

Genetic Features of Mitochondrial Respiratory Chain Disorders

AGNÈS RÖTIG and ARNOLD MUNNICH

INSERM U393 and Service de Génétique, Hôpital Necker-Enfants Malades, Paris, France.

Abstract. Oxidative phosphorylation, *i.e.*, ATP synthesis by the oxygen-consuming respiratory chain (RC), supplies most organs and tissues with a readily usable energy source, being functional before birth. Consequently, RC deficiencies can theoretically give rise to any symptom, in any organ or tissue, at any age and with any mode of inheritance, because of the twofold genetic origin of RC components (nuclear DNA and mitochondrial DNA). It was long wrongly considered that RC disorders originate from mutations of mitochondrial DNA,

because for a long time only mutations or deletions of mitochondrial DNA were identified. However, the number of known disease-causing mutations in nuclear genes is steadily growing. These genes encode the various subunits of each complex, ancillary proteins functioning at different stages of holoenzyme biogenesis, including transcription, translation, chaperoning, addition of prosthetic groups, and protein assembly, and various enzymes involved in mitochondrial DNA metabolism.

The mitochondrial respiratory chain (RC) catalyzes the oxidation of fuel molecules and the concomitant energy transduction into ATP via five complexes, which are embedded in the inner mitochondrial membrane (1) (Figure 1). Complex I [NADH-coenzyme Q (CoQ) reductase] carries reducing equivalents from NADH to CoQ (ubiquinone) and consists of >40 different polypeptides. Complex II (succinate-CoQ reductase) carries reducing equivalents from FADH₂ to CoQ and contains four polypeptides, including the FAD-dependent succinate dehydrogenase and iron-sulfur proteins. Complex III (reduced CoQ-cytochrome *c* reductase) carries electrons from CoQ to cytochrome *c*. It contains 11 subunits. Complex IV [cytochrome *c* oxidase (COX)], the terminal oxidase of the RC, catalyzes the transfer of reducing equivalents from cytochrome *c* to molecular oxygen. It is composed of two cytochromes (cytochromes *a* and *a*₃), two copper atoms, and 13 different protein subunits.

During the oxidation process, electrons are transferred to oxygen via the energy-transducing complexes of the RC, *i.e.*, complexes I, III, and IV for NADH-producing substrates; complexes II, III, and IV for succinate; and complexes III and IV for FADH₂ derived from the β -oxidation pathway via the electron transfer flavoprotein and the electron transfer flavoprotein-CoQ oxidoreductase system. CoQ, a highly hydrophobic quinone, and cytochrome *c*, a low-molecular weight hemoprotein, act as “shuttles” between the complexes. The free

energy generated from the redox reactions is converted into a transmembrane proton gradient. Protons are pumped through complexes I, III, and IV of the RC, which creates a charge differential. Complex V (ATP synthase) allows protons to flow back into the mitochondrial matrix and uses the released energy to synthesize ATP. Three ATP molecules are produced for each NADH molecule oxidized.

Mitochondrial Genetic Features

Mitochondrial RC

The mitochondrial RC is composed of approximately 100 different proteins. Only 13 of the proteins are encoded by mitochondrial genes; the others are encoded by nuclear genes. All complexes of the RC except complex II have a double genetic origin, and one to seven subunits of these complexes are mitochondrially encoded (Table 1). Moreover, it is hypothesized that several hundred nuclear genes are also needed for various functions of the RC. Mitochondrial proteins represent >3% of all cellular proteins.

Mitochondrial DNA

Human mitochondrial DNA (mtDNA) is a 16,569-bp closed circular molecule (2) (Figure 2). Mitochondria form large reticular networks and contain several molecules of mtDNA. Each molecule contains 37 genes, encoding large and small RNA (12S rRNA and 16S rRNA), 22 tRNA, and 13 key RC subunits (3). *ND1* to *ND6* encode subunits of complex I, cytochrome *b* is the only mitochondrially encoded subunit of complex III, *COXI* to *COXIII* encode subunits of complex IV, and *ATP6* and *ATP8* encode subunits of ATPase (complex V).

Mitochondria have independent replication, transcription, and translation systems. The mitochondrial genome is replicated in two phases. The replication starts at the heavy-strand replication origin and continues clockwise around the mtDNA. When the light-strand replication origin is exposed as a single

Correspondence to Dr. Agnès Rötig, INSERM U393 and Service de Génétique, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France. Phone: 33-0-144495161; Fax: 33-0-147348514; E-mail: roetig@necker.fr

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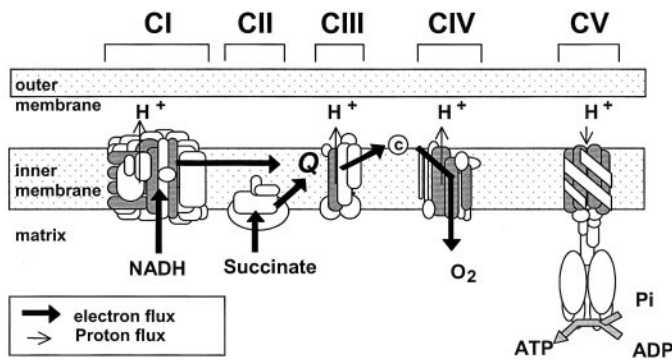


Figure 1. Mitochondrial respiratory chain. CI to CV, complexes I to V; Q, ubiquinone; Pi, inorganic phosphate; c, cytochrome c.

Table 1. Genetic origin of RC subunits^a

	No. of Subunits	No. of Mitochondrial Genes	No. of Nuclear Genes
Complex I	30	7	34
Complex II	4	0	4
Complex III	11	1	10
Complex IV	13	3	10
Complex V	14	2	12
Total		13	70

^a RC, respiratory chain.

strand, the second strand is replicated in the opposite direction, starting from the light-strand replication origin (4). Therefore, replication is bidirectional but asynchronous. A new model of mtDNA replication in mammals was recently proposed. The mtDNA replication begins from multiple origins and proceeds via a strand-coupled mechanism (5). The two mtDNA strands are transcribed from specific promoters into polycistronic RNA, which is further processed into rRNA, tRNA, and mRNA. The mitochondrial mRNA are translated in the mitochondrial matrix with nuclearly encoded machinery but following a specific genetic code, which is different from the nuclear code.

During cell division, mitochondria are randomly partitioned into the daughter cells (mitotic segregation). Usually all of the mtDNA molecules are identical, but sometimes a mixture of wild-type and mutant mtDNA is encountered. This situation is called heteroplasmy, whereas homoplasmy refers to the occurrence of only one type of mtDNA (Figure 2). In heteroplasmic cells, the mtDNA genotype can shift during cell replication. Consequently, some lineages drift toward wild-type mtDNA and become homoplasmic, whereas others remain heteroplasmic.

The mitochondrial genome is maternally transmitted. The mother transmits her mtDNA to all of her progeny, male and female, and her daughters transmit their mtDNA to the next generation. Theoretically, male subjects never transmit their mtDNA.

Nuclear Genes

Genes Encoding RC Subunits. The majority of RC proteins are encoded by nuclear genes. Table 1 indicates the genetic origin of the RC subunits. The nuclear gene-encoded proteins are translated in the cytosol and transported across one or both mitochondrial membranes. These nuclear genes are spread among the human chromosomes, on both autosomes and sex chromosomes. For example, 33 genes for complex I subunits were mapped to various autosomes, one to the X chromosome, and seven to mtDNA. Several of these nuclear genes have one or more pseudogenes (nonexpressed copies), which can complicate mutation screening among patients.

Genes Involved in RC Assembly. The double genetic origin of the RC indicates tightly regulated communication between mitochondria and the cytosolic and nuclear compartments. In addition to the structural components of the RC, a large number of nuclearly encoded proteins are involved in the assembly and maintenance of the complexes. Most of these genes were first identified in yeast, a model organism for mitochondrial function. Analyses of yeast mutants with disturbances in RC assembly have led to the identification of many nuclear products involved in protein folding, stabilization, membrane translocation, and cofactor addition (6) (Figure 3). To date, 350 such genes have been identified in yeast, and some have human counterparts. The exact function of the corresponding proteins is not always clear. It should be emphasized that some of the yeast genes are involved in mitochondrial gene splicing. These genes have no obvious human counterpart, because the human mtDNA does not contain an intron.

Genes Involved in mtDNA Metabolism and Maintenance. Mitochondria possess specific replication, transcription, and translation machinery. All of the proteins involved in these processes are encoded by nuclear genes, translated in the cytosol, and then translocated to the mitochondria. Only the two rRNA (12S rRNA and 16S rRNA) and the 22 tRNA are mitochondrially encoded. To date, >100 genes that result in mtDNA loss when defective have been identified in yeast (7). The proteins involved in mammalian mtDNA maintenance are those directly involved in mtDNA processing, such as DNA polymerase γ , helicase, and mitochondrial transcription factor. It has long been claimed that no repair mechanisms exist in mitochondria; however, recent evidence suggests that specific DNA repair mechanisms exist in these organelles (8–10).

Mitochondrial translation requires ribosomal proteins and tRNA synthetases. In total, approximately 100 different proteins are involved in the translation of the 13 proteins encoded by the mitochondrial genome, emphasizing the considerable investment required to maintain the mitochondrial genetic system.

Finally, a large number of other nuclear genes encode proteins that are not directly related to RC assembly or mtDNA maintenance but can interact with those components. Mutations in these genes can thus give rise to an abnormal RC. Among these proteins are chaperones, proteases, proteins involved in mitochondrial inheritance or morphologic features, antioxidant enzymes, and various carriers of iron, phosphate,

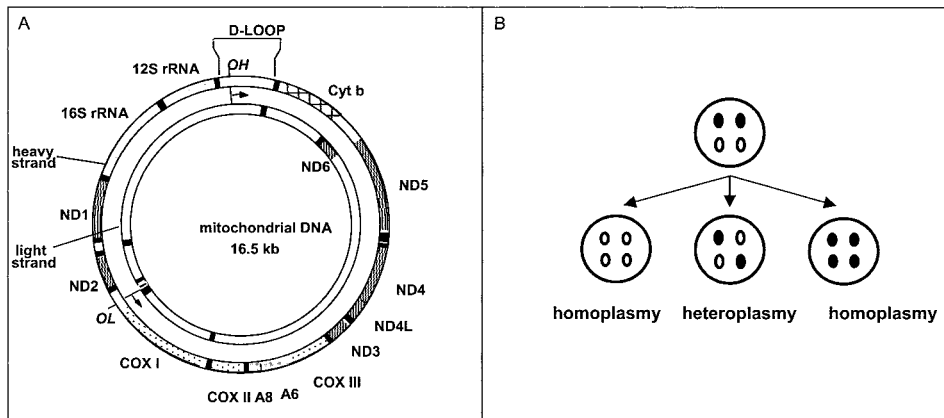


Figure 2. Mitochondrial DNA (mtDNA) (A) and heteroplasmy (B). OH, heavy-strand replication origin; OL, light-strand replication origin; Cyt b, cytochrome b.

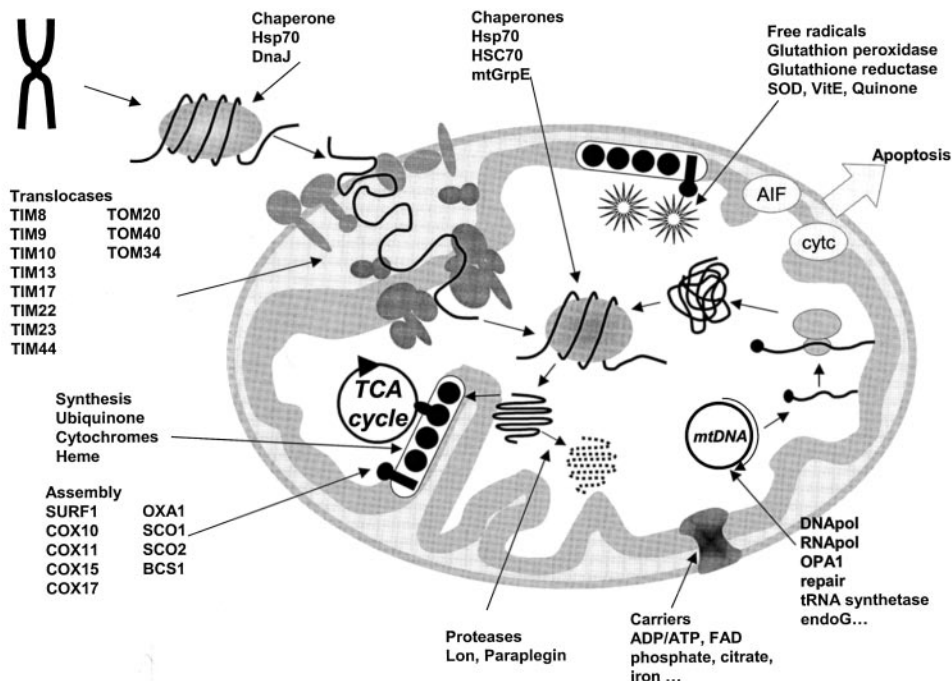


Figure 3. Some mitochondrial proteins. TCA, tricarboxylic acid; SOD, superoxide dismutase; cytc, cytochrome c; DNAPol, DNA polymerase; RNAPol, RNA polymerase; VitE, vitamin E; AIF, apoptosis inducing factor.

and citrate. It is commonly assumed that mitochondria contain approximately 1000 different proteins, with their genes being possible candidate genes for mitochondrial disorders.

Mitochondrial RC Disorders

Presentations. Oxidative phosphorylation, *i.e.*, ATP synthesis by the RC, is a ubiquitous metabolic pathway that supplies most organs and tissues with energy. Consequently, RC deficiencies can theoretically give rise to any symptom, in any organ or tissue, at any age and with any mode of inheritance, because of the double genetic origin (nuclear DNA and mtDNA) of respiratory enzymes.

In the past few years, it has become increasingly clear that genetic defects of oxidative phosphorylation account for a

large variety of clinical symptoms in childhood (11). Overall, the diagnosis of RC deficiency is difficult to consider when the first symptom occurs. The diagnosis becomes easier when two seemingly unrelated symptoms are observed. Renal involvement is noted for 5% of patients with RC deficiencies. The first symptoms develop in the neonatal period or before the age of 2 yr. The most common manifestation is a proximal tubulopathy with de Toni-Debré-Fanconi syndrome (12,13). Other renal presentations have been reported, including glomerular disease with nephrotic syndrome and chronic tubulointerstitial nephropathy (14,15). Plasma lactate levels and lactate/pyruvate (L/P) ratios are consistently normal but, interestingly, abnormal urinary lactate and Krebs cycle intermediate levels indicate a RC deficiency. The de Toni-Debré-Fanconi syndrome is

characterized by an impairment of proximal tubular reabsorption, leading to urinary losses of amino acids, glucose, proteins, ions, and water. With RC deficiencies, the syndrome is frequently limited to mild aminoaciduria and is occasionally responsible for metabolic acidosis. When available, renal biopsies demonstrate nonspecific anomalies of the tubular epithelium, with dilation or obliteration by casts, dedifferentiation or atrophy, and occasionally giant mitochondria. Glomerular disease with nephrotic syndrome has been observed among patients with pathologic evidence of focal segmental glomerulosclerosis. In addition, tubulointerstitial nephritis has been occasionally observed among patients with chronic renal insufficiency. Renal biopsies demonstrated diffuse interstitial fibrosis, with tubular atrophy and sclerosed glomeruli. Finally, the mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome mutation has been observed in several families with cardiomyopathy, diabetes mellitus, sensorineural deafness, and kidney failure unrelated to diabetes mellitus (16–19). Various examples of renal involvement encountered in our experience are presented in Table 2.

Metabolic Screening. The current screening protocol for RC deficiencies includes the determination of plasma levels of lactate, pyruvate, and ketone bodies and their molar ratios, as indices of the oxidation/reduction status in cytoplasm and mitochondria, respectively. Persistent hyperlactatemia (>2.5 mM) and elevated L/P (>20) and ketone body molar ratios strongly suggest a RC deficiency (particularly in the postabsorptive period). In addition, investigation of the redox status

in plasma can facilitate discrimination between the different forms of congenital lactic acidosis, on the basis of L/P and ketone body molar ratios *in vivo*. Impairments of oxidative phosphorylation usually result in L/P ratios of >20 and ketone body ratios of >2, whereas defects in the pyruvate dehydrogenase complex result in low L/P ratios (<10). When baseline screening test results are inconclusive, other tests should be performed (11).

When screening test results are negative, RC deficiencies may be misdiagnosed. Therefore, the evaluation of patients at risk of RC deficiency should include the systematic screening of all possible target organs and tissues, regardless of the presenting symptom, because multiple-organ involvement is an important diagnostic clue in RC deficiencies.

Biochemical Defects. Diagnostic tests include polarographic and spectrophotometric studies, each providing an independent clue to the diagnosis of RC deficiencies. Polarographic studies consist of the measurement, with a Clarke electrode, of oxygen consumption by mitochondria-enriched fractions in the presence of various oxidative substrates (*e.g.*, malate plus pyruvate, glutamate, succinate, or palmitate) (20). Measurements of oxygen consumption by intact or detergent-permeabilized circulating lymphocytes (isolated from 10 ml of blood on a Percoll cushion) and cultured cells (lymphoblastoid cell lines or skin fibroblasts) are also feasible and represent a noninvasive, easily reproducible, diagnostic test. The only limitation of these techniques is the absolute requirement for fresh specimens; polarographic studies are not possible with frozen material.

Table 2. Renal involvement in mitochondrial RC disorders^a

Kidney Disease	Clinical Outcome	RC Deficiency/Mutant Gene	Reference
Kidney failure	Deafness, diabetes mellitus, hypertrophic cardiomyopathy, migraine-like headaches, general weakness	tRNA ^{Leu} mutation	(19)
Tubulointerstitial nephritis	RP, mental retardation	mtDNA deletion	(15)
Kidney failure	Sensorineural deafness	<i>CI</i>	Unpublished
Nephrotic syndrome	Cardiomyopathy, RP, deafness, cortical atrophy, seizures	<i>CI</i>	Unpublished
Nephrotic syndrome	Myopathic features, ptosis, pyramidal syndrome, deafness	<i>CI</i>	Unpublished
Nephrotic syndrome	Sensorineural deafness, nystagmus, cataracts, retinal dystrophy, muscle weakness, mental retardation	Ubiquinone synthesis deficiency	(61)
Proximal tubulopathy	Myopathy, encephalopathy	<i>CIII</i>	Unpublished
Proximal tubulopathy	Rickets, anorexia, diarrhea, diabetes mellitus, deafness, cerebellar ataxia, myoclonic jerks, blindness, liver involvement	mtDNA duplication	(12)
Proximal tubulopathy	IDDM	mtDNA deletion	Unpublished
Proximal tubulopathy	GH deficiency, Pearson syndrome	mtDNA deletion	(37)
Proximal tubulopathy	Trichothiodystrophy, pyramidal syndrome, psychomotor retardation	<i>CIII</i>	Unpublished
Proximal tubulopathy	Leukodystrophy	<i>COX10</i>	(48)
Proximal tubulopathy	Liver failure	<i>BCS1</i>	(53)

^a mtDNA, mitochondrial DNA; IDDM, insulin-dependent diabetes mellitus; RP, retinitis pigmentosa; GH, growth hormone.

Spectrophotometric studies consist of isolated or combined respiratory enzyme assays with specific electron donors and acceptors. Such studies do not require the isolation of mitochondrial fractions and can be performed with tissue homogenates. Therefore, the amount of material required for enzyme assays (1 to 20 mg) is very small, and samples can be easily derived from needle biopsies of liver or kidney, from endomyocardial biopsies, or from pellets of lymphocytes or cultured skin fibroblasts. Samples should be immediately frozen and kept dry in liquid nitrogen (or at -80°C) (20).

The question of which tissue should be investigated deserves particular attention. In principle, the relevant tissue is the one that clinically expresses the disease. Whatever the affected organ, it is necessary to obtain skin biopsies for subsequent investigations with cultured fibroblasts (even after death).

Complex I, complex IV, and complex I plus IV deficiencies demonstrate comparable frequencies (approximately 25%). A variety of neuromuscular and non-neuromuscular symptoms have been observed, but truncal hypotonia, growth retardation, cardiomyopathy, encephalopathy, and liver failure are the most frequent symptoms. No obvious correlation between the type of RC deficiency and the clinical presentation can be noted (21).

Genetic Features of Mitochondrial RC Disorders

Heterogeneity of Mutations. It was long thought that mitochondrial RC disorders originate from mutations of mtDNA, because for a long time only mutations or deletions of mtDNA were identified. However, mitochondrial RC disorders are actually genetically heterogeneous. The RC is composed of approximately 100 polypeptides, encoded by as many different genes. These genes are either nuclear or mitochondrial genes. Moreover, the biogenesis and assembly of all of these polypeptides require several dozen nuclear genes, some of which are known only in yeast. Mitochondrial RC disorders can result from mutation of one of these hundreds of genes. Therefore, all modes of inheritance can be encountered in mitochondrial RC disorders, including mtDNA or nuclear gene mutations in sporadic cases, maternal transmission of mtDNA mutations, and autosomal recessive, dominant, and X-linked inheritance. Unfortunately, the disease-causing mutations have been identified for only a few patients (10 to 15%).

mtDNA Mutations

Types of Mutations Pathologic alterations of mtDNA fall into three major classes, *i.e.*, point mutations, deletion-duplications, and copy number mutations (depletions). In most cases, mtDNA mutations are heteroplasmic, because both normal and mutant mtDNA are present. In cells with mutant and wild-type molecules, the phenotype reflects the proportion of mutant mtDNA molecules and the extent to which the cell type relies on mitochondrial function. Point mutations include amino acid substitutions and protein synthesis mutations (tRNA or rRNA).

Point Mutations in Mitochondrial Protein Synthesis Genes

The A3243G mutation in the tRNA^{Leu} gene is responsible for the MELAS syndrome (22). The MELAS syndrome is characterized by a childhood onset of intermittent hemicranial head-

aches, vomiting, proximal limb weakness, recurrent neurologic deficits resembling the effects of strokes (hemiparesis, cortical blindness, and hemianopsia), lactic acidosis, and occasionally ragged red fibers in muscle biopsies. Brain computed tomographic scans demonstrate low-density areas (usually posterior), which may affect both white and gray matter but are not always correlated with clinical symptoms or vascular territories. The pathogenesis of stroke-like episodes in the MELAS syndrome has been ascribed to either cerebral blood flow disruptions or acute metabolic decompensation in biochemically deficient areas of the brain.

The A8344G missense mutation in the mitochondrial tRNA^{Lys} gene accounts for 80% of cases of myoclonic epilepsy with ragged red fibers (23). This disease is characterized by encephalomyopathy with myoclonus, ataxia, hearing loss, muscle weakness, and generalized seizures.

Syndromic forms of sensorineural hearing loss are not exceptional. The association of diabetes mellitus and deafness is frequently attributed to the A3243G mutation in the tRNA^{Leu} gene, which is also known to account for the MELAS syndrome (24). Hearing loss usually develops after the onset of diabetes mellitus. The deafness-ataxia-myoclonus syndrome is caused by a single-nucleotide insertion in the tRNA^{Ser} gene (C7472) (25). The deafness-palmoplantar keratoderma syndrome is a recently identified syndrome ascribed to a maternally inherited mutation affecting tRNA^{Ser} stability (26); the 7445 mutation is adjacent to the 3'-end of tRNA^{Ser} on the light strand and a silent change in the stop codon of the *COXI* gene on the heavy strand.

Mutations in Protein-Coding Genes The most frequent mutations in mitochondrial genes encoding structural proteins have been observed in Leber hereditary optic neuropathy and in neurogenic muscle weakness-ataxia-retinitis pigmentosa (NARP) or Leigh syndrome. These mutations are recurrent mutations, because numerous unrelated patients demonstrated the mutations. Other mutations in several genes have also been described but are most often restricted to a specific patient or family. All of these mutations are presented in Table 3.

Neurogenic muscle weakness-ataxia-retinitis pigmentosa and variable sensory neuropathy-seizures-mental retardation syndromes are attributable to an amino acid change in the *ATP6* gene (T8993G) (27). Leber hereditary optic neuropathy is associated with rapid bilateral central vision loss attributable to optic nerve death. Cardiac dysrhythmia is frequently associated with the disease, but no evidence of skeletal muscle pathologic conditions or gross structural mitochondrial abnormalities has been documented. The median age of vision loss is 20 to 24 yr, but vision loss can occur at any age between adolescence and late adulthood. Expression among maternally related individuals is variable, and there is a bias toward male subjects being affected. To date, the disease has been associated with 18 missense mutations in mtDNA, which can act autonomously or in association with each other to cause the disease (28).

Complex I deficiency is the most frequent cause of mitochondrial disorders, representing $>30\%$ of the cases. Truncal hypotonia, prenatal and postnatal growth retardation, enceph-

Table 3. mtDNA mutations in protein-coding genes^a

Gene	Mutation	Amino Acid	Clinical Presentation	Reference
Complex I gene mutations				
<i>ND1</i>	G3460A	A52T	LHON	(62)
<i>ND1</i>	3902 to 3908 inversion	D199G, L200K, A201V	Exercise intolerance	(62)
<i>ND2</i>	G5244A	G259S	LHON	(62)
<i>ND4</i>	G11,778A	R340H	LHON	(62)
<i>ND4</i>	G11,832A	W358X	Isolated myopathy	(62)
<i>ND5</i>	G13,513A	D393N	MELAS MELAS/LHON	(62)
<i>ND5</i>	G13,730A	G465E	LHON	(62)
<i>ND5</i>	A13,514G	D393G	MELAS	(62)
<i>ND6</i>	G14,459A	A72V	LHON/dystonia	(62)
<i>ND6</i>	T14,484C	M64V	LHON	(62)
Complex III gene mutations				
<i>Cyt b</i>	4-bp deletion (14,784 + 4)	Frameshift L13	MELAS + parkinsonism	(62)
<i>Cyt b</i>	G14,846A	G34S	Exercise intolerance	(32)
<i>Cyt b</i>	G15,084A	W113X	Exercise intolerance	(32)
<i>Cyt b</i>	G15,168A	W141X	Exercise intolerance	(32)
<i>Cyt b</i>	G15,242A	G166X	Encephalomyopathy	(62)
<i>Cyt b</i>	G15,243A	G166E	Cardiomyopathy	(31)
<i>Cyt b</i>	G15,059A	G190X	Myoglobinuria, myopathy	(62)
<i>Cyt b</i>	G15,498A	G251D	Hystiocytoid cardiomyopathy, hepatic steatosis, tubular necrosis	(62)
<i>Cyt b</i>	24-bp deletion (15,498 to 15,521)	8-amino acid deletion (251 to 258)	Exercise intolerance	(32)
<i>Cyt b</i>	G15,615A	G290D	Exercise intolerance	(62)
<i>Cyt b</i>	G15,723A	W326X	Exercise intolerance	(32)
<i>Cyt b</i>	G15,762A	G339E		(62)
Complex IV gene mutations				
<i>COXI</i>	5-bp deletion	Frameshift	Motor neuron-like degeneration	(64)
<i>COXI</i>	G5920A	W6X	Myoglobinuria	(62)
<i>COXI</i>	T6721C	M273T	Sideroblastic anemia	(63)
<i>COXI</i>	T6742C	I280T	Sideroblastic anemia	(63)
<i>COXI</i>	G6930A	G343X	Multisystem disorder	(62)
<i>COXII</i>	T7587C	M1T	Encephalomyopathy	(62)
<i>COXII</i>	T7671A	M29K	Myopathy	(62)
<i>COXIII</i>	T9557C	F251L	MELAS	(62)
<i>COXIII</i>	15-bp deletion (9514 to 9528)	5-amino acid deletion (94 to 98)	Myoglobinuria	(65)
<i>COXIII</i>	9537Cins	Frameshift	Leigh-like	(62)
<i>COXIII</i>	G9952A	W249X	Encephalopathy, myopathy	(62)
Complex V gene mutations				
<i>ATP6</i>	T8993G	L156R	NARP/Leigh syndrome	(27)
<i>ATP6</i>	T9176C	L217P	NARP/Leigh syndrome	(62)

^a *Cyt b*, cytochrome *b*; LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; NARP, neurogenic muscle weakness, ataxia, and retinitis pigmentosa.

alopathy, and liver failure are the main clinical features. The systematic sequencing of mitochondrial complex I genes demonstrated that approximately 20% of complex I-deficient patients harbored point mutations in one of these genes (29).

Complex III deficiency represents a relatively rare cause of respiratory enzyme dysfunction (30). In our experience, among all RC enzyme-deficient patients, only 7% exhibited a complex III deficiency (21). The clinical presentation of complex III-deficient patients is very heterogeneous, including myopathy, encephalomyopathy, multiple-organ disorders, cardiomyopathy, tubulopathy, and intrauterine growth retardation (21,30,31). This complex contains 11 subunits, and only one, cytochrome *b*, is of mitochondrial origin. To date, 12 cytochrome *b* mutations have been observed in association with various clinical presentations. Interestingly, most of the patients (eight of 12 patients) presented with the predominant feature of severe exercise intolerance, sometimes including muscle weakness and/or myoglobinuria (32). Two patients presented with cardiomyopathy, one patient presented with encephalomyopathy, and one patient presented with a MELAS and akinetic rigid syndrome.

COX deficiency is one of the most frequent causes of RC disorders in childhood and is clinically heterogeneous, with phenotypes including encephalomyopathy, Leigh syndrome (33,34), fatal or benign infantile myopathies, liver failure (35), and myoglobinuria. In the past 6 yr, mtDNA mutations have been identified among patients with various clinical presentations. These mutations have been observed in the three mitochondrial COX genes (*COXI*, *COXII*, and *COXIII*). They have been always associated with single pedigrees and are thus individual mutations. Most of the patients with mtDNA *COX* mutations presented with muscular or neuromuscular symptoms. However, *COXI* mutations were also observed in two cases of acquired sideroblastic anemia.

Most of these mutations are maternally inherited and heteroplasmic, but they are associated with a striking variety of clinical phenotypes, depending on the proportion of mutant mtDNA inherited by different maternal relatives. Within a particular pedigree, clinical presentations may range from migraine headaches and attention deficit disorders to the full MELAS syndrome. Maternal relatives of patients are generally healthy as long as they have no more than 85% mutant mtDNA. When the percentage of mutant mtDNA increases above this level, there are increasingly serious consequences with respect to the clinical phenotype, illustrating the distinct threshold for protein synthesis mutants.

Large-Scale mtDNA Rearrangements The second class of mtDNA diseases involves deletions-duplications of the mitochondrial genome. Although the sizes and positions of the deletions differ markedly among patients, the deletions usually encompass several coding genes and tRNA genes. They are usually sporadic, heteroplasmic, and unique and frequently occur between directly repeated sequences, suggesting that they are caused by *de novo* rearrangements during oogenesis or early development.

Kearns-Sayre syndrome is a multisystem disorder character-

ized by the unvarying triad of onset before the age of 20 yr, progressive external ophthalmoplegia, and pigmentary retinal degeneration, plus at least one of the following: complete heart block, cerebrospinal fluid protein levels of >100 mg/dl, or cerebellar ataxia. Large-scale heteroplasmic mtDNA deletions are frequently detected in skeletal muscle (36).

Pearson syndrome is a syndrome of refractory sideroblastic anemia, with variable neutropenia and thrombocytopenia, vacuolization of marrow precursors, and exocrine pancreatic dysfunction. Severe transfusion-dependent macrocytic anemia begins in early infancy (before 1 yr of age), and the disease is fatal before 3 yr of age in 62% of cases. The patients who survive spontaneously recover from their myelodysplasia but usually develop Kearns-Sayre syndrome. Large-scale heteroplasmic mtDNA deletions are present in all tissues, with the ratio between normal and deleted genomes being related to the expression of the disease (37).

Progressive external ophthalmoplegia is a mitochondrial myopathy with progressive muscle weakness and external ophthalmoplegia. Ataxia, episodic ketoacidotic coma, and early death have also been reported. Large-scale mtDNA deletions are observed in skeletal muscle (38).

Finally, mtDNA deletions or deletions-duplications have been identified among several patients with multiple-organ involvement, including tubulopathy, deafness, diabetes mellitus, optic atrophy, myopathy, and encephalopathy (12). It is worth noting that the most common deletion (4977 bp), which was observed for 30% of patients with a unique deletion and is flanked by 13-bp direct repeats, was simultaneously observed in Pearson syndrome and Kearns-Sayre syndrome and was subsequently observed in progressive external ophthalmoplegia. Similarly, identical mtDNA duplications have been observed in strikingly different conditions such as Pearson syndrome and proximal tubulopathy. Therefore, no correlation between the clinical presentation and the nature or extent of the rearrangements can be noted. The observation of progressive organ involvement should prompt consideration of the diagnosis of mtDNA rearrangement, with Southern blot analyses of total DNA. Although the vast majority of mtDNA rearrangements are sporadic, pedigrees in which mtDNA deletions-duplications are present in close maternal relatives have occasionally been observed (12,39). This indicates that maternal transmission of rearranged molecules may occur, although germline transmission is limited. Rare cases of multiple mtDNA deletions are associated with autosomal dominant transmission (40), which suggests that a mutation occurred in a nuclear gene essential for replication or maintenance of the mitochondrial genome.

mtDNA Copy Number Mutations The last class of mtDNA diseases involves mtDNA depletions attributable to copy number mutations. Rare cases of fatal infantile respiratory system, muscle, liver, or kidney failure have been ascribed to mtDNA depletions and are consistent with autosomal recessive inheritance (41). In these patients, there is a marked (sometimes tissue-specific) deficiency in mtDNA levels but not in nuclear gene levels. The patients also exhibit multiple RC deficiencies.

Nuclear DNA Mutations

Approaches for Identification of Gene Mutations The number of disease-causing mutations identified in nuclear genes is steadily growing, and such mutations presumably underlie the vast majority of RC deficiencies. It is worth noting that mtDNA deletions and mutations account for no more than 10 to 15% of cases, at least among pediatric patients. Therefore, in most cases, nuclear gene defects are presumably responsible for the RC deficiency.

Correct RC functioning requires not only the presence of the various subunits of each complex but also ancillary proteins involved in different stages of holoenzyme biogenesis, including transcription, translation, chaperoning, addition of prosthetic groups, and assembly of the proteins, as well as various enzymes involved in mtDNA metabolism. All of the proteins involved in these processes are not known, but several have been identified in the yeast *Saccharomyces cerevisiae* (6). Some of the proteins have known human orthologues. Because of the large number of these genes, a systematic study is not feasible. Therefore, various approaches for identification of the nuclear assembly genes have been developed by different groups.

The first approach is the study of candidate genes. Genes encoding the various subunits of a specific complex are obvious candidate genes for deficiencies of that complex. This approach led to the identification of disease-producing mutations in complex I and II subunits and in assembly proteins for complex III (*BCS1*) and complex IV (*SCO2*). However, because of the large number of possible candidate genes, this approach most often remains laborious.

The second approach involves chromosomal transfer experiments, allowing identification of the chromosomes that complement the mitochondrial defects in patient cell lines. The rescuing chromosome is thought to contain the functional copy of the gene that is mutated in the patient. Further experiments using deleted versions of the chromosome and genetic markers allow reduction of the genetic interval studied and identification of the candidate gene.

The third approach, based on linkage analyses of informative families, has resulted in the identification of disease-causing mutations in cases of multiple RC deficiencies (*ANT1* and thymidine phosphorylase) and isolated COX deficiencies (*SCO1* and *COX10*). However, the scarcity of informative families limits the effectiveness of this approach.

Defects in Structural RC Genes The various nuclear genes encoding the RC subunits are almost all known and mapped. Mutations in some of these genes have been identified for several patients. Interestingly, most of these mutations have been observed in complex I genes; despite the concerted efforts of a number of groups, very few mutations in genes encoding other complex subunits have been observed.

The first mutation in a gene encoding a RC subunit was reported in 1995 (42), for two sisters with Leigh syndrome and complex II deficiency. Leigh subacute necrotizing encephalomyopathy is a devastating encephalopathy characterized by recurrent attacks of psychomotor regression, with pyramidal and extrapyramidal symptoms, leukodystrophy, and brainstem

dysfunction. The pathologic hallmarks are focal, symmetrical, necrotic lesions in the thalamus, the brainstem, and the posterior columns of the spinal cord. Microscopically, these spongiform lesions exhibit demyelination, vascular proliferation, and astrocytosis. The pathogenic mutation was in the gene encoding the flavoprotein of complex II (42). It is worth noting that all four subunits of this complex are nuclearly encoded, which suggests Mendelian inheritance of these deficiencies. Mutations in the same gene were subsequently reported for another patient with Leigh syndrome (43). However, only a few cases of complex II deficiency have been molecularly characterized, despite systematic study of the four genes encoding the complex II subunits, which suggests that these deficiencies could be attributable to mutations in assembly proteins. Interestingly, mutations in the genes encoding subunits B, C, and D of complex II have been observed in hereditary paraganglioma and pheochromocytoma, which suggests that housekeeping genes can be involved in carcinogenesis, through an unknown mechanism (44).

A pioneering work by Smeitink and colleagues (29) in the Netherlands first identified the molecular bases of complex I deficiencies. This complex is the largest RC complex and consists of seven mitochondrially encoded proteins and at least 35 nuclearly encoded proteins. Complex I deficiency is one of the most common causes of mitochondrial diseases. Screening of the various structural complex I genes by several groups allowed identification of mutations in conserved subunits of this complex. That work demonstrated that approximately 40% of complex I deficiencies are related to mutations in those genes. The complex I mutations are presented in Table 3. Most of the patients presented with Leigh or Leigh-like syndrome, but cardiomyopathy was also reported.

Only one patient with a complex III deficiency has been noted to have a mutation in a complex III structural gene. This mutation (a 4-bp deletion) occurred in the *UQCRB* gene, which encodes the human ubiquinone-binding protein of the complex (45). Finally, no mutations in any of the nuclear genes for complex IV or V have been described, although several mutations in the mitochondrial genes have been reported.

Mutations in Genes Involved in RC Assembly The first identification of mutations in an assembly gene resulted from chromosomal transfer experiments in Leigh syndrome associated with COX deficiency. The rescue of patient cell lines with exogenous chromosome 9 first demonstrated that the mutant gene mapped to that chromosome. The use of deleted versions of chromosome 9 and the study of genetic markers allowed identification of *SURF1* as the disease-related gene (46). The exact function of the *SURF1* gene is not clearly understood, but its mutant form results in COX assembly defects. *SURF1* represents a major gene for Leigh syndrome associated with COX deficiency, because 25 to 75% of patients with Leigh syndrome and COX deficiency demonstrated *SURF1* mutations (47). Five other COX assembly genes have been demonstrated to be responsible for COX deficiencies. Mutations in the *COX10* gene, which encodes a heme A farnesyl transferase, an enzyme required for correct maturation of the heme group of COX, are responsible for tubulopathy and leukodystrophy

Table 4. Nuclear genes of mitochondrial disorders^a

Gene	Function	Enzyme Deficiency	Clinical Features	Reference
Structural RC genes				
<i>FpSDH</i>	Complex II subunit	SDH (complex II)	Leigh syndrome	(42)
<i>NDUFV1</i>	Complex I subunit	Complex I	Leukodystrophy and myoclonic epilepsy	(66)
<i>NDUFS8</i>	Complex I subunit	Complex I	Leigh syndrome	(67)
<i>AQDQ</i>	Complex I subunit	Complex I	Multivisceral involvement	(68)
<i>NDUFS7</i>	Complex I subunit	Complex I	Leigh syndrome	(29)
<i>NDUFS1</i>	Complex I subunit	Complex I	Leigh syndrome	(69)
<i>NDUFV2</i>	Complex I subunit	Complex I	Cardiomyopathy, encephalopathy	(70)
Genes involved in RC assembly				
<i>SURF1</i>	COX assembly	COX	Leigh syndrome	(46)
<i>COX10</i>	Heme maturation	COX	Tubulopathy and leukodystrophy	(48)
<i>SCO1</i>	Copper metabolism	COX	Hepatopathy and ketoacidotic coma	(49)
<i>SCO2</i>	Copper metabolism	COX	Cardiomyopathy	(50)
<i>COX15</i>	Heme maturation		Cardiomyopathy	(51)
<i>LRPPRC</i>		COX	Leigh syndrome	(52)
<i>BCS1</i>	Complex III assembly	Complex III	Tubulopathy and liver failure, GRACILE syndrome	(53)
				(54)
<i>HSP60</i>	Chaperone		Spastic paraplegia (SPG13)	(71)
<i>OPA1</i>	Dynamin-related protein		Dominant optic atrophy	(72)
<i>ABC7</i>	Iron carrier		Sideroblastic anemia	(73)
frataxine	Fe-S cluster assembly	RC deficiency	Friedreich's ataxia	(74)
<i>WD</i>	Copper-transporting ATPase		Wilson's disease	(75)
paraplegin	Chaperone-like protein	Multiple deficiency	Spastic paraplegia	(76)
<i>DDP</i>	Mitochondrial import machinery		Deafness, dystonia, mental deficiency, and blindness	(77)
Genes involved in mtDNA stability				
<i>TP</i>	Thymidine phosphorylase	Multiple mtDNA deletion	MNGIE	(60)
<i>G4.5</i>	Tafazzin	Multiple RC deficiency	Barth syndrome	(78)
Twinkle		Multiple deficiency, mtDNA deletions	PEO	(57)
<i>ANT1</i>	ADP/ATP translocator		PEO	(55)
<i>POLG</i>	Polymerase γ		PEO	(56)
<i>TK2</i>	Thymidine kinase	mtDNA depletion		(59)
<i>DGUOK</i>	Deoxyguanosine kinase	mtDNA depletion		(58)

^a SDH, succinate dehydrogenase; GRACILE, growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death; PEO, progressive external ophthalmoplegia; MNGIE, mitochondrial neurogastrointestinal encephalopathy; SPG13, spastic paraplegia; COX, cytochrome *c* oxidase.

(48). Mutations in the *SCO1* (49) and *SCO2* (50) genes, both of which are involved in mitochondrial copper metabolism, give rise to hepatopathy and ketoacidotic coma (*SCO1*) and cardiomyopathy (*SCO2*). Mutations in the *COX15* gene, which is necessary for the final step in heme A biosynthesis, lead to hypertrophic cardiomyopathy (51). Finally, mutations in *LRP-PRC* cause Leigh syndrome, French-Canadian type (52). The variability of the clinical phenotypes associated with mutations in different COX assembly factors remains a major unresolved issue in these disorders. All of these genes are housekeeping genes, and their mutations cause severe isolated COX deficiency, with abnormal patterns of COX subunits. Therefore, it can be hypothesized that the different clinical presentations are related to different patterns of expression of the genes during embryonic or fetal life.

Only one gene involved in complex III assembly has been identified in human subjects. This gene, *BCS1*, allows assembly of the iron-sulfur protein subunit in the complex. *BCS1* mutations have been identified in two clinical entities associated with complex III deficiency; one group of patients presented with tubulopathy and liver failure (53), whereas another group of patients defined the syndrome of growth retardation, aminoaciduria, cholestasis, iron overload, lacticidosis, and early death (54). No genes involved in complex I, II, and IV assembly have been identified in human subjects.

Recently, mutations in nuclear genes encoding mitochondrial proteins were demonstrated to cause diseases that were not regarded as “mitochondrial” until the genes were identified. Therefore, Friedreich’s ataxia, hereditary spastic paraplegia, dominant optic atrophy, and deafness-dystonia syndrome are now considered mitochondrial disorders (Table 4).

Mutations in Genes Involved in mtDNA Stability In these disorders, a primary nuclear gene defect causes secondary mtDNA deletion or loss. These diseases clinically resemble those caused by mtDNA mutations but exhibit Mendelian inheritance. Autosomal dominant external ophthalmoplegia is associated with multiple mtDNA deletions and is attributable to mutations in *ANT1* (mitochondrial adenine nucleotide translocator) (55), mtDNA polymerase γ (56), and Twinkle (mtDNA helicase) (57).

mtDNA depletion syndrome is a severe disease of childhood with tissue-specific reductions in mtDNA copy numbers and decreased activity of the mtDNA-encoded RC complexes. Mutations in two nuclear genes, encoding mitochondrial deoxyguanosine kinase (*DGUOK*) and thymidine kinase 2 (*TK2*), both of which are involved in the salvage pathway for dNTP for mtDNA synthesis, are responsible for mtDNA depletion syndrome (58,59).

Mitochondrial neurogastrointestinal encephalopathy syndrome is a multisystem disorder clinically characterized by onset between the second and fifth decades of life, ptosis, progressive external ophthalmoplegia, gastrointestinal dysmotility, diffuse leukoencephalopathy, peripheral neuropathy, and myopathy. The patients may have multiple mtDNA deletions and/or mtDNA depletion. The disease-causing gene (*TP*) encodes thymidine phosphorylase (60). Mutations in this gene

could affect the balance of the intramitochondrial dNTP pool and lead to the generation of mtDNA deletions and depletion.

Conclusion

The genetic investigation of mitochondrial RC disorders requires determination of an extensive pedigree, reporting on minor signs among relatives. This information is of particular importance for establishing which molecular studies should be performed first. For example, maternal inheritance points toward mtDNA mutations. Sporadic cases and cases consistent with autosomal recessive inheritance (consanguineous parents) should be tested for nuclear gene mutations. The number of identified disease-causing mutations in nuclear genes is steadily increasing, and such mutations presumably underlie the vast majority of RC deficiencies. It is worth bearing in mind that mtDNA deletions and mutations account for no more than 10 to 15% of cases, at least among pediatric patients. Therefore, in most cases, nuclear gene defects are presumably responsible for the RC deficiencies. Determination of genotype/phenotype relationships for nuclearly encoded mitochondrial disorders is complicated by a number of factors, including the following. (1) Several hundred nuclear genes may be involved. (2) Functional similarities do not provide any clue. For example, mutations in the COX assembly genes *SURF1*, *COX10*, *COX15*, *SCO1*, and *SCO2* all result in distinct clinical presentations. (3) Unpredictable functional consequences may result from mutations in some of these genes, e.g., multiple mtDNA deletions resulting from mutations in the *ANT1* gene. Therefore, elucidation of the genetic bases of RC deficiencies of nuclear origin promises to be a difficult task, which will need to take advantage of new strategies and technologies that have yet to be defined.

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