate blood and tissue levels of the CoQ10. Whether other manifestations observed in MELAS patients, such as dementia, cardiomyopathy, cardiac conduction abnormalities, and ophthalmological complications such as retinitis pigmentosa or ophthalmoparesis, respond to CoQ10 administration remains unclear at present. As discussed above, Kearns-Sayre and CPEO patients have a diverse array of clinical manifestations. Those that appear to respond to CoQ10 administration are fatigability and the metabolic abnormalities associated with mitochondrial myopathy, the intention tremor caused by cerebellar degeneration, cardiac conduction disturbances, and respiratory failure due to weakness of the diaphragm. A genetically heterogeneous population of patients with Kearns-Sayre syndrome or CPEO syndromes was included in a multicenter, double-blind study of CoQ10 at a dose of 2 mg/kg per day. Only a subset of these patients harbored mtDNA deletion mutations. However, despite the genetic heterogeneity of the patient population, a decrease in postexercise lactate levels was observed in ~ 30% of individuals, and intention tremor improved in ~ 10% of individuals with cerebellar ataxia (23).

Patients with Kearns-Sayre syndrome or CPEO syndromes can experience abnormal conduction from the sinoatrial node to the ventricular muscle fibers (detected as a prolonged PR interval on electrocardiograms), which represents degeneration in the cardiac conduction pathway. This manifestation can result in severe cardiac rhythm disturbances and often requires a cardiac pacemaker to maintain the cardiac rate at acceptable levels. Administration of CoQ10 at doses ranging from 60–150 mg/day resulted in significant improvement of cardiac conduction in patients with Kearns-Sayre syndrome or CPEO syndromes (16, 22, 138, 139). The final manifestation of Kearns-Sayre and CPEO patients that has responded to CoQ10 administration is the respiratory failure caused by diaphragm weakness. Patients who received either 300 mg/kg per

day + 6 g/day of sodium succinate or 3 mg/kg per day of CoQ10 experienced resolution of their respiratory failure (43, 175). As in patients with MELAS, CoQ10 supplementation generally has no effect on the ptosis, ophthalmoplegia, cardiomyopathy, and retinitis pigmentosa associated with Kearns-Sayre syndrome and CPEO syndromes. Due to the complexities of CoQ10 absorption, particularly in pediatric groups, analysis of CoQ10 blood levels can be helpful in optimizing the dose. Based on our clinical experience with CoQ10 dosing and on the doses reported in patients who experienced positive therapeutic responses, we administer CoQ10 at a dose of 4.3 mg/kg per day to both pediatric and adult patients.

Vitamins

MENADIONE AND PHYLLOQUINONE Two vitamin K compounds, menadione (vitamin K_3) and phylloquinone (vitamin K_1 or phytonadione), have been administered in conjunction with ascorbate (vitamin C) to donate electrons directly to cytochrome c (5, 45). The use of vitamin K and ascorbate to enhance ATP production is based on studies in yeast (24, 134, 164, 216), rats (33, 40), and human fibroblasts (219, 220) with various types of OXPHOS defects. Evidence supporting the clinical efficacy of vitamin K and ascorbate treatment in patients with OXPHOS diseases is limited. The administration of menadione (40–80 mg/day) and ascorbate (4 g/day) to a patient with a mitochondrial myopathy due to a Complex III defect resulted in improved cellular phosphate metabolism as measured by 31P-nuclear magnetic resonance (NMR) (5, 45).

Water-soluble menadione must be alkylated to menaquinone-4 to be both lipophilic and biologically active (200). In contrast, phylloquinone is already in a lipid-soluble and biologically active form. Although both are vitamin K derivatives with a relatively short half-life, phylloquinone appears to have better tissue retention and to reach a greater concentration in the mitochondria than menadione (200, 205). Because menadione distribution in the US was recently discontinued, phylloquinone (vitamin K_1) can be used for patient treatment at a dose of 0.36 mg/kg per day in conjunction with ascorbate at 57 mg/kg per day.

Thiamine and riboflavin Thiamine and riboflavin have been given to some OXPHOS disease patients. As a cofactor of pyruvate dehydrogenase, thiamine has been used to stimulate production of NADH, which can then enter OXPHOS at Complex I. A patient with a CPEO syndrome showed improved plasma lactate and pyruvate levels when treated with a thiamine dose of 300 mg/day (106). After conversion to flavin monophosphate and flavin adenine dinucleotide, riboflavin (vitamin B_2) functions as a cofactor for electron transport in Complex I, Complex II, and the electron transfer flavoprotein. Improved

exercise capacity was noted in a mitochondrial myopathy patient with Complex I dysfunction following the administration of 100 mg/day of riboflavin (7).

Metabolic Intermediates

SUCCINATE Succinate is a tricarboxylic acid cycle intermediate that donates electrons directly to Complex II. Preliminary evidence for a positive therapeutic effect of succinate has been reported in two cases. In one of our Kearns-Sayre/CPEO syndrome patients with the 4.9-kb mtDNA deletion and an associated defect of Complex I, IV, and V, respiratory failure resolved on a regimen of 300 mg/day CoQ10 and 6 g/day sodium succinate (175). However, it is difficult to separate the effects of CoQ10 and succinate in this patient. Similarly, a MELAS patient reportedly had a decreased frequency of stroke-like episodes when treated with sodium succinate at 6 gm/day (96). Due to the high sodium content of succinate preparations, the administration of this compound to patients with cardiomyopathy requires careful supervision.

Pharmaceutical Therapies

IDEBENONE Idebenone is a novel quinone compound that has been used to treat neurological dysfunction caused by ischemia (127) and inhibition of lipid peroxidation (198). Based on the proposed physiological effects and the structural similarity to CoQ10, this compound has been administered at doses of 90 mg/day (12) to treat patients with MELAS (79, 80, 223). Improvements in clinical and metabolic abnormalities were observed in these patients. A single patient with LHON caused by the MTND4*LHON11778 mutation was also treated with idebenone (90 mg/day) for a year and during that time experienced a mild improvement of visual acuity (110). Although idebenone is not approved for patient use in the US, further investigation of the efficacy of this compound in OXPHOS diseases is warranted.

CORTICOSTEROIDS Corticosteroids have been reported to produce positive effects in MELAS (53, 65, 117, 171, 183), MERRF (123), and mitochondrial myopathy patients (111, 142). The mechanism for clinical improvement is unclear, and the use of corticosteroids in OXPHOS diseases is not advised. Initially, some patients respond favorably to these drugs, but these individuals may become dependent on them and develop the sequellae of chronic steroid use and may undergo clinical deterioration when the drug is reduced or withdrawn (9, 171, 183).

DICHLOROACETATE(DCA) DCA has been studied for many years for its ability to reduce blood glucose in chemically or surgically induced diabetes mellitus (100, 112, 166, 190). The primary metabolic effect of DCA is to stimulate

pyruvate dehydrogenase (PDH) function (218) by inhibiting PDH kinase, the enzyme that normally phosphorylates and inactivates PDH (149, 217, 218). Hence, in conditions that result in the accumulation of lactate and alanine (compounds in equilibrium with pyruvate), activation of PDH decreases the release of these compounds from peripheral tissues and enhances their oxidative metabolism by liver (54, 169, 186–188). This process interrupts the Cori and alanine cycles, which allow lactate and alanine to be utilized by the liver for glycogen synthesis.

DCA has been used in pediatric and adult patients to treat lactic acidosis. In infants and children, oral or i.v. doses ranging from 15–200 mg/kg per day have been administered without adverse effect and generally have been associated with at least a 20% drop in blood lactate. Adults have shown a similar response when doses of 35 or 50 mg/kg were administered (19, 191, 192). However, a large multicenter, placebo-controlled, randomized trial of DCA in adult patients from a critical care unit with various types of shock (septic, cardiogenic, hemorrhagic, multisystems failure, etc) failed to show any benefit from a daily dose of 50 mg/kg of parenteral DCA (193).

Whether DCA may be beneficial in patients with heritable forms of lactic acidosis is unknown. A patient with MELAS who had auditory hallucinations experienced a normalization of lactate in the blood and CSF and a resolution of his psychiatric symptoms when DCA was administered orally at doses of 12.5–100 mg/kg per day (162). Currently DCA is only available under research protocols. Its effect on the morbidity and mortality of OXPHOS diseases has not yet been determined. More intensive trials will be required to resolve these issues.

CHLORAMPHENICOL The hypermetabolism of Luft's disease has been reduced both by the induction of hypothyroidism and by the inhibition of mtDNA protein synthesis with chloramphenicol (3, 44, 107). However, the long-term clinical benefit of this treatment is unclear. Moreover, chronic chloramphenicol therapy can induce pancytopenia and, in some instances, an aplastic anemia.

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