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REVIEW ARTICLE

Mitochondrial disorders

Massimo Zeviani[1] and Stefano Di Donato[2]

[1]*Division of Molecular Neurogenetics and* [2]*Division of Biochemistry and Genetics, Istituto Nazionale Neurologico, Milan, Italy*

Correspondence to: Massimo Zeviani, Stefano Di Donato, Istituto Nazionale Neurologico 'C. Besta', Via Celoria 11 20133 Milan, Italy
E-mail: zeviani@istituto-besta.it, didonato@istituto-besta.it

Summary

In the medical literature the term 'mitochondrial disorders' is to a large extent applied to the clinical syndromes associated with abnormalities of the common final pathway of mitochondrial energy metabolism, i.e. oxidative phosphorylation (OXPHOS). Faulty oxidative phosphorylation may be due to overall dysfunction of the respiratory chain, a heteromultimeric structure embedded in the inner mitochondrial membrane, or can be associated with single or multiple defects of the five complexes forming the respiratory chain itself. From the genetic standpoint, the respiratory chain is a unique structure of the inner mitochondrial membrane formed by means of the complementation of two separate genetic systems: the nuclear genome and the mitochondrial genome. The nuclear genome encodes the large majority of the protein subunits of the respiratory complexes and most of the mitochondrial DNA (mtDNA) replication and expression systems, whereas the mitochondrial genome encodes only 13 respiratory complex subunits, and some RNA components of the mitochondrial translational apparatus. Accordingly, mitochondrial disorders due to defects in OXPHOS include both mendelian-inherited and cytoplasmic-inherited diseases. This review describes human genetic diseases associated with mtDNA and nuclear DNA mutations leading to impaired OXPHOS.

Keywords: respiratory chain; oxidative phosphorylation; mitochondrial DNA mutations; nuclear DNA mutations

Abbreviations: adPEO = autosomal dominant progressive external ophthalmoplegia; CoQ10 = coenzyme Q10; COX = cytochrome *c* oxidase; KSS = Kearns–Sayre syndrome; LHON = Leber's hereditary optic neuropathy; MELAS = mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF = myoclonic epilepsy with ragged red fibres; MDS = mitochondrial DNA depletion syndrome; mETC = mitochondrial electron transport chain; MNGIE = mitochondrial neurogastro-intestinal encephalomyopathy; mtDNA = mitochondrial DNA; NARP = neuropathy ataxia and retinitis pigmentosa; OXPHOS = oxidative phosphorylation; PEO = progressive external ophthalmoplegia; RRFs = ragged red fibres; SDH = succinate dehydrogenase; SNHL = non-syndromic and aminoglycoside-induced sensorineural hearing loss; TK = thymidine kinase 2; TP = thymidine phosphorylase.

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Introduction

Neurological syndromes are the most frequent clinical presentations of mitochondrial disorders, a group of human diseases characterized by defects of the mitochondrial energy output.

Mitochondria are cytoplasmic, double-membrane organelles, the main role of which is to synthesize ATP, the universal energy 'currency' of the cell. Because of the remarkable expansion of knowledge on the molecular characterization of human disorders associated with the energy pathways of mitochondria, the term 'mitochondrial disorders' is nowadays restricted to indicate only the clinical syndromes associated with abnormalities of oxidative phosphorylation (OXPHOS).

The respiratory chain is composed of five enzymatic multiheteromeric complexes (I, II, III, IV and V), embedded in the inner membrane of mitochondria. The protein subunits of the respiratory chain complexes are assembled together and with prosthetic groups and metal-containing reactive centres by a set of chaperones and assembly factors, some of which are specific to each complex. Coenzyme Q (a lipoidal quinone) and cytochrome *c* are also involved in mitochondrial respiration, serving as 'electron shuttles' between the complexes (Wallace, 1999).

The formation of the respiratory chain is under the control of two separate genetic systems, the nuclear genome and the

mitochondrial genome [mitochondrial DNA (mtDNA)]. In particular, four of the five respiratory chain complexes (I, III, IV and V) contain both nuclear-encoded and mtDNA-encoded polypeptides (Fig. 1).

In terms of function, the first two linked events of respiration, i.e. electron transfer and proton pumping, are carried out by the mitochondrial electron transport chain (mETC), a functional supramolecular structure located in the lipid bilayer of the membrane, and composed of four complexes (complex I–IV). In humans, complex I or NADH-ubiquinone oxidoreductase, which accomplishes the oxidation of NADH derived by the oxidation of fatty acids, pyruvate and amino acids, contains seven subunits which are encoded by the mtDNA (subunits ND1–ND6 and ND4L), plus at least 39

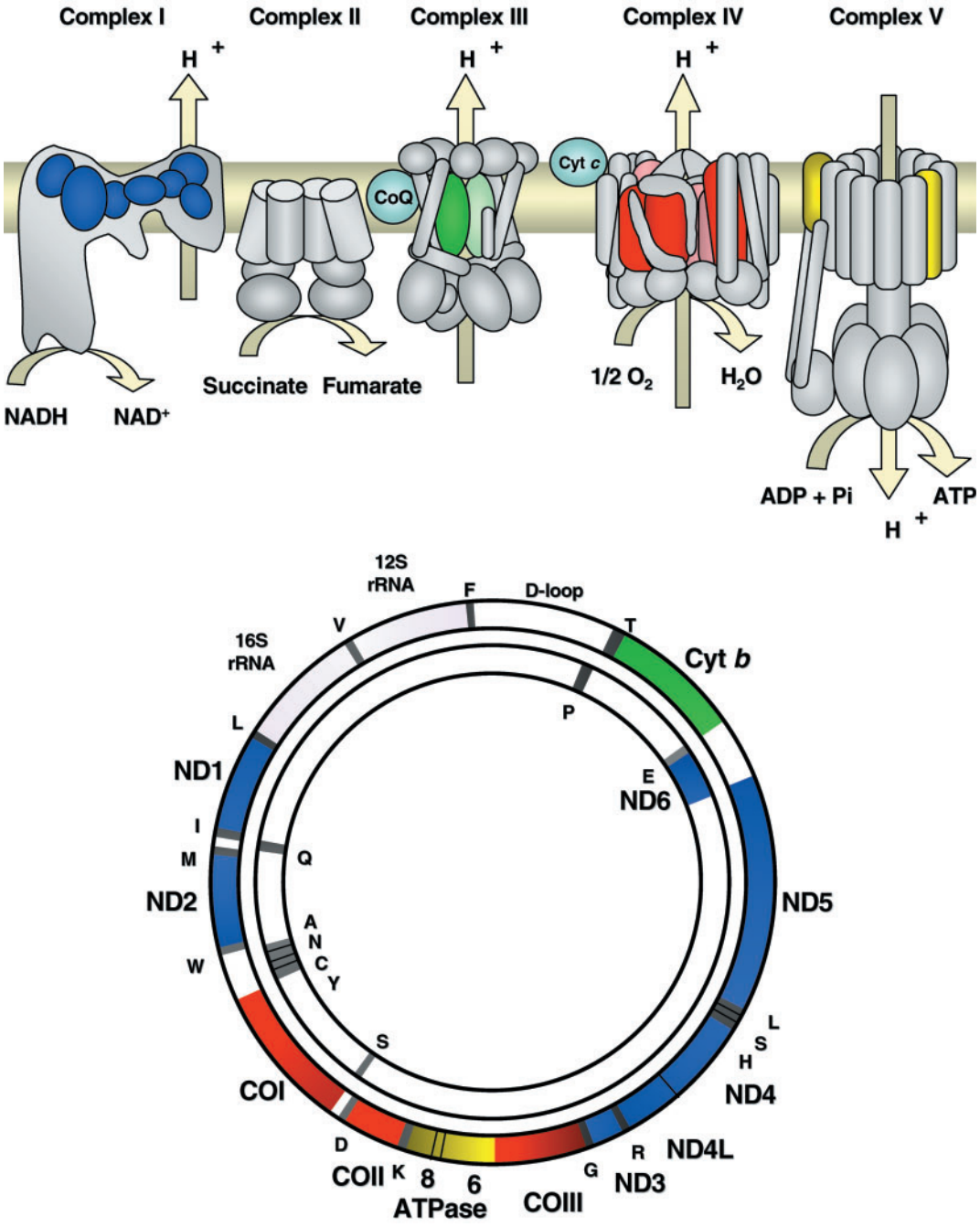

Fig. 1 Creative drawing of the respiratory chain and human mitochondrial DNA. *Top*: respiratory chain complexes. Mitochondrially encoded subunits, embedded in the midst of nuclear-encoded subunits, are shown in different colours: complex I subunits = blue; complex III subunit = green; complex IV subunits = red; complex V subunits = yellow. Pi = inorganic posphate; Cyt *c* = cytochrome *c*; CoQ = coenzyme Q. *Bottom*: mtDNA. *myt* genes: complex I genes = blue; complex III *cytb* gene = green; complex IV genes = red; complex V genes = yellow. *syn* genes: tRNA genes = grey; rRNA genes = purple. Cyt *b* = cytochrome *b*; COI = complex I; COII = complex II; COIII = complex III. (Courtesy of Dr Loredana Lamantea, Division of Molecular Neurogenetics).

nuclear-encoded subunits of complex I (Smeitink, 2001; Carroll *et al*., 2003). Complex II or succinate-ubiquinone oxidoreductase, which accomplishes the oxidation of FADH2 derived from fatty acid and the Krebs' cycle, is composed of only four subunits, all encoded by the nuclear genome. Complex III or ubiquinol-ferricytochrome *c* oxidoreductase holds one subunit, cytochrome *b*, encoded by the mitochondrial genome and 10 subunits encoded by the nuclear genome. Complex IV or cytochrome *c* oxidase (COX) is composed of 13 subunits, three of which are encoded by mtDNA (COX I–III) and the other 10 by nuclear DNA. In addition, mETC contains two highly hydrophobic, mobile, small electron carriers, coenzyme Q10 and cytochrome *c*, both synthesized by nuclear genes (Fig. 1). In substance the mETC is especially built to accept electrons from NADH and FADH2, transfer them through a series of oxidation–reduction reactions to molecular oxygen to produce water and to simultaneously coupling this exergonic reaction to the translocation of protons across the inner membrane (Saraste, 1999; Di Donato, 2000).

Synthesis of ATP from ADP is the second fundamental reaction of the mitochondrial respiratory chain, a process performed by complex V or ATP synthase. ATP synthase is also a genetic mosaic, since it is composed of two mtDNA-encoded subunits (ATPase 6 and 8), and at least 13 nuclear DNA-encoded subunits (Fig. 1). As mentioned, the proton electrochemical gradient generated at the mETC level during electron transfer to oxygen creates a polarization of the inner membrane which is changed back by the proton flux through a proton channel which resides in the F_0 component of ATP synthase. The proton flux drives the condensation of ADP and inorganic phosphate into ATP (Saraste, 1999; Wallace, 1999). Electron transfer across the mETC and ATP synthesis are coupled, or linked. In fact, the respiratory chain works as a proton pump which generates a proton gradient and a membrane potential of about 180 mV across the inner membrane with a negative polarity at the matrix side of the inner membrane. The proton gradient is utilized by the ATP synthase to phosphorylate matrix ADP. During this process the proton gradient is decreased and this activates respiration, i.e. electron transfer (Saraste, 1999). Hence, the fundamental reaction of life, i.e. oxygen activation and the conservation of energy in cell respiration, is essentially a function of the integrity of the inner membrane respiratory chain (Babcock and Wilkstrom, 1992).

Notably, energy production in mitochondria requires not only a full assembly of functional protein at the level of the inner mitochondrial membrane, but also a bidirectional flow of information between the nuclear genome and the mitochondrial genome to adjust energy production in tissues to different energetic demands (Poyton and McEwan, 1996). Accordingly, many different mutations in mtDNA- and nuclear DNA-encoding subunits, components or regulators of the respiratory chain function can produce a wide range of OXPHOS diseases (DiMauro and Schon, 2003; Zeviani and Carelli, 2003).

Clinical aspects

Given the complexity of mitochondrial genetics and biochemistry, the clinical manifestations of mtDNA disorders are extremely heterogenous. They range from lesions of single tissues or structures, such as the optic nerve in Leber's hereditary optic neuropathy (LHON), or the cochlea in maternally inherited non-syndromic deafness, to more widespread lesions including myopathies, encephalomyopathies, cardiopathies, or complex multisystem syndromes with onset ranging from neonatal to adult life (Table 1).

Adult patients usually show signs of myopathy associated with variable involvement of the CNS (ataxia, hearing loss, seizures, polyneuropathy, pigmentary retinopathy and, more rarely, movement disorders). Some patients complain only of muscle weakness and/or wasting with exercise intolerance (Zeviani and Carelli, 2003). Several morphological and biochemical hallmarks characterize many, albeit not all, of these syndromes. The best known morphological finding is perhaps the transformation of scattered muscle fibres into 'ragged red fibres' (RRFs) (Fig. 2A). RRFs are characterized by the accumulation of abnormal mitochondria under the sarcolemmal membrane (Fig. 2D). The latter phenomenon is clearly demonstrated by an intense subsarcolemmal reaction to a respiratory chain-specific mitochondrial enzyme such as succinate dehydrogenase (SDH) (Fig. 2C). Another common finding is the presence of muscle fibres that stain negative to the histochemical reaction to COX (respiratory complex IV) (Fig. 2B). However, typical 'mitochondrial' clues may

Table 1 *Phenotypic expression of mitochondrial diseases*

Neurological manifestations	Systemic manifestations
Neuromuscular	Heart
Ophtalmoplegia	Cardiomyopathy
Myopathy	Cardiac conduction defects
Exercise intolerance	
Peripheral sensory–motor	Endocrine system
neuropathy	Diabetes
CNS	Exocrine pancreas dysfunction
Myelopathy	Hypoparathyrodism
Headache	Multiple endocrinopathy
Stroke	Short stature
Seizures	
Dementia	Blood
	Pancytopenia
Movement disorders	Sideroblastic anaemia
Ataxia	
Dystonia	Mesenchymal organs
Parkinsonism	Hepatopathy
Myoclonus	Nephropathy
	Intestinal pseudo-obstruction
Eye	
Blindness	Metabolism
Optic neuropathy	Metabolic acidosis
Pigmentary retinopathy	Nausea and vomiting
Cataract	
Ear	
Sensorineural deafness	

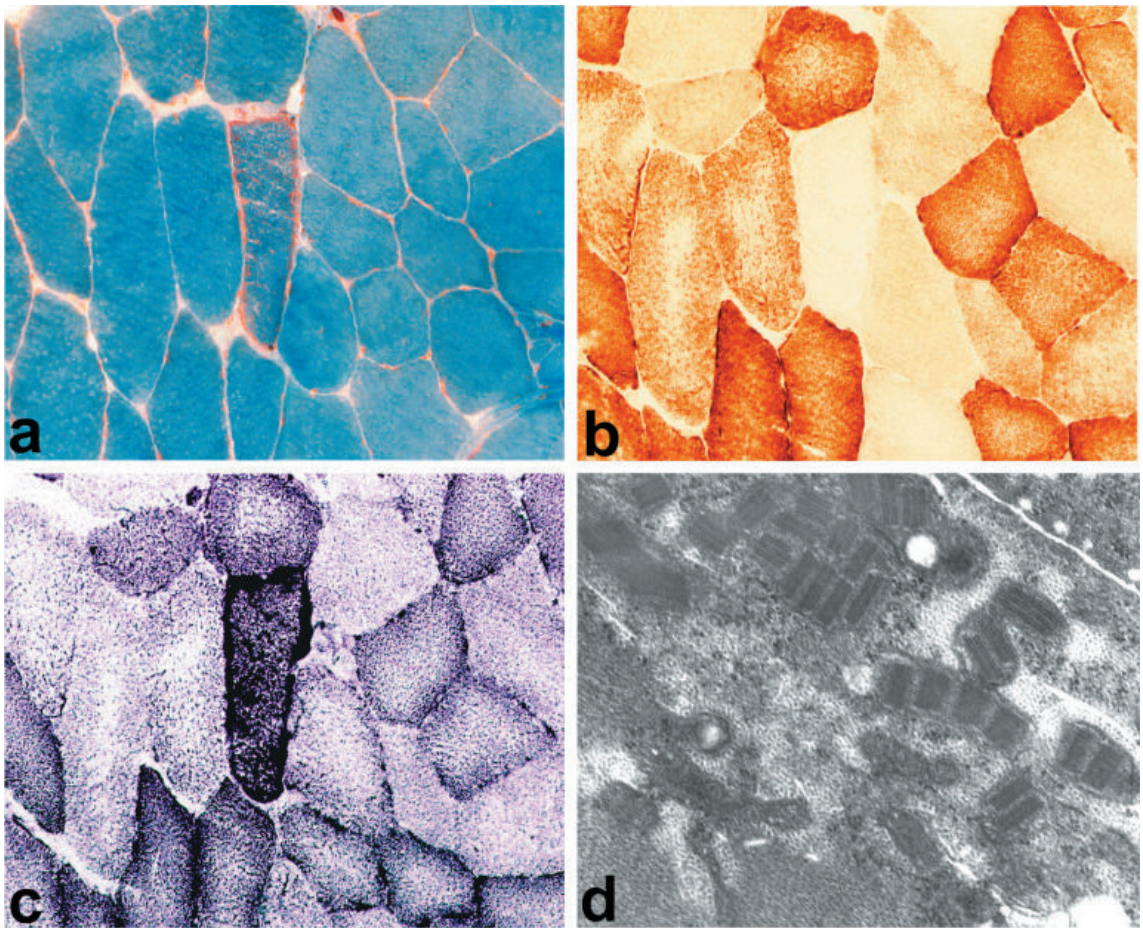

Fig. 2 (**A**–**C**) Serial transverse sections of a biopsy from the vastus lateralis muscle of a 18-year-old patient with MERRF: modified Gomori trichrome stain (**A**); COX stain (**B**); SDH stain (**C**). Note absent COX activity, and increased SDH activity in the RRF at the centre of the figure. (**D**) Transverse section though the periphery of the RRF in (**A**) shows numerous enlarged mitochondria, many of which contain paracrystalline inclusions. (Courtesy of Dr Marina Mora, Laboratory of Muscle Biology).

be absent in otherwise demonstrated mitochondrial disorders. This is the case for LHON, for neuropathy ataxia and retinitis pigmentosa (NARP), and it is also true in many paediatric cases.

In paediatric patients the most frequent clinical features are severe psychomotor delay, generalized hypotonia, lactic acidosis and signs of cardiorespiratory failure (Zeviani and Carelli, 2003). Clinical presentations include fatal multisystem syndromes, encephalomyopathies, or isolated myopathies sometimes associated with cardiopathies. The most common, and better characterized, early onset mitochondrial encephalopathy is Leigh syndrome or subacute necrotizing encephalomyelopathy. Affected infants show severe psychomotor delay, cerebellar and pyramidal signs, dystonia, seizures, respiratory abnormalities, uncoordination of ocular movements and recurrent vomiting. Focal symmetric lesions are found at necropsy, and by MRI, in the brainstem, thalamus and posterior columns of the spinal chord (Leigh, 1951). RRFs are consistently absent in the muscle biopsy.

Leigh syndrome is clearly a genetically heterogeneous entity. In some cases it is attributable to mtDNA mutations, in others to an autosomal recessive defect of a nuclear gene, encoding structural subunits or assembly factors of the respiratory chain complexes. In yet other cases, the defect is X-linked or sporadic, as in the case of the defect of the E1α subunit of pyruvate dehydrogenase complex. However, all defects described to date in patients with Leigh syndrome affect the terminal oxidative metabolism and are likely to impair energy production (DiMauro and DeVivo, 1996). The typical neuropathological findings of Leigh syndrome are therefore the expression of the damage produced by impaired oxidative metabolism on the developing brain, irrespective of the specific biochemical or genetic causes.

Finally, it is important to emphasize that molecular investigation still fails to identify the responsible gene defect in ~50% of adult patients affected by mitochondrial disease, as demonstrated by specific biochemical and/or morphological evidence. The percentage of undiagnosed cases increases to 80–90% for paediatric disorders. These figures illustrate the formidable task still facing investigators working on the elucidation of the genetic basis of mitochondrial disorders. Since the sequence of the entire mtDNA is now available in several research centres worldwide, most of these cases, which still

await molecular characterization, are likely to be due to mutations in (unknown) nuclear genes related to OXPHOS.

Defects of the mtDNA genes

Gene organization of the mitochondrial genome

Human mtDNA is a 16.5 kb circular minichromosome, composed of two complementary strands, the heavy and light strands. All of the coding sequences are contiguous with each other with no introns (Anderson *et al*., 1981). The only non-coding stretch of mtDNA is the displacement-loop (D-loop), a region of about 1 kb which contains the promoters for light and heavy strand transcription (Fig. 1). Replication of mtDNA was believed to proceed asynchronously and asymmetrically, starting from two spatially separated replication origins, one for each strand. This model, proposed by Clayton (1991), has recently been challenged by experimental evidence supporting the existence of conventional, strand-coupled replication of mammalian mtDNA (Holt *et al*., 2000; Yang *et al*., 2002).

Since the mtDNA genetic code differs from the universal code, expression of mtDNA genes must rely upon mitochondrion-specific protein synthesis, carried out through the interplay of nuclear-encoded transcriptional and translational factors with tRNAs and rRNAs synthesized *in situ* from the corresponding mitochondrial genes. Thus, human mtDNA contains both protein-encoding genes (analogous to the yeast *mit* genes), and protein synthesis genes (analogous to the yeast *syn* genes). An important progress in the understanding of the mitochondrial transcriptional machinery has been the discovery that two novel transcriptional factors, TFB1M and TFB2 M, cooperate with mitochondrial RNA polymerase and mitochondrial transcription factor A to carry out basal transcription of mammalian mtDNA (Falkenberg *et al*., 2002).

The 13 *mit* genes specify seven ND subunits of NADH-ubiquinone reductase, three subunits of COX (complex IV), subunits 6 and 8 of ATPsynthase (complex V), and apocytochrome *b*, which is part of ubiquinol-cytochrome *c* reductase (complex III). SDH-ubiquinone reductase (complex II) is composed of four subunits, all encoded by nuclear genes. The *syn* genes of mtDNA encode two rRNAs (12 and 16S rRNA) and 22 tRNAs that are involved in protein translation of the *mit* gene products. (see Fig. 1).

Clinical genetics

The genetics of mtDNA differs from that of nuclear DNA in the following unique properties (Zeviani *et al*., 2003).

The mitochondrial genome is maternally inherited. Paternal mtDNA does not contribute to mitochondrial inheritance despite a few sperm mitochondria entering the egg (Schwartz and Vissing, 2002). Only the mother transmits her oocyte mtDNA to all of her offspring, and her daughters transmit their mtDNA to the next generation (Giles *et al*., 1980; Ankel-Simons and Cummins, 1996). Mitochondria are polyploid. Each human cell has hundreds of mitochondria, each containing 2–10 mtDNA molecules. At cell division, mitochondria and their genomes are randomly distributed to daughter cells.

Normally, the mitochondrial genotype of an individual is composed of a single mtDNA species, a condition known as homoplasmy. However, the intrinsic propensity of mtDNA to mutate randomly can occasionally determine a transitory condition known as heteroplasmy, where the wild-type and the mutant genomes co-exist intracellularly. Because of mitochondrial polyploidy, during mitosis the two mtDNA species are stochastically distributed to daughter cells (Jenuth *et al*., 1996). This phenomenon can account for the drastic change in mutation loads observed in different generations of families carrying heteroplasmic mtDNA, and increases the remarkable variability in the phenotypic presentations of mitochondrial disorders. Because of mitotic mtDNA segregation and polyploidy, a threshold effect dictates the phenotypic expression of a mtDNA-associated character (Jenuth *et al*., 1997). For a given heteroplasmic mutation, only when mutated gene copies accumulate over a certain threshold, the deleterious effects of the mutation will no longer be complemented by the co-existing wild-type mtDNA, and will be expressed phenotypically as a cellular dysfunction leading to disease (Thorbum and Dahl, 2001). A major breakthrough in the understanding of mitochondrial disorders has been the discovery of an impressive number of mutations of mtDNA (available from: http://www.mitomap.org/).

The variability in clinical manifestations of mtDNA stems from a number of factors, including the nature of the mutation, i.e. its intrinsic pathogenicity, and the gene specifically affected, the mutation load and its tissue distribution, and the relative reliance of each organ system on the mitochondrial energy supply. In general, the visual and auditory systems, the CNS and PNS, the heart, muscle, endocrine pancreas, kidney and liver are, in that order, the organs most sensitive to OXPHOS failure (Table 1). However, almost 15 years after the first reports on human mtDNA mutations, and the many more that have been discovered afterwards, the intimate molecular and cellular mechanisms which link a given mtDNA change to a specific clinical presentation are still largely unknown (Zeviani and Carelli, 2003).

Mutations of mtDNA are divided into large-scale rearrangements (i.e. partial deletions or duplications) and inherited point mutations. Both groups have been associated with well-defined clinical syndromes. While large-scale rearrangements are usually sporadic, point mutations are usually maternally inherited. Similar to rho^0-petite phenotype in yeast, large-scale rearrangements include several genes and are invariably heteroplasmic. In contrast, point mutations may be heteroplasmic or homoplasmic, and affect individual *mit* or *syn* genes (Table 2).

Large-scale rearrangements of mtDNA

Single, large-scale rearrangements of mtDNA can be single partial deletions, or partial duplications. Rearranged molecules, lacking a portion of the mitochondrial genome, can be detected as an independent mtDNA species (single mtDNA deletion) or

Table 2 *Mitochondrial OXPHOS diseases due to mtDNA mutations*

Large-scale rearrangements of mtDNA	Phenotype	mtDNA mutation
KSS	Ataxia, neuropathy, PEO, pigmentary retinal degeneration, cardiomyopathy and conduction block, short stature, high CSF protein	Single deletions or duplications (mostly sporadic)
Pearson's syndrome	Frequent death in infancy. Refractory sideroblastic anaemia with vacuolization of marrow precursors.	
PEO	Late-onset bilateral ptosis and ophtalmoplegia, proximal muscle weakness and wasting, and exercise intolerance	
Point mutations of mtDNA		
MELAS	Stroke-like episodes due to focal brain lesions in the parieto-occipital lobes, lactic acidosis and/or RRFs	Heteroplasmic point mutations (maternally inherited)
MERRF	Myoclonus, epilepsy, muscle weakness and wasting with RRFs, cerebellar ataxia, deafness and dementia	
NARP	Ataxia, pigmentary retinopathy, peripheral neuropathy and distal neurogenic weakness	
Hearing loss–ataxia–myoclonus	Syndromic hearing loss, myoclonus epilepsy, ataxia, myopathy	
LHON	Loss of central vision, large centro-caecal absolute scotoma, circumpapillary telangiectatic microangiopathy	Homoplasmic point mutations (maternally inherited)
SNHL	Non-syndromic and aminoglycoside-induced hearing loss	

joined to a wild-type molecule in a 1 : 1 ratio, as partially duplicated mtDNA. Frequently, a mixture of the two rearrangements co-exists in the same cell or tissue (Zeviani *et al*., 1988; Poulton *et al*., 1989).

Three main clinical phenotypes are associated with these mutations: Kearns–Sayre syndrome (KSS), sporadic progressive external ophthalmoplegia (PEO) and Pearson's syndrome (Table 2).

KSS is a (usually) sporadic disorder characterized by the triad of: (i) chronic progressive external ophthalmoplegia; (ii) onset before age of 20 years; and (iii) pigmentary retinopathy. Cerebellar syndrome, heart block, increased CSF protein content, diabetes and short stature are also part of the syndrome. Patients with this disease invariably show RRFs in muscle biopsy (Mita *et al*., 1989). KSS is characterized by neuroradiological abnormalities affecting the deep structures of the brain and the subcortical white matter (Barkovich *et al*., 1993).

Single deletions/duplications can also result in milder phenotypes as PEO, characterized by late-onset progressive external ophtalmoplegia, proximal myopathy and exercise intolerance. In both KSS and PEO, diabetes mellitus and hearing loss are frequent additional features, that may occasionally precede, by years, the onset of neuromuscular symptoms (Shoffner *et al*., 1989).

Finally, large-scale single deletions/duplications of mtDNA may cause Pearson's bone-marrow–pancreas syndrome, a rare disorder of early infancy characterized by connatal sideroblastic pancytopenia and, less frequently, severe exocrine pancreatic insufficiency with malabsorption (Rotig *et al*., 1990). Interestingly, infants surviving into childhood or adolescence may develop the clinical features of KSS (Shanske *et al*., 2002).

The majority of single large-scale rearrangements of mtDNA are sporadic and are therefore believed to be the result of the clonal amplification of a single mutational event, occurring in the maternal oocyte or early during the development of the embryo (Schon *et al*., 1989; Chen *et al*., 1995). It is not yet understood why in multisystem disorders such as KSS, in which D-mtDNAs are virtually ubiquitous, mutations are not transmitted through female gametes to the progeny. One possibility is that the germinal cells containing deleted genomes are not viable for gametogenesis and/or fertilization. However, mother-to-offspring transmission has occasionally been documented in KSS/PEO. Hence, the recurrency risk for these mtDNA abnormalities can no longer be considered absent. Until a reliable epidemiological survey of PEO or KSS due to single rearrangements of mtDNA is available, we suggest a prudential figure of 5% recurrency risk in the genetic counselling of affected women (Chinnery *et al*., 2000).

Molecular pathogenesis

The relative amount and tissue distribution of the molecular lesion dictate the onset and severity of the disease.

Transmitochondrial cybrids, obtained by introducing deleted mtDNAs into mtDNA-less rho^0 cells, showed impaired respiration (Hayashi *et al*., 1991). A threshold of >60% rearranged mtDNA molecules is enough for OXPHOS failure to occur. The more widespread is the tissue distribution of the lesion, the more severe is the clinical syndrome, from PEO, to KSS, to Pearson's syndrome. This notion is also relevant for the diagnosis: for instance, deletions are confined to the muscle biopsy in PEO, but in KSS they can also be found in blood, albeit in lesser amounts, while in Pearson's syndrome the amount is comparable in blood and muscle.

Most rearrangements occur across direct repeats of variable length (Schon *et al*., 1989; Mita *et al*., 1989), suggesting a mechanism based on illegitimate homologous recombination.

Defective OXPHOS of mitochondria containing D-mtDNA is due to the loss of both *mit* and *syn* genes contained within the deletion. In particular, because the lack of tRNA genes results in incompetency for translation (Mariotti *et al*., 1994), mitochondria containing only D-mtDNA are rho^0-mutants, which cannot synthesize functional OXPHOS enzymes. However, partial correction of the rho^0-phenotype can be accomplished through complementation by mRNAs and tRNAs synthesized from wild-type mtDNA, provided that D-mtDNA and wild-type mtDNA co-segregate in the same organelles.

Point mutations of mtDNA

In contrast to large-scale rearrangements, mtDNA point mutations are usually maternally inherited. Given the very high mutational rate of mtDNA and the presence of numerous 'private' or population-specific polymorphisms, the distinction between non-deleterious and pathogenic mutations may not be easy. The following features are frequently present in pathogenic mutations: (i) high conservation of the affected nucleotide/amino acid or loss of function of the gene product (e.g. a stop mutation in a *mit* gene); (ii) segregation with phenotype; (iii) quantitative correlation between phenotype and heteroplasmy, if present; and (iv) identification of the mutation in affected families from ethnically distinct human populations (Zeviani and Carelli, 2003).

Point mutations involving tRNA *syn* genes cause a reduced availability of functional tRNAs that may impair the overall mitochondrial protein synthesis. Marked reduction of both mitochondrial protein synthesis and respiration has been documented for some mutations, when a threshold of 80–90% of mutant mtDNA is reached. Mutations involving protein-encoding *mit* genes affect specifically the function of the respiratory chain complexes to which the corresponding protein belongs (Mariotti *et al*., 1994).

It is worth mentioning that the clinical and biochemical variability of many mtDNA mutations may be due to different mitochondrial and/or nuclear 'gene backgrounds'. For instance, the fate and expression of mutations in cultures appears to be strongly influenced by the different nuclear backgrounds of the cell types (Dunbar *et al*., 1995). It has also been proposed that nucleotide changes in mtDNA that are not intrinsically pathogenic may predispose to, modulate the effects of, or reflect a propensity for the occurrence of deleterious mutations. In turn, deleterious mutations may promote the accumulation of somatic changes, through the generation of OXPHOS-related mutagens. This phenomenon could trigger a positive feed-back loop contributing to the progression of the mitochondrial dysfunction (Luft, 1994). Given their different pathophysiology and genetic features, the most frequent heteroplasmic and homoplasmic mtDNA point mutations will be discussed separately (Table 2).

Heteroplasmic point mutations

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

This is defined by the presence of (i) stroke-like episodes due to focal brain lesions, often localized in the parieto-occipital lobes; and (ii) lactic acidosis and/or RRFs. Other signs of CNS involvement include dementia, recurrent headache and vomiting, focal or generalized seizures, pigmentary retinopathy and deafness. Ataxia can be observed in some patients. Diabetes, intestinal pseudo-obstructions and cardiomyopathy may complicate single cases (Hirano *et al*., 1992).

Infarct-like lesions widespread in the cerebral cortex are associated with diffuse fibrillary gliosis in the cerebral and cerebellar white matter. Multiple focal lesions with demyelination and numerous spheroids have been reported in the pontocerebellar fibres, together with marked degeneration of the posterior columns and spinocerebellar tracts (Mizukami *et al*., 1992). Electron microscopic examination shows accumulations of abnormal mitochondria in smooth muscle cells and endothelium of the cerebral and cerebellar blood vessels, suggesting a 'mitochondrial angiopathy'. However, the presence of diffuse, prominent white matter gliosis of the CNS and cerebellar cortical degeneration of granular cell type may indicate morphologically widespread cellular dysfunction, not restricted to either neuronal or vascular derangement (Mizukami *et al*., 1992; Tsuchiya *et al*., 1999). MRI examination typically shows that the signal abnormalities in the brain do not correspond to well-defined vascular territories (Barkovich *et al*., 1993). The stroke-like lesions may be transient and resolve after a few months. The recurrent occurrence of stroke-like episodes eventually leads to permanent lesions.

MELAS was first associated with a heteroplasmic point mutation in the tRNA$^{Leu(UUR)}$, an A→G transition at position 3243 (Goto *et al*., 1990). Many other MELAS-associated point mutations were later reported, although the 3243A>G remains by far the most frequent one (Mitomap available from: http://www.mitomap.org/). The genotype–phenotype correlation of the A3243G mutation is rather loose, since the observed clinical manifestations are not limited to the full-blown MELAS syndrome. For instance, the 3243A>G

mutation has been detected in several patients (and families) with maternally inherited PEO, isolated myopathy alone, cardiomyopathy, or in pedigrees with maternally inherited diabetes mellitus and deafness (Chinnery and Turnbull, 1999; Leonard and Shapira, 2000*a*; DiMauro and Schon, 2003). Biochemically, complex I is frequently the most affected respiratory chain activity in MELAS, while complex IV is often normal. This accounts for the observation that, in contrast to other mitochondrial syndromes, RRFs in A3243G-MELAS (but not in A3243G-PEO) specimens display a robust histochemical reaction to COX. A specific link between defective complex I and the MELAS phenotype is suggested by the recent identification of mutations in ND genes associated with this phenotype, or with MELAS/LHON overlap presentations (Corona *et al*., 2001).

Myoclonic epilepsy with ragged red fibres (MERRF)

This is a maternally inherited neuromuscular disorder characterized by myoclonus, epilepsy, muscle weakness and wasting with RRFs, cerebellar ataxia, deafness and dementia (Shoffner *et al*., 1990). Symmetric lipomatosis, especially in the trunk, is a frequent, intriguing sign in MERRF, that can anticipate the onset of neurological symptoms by several years.

Neuronal loss and gliosis of the cerebellar dentate nuclei and inferior olives have been reported in MERRF (Lombes *et al*., 1989; Oldfors *et al*., 1995) and confirmed by neuroimaging studies (Berkovic *et al*., 1989).

The most commonly observed mutation of mtDNA associated with MERRF is an A→G transition at nt 8344 in the $tRNA^{Lys}$ gene (Wallace *et al*., 1988*a*). Different mutations in the same gene have been reported in association with MERRF (Silvestri *et al*., 1992), MERRF/MELAS overlap syndrome (Zeviani *et al*., 1993) or other complex phenotypes. Complex IV deficiency is the most prominent biochemical finding in 8344A>G-positive MERRF muscle, although complex I can be affected too. COX-depleted RRFs are invariably detected in the muscle biopsy (Fig. 2).

Clinical, biochemical and molecular investigation of large pedigrees shows a positive correlation between the severity of the disease, age at onset, mtDNA heteroplasmy and reduced activity of respiratory chain complexes in skeletal muscle. However, even though the genotype–phenotype correlation between MERRF syndrome and the A8344G mutation is tighter than that of other mutations (Hammans *et al*., 1993), the A8344G transition has also been reported in phenotypes as different as Leigh's syndrome, isolated myoclonus, familial lipomatosis, isolated myopathy and a variant neurological syndrome characterized by ataxia, myopathy, hearing loss and neuropathy (Austin *et al*., 1998; Mitomap available from: http://www.mitomap.org/). MERRF must be considered in the differential diagnosis of progressive myoclonus epilepsies, including Ramsay–Hunt syndrome and Unverricht–Lundborg disease (Berkovic *et al*., 1993).

Neurogenic weakness, ataxia and retinitis pigmentosa (NARP)

This is a maternally inherited syndrome in which the cardinal manifestations include ataxia, pigmentary retinopathy and peripheral neuropathy (Holt *et al*., 1990). MRI examination of NARP patients has revealed the presence of moderate, diffuse cerebral and cerebellar atrophy, and, in the most severely affected patients, symmetric lesions of the basal ganglia (Barkovich *et al*., 1993; Uziel *et al*., 1997).

NARP is associated with a heteroplasmic T→G transversion at position 8993 in the ATPase 6 subunit gene (Holt *et al*., 1990). A transition in the same position (8993T>C) has later been described in patients affected by a mild variant of NARP (de Vries *et al*., 1993). RRFs are consistently absent in the muscle biopsy. The degree of heteroplasmy is correlated with the severity of the disease. For instance, when the percentage of mutant mtDNA is >95%, patients show the clinical, neuroradiological and neuropathological findings of maternally inherited Leigh's syndrome (Tatuch *et al*., 1992). NARP/maternally inherited Leigh's syndrome phenotypes have been described in association with other mutations of the ATPase 6 gene, e.g. mutation 9176T→C (Dionisi-Vici *et al*., 1998). NARP and maternally inherited Leigh's syndrome may co-exist in the same family. Impairment of ATP synthesis has been reported in cell cultures harbouring the T8993G mutation, as well as in tissue-derived mitochondria, showing a strict correlation with the mutation load (Carelli *et al*., 2002*b*).

Hearing loss–ataxia–myoclonus

This syndrome was originally reported in a large Italian pedigree (Tiranti *et al*., 1995). The responsible mutation, 7472insC, affects the $tRNA^{Ser(UCN)}$ gene. This mutation has later been reported in several families, in which affected members showed a wide range of clinical manifestations, from isolated hearing loss, to epilepsia partialis continua and ataxia, to overt MERRF (Jaksch *et al*., 1998). Given the increasing frequency at which the 7472insC has been found, the search for this mutation should become part of the routine screening of mitochondrial encephalomyopathies and/or maternally inherited hearing loss (Hutchin and Cortopassi, 2000).

Other syndromes

In spite of the enormous variability of the clinical presentations associated with heteroplasmic mtDNA point mutations, the accumulation of a remarkable amount of clinical and genetic data makes it possible now to establish a tentative correlation between specific mutations, or mutations clustered in specific mtDNA genes, and different clinical presentations. For instance, several mutations in $tRNA^{Ser(UCN)}$, including the 7472insC, may present with hearing loss as the only or predominant symptom, suggesting an exquisite sensitivity of the cochlear receptor and auditory system to the functional impairment of this particular mt-tRNA gene (Hutchin and

Cortopassi, 2000). The pathogenetic mechanisms underlying this well-established observation are presently unknown. Likewise, mutations in *tRNA^{Ile}* are mainly associated with cardiomyopathy (Santorelli *et al*., 2001) or PEO, while mutations in cytochrome *b* are mainly associated with isolated myopathy with high serum creatine kinase or myoglobinuria (Andreu *et al*., 1999). Cytochrome *b* mutations are usually restricted to skeletal muscle and, in contrast to most of the other point mutations, are not transmitted maternally. A number of different clinical presentations, ranging from infantile Leigh syndrome to adult-onset motor neuron disease, to complex multisystem disorders, have been reported with different point mutations of the three genes encoding complex IV, while point mutations of the genes encoding complex I subunits are usually associated with MELAS, LHON or overlap syndromes (Mitomap available from: http://www.mitomap.org/). However, an increasing number of Leigh-like, infantile encephalopathic syndromes have been reported recently in association with the 13513G >A mutation in ND5 (Chol *et al*., 2003).

Homoplasmic mtDNA mutations

General features

In contrast to many heteroplasmic mutations, the clinical expression of disorders associated with homoplasmic mutations is often stereotypical and mainly restricted to a single tissue. In this group of disorders, the presence of a pathogenic mtDNA mutation is necessary but not sufficient to induce disease (Table 2). As a consequence, penetrance is incomplete and possibly controlled by environmental factors, additional mitochondrial polymorphisms, or the effect of nuclear gene(s) (Howell and Mackey, 1998). However, the specific molecular mechanisms underlying these contributions are still largely unkown.

LHON

This was the first maternally inherited disease to be associated with a mtDNA point mutation (Wallace *et al*., 1988*b*). LHON typically affects young adults, more often males. Visual acuity deteriorates over a period of days/weeks as a consequence of rapid, painless loss of central vision in one eye, usually followed by the other eye. Stable residual values at or below 20/200 are reached in a few months, associated with a large centro-caecal absolute scotoma. Characteristic fundus changes include circumpapillary telangiectatic microangiopathy with tortuosity of peripapillary arterioles, swelling of the nerve fibre layer and hyperaemic optic disc, and absence of leakage on fluorescein angiography (Smith *et al*., 1973; Nikoskelainen *et al*., 1983). Axonal loss in the papillomacular bundle, leading to an early and prevalent temporal atrophy of the optic disc, is a pathognomonic feature of LHON (Kwittken and Barest, 1958; Smith *et al*., 1973).

Histopathological investigations show loss of retinal ganglion cell and nerve fibre layers, while the remaining layers appear virtually normal. Ultrastructural investigations in genetically proven LHON optic nerves showed degenerative features in both axoplasm and myelin sheaths. Patchy accumulations of mitochondria suggested an impairment of axoplasmic transport. Variability in myelin thickness was also evident, some axons being almost denuded of myelin sheath. Morphometric investigation showed a preferential loss of the smallest axons, corresponding to the P-cell population which provides central vision (Sadun *et al*., 2000; Carelli *et al*., 2002*a*).

Approximately 90% of the worldwide LHON patients carry one of the three most frequent mtDNA mutations associated with LHON, namely the 11778G>A, 3460A>G and 14484T>C mutations (Wallace *et al*., 1988*b*; Howell *et al*., 1991; Chinnery *et al*., 2001). A further group of rare, but well-established pathogenic mutations have been found only in a few families; also, prognosis depends on the type of mutation (Mackey and Howell, 1992; Kim *et al*., 2002). Other mutations, found only in single cases or families, still await confirmatory identification from multiple independent cases.

All the LHON mutations which have been proved to be pathogenic affect different mtDNA-encoded subunits of complex I. Mutations are usually homoplasmic, although heteroplasmy can occasionally be found in some families or singleton cases.

Variable expression of LHON may be due to the association of pathogenic mutations with specific mtDNA haplogroups. For instance, the European-specific haplogroup J is found more frequently in 11778- or 14484-positive LHON patients than in ethnically matched control populations, suggesting that this haplogroup may increase the penetrance of the disease (Brown *et al*., 2002; Hofman *et al*., 1997; Torroni *et al*., 1997). Environmental factors seem also to play a role as risk factors, in particular tobacco smoke (Tsao *et al*., 1999). Finally, a nuclear modifier is thought to be a major determinant for both disease expression and male prevalence. However, search for an X-linked nuclear modifier has been unsuccessful to date (Chalmers *et al*., 1996).

Additional puzzling features of LHON are the exquisite tissue specificity and the subtle and ill-defined biochemical abnormalities found in this condition. The unique anatomical and physiological features of the optic nerve may explain its vulnerability to the decreased bioenergetic efficiency and increased oxidative stress associated with LHON mutations (Bristow *et al*., 2002; Wong *et al*., 2002).

LHON-like optic atrophy may be part of more complex syndromes including dystonia, Leigh syndrome and MELAS (Shoffner *et al*., 1995). Private or infrequent mutations, again affecting complex I subunit genes, have been reported in these cases (Carelli *et al*., 2002*a*).

Non-syndromic and aminoglycoside-induced sensorineural hearing loss (SNHL)

This has been both associated with a unique, maternally inherited point mutation at position 1555 (A→G) of the 12S rRNA

gene (Prezant *et al*., 1993). Similar to LHON, this mutation is almost invariably homoplasmic, and variable penetrance and clinical severity have been documented (Jaber *et al*., 1992; Estivill *et al*., 1998) A two-locus model, including a primary mitochondrial mutation associated with a nuclear modifier gene, was suggested to explain incomplete penetrance. Bykhovskaya and colleagues reported the identification of a locus on chromosome 8 for a putative nuclear modifier gene, but this finding has not been confirmed by other studies (Bykhovskaya *et al*., 2000; Finnila and Majamaa, 2003). In addition, a paraomomycin resistance mutation in yeast, homologous to the human 1555 mutation, expresses a respiratory-deficient phenotype only in the presence of a nuclear mutation in one of two genes, *Mss1* and *Mto1* (Hu *et al*., 1991). The human analogues of *Mss1* and *Mto1* are obvious candidates as nuclear modifier genes in the 1555-related SNHL. The 1555 mutation affects a highly conserved region of the 12S rRNA gene, homologous to the bacterial domain that binds aminoglycosides, and increases the similarity of the human 12S rRNA to its bacterial counterpart. The growth rate of mutant cells is markedly reduced when they are exposed to aminoglycosides, confirming their sensitivity to this drug (Inoue *et al*., 1996). However, 1555-positive subjects who were never exposed to aminoglycosides can also become deaf. Therefore, the 1555 mutation is now considered as a frequent genetic cause of both non-syndromic and aminoglycoside-induced post-lingual SNHL. The hair cells of the cochlea are very energy dependent and local gene expression may also play a relevant role in the strict tissue specificity observed with the 1555 mutation and with other mutations of mtDNA which are predominantly characterized by hearing loss. A second homoplasmic mutation (1494C>T) in the mtDNA 12S rRNA gene has recently been associated with maternally inherited, aminoglycoside-induced, non-syndromic deafness in a large Chinese family (Zhao *et al*., 2004).

Other homoplasmic mutations

Homoplasmic mutations are frequently found during systematic screening of mtDNA in mitochondrial patients, but their pathogenic significance remains uncertain. A well-documented case is a mutation at position 1624 in the $tRNA^{Val}$ gene (McFarland *et al*., 2002). This homoplasmic mutation was found in a clinically normal woman, who had six stillbirths and one surviving child with Leigh syndrome, from different partners. Biochemical investigations demonstrated a profound respiratory chain deficiency in both the apparently healthy woman and her child. A second, homoplasmic mutation (in $tRNA^{Ile}$) has later been identified in a family composed of a healthy mother and three affected daughters. Both primary fibroblast cell cultures and transmitochondrial cybrid derivatives from several members of this family showed a profound defect in complexes I and IV (Limongelli *et al*., 2004). A third mutation, again in the $tRNA^{Ile}$ gene (4300G>A), has been found in a few families with maternally inherited congestive cardiomyopathy of variable penetrance (Casali *et al*., 1995). Interestingly, this mutation was shown to be associated with very low steady-state levels of the $tRNA^{Ile}$ transcript in heart, but not in skeletal muscle. Accordingly, a severe, combined defect in complexes I and IV was detected in the heart muscle, but not in the skeletal muscle of an index case (Taylor *et al*., 2003).

These results strongly support the novel concept that homoplasmic mutations in tRNA genes can be responsible for mitochondrial disorders characterized by extremely variable penetrance. Homoplasmic pathogenic mutations in the mitochondrial genome represent a potentially vast, still largely overlooked and poorly understood area of mitochondrial medicine, and will stand as a new challenge in the nosological and physiopathological definition of these disorders in the future.

Nuclear gene mutations

A clinical–genetic classification can now be proposed for these defects, as follows (Leonard and Schapira, 2000*b*; DiMauro and Schon, 2003; Zeviani *et al*., 2003): (i) disorders due to gene defects altering the stability of mtDNA (Table 3); (ii) disorders due to nuclear gene defects encoding structural components or assembly factors of the OXPHOS complexes (Table 3); (iii) disorders due to defects in non-protein components of the respiratory chain (Table 3) and (iv) disorders due to gene defects encoding proteins indirectly related to OXPHOS (Table 4).

Disorders due to gene defects altering the stability of mtDNA

Autosomal dominant progressive external ophthalmoplegia (adPEO) is a mendelian disorder characterized by the accumulation of multiple deletions of mtDNA in patient's tissues (Zeviani *et al*., 1989). The typical clinical feature of adPEO is progressive muscle weakness, most severely affecting the external eye muscles. Skeletal muscle shows RRFs and a mild reduction in the activities of respiratory chain enzymes. Ataxia, depression, hypogonadism, hearing loss, peripheral neuropathy and cataract are present in some families (Servidei *et al*., 1991; Hirano *et al*., 2001).

Most of the adPEO families carry heterozygous mutations in one of three genes: *ANT1*, encoding the muscle-heart-specific mitochondrial adenine nucleotide translocator (Kaukonen *et al*., 2000), *Twinkle*, encoding a putative mtDNA helicase (Spellbrink *et al*., 2001), and *POLG1*, encoding the catalytic subunit of the mtDNA-specific polymerase gamma (Van Goethem *et al*., 2001). Mutations in both *POLG1* alleles were also found in autosomal recessive PEO sibships with multiple affected members and in apparently sporadic cases (Lamantea *et al*., 2002). A prevalent mutation, the Y955C, dramatically reduces the apparent binding affinity for nucleoside triphosphates *in vitro* and also the accuracy for base pair substitutions (Ponomarev *et al*., 2002).

Table 3 *Mitochondrial OXPHOS diseases due to nuclear mutations*

	Phenotype	Disease
Genes controlling the stability of mtDNA		
ANT1	Multiple deletions mtDNA, PEO, muscle weakness, ataxia, depression, hypogonadism, hearing loss, peripheral neuropathy	adPEO
Twinkle		
POLG1		
TP (autosomal dominant or recessive)	Multiple deletion/depletion mtDNA, ophthalmoparesis, peripheral neuropathy, leucoencephalopathy, and gastrointestinal symptoms with intestinal dismotility	MNGIE
TK2	Fatal infantile congenital myopathy with or without a DeToni–Fanconi renal syndrome	MDS
DGUOK	Fatal infantile hepatopathy leading to rapidly progressive liver failure	
Deoxynucleotide carrier	Congenital microcephaly of Amish	
Genes encoding protein respiratory chain components		
Complex I *NDUFS1*	Leigh syndrome, complex I deficiency	Autosomal recessive mutations
Complex I *NDUFS2*	Cardiomyopathy–encephalomyopathy	Autosomal recessive mutations
Complex I *NDUSFS4*	Leigh-like syndrome	Autosomal recessive mutations
Complex I *NDUFS7*	Leigh syndrome	Autosomal recessive mutations
Complex I *NDUFS8*	Leigh syndrome	Autosomal recessive mutations
Complex I *NDUFV1*	Leigh syndrome, leucodystrophy, myoclonus	Autosomal recessive mutations
Complex II *SDHA*	Leigh syndrome	Autosomal recessive mutations
Complex II *SDHB*	Phaeochromocytoma, cervical paraganglioma	Autosomal dominant or sporadic
Complex II *SDHC* and *SDHD*	Hereditary paraganglioma	Autosomal recessive mutations
Complex III *UQCRB* gene subunit VII	Hypokalaemia and lactic acidosis	Autosomal recessive homozygous deletion
Defects of non-protein respiratory chain constituents		
Coenzyme Q deficiency	Ataxia, seizures, myopathy	(?)
Tafazzin (cardiolipin acyltransferase?)	Barth syndrome	X-linked recessive
Genes encoding respiratory chain assembly components		
SURF1	COX^- Leigh syndrome	Autosomal recessive mutations
SCO1	COX^- hepathopathy and ketoacidotic coma	Autosomal recessive mutations
SCO2	COX^- infantile cardiomyopathy	Autosomal recessive mutations
COX10	COX^- leucodystrophy and renal tubulopathy	Autosomal recessive mutations
COX15	COX^- hypertrophic cardiomyopathy	Autosomal recessive mutations
BCS1L	Complex III-deficient encephalopathy, liver failure, renal tubulopathy	Autosomal recessive mutations
LRPPRC (mRNA-binding protein)	COX^- Leigh syndrome	Autosomal recessive mutations
ATP12	Complex V deficiency–encephalopathy	Autosomal recessive mutations

Table 4 *Mitochondrial diseases due to nuclear mutations of genes indirectly involved in OXPHOS*

Disease	Phenotype	Nuclear DNA mutation
Freidreich's ataxia (*FRDA1* gene)	Ataxia, loss of DTR, sensory neuropathy, Babinski sign, cardiomyopathy, diabetes	Autosomal recessive mutation in the *frataxin* gene (iron handler iron–sulfur cluster assembly)
X-linked ataxia and sideroblastic anaemia	Ataxia, sideroblastic anaemia	Autosomal recessive mutation in the *ABC7* iron exporter
Hereditary spastic paraplegia	Spastic paraplegia	Autosomal recessive mutation in the *SPG7* gene encoding a metalloprotease
X-linked deafness–dystonia syndrome	Deafness and dystonia	X-linked recessive mutation in the *DDP1* gene encoding protein mitochondrial transporter
Autosomal dominant optic atrophy (*OPA1* gene)	Optic atrophy and visual failure	Autosomal dominant mutations in the *OPA1* gene encoding a dynamin-related protein

Another disease in this series, mitochondrial neurogastro-intestinal encephalomyopathy (MNGIE), is a devastating disorder of juvenile onset, characterized by ophthalmoparesis, peripheral neuropathy, leucoencephalopathy and gastrointestinal symptoms with intestinal dismotility, and histologically abnormal mitochondria in muscle (Hirano *et al*., 1994). Mutations in the gene encoding thymidine phosphorylase (TP), leading to loss of activity of the enzyme, are associated with MNGIE (Nishino *et al*., 1999). TP is an important factor involved in the control and maintenance of the pyrimidine nucleoside pool of the cell. Defects of TP are thought to produce an excess of dTTP, resulting in the imbalance of dNTP pools that can ultimately affect both the rate and fidelity of mtDNA replication. This is reflected by the molecular phenotype of MNGIE, which is characterized by both multiple deletions and partial depletion of muscle mtDNA (Nishino *et al*., 2000).

mtDNA depletion syndrome (MDS) is a heterogeneous group of disorders characterized by a reduction in mtDNA copy number (Moraes *et al*., 1991). Clinically, they include a fatal infantile congenital myopathy with or without DeToni–Fanconi renal syndrome, fatal infantile hepatopathy leading to rapidly progressive liver failure, and late infantile or childhood myopathy, with onset after 1 year of age, characterized by a progressive myopathy causing respiratory failure and death by 3 years of age.

The presence of affected siblings born from healthy parents suggested an autosomal recessive mode of inheritance, possibly affecting a nuclear gene involved in the control of the mtDNA copy number. An important contribution to the elucidation of the genetic bases of mtDNA depletion has recently come from studies on selected families. MDS has been linked to mutations in two genes involved in dNTP metabolism: thymidine kinase 2 (*TK2*) and deoxy-guanosine kinase, which are responsible for the myopathic form and the hepatoencephalopathic form of MDS, respectively (Mandel *et al*., 2001; Saada *et al*., 2001). The first reports on these genes have later been confirmed by studies on larger cohorts of MDS patients (Mancuso *et al*., 2002; Salviati *et al*., 2002*a*). Correction of the original *TK2* gene sequence and biochemical investigations *in vitro* on the kinetic properties of mutant TK2 proteins have also been reported (Spinazzola *et al*., 2002). However, defects in *TK2* or guanosine kinase are responsible for only a minor fraction of MDS cases, indicating that the condition is genetically heterogeneous. Both guanosine kinase and *TK2* genes are involved in the formation of the mitochondrial nucleotide pool, as is TP, responsible for MNGIE. Biochemical investigations in patients' cells do suggest that derangement of balanced availability of dNTPs can affect mtDNA integrity and maintenance (Spinazzola *et al*., 2002). However, the pathogenetic relationship between reduction of TP activity, increased levels of thymidine in blood and accumulation of mtDNA lesions remains unclear. This issue is further complicated by the absence of mtDNA abnormality recently reported in knockout mice deficient in either the *TP* gene or in both *TP* and uridine phosphorylase genes (Haraguchi *et al*., 2002).

Adding interest to the role of nucleotide supply in mitochondrial biogenesis and disease is the discovery that a recently identified mitochondrial deoxynucleotide carrier is responsible for a rare form of congenital microcephaly, found in interrelated Old Order Amish. These data indicate that mitochondrial deoxynucleotide transport may be essential for fetal brain development (Rosenberg *et al*., 2002).

Genes encoding protein subunits of the respiratory complexes

Isolated deficiency of complex I is relatively frequent among mitochondrial disorders. The primary genetic defect may be either at the mtDNA or at the nuclear DNA level. There are seven mtDNA-encoded and at least 39 nuclear-encoded subunits of complex I (Carroll *et al*., 2003), for a total of 46 genes, which represents a truly formidable challenge for a systematic genetic screening even in highly selected patients. Nevertheless, several disease-associated complex I mutations have been discovered recently (Triepels *et al*., 2001) (Table 3). In most of these cases, the clinical presentation is that of an early onset progressive neurological disorder with lactic acidosis, most often Leigh syndrome, occasionally complicated by cardiomyopathy, or multisystem involvement (Morris *et al*., 1996). However, no mutation in structural genes has been found in many cases of complex I deficiency, suggesting that still unknown assembly factors for complex I, or other gene products involved in its formation and activity may be responsible for these forms (Smeitink *et al*., 2001).

Complex II is an FAD-dependent enzyme at a cross-point between OXPHOS and Krebs cycle pathways. It is composed of four protein subunits, all encoded by nuclear genes (*SDH-A*, *-B*, *-C*, *-D*). Mutations in *SDHA*, the largest subunit of complex II, are a rare cause of Leigh syndrome or late-onset neurodegenerative disease (Bougeron *et al*., 1995). However, the most interesting discovery concerning defects of complex II is their association with inherited paragangliomas (Baysal, 2002). In 10–15% of the cases, these usually benign neuroectodermal tumours are inherited in an autosomal dominant fashion with incomplete penetrance. It now appears that mutations in *SDHB*, *SDHC* and *SDHD* are responsible for the majority of familial paragangliomas and also for a significant fraction of non-familial tumours, including phaeochromocytomas (tumours of the adrenal medulla) (Baysal *et al*., 2002). The inactivation of the *SDHD* gene is associated with stimulation of the angiogenic pathway, a mechanism that could be involved in the pathogenesis of neoplasm (Gimenez-Roqueplo *et al*., 2001). Finally, the first mutation in a nuclear gene encoding a subunit of complex III has recently been identified in an infant with hypoglycaemic episodes and lactic acidosis. A homozygous 4-bp deletion in the *UQCRB* gene, encoding subunit QP-C (or subunit VII), was associated with an isolated defect of complex III and reduced amount of cytochrome *b* content in isolated mitochondria (Haut *et al*., 2003).

Genes involved in the assembly of respiratory complexes

This group comprises, so far, defects of genes encoding assembly factors of COX (complex IV), ubiquinol-cytochrome *c* reductase (complex III) and ATP synthase (complex V).

Human COX is composed of 13 subunits: the three largest ones are encoded by mtDNA genes, while the remaining subunits are encoded by nuclear genes. In infancy, the most frequent manifestation of isolated, profound COX deficiency is Leigh syndrome, although other phenotypes, including leucoencephalopathy, severe cardiomyopathy or complex encephalocardiomyopathies have also been reported (Shoubridge, 2001). COX defects have been associated with mutations of mtDNA tRNA genes, and also with a few mutations in mtDNA genes encoding COX subunits. No mutation in any of the nucleus-encoded subunits of COX has been reported, while all of the nuclear gene defects of COX so far identified are due to mutations in assembly factors of the enzyme, including SURF1, SCO1, SCO2, COX10 and COX15. SURF1 is a 30 kDa hydrophobic protein located in the inner membrane of mitochondria. Mutations in *SURF1* are relatively frequent, accounting for the majority of the Leigh syndrome cases due to COX deficiency (Tiranti *et al*., 1998). Absence of *SURF1* causes the accumulation of early assembly intermediates and the drastic reduction of fully assembled COX (Tiranti *et al*., 1999). This phenomenon has been observed in different organisms carrying null mutations of *SURF1*, including yeast strains (Nijtmans *et al*., 2001; Barientos *et al*., 2002), human patients (Tiranti *et al*., 1999) and, more recently, *SURF1* knockout mice (Agostino *et al*., 2003).

Mutations in other COX assembly genes are much rarer and, in some cases, they have been reported in only a few families or singleton cases.

Human SCO1 and SCO2 are nuclear-encoded copper-binding proteins, presumed to be responsible for the insertion of Cu into the COX holoenzyme. While mutations in *SCO1* were found in only one family (Valnot *et al*., 2000*a*), mutations in *SCO2* are more frequent (Papadopoulou *et al*., 1999). The usual clinical presentation is that of an early-onset, fatal cardio-encephalo-myopathy with COX deficiency, but clinical variants have been reported resembling early-onset (type 1) spinal muscular atrophy (Salviati *et al*., 2002*b*). Studies in yeast, bacteria and, more recently, humans, have shown that Cu supplementation can restore COX activity in cells harbouring mutations in genes involving Cu transport, including *SCO2* (Jaksch *et al*., 2001; Salviati *et al*., 2002*c*).

Also the product of the *COX10* gene, mapping like the *SCO1* gene on chromosome 17p13, is involved in a crucial step of COX maturation. COX10 encodes haem A: farnesyltransferase, which catalyses the first step in the conversion of protohaem to the haem A prosthetic groups of the enzyme. A homozygous missense mutation in the *COX10* gene was found in the affected members of a consanguineous family with an isolated COX defect leading to an early-onset leucoencephalopathy (Valnot *et al*., 2000*b*).

Similar to COX10, COX15 is involved in the synthesis of haem A, the prosthetic group for COX. Antonicka and colleagues recently identified the first deleterious mutations in *COX15*, in a patient with fatal, infantile hypertrophic cardiomyopathy (Antonicka *et al*., 2002). This study establishes COX15 as an additional cause, along with SCO2, of fatal infantile, hypertrophic cardiomyopathy associated with isolated COX deficiency. However, mutations in *COX15* may also cause Leigh syndrome.

Finally, mutations in *LRPPRC* (leucine-rich motif-PPR containing) have been found in infants with a COX-deficiency syndrome. Sequence analysis identified two mutations on two independent haplotypes, providing definitive genetic proof that genetic mutation in *LRPPRC* is a cause of Leigh syndrome (Mootha *et al*., 2003). LRPPRC encodes an mRNA-binding protein which is likely to be involved with mtDNA transcript processing, suggesting an additional mechanism of mitochondrial pathophysiology.

Complex III catalyses electron transfer from succinate and nicotinamide adenine dinucleotide-linked dehydrogenases to cytochrome *c*. Complex III is made up of 11 subunits, of which all but one (cytochrome *b*) are encoded by nuclear DNA. Although several pathogenic mutations in the gene encoding mitochondrial cytochrome *b* have been described (Andreu *et al*., 1999), mutations in only one nuclear DNA-encoded subunit of complex III (subunit VII or QP-C) has been reported in a single infant patient affected by hypoglycaemia and lactic acidosis. BCS1L, a mitochondrial inner-membrane protein, is a chaperone necessary for the assembly of mitochondrial respiratory chain complex III. Mutations in *BCS1L* have been shown in infantile cases of complex III deficiency associated with neonatal proximal tubulopathy, hepatic involvement and encephalopathy (de Lonlay *et al*., 2001), also called GRACILE (growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis and early death) syndrome (Visapaa *et al*., 2002).

Finally, the first mutation in ATP12, an assembler of mitochondrial ATP synthase, has been identified in a single infant patient with lactic acidosis, dysmorphic features and rapidly progressive encephalopathy. Deficiency of complex V activity was associated with marked reduction of immunodetectable complex V subunits in both muscle and liver mitochondria (De Meirleir *et al*., 2004).

Defects of non-protein constituents of mitochondria

Coenzyme Q10 (CoQ10) deficiency

CoQ10, or ubiquinone, is a lipophilic component of the electron transport chain, which transfers to complex III (ubiquinone-cytochrome *c* reductase) electrons derived from complex I, complex II, and from the oxidation of fatty acids and branched-chain amino acids via flavin-linked dehydrogenases. CoQ10 also plays a role as an antioxidant and as a membrane stabilizer.

Primary CoQ10 deficiency was first described (Ogasahara *et al*., 1989) in two sisters aged 14 and 12 years with abnormal fatiguability and slowly progressive weakness of proximal limb and trunk muscles, seizures and myoglobinuria. In muscle specimens from both patients, all type-1 RRFs also showed marked lipid excess. Biochemical analysis of the respiratory chain in muscle mitochondria revealed normal activities of complexes I, II, III and IV, while the combined activities of complexes I–III and II–III were reduced. These results pointed to a defect of CoQ10 which was confirmed in both sisters by direct assay of CoQ10. Treatment with oral CoQ10 improved the muscle weakness, ataxia, learning disability and lactic acidosis in both sisters. A similar syndrome characterized by the triad of recurrent myoglobinuria, brain involvement (seizures, ataxia, mental retardation) and RRFs/lipid storage in muscle has been also reported (Sobreira *et al*., 1997).

A more widespread genetic defect of the respiratory chain associated with severe coenzyme Q deficiency was described in two siblings in whom coenzyme Q deficiency was generalized and present in muscle, blood cells and skin fibroblasts resulting in severe encephalomyopathy and renal failure. Both children had substantial improvement under oral ubidecarenone supplementation (Rotig *et al*., 2000). A variant phenotype with clinical and MRI features of an adult-onset Leigh syndrome has also recently been described in two sisters (Van Maldergem *et al*., 2002).

Finally, a syndrome characterized by low coenzyme Q in muscle, unexplained cerebellar ataxia, pyramidal signs, and seizures, unspecific myopathic change and no myoglobinuria has been reported (Musumeci *et al*., 2001). Irrespective of the genetic causes of this defect, which are presently unknown, early recognition of coenzyme Q deficiency is important, because supplementation of CoQ10 can lead to substantial clinical improvement.

Barth's syndrome

An abnormality of cardiolipin metabolism has been found in Barth syndrome (X-linked mitochondrial myopathy, cardiopathy, neutropenia, short stature and 3-methyl glutaconic aciduria). The product of the mutated gene in Barth syndrome, called tafazzin (Bione *et al*., 1996), is homologous to phospholipid acyltransferases. Cardiolipin is a major component of the phospholipid milieu of the mitochondrial inner membrane (Valianpour *et al*., 2002) where it plays a modulatory role on the activities of several respiratory chain complexes, including complexes I and IV.

Genes encoding mitochondrial factors indirectly related to OXPHOS

Other neurodegenerative disorders have been attributed to mutations in several mitochondrial proteins, which are not obviously linked to overt OXPHOS defects, yet indirectly related to respiration and energy production (Di Donato, 2000) (Table 4). This observation further broadens the concept of mitochondrial disease and extends the possible involvement of mitochondrial energy metabolism in a previously unsuspected large number of important clinical phenotypes. This group includes paraplegin, a mitochondrial metalloprotease associated with autosomal recessive spastic paraplegia (Casari *et al*., 1998); ABC7, an iron mitochondrial exporter, which controls the generation of cytosolic iron–sulfur proteins and is responsible of X-linked sideroblastic anaemia and ataxia (Allikmets *et al*., 1999); frataxin, a mitochondrial protein which is responsible for Friedreich's ataxia, also putatively involved in iron handling and iron–sulfur protein manteinance (Campuzano *et al*., 1996; Puccio *et al*. 2001); and DDP1, a component of the import machinery for mitochondrial carrier proteins, which is responsible of X-linked deafness–dystonia syndrome, the Mohr–Tranebjaerg syndrome (Koehler *et al*., 1999; Roesch *et al*., 2002).

Mutations in *OPA1*, a gene encoding a dynamin-related protein embedded in the mitochondrial inner membrane (Olichon *et al*., 2003), have been found in autosomal dominant optic neuropathy of the Kjehr type (Delettre *et al*., 2002). Haplo-insufficiency of the gene seems to be a common pathogenetic mechanism in *OPA1* mutations. In addition, polymorphisms in the *OPA1* gene have been associated with another ocular condition, normal tension glaucoma (Aung *et al*., 2002; Buono *et al*., 2002). Down-regulation of *OPA1* gene expression in HeLa cells by RNA interference experiments induced fragmentation of the mitochondrial network, concomitant dissipation of membrane potential, disorganization of the cristae, release of cytochrome *c* and activation of caspase-dependent apoptosis (Olichon *et al*., 2003). These findings on *OPA1* are similar to those showing that cells carrying LHON-associated mtDNA mutations are more prone to apoptosis (Ghelli *et al*., 2003), and suggest the existence of a common pathogenetic mechanism for these hereditary optic neuropathies.

Finally, to further expand the spectrum of neurodegenerative disorders associated with impairment of mitochondrial biogenesis and OXPHOS stands the recent observation that missense mutations in *MFN2*, a gene encoding mitofusin 2, lead to Charcot–Marie–Tooth neuropathy type 2A (Züchner *et al*., 2004). Mitofusins are GTPase proteins regulating the fission–fusion dynamics of the mitochondrial network. This is a fundamental process in mitochondrial biogenesis, required for establishing a uniform membrane potential of the organelles, for even energy supply throughout the cell.

New strategies for the discovery of disease loci and genes

Identification of nuclear OXPHOS disease genes is complicated by the scarcity of large-size families and consanguineous families, and by the great heterogeneity of the disorders, that may prevent the possibility of carrying out a genome-wide search for disease loci based on traditional strategies, including linkage analysis and homozygosity

mapping. Therefore, new strategies based, for instance, on functional complementation of OXPHOS phenotype expressed in cell culture have been applied in several cases to elucidate the genetic aetiology of these disorders. An interesting, successful development of these strategies has recently been reported (de Lonley *et al*., 2002). A functional complementation approach was developed by: (i) growing the patient's fibroblasts in a highly selective medium; and (ii) transferring human chromosome fragments into respiratory chain-deficient fibroblasts by microcell-mediated transfer. In the absence of carbohydrates in the culture medium, OXPHOS-deficient cells rapidly disappeared unless they were rescued by a chromosome fragment carrying the disease gene. This method, applied on two cell lines with complex II or complex I + IV defects, allowed the mapping of the disease-causing genes to small intervals (4 and 12 Mb) on chromosomes 12p13 and 7p21, respectively. This approach makes the physical mapping of the disease genes feasible in sporadic cases of OXPHOS deficiency.

The availability of the entire genome of the yeast *Saccharomyces cerevisiae*, and the near completion of the human genome project, including the establishment of expression profiles of human gene clusters in different tissues, will make it possible to use high-throughput strategies, which combine *in vitro* and *in silico* investigations, to assess the human mitochondrial proteome and identify new disease genes. Validation of these new strategies has actually been provided by two recently published papers. A first strategy (Steinmetz *et al*., 2002) exploited the high similarity between yeast and human mitochondria to perform a systematic functional screen based on both database interrogation and microarray expression analysis on the whole-genome pool of yeast deletion mutants. Human orthologues were then identified, many of which encode novel proteins, and some of them were linked to heritable diseases using genomic map positions. A second strategy exploited the availability of whole-genome data sets of RNA and protein expression to identify the gene causing Leigh syndrome, French-Canadian type, a human COX deficiency that maps to chromosome 2p16–21. By intersecting information derived from RNA expression data sets and a large survey of organellar proteomics with the relevant genomic region, a single clear candidate gene was identified and then proven to be mutated in affected individuals (Mootha *et al*., 2003). A similar strategy has been adopted more recently to identify the gene responsible for ethylmalonic encephalopathy, a complex mitochondrial disorder of infancy characterized by persistent lactic acidosis, abnormal excretion of ethylmalonic acid, progressive neurodegenerative lesions in the brainstem, persistent diarrhoea, peripheral vasculopathy and cytochrome *c* oxidase deficiency in skeletal muscle (Tiranti *et al*., 2004).

Animal models

Mice carrying mtDNA with pathogenic mutations would provide a system in which to study how mutant mtDNAs are transmitted and distributed in tissues, resulting in expression of mitochondrial diseases. The first mouse carrying a heteroplasmic mtDNA deletion has been obtained by isolating respiration-deficient cybrids with mtDNA carrying a deletion and introducing this mtDNA into fertilized eggs (Inoue *et al*., 2000). The mutant mtDNA was transmitted maternally, and its accumulation induced mitochondrial dysfunction in various tissues. Moreover, most of these mice died because of renal failure, suggesting the involvement of mtDNA mutations in the pathogeneses of new diseases.

Two interesting mouse models lacking mtDNA in selected tissues have been developed by knocking out mtTFA, the main transcription/replication activator of mtDNA. Conditional mice were developed lacking mtTFA and mtDNA in frontal cortex neurons during embryonic development (Wang *et al*., 1999). Knockout mice survived for several months before dying from massive apoptosis of cortical neurons. A similar strategy was also adopted to create conditional mtDNA-less mice in skeletal muscle, which developed a typical mitochondrial myopathy with RRFs and COX depletion (Li *et al*., 2000).

The importance of mitochondrial defects in degenerative diseases and ageing has been demonstrated using different mouse models of mitochondrial disease (Melov *et al*., 1999), including knockouts for Ant1 the adenine nucleotide translocator, Surf1 and the mitochondrial manganese superoxide dismutase. More recently, a mtDNA mutation imparting chloramphenicol resistance to mitochondrial protein synthesis has been transferred into mice and resulted in growth retardation and cardiomyopathy (Levy *et al*., 1999).

Treatment

No effective therapy is available for mitochondrial disorders. This is due to the lack, until recently, of suitable animal models, as well as to the rarity and heterogeneity of the disorders. However, several supportive measures, such as improvement of nutrition, surgical correction of ptosis, treatment of seizures and other complications, correction of lactic acidosis, etc., can ameliorate specific problems and improve the quality of life in several cases.

Gene therapy

A number of experimental strategies are currently being pursued. These include the introduction of modified genes or gene products into mitochondria via the protein import machinery (Manfredi *et al*., 2002) and inhibition of replication of mutant mtDNA by sequence-specific antigenomic peptide-nucleic acids (Taylor *et al*., 1997). These approaches are not yet clinically relevant. In selected, isolated myopathy cases, reduction of heteroplasmic mutant load was obtained by controlled muscle fibre damage and regeneration by mutation-free satellite cells, using myotoxic drugs (Irwin *et al*., 2002). However, this treatment was not effective in improving ptosis in five patients with PEO.

Metabolic therapy

Creatine, the latest compound proposed for treatment of mitochondrial disorders, is the substrate for the synthesis of phosphocreatine, the most abundant energy storage compound in muscle, heart and brain. An open trial of 81 patients with various neuromuscular disorders (including 17 with mitochondrial diseases) showed significant improvement of ischaemic isometric handgrip strength and non-ischaemic isometric dorsiflexion torque. Another placebo-controlled, double-blind, randomized crossover trial in 16 patients with chronic progressive external ophthalmoplegia or mitochondrial myopathy, however, did not find significant effects on exercise performance, eye movements, or activities of daily life (Chinnery and Turnbull, 2001). Taken together, these data suggest that creatine may be effective in some, but not all mitochondrial diseases. As creatine is virtually free of adverse effects, its administration may be warranted in patients with muscle weakness even before a large controlled trial resolves the issue of its efficacy. While CoQ10 is not effective in mtDNA-associated mitochondrial disease (Bresolin *et al*., 1990) it leads to marked improvement in 'primary' CoQ10 deficiency (see above). Idebenone, a shorter chain analogue of CoQ10, appears to be effective in halting or even improving the hypertrophic cardiomyopathy in Friedreich's ataxia (Rustin *et al*., 1999; Mariotti *et al*., 2003). Further trials are currently underway to confirm this exciting preliminary observation.

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References

Agostino A, Invernizzi F, Tiveron C, Fagiolari G, Prelle A, Lamantea E, et al. Constitutive knockout of Surf1 is associated with high embryonic lethality, mitochondrial disease and cytochrome c oxidase deficiency in mice. Hum Mol Genet 2003; 12: 399–413.

Allikmets R, Raskind WH, Hutchinson A, Schueck ND, Dean M, Koeller DM. Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A). Hum Mol Genet 1999; 8: 743–9.

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. Nature 1981; 290: 457–65.

Andreu AL, Hanna MG, Reichmann H, Bruno C, Penn AS, Tanji K, et al. Exercise intolerance due to mutations in the cytochrome b gene of mitochondrial DNA. N Engl J Med 1999; 341: 1037–44.

Ankel-Simons F, Cummins JM. Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. Proc Natl Acad Sci USA 1996; 93: 13859–63.

Antonicka H, Mattman A, Carlson CG, Glerum DM, Hoffbuhr KC, Leary SC, et al. Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy. Am J Hum Genet 2003; 72: 101–14.

Aung T, Ocaka L, Ebenezer ND, Morris AG, Krawczak M, Thiselton DL, et al. A major marker for normal tension glaucoma: association with polymorphisms in the OPA1 gene. Hum Genet 2002; 110: 52–6.

Austin SA, Vriesendorp FJ, Thandroyen FT, Hecht JT, Jones OT, Johns DR. Expanding the phenotype of the 8344 transfer RNAlysine mitochondrial DNA mutation. Neurology 1998; 51: 1447–50.

Babcock GT, Wikström M. Oxygen activation and the conservation of energy in cell respiration. Nature 1992; 356: 301–9.

Barkovich A, Good W, Koch T, Berg B. Mitochondrial disorders: analysis of their clinical and imaging characteristics. AJNR Am J Neuroradiol 1993; 14: 1119–37.

Baysal BE. Hereditary paraganglioma targets diverse paraganglia. J Med Genet 2002; 39: 617–22.

Baysal BE, Willett-Brozick JE, Lawrence EC, Drovdlic CM, Savul SA, McLeod DR, et al. Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. J Med Genet 2002; 39: 178–83.

Berkovic SF, Carpenter S, Evans A, Karpati G, Shoubridge EA, Andermann F, et al. Myoclonus epilepsy and ragged-red fibres (MERRF). A clinical, pathological, biochemical, magnetic resonance spectrographic and positron emission tomographic study. Brain 1989; 112: 1231–60.

Berkovic SF, Cochius J, Andermann E, Andermann F. Progressive myoclonus epilepsies: clinical and genetic aspects. Epilepsia 1993; 34 Suppl 3: S19–30.

Bione S, D'Adams P, Maestrini E, Gedeon AK, Bolhuis PA, Toniolo D. A novel X-linked gene, G4.5 is responsible for Barth syndrome. Nat Genet 1996; 12: 385–9.

Bourgeron T, Rustin P, Chretien D, Birch-Machin M, Bourgeois M, Viegas-Pequignot E, et al. Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. Nat Genet 1995; 11: 144–9.

Bresolin N, Doriguzzi C, Ponzetto C, Angelini C, Moroni I, Castelli E, et al. Ubidecarenone in the treatment of mitochondrial myopathies: a multicenter double-blind trial. J Neurol Sci 1990; 100: 70–8.

Bristow EA, Griffiths PG, Andrews RM, Johnson MA, Turnbull DM. The distribution of mitochondrial activity in relation to optic nerve stricture. Arch Ophthalmol 2002; 120: 791–6.

Brown MD, Starikovskaya E, Derbeneva O, Hosseini S, Allen JC, Mikhailovskaya IE, et al. The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup J. Hum Genet 2002; 110: 130–8.

Buono LM, Foroozan R, Sergott RC, Savino PJ. Is normal tension glaucoma actually an unrecognized hereditary optic neuropathy? New evidence from genetic analysis. Curr Opin Ophthalmol 2002; 13: 362–70.

Bykhovskaya Y, Estivill X, Taylor K, Hang T, Hamon M, Casano RA, et al. Candidate locus for a nuclear modifier gene for maternally inherited deafness. Am J Hum Genet 2000; 66: 1905–10.

Campuzano V, Montermini L, Molto MD, Pianese L, Cossee M, Cavalcanti F, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 1996; 271: 1423–7.

Carelli V, Ross-Cisneros FN, Sadun AA. Optic nerve degeneration and mitochondrial dysfunction: genetic and acquired optic neuropathies. Neurochem Int 2002a; 40: 573–84.

Carelli V, Baracca A, Barogi S, Pallotti F, Valentino ML, Montagna P, et al. Biochemical–clinical correlation in patients with different loads of the mitochondrial DNA T8993G mutation. Arch Neurol 2002b; 59: 264–70.

Carroll J, Fearnley IM, Shannon RJ, Hirst J, Walker JE. Analysis of the subunit composition of complex I from bovine heart mitochondria. Mol Cell Proteomics 2003; 2: 117–26.

Casali C, Santorelli FM, D'Amati G, Bernucci P, DeBiase L, DiMauro S. A novel mtDNA point mutation in maternally inherited cardiomyopathy. Biochem Biophys Res Commun 1995; 213: 588–93.

Casari G, De Fusco M, Ciarmatori S, Zeviani M, Mora M, Fernandez P, et al. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. Cell 1998; 93: 973–83.

Chalmers RM, Davis MB, Sweeney MG, Wood NW, Harding AE. Evidence against an X-linked visual loss susceptibility locus in Leber hereditary optic neuropathy. Am J Hum Genet 1996; 59: 103–8.

Chen X, Prosser R, Simonetti S, Sadlock J, Jagiello G, Schon EA. Rearranged mitochondrial genomes are present in human oocytes. Am J Hum Genet 1995; 57: 239–47.

Chinnery PF, Turnbull DM. Mitochondrial DNA and disease. Lancet 1999; 354 Suppl I: SI17–21.

Chinnery PF, Turnbull DM. Epidemiology and treatment of mitochondrial disorders. Am J Med Genet 2001; 106: 94–101.

Chinnery PF, Johnson MA, Wardell TM, Singh-Kler R, Hayes C, Brown DT, et al. The epidemiology of pathogenic mitochondrial DNA mutations. Ann Neurol 2000; 48: 188–93.

Chinnery PF, Brown DT, Andrews RM, Singh-Kler R, Riordan-Eva P, Lindley J, et al. The mitochondrial ND6 gene is a hot spot for mutations that cause Leber's hereditary optic neuropathy. Brain 2001; 124: 209–18.

Chol M, Lebon S, Benit P, Chretien D, de Lonlay P, Goldenberg A, et al. The mitochondrial DNA G13513A MELAS mutation in the NADH dehydrogenase 5 gene is a frequent cause of Leigh-like syndrome with isolated complex 1 deficiency. J Med Genet 2003; 40: 188–91.

Clayton DA. Replication and transcription of vertebrate mitochondrial DNA. Annu Rev Cell Biol 1991; 7: 453–78.

Corona P, Antozzi C, Carrara F, D'Incerti L, Lamantea E, Tiranti V, et al. A novel mtDNA mutation in the ND5 subunit of complex I in two MELAS patients. Ann Neurol 2001; 49: 106–10.

de Lonlay P, Valnot I, Barrientos A, Gorbatyuk M, Tzagoloff A, Taanman JW, et al. A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. Nat Genet 2001; 29: 57–60.

de Lonlay P, Mugnier C, Sanlaville D, Chantrel-Groussard K, Benit P, Lebon S, et al. Cell complementation using Genebridge 4 human:rodent hybrids for physical mapping of novel mitochondrial respiratory chain deficiency genes. Hum Mol Genet 2002; 11: 3273–81.

De Meirleir L, Seneca S, Lissens W, De Clercq I, Eyskens F, Gerlo E, et al. Respiratory chain complex V deficiency due to a mutation in the assembly gene ATP12. J Med Genet 2004; 41: 120–4.

de Vries DD, van Engelen BG, Gabreels FJ, Ruitenbeek W, van Oost BA. A second missense mutation in the mitochondrial ATPase 6 gene in Leigh's syndrome. Ann Neurol 1993; 34: 410–12.

Delettre C, Lenaers G, Pelloquin L, Belenguer P, Hamel CP. OPA1 (Kjer type) dominant optic atrophy: a novel mitochondrial disease. Mol Genet Metab 2002; 75: 97–107.

Di Donato S. Disorders related to mitochondrial membranes: pathology of the respiratory chain and neurodegeneration. J Inherit Metab Dis 2000; 23: 247–63.

DiMauro S, De Vivo DC. Genetic heterogeneity in Leigh syndrome. Ann Neurol 1996; 40: 5–7.

DiMauro S, Schon E Mitochondrial respiratory-chain diseases. N Engl J Med 2003; 348: 2656–68.

Dionisi-Vici C, Seneca S, Zeviani M, Fariello G, Rimoldi M, Bertini E, et al. Fulminant Leigh syndrome and sudden unexpected death in a family with the T9176C mutation of the mitochondrial ATPase 6 gene. J Inherit Metab Dis 1998; 21: 2–8.

Dunbar DR, Moonie PA, Jacobs HT, Holt IJ. Different cellular backgrounds confer a marked advantage to either mutant or wild-type mitochondrial genomes. Proc Natl Acad Sci USA 1995; 92: 6562–6.

Estivill X, Govea N, Barcelo E, Badenas C, Romero E, Moral L, et al. Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment with aminoglycosides. Am J Hum Genet 1998; 62: 27–35.

Falkenberg M, Gaspari M, Rantanen A, Trifunovic A, Larsson NG, Gustafsson CM. Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. Nat Genet 2002; 31: 289–94.

Finnila S, Majamaa K. Lack of a modulative factor in locus 8p23 in a Finnish family with nonsyndromic sensorineural hearing loss associated with the 1555A>G mitochondrial DNA mutation. Eur J Hum Genet 2003; 11: 652–8.

Ghelli A, Zanna C, Porcelli AM, Schapira AH, Martinuzzi A, Carelli V, et al. Leber's hereditary optic neuropathy (LHON) pathogenic mutations induce mitochondrial-dependent apoptotic death in transmitochondrial cells incubated with galactose medium. J Biol Chem 2003; 278: 4145–50.

Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci USA 1980; 77: 6715–19.

Gimenez-Roqueplo AP, Favier J, Rustin P, Mourad JJ, Plouin PF, Corvol P, et al. The R22X mutation of the SDHD gene in hereditary paraganglioma abolishes the enzymatic activity of complex II in the mitochondrial respiratory chain and activates the hypoxia pathway. Am J Hum Genet 2001; 69: 1186–97.

Goto Y, Nonaka I, Horai S. A mutation in $tRNA^{Leu(UUR)}$ gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 1990; 348: 651–3.

Hammans SR, Sweeney MG, Brockington M, Lennox GG, Lawton NF, Kennedy CR, et al. The mitochondrial DNA transfer RNA(Lys)A→G(8344) mutation and the syndrome of myoclonic epilepsy with ragged red fibres (MERRF). Relationship of clinical phenotype to proportion of mutant mitochondrial DNA. Brain 1993; 116: 617–32.

Haraguchi M, Tsujimoto H, Fukushima M, Higuchi I, Kuribayashi H, Utsumi H, et al. Targeted deletion of both thymidine phosphorylase and uridine phosphorylase and consequent disorders in mice. Mol Cell Biol 2002; 22: 5212–21.

Haut S, Brivet M, Touati G, Rustin P, Lebon S, Garcia-Cazorla A, et al. A deletion in the human QP-C gene causes a complex III deficiency resulting in hypoglycaemia and lactic acidosis. Hum Genet 2003; 113: 118–22.

Hayashi J, Ohta S, Kikuchi A, Takemitsu M, Goto Y, Nonaka I. Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking mitochondrial DNA results in mitochondrial dysfunction. Proc Natl Acad Sci USA 1991; 88: 10614–18.

Hirano M, Ricci E, Koenigsberger MR, Defendini R, Pavlakis SG, DeVivo DC, et al. MELAS: an original case and clinical criteria for diagnosis. Neuromuscul Disord 1992; 2: 125–35.

Hirano M, Silvestri G, Blake DM, Lombes A, Minetti C, Bonilla E, et al. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. Neurology 1994; 44: 721–7.

Hirano M, Marti R, Ferreiro-Barros C, Vila MR, Tadesse S, Nishigaki Y, et al. Defects of intergenomic communication: autosomal disorders that cause multiple deletions and depletion of mitochondrial DNA. Semin Cell Dev Biol 2001; 12: 417–27.

Hofmann S, Jaksch M, Bezold R, Mertens S, Aholt S, Paprotta A, et al. Population genetics and disease susceptibility: characterization of central European haplogroups by mtDNA gene mutations, correlation with D loop variants and association with disease. Hum Mol Genet 1997; 6: 1835–46.

Holt IJ, Harding AE, Petty RHK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 1990; 46: 428–33.

Holt IJ, Lorimer HE, Jacobs HT. Coupled leading- and lagging-strand synthesis of mammalian mitochondrial DNA. Cell 2000; 100: 515–24.

Howell N, Mackey DA. Low-penetrance branches in matrilineal pedigrees with Leber hereditary optic neuropathy. Am J Hum Genet 1998; 63: 1220–4.

Howell N, Bindoff LA, McCullough DA, Kubacka I, Poulton J, Mackey D, et al. Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. Am J Hum Genet 1991; 49: 939–50.

Hu DN, Qui WQ, Wu BT, Fang LZ, Zhou F, Gu YP, et al. Genetic aspects of antibiotic induced deafness: mitochondrial inheritance. J Med Genet 1991; 28: 79–83.

Hutchin TP, Cortopassi GA. Mitochondrial defects and hearing loss. Cell Mol Life Sci 2000; 57: 1927–37.

Inoue K, Takai D, Soejima A, Isobe K, Yamasoba T, Oka Y, et al. Mutant mtDNA at 1555 A to G in 12S rRNA gene and hypersusceptibility of mitochondrial translation to streptomycin can be co-transferred to ρ HeLa cells. Biochem Biophys Res Commun 1996; 223: 496–501.

Inoue K, Nakada K, Ogura A, Isobe K, Goto Y, Nonaka I, et al. Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes. Nat Genet 2000; 26: 176–81.

Irwin W, Fontaine E, Agnolucci L, Penzo D, Betto R, Bortolotto S, et al. Bupivacaine myotoxicity is mediated by mitochondria. J Biol Chem. 2002; 277: 12221–7.

Jaber L, Shohat M, Bu X, Fischel-Ghodsian N, Yang HY, Wang SJ, et al. Sensorineural deafness inherited as a tissue specific mitochondrial disorder. J Med Genet 1992; 29: 86–90.

Jaksch M, Klopstock T, Kurlemann G, Dorner M, Hofmann S, Kleinle S, et al. Progressive myoclonus epilepsy and mitochondrial myopathy associated with mutations in the tRNA(Ser(UCN)) gene. Ann Neurol 1998; 44: 635–40.

Jaksch M, Paret C, Stucka R, Horn N, Muller-Hocker J, Horvath R, et al. Cytochrome c oxidase deficiency due to mutations in SCO2, encoding a mitochondrial copper-binding protein, is rescued by copper in human myoblasts. Hum Mol Genet 2001; 10: 3025–35.

Jenuth JP, Peterson AC, Fu K, Shoubridge EA. Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. Nat Genet 1996; 14: 146–51.

Jenuth JP, Peterson AC, Shoubridge EA. Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. Nat Genet 1997; 16: 93–5.

Kaukonen J, Juselius JK, Tiranti V, Kyttala A, Zeviani M, Comi GP, et al. Role of adenine nucleotide translocator 1 in mtDNA maintenance. Science 2000; 289: 782–5.

Kim JY, Hwang J-M, Park SS. Mitochondrial DNA C4171A/ND1 is a novel primary causitive mutation of Leber's hereditary optic neuropathy with a good prognosis. Ann Neurol 2002; 51: 630–4.

Koehler CM; Leuenberge D, Merchant S, Renold A, Junne T, Schatz, G. Human deafness dystonia syndrome is a mitochondrial disease. Proc Natl Acad Sci USA 1999; 96: 2141–6.

Kwittken J, Barest HD. The neuropathology of hereditary optic atrophy (Leber's disease); the first complete anatomic study. Am J Pathol 1958; 34: 185–207.

Lamantea E, Tiranti V, Bordoni A, Toscano A, Bono F, Servidei S, et al. Mutations of mitochondrial DNA polymerase gammaA are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. Ann Neurol 2002; 52: 211–19.

Leigh D Subacute necrotizing encephalomyelopathy in an infant. J Neurochem 1951; 14: 216–21.

Leonard JV, Schapira AVH. Mitochondrial respiratory chain disorders I: mitochondrial DNA defects. Lancet 2000a; 355: 299–304.

Leonard JV, Schapira AVH. Mitochondrial respiratory chain disorders II: neurodegenerative disorders and nuclear gene defects. Lancet 2000b; 355: 389–94.

Levy SE, Waymire KG, Kim YL, MacGregor GR, Wallace DC. Transfer of chloramphenicol-resistant mitochondrial DNA into the chimeric mouse. Transgenic Res 1999; 8: 137–45.

Li H, Wang J, Wilhelmsson H, Hansson A, Thoren P, Duffy J, et al. Genetic modification of survival in tissue-specific knockout mice with mitochondrial cardiomyopathy. Proc Natl Acad Sci USA 2000; 97: 3467–72.

Limongelli A, Schaefer J, Jackson S, Invernizzi F, Kirino Y, Suzuki T, et al. Variable penetrance of a familial progressive necrotising encephalopathy due to a novel tRNA(Ile) homoplasmic mutation in the mitochondrial genome. J Med Genet 2004; 41: 342–349.

Lombes A, Mendell JR, Nakase H, Barohn RJ, Bonilla E, Zeviani M, et al. Myoclonic epilepsy and ragged-red fibers with cytochrome oxidase deficiency: neuropathology, biochemistry, and molecular genetics. Ann Neurol 1989; 26: 20–33.

Luft R. The development of mitochondrial medicine. Proc Natl Acad Sci USA 1994; 91: 8731–8.

Mackey D, Howell N. A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual mitochondrial genetic etiology. Am J Hum Genet 1992; 51: 1218–28.

Mancuso M, Salviati L, Sacconi S, Otaegui D, Camano P, Marina A, et al. Mitochondrial DNA depletion: mutations in thymidine kinase gene with myopathy and SMA. Neurology 2002; 59: 1197–202.

Mandel H, Szargel R, Labay V, Elpeleg O, Saada A, Shalata A, et al. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. Nat Genet 2001; 29: 337–41.

Manfredi G, Fu J, Ojaimi J, Sadlock JE, Kwong JQ, Guy J, et al. Rescue of a deficiency in ATP synthesis by transfer of MTATP6, a mitochondrial DNA-encoded gene, to the nucleus. Nat Genet 2002; 30: 394–9.

Mariotti C, Tiranti V, Carrara F, Dallapiccola B, DiDonato S, Zeviani M. Defective respiratory capacity and mitochondrial protein synthesis in transformants cybrids harboring the $tRNA^{Leu(UUR)}$ mutation associated with maternally inherited myopathy and cardiomyopathy. J Clin Invest 1994; 93: 1102–107.

Mariotti C, Solari A, Torta D, Marano L, Fiorentini C, Di Donato S. Idebenone treatment in Friedreich patients: one-year-long randomized placebo-controlled trial. Neurology 2003; 60: 1676–98.

McFarland R, Clark KM, Morris AA, Taylor RW, Macphail S, Lightowlers RN, et al. Multiple neonatal deaths due to a homoplasmic mitochondrial DNA mutation. Nat Genet 2002; 30: 145–6.

Melov S, Coskun PE, Wallace DC. Mouse models of mitochondrial disease, oxidative stress, and senescence. Mutat Res 1999; 434: 233–42.

Mita S, Schmidt B, Schon EA, DiMauro S, Bonilla E. Detection of 'deleted' mitochondrial genomes in cytochrome-c oxidase-deficient muscle fibers of a patient with Kearns–Sayre syndrome. Proc Natl Acad Sci USA 1989; 86: 9509–13.

Mizukami K, Sasaki M, Suzuki T, Shiraishi H, Koizumi J, Ohkoshi N, et al. Central nervous system changes in mitochondrial encephalomyopathy: a light and electron microscopic study. Acta Neuropathol (Berl) 1992; 83: 449–52.

Mootha VK, Lepage P, Miller K, Bunkenborg J, Reich M, Hjerrild M, et al. Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics. Proc Natl Acad Sci USA 2003; 100: 605–10.

Moraes CT, Shanske S, Tritschler HJ, Aprille JR, Andreetta F, Bonilla E, et al. mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. Am J Hum Genet 1991; 48: 492–501.

Morris AA, Leonard JV, Brown GK, Bidouki SK, Bindoff LA, Woodward CE, et al. Deficiency of respiratory chain complex I is a common cause of Leigh disease. Ann Neurol 1996; 40: 25–30.

Musumeci O, Naini A, Slonim AE, Skavin N, Hadjigeorgiou GL, Krawiecki N, et al. Familial cerebellar ataxia with muscle coenzyme Q10 deficiency. Neurology 2001; 56: 849–55.

Nijtmans LG, Artal Sanz M, Bucko M, Farhoud MH, Feenstra M, Hakkaart GA, et al. Shy1p occurs in a high molecular weight complex and is required for efficient assembly of cytochrome c oxidase in yeast. FEBS Lett 2001; 498: 46–51.

Nikoskelainen E, Hoyt WF, Nummelin K. Ophthalmoscopic findings in Leber's hereditary optic neuropathy. II. The fundus findings in the affected family members. Arch Ophthalmol 1983; 101: 1059–68.

Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. Science 1999; 283: 689–92.

Nishino I, Spinazzola A, Papadimitriou A, Hammans S, Steiner I, Hahn CD, et al. Mitochondrial neurogastrointestinal encephalomyopathy: an autosomal recessive disorder due to thymidine phosphorylase mutations. Ann Neurol 2000; 47: 792–800.

Ogasahara S, Engel AG, Frens D, Mack D. Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. Proc Natl Acad Sci USA 1989; 86: 2379–82.

Oldfors A, Holme E, Tulinius M, Larsson NG. Tissue distribution and disease manifestations of the tRNA(Lys) A→G(8344) mitochondrial DNA mutation in a case of myoclonus epilepsy and ragged red fibres. Acta Neuropathol (Berl) 1995; 90: 328–33.

Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, et al. Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J Biol Chem 2003; 278: 7743–6.

Papadopoulou LC, Sue CM, Davidson MM, Tanji K, Nishino I, Sadlock JE, et al. Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. Nat Genet 1999; 23: 333–7.

Ponamarev MV, Longley MJ, Nguyen D, Kunkel TA, Copeland WC. Active site mutation in DNA polymerase gamma associated with progressive external ophthalmoplegia causes error-prone DNA synthesis. J Biol Chem 2002; 277: 15225–8.

Poulton J, Deadman ME, Gardiner RM. Duplications of mitochondrial DNA in mitochondrial myopathy. Lancet 1989; 1: 236–40.

Poyton RO, McEwen JE. Crosstalk between nuclear and mitochondrial genomes. Annu Rev Biochem 1996; 65: 563–607.

Prezant TR, Agapian JV, Bohlman MC, Bu X, Oztas S, Qiu WQ, et al. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet 1993; 4: 289–94.

Puccio H, Simon D, Cossee M, Criqui-Filipe P, Tiziano F, Melki J, et al. Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe–S enzyme deficiency followed by intramitochondrial iron deposits. Nat Genet 2001; 27: 181–6.

Roesch K, Curran SP, Tranebjaerg L, Koehler CM. Human deafness dystonia syndrome is caused by a defect in assembly of the DDP1/TIMM8a–TIMM13 complex. Hum Mol Genet 2002; 11: 477–86.

Rosenberg MJ, Agarwala R, Bouffard G, Davis J, Fiermonte G, Hilliard MS, et al. Mutant deoxynucleotide carrier is associated with congenital microcephaly. Nat Genet 2002; 32: 175–9.

Rotig A, Cormier V, Blanche S, Bonnefont JP, Ledeist F, Romero N, et al. Pearson's marrow–pancreas syndrome. A multisystem mitochondrial disorder in infancy. J Clin Invest 1990; 86: 1601–8.

Rotig A, Appelkvist EL, Geromel V, Chretien D, Kadhom N, Edery P, et al. Quinone-responsive multiple respiratory chain dysfunction due to widespread coenzyme Q10 deficiency. Lancet 2000; 356: 391–5.

Rustin P, von Kleist-Retzow JC, Chantrel-Groussard K, Sidi D, Munnich A, et al. Effect of idebenone on cardiomyopathy in Friedreich's ataxia: a preliminary study. Lancet 1999; 354: 477–9.

Saada A, Shaag A, Mandel H, Nevo Y, Eriksson S, Elpeleg O. Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. Nat Genet 2001; 29: 342–4.

Sadun AA, Win PH, Ross-Cisneros FN, Walker S, Carelli V. Leber's hereditary optic neuropathy differentially affects smaller axons in the optic nerve. Trans Am Ophthalmol Soc 2000; 98: 223–32.

Salviati L, Sacconi S, Mancuso M, Otaegui D, Camano P, Marina A, et al. Mitochondrial DNA depletion and dGK gene mutations. Ann Neurol 2002a; 52: 311–17.

Salviati L, Hernandez-Rosa E, Walker WF, Sacconi S, DiMauro S, Schon EA, et al. Copper supplementation restores cytochrome c oxidase activity in cultured cells from patients with SCO2 mutations. Biochem J 2002b; 363: 321–7.

Salviati L, Sacconi S, Rasalan MM, Kronn DF, Braun A, Canoll P, et al. Cytochrome c oxidase deficiency due to a novel SCO2 mutation mimics Werdnig–Hoffmann disease. Arch Neurol 2002c; 59: 862–5.

Santorelli FM, Tessa A, D'amati G, Casali C. The emerging concept of mitochondrial cardiomyopathies. Am Heart J 2001; 141: E1.

Saraste M. Oxidative phosphorylation at the fin de siecle. Science 1999; 283: 1488–93.

Schon EA, Rizzuto R, Moraes CT, Nakase H, Zeviani M, DiMauro S. A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. Science 1989; 244: 346–9.

Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. N Engl J Med 2002; 347: 576–80.

Servidei S, Zeviani M, Manfredi G, Ricci E, Silvestri G, Bertini E, et al. Dominantly inherited mitochondrial myopathy with multiple deletions of mitochondrial DNA: clinical, morphologic, and biochemical studies. Neurology 1991; 41: 1053–9.

Shanske S, Tang Y, Hirano M, Nishigaki Y, Tanji K, Bonilla E, et al. Identical mitochondrial DNA deletion in a woman with ocular myopathy and in her son with Pearson syndrome. Am J Hum Genet 2002; 71: 679–683.

Shoffner JM, Lott MT, Voljavec AS, Soueidan SA, Costigan DA, Wallace DC. Spontaneous Kearns–Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. Proc Natl Acad Sci USA 1989; 86: 7952–56.

Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA $tRNA^{Lys}$ mutation. Cell 1990; 61: 931–7.

Shoffner JM, Brown MD, Stugard C, Jun AS, Pollock S, Haas RH, et al. Leber's hereditary optic neuropathy plus dystonia is caused by a mitochondrial DNA point mutation. Ann Neurol 1995; 38: 163–9.

Shoubridge EA. Cytochrome c oxidase deficiency. Am J Med Genet 2001; 106: 46–52.

Silvestri G, Moraes CT, Shanske S, Oh SJ, DiMauro S. A new mtDNA mutation the $tRNA^{Lys}$ gene associated with myoclonic epilepsy and ragged-red fibers (MERRF). Am J Hum Genet 1992; 51: 1213–17.

Smeitink J, van den Heuvel L, DiMauro S. The genetics and pathology of oxidative phosphorylation. Nat Rev Genet 2001; 2: 342–52.

Smith JL, Hoyt WF, Susac JO. Ocular fundus in acute Leber optic neuropathy. Arch Ophthalmol 1973; 90: 349–54.

Sobreira C, Hirano M, Shanske S, Keller RK, Haller RG, Davidson E, et al. Mitochondrial encephalomyopathy with coenzyme Q10 deficiency. Neurology 1997; 48: 1238–43.

Spelbrink JN, Li FY, Tiranti V, Nikali K, Yuan QP, Tariq M, et al. Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. Nat Genet 2001; 28: 223–31.

Spinazzola A, Marti R, Nishino I, Andreu AL, Naini A, Tadesse S, et al. Altered thymidine metabolism due to defects of thymidine phosphorylase. J Biol Chem. 2002; 277: 4128–33.

Steinmetz LM, Scharfe C, Deutschbauer AM, Mokranjac D, Herman ZS, Jones T, et al. Systematic screen for human disease genes in yeast. Nat Genet 2002; 31: 400–4.

Tatuch Y, Christodoulou J, Feigenbaum A, Clarke JT, Wherret J, Smith C, et al. Heteroplasmic mtDNA mutation (T→G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. Am J Hum Genet 1992; 50: 852–8.

Taylor RW, Chinnery PF, Turnbull DM, Lightowlers RN. Selective inhibition of mutant human mitochondrial DNA replication *in vitro* by peptide nucleic acids. Nat Genet 1997; 15: 212–15.

Taylor RW, Giordano C, Davidson MM, d'Amati G, Bain H, Hayes CM, et al. A homoplasmic mitochondrial transfer ribonucleic acid mutation as a cause of maternally inherited hypertrophic cardiomyopathy. J Am Coll Cardiol 2003; 41: 1786–96.

Thorburn DR, Dahl HH. Mitochondrial disorders: genetics, counseling, prenatal diagnosis and reproductive options. Am J Med Genet 2001; 106: 102–14.

Tiranti V, Chariot P, Carella F, Toscano A, Soliveri P, Girlanda P, et al. Maternally inherited hearing loss, ataxia and myoclonus associated with a novel point mutation in mitochondrial $tRNA^{Ser(UCN)}$ gene. Hum Mol Genet 1995; 4: 1421–7.

Tiranti V, Hoertnagel K, Carrozzo R, Galimberti C, Munaro M, Granatiero M, et al. Mutations of SURF-1 in Leigh disease associated with cytochrome c oxidase deficiency. Am J Hum Genet 1998; 63: 1609–21.

Tiranti V, Galimberti C, Nijtmans L, Bovolenta S, Perini MP, Zeviani M. Characterization of SURF-1 expression and Surf-1p function in normal and disease conditions. Hum Mol Genet. 1999; 8: 2533–40.

Tiranti V, D'Adamo P, Briem E, Ferrari G, Mineri R, Lamantea E, et al. Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene

encoding a mitochondrial matrix protein. Am J Hum Genet 2004; 74: 239–52.

Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, et al. Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am J Hum Genet 1997; 60: 1107–21.

Triepels RH, van Den Heuvel LP, Trijbels JM, Smeitink JA. Respiratory chain complex I deficiency. Am J Med Genet 2001; 106: 37–45.

Tsao K, Aitken PA, Johns DR. Smoking as an aetiological factor in a pedigree with Leber's hereditary optic neuropathy. Br J Ophthalmol 1999; 83: 577–81.

Tsuchiya K, Miyazaki H, Akabane H, Yamamoto M, Kondo H, Mizusawa H, et al. MELAS with prominent white matter gliosis and atrophy of the cerebellar granular layer: a clinical, genetic, and pathological study. Acta Neuropathol (Berl) 1999; 97: 520–4.

Uziel G, Moroni I, Lamantea E, Fratta GM, Ciceri E, Carrara F, et al. Mitochondrial disease associated with the T8993G mutation of the mitochondrial ATPase 6 gene: a clinical, biochemical, and molecular study in six families. J Neurol Neurosurg Psychiatry 1997; 63: 16–22.

Valianpour F, Wanders RJ, Overmars H, Vreken P, Van Gennip AH, Baas F, et al. Cardiolipin deficiency in X-linked cardioskeletal myopathy and neutropenia (Barth syndrome, MIM 302060): a study in cultured skin fibroblasts. J Pediatr 2002; 141: 729–33.

Valnot I, Osmond S, Gigarel N, Mehaye B, Amiel J, Cormier-Daire V, et al. Mutations of the SCO1 gene in mitochondrial cytochrome c oxidase deficiency with neonatal-onset hepatic failure and encephalopathy. Am J Hum Genet 2000a; 67: 1104–9.

Valnot I, von Kleist-Retzow JC, Barrientos A, Gorbatyuk M, Taanman JW, Mehaye B, et al. A mutation in the human heme A:farnesyltransferase gene (COX10) causes cytochrome c oxidase deficiency. Hum Mol Genet 2000b; 9: 1245–9.

Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C. Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. Nat Genet 2001; 28: 211–12.

Van Maldergem L, Trijbels F, DiMauro S, Sindelar PJ, Musumeci O, Janssen A, et al. Coenzyme Q-responsive Leigh's encephalopathy in two sisters. Ann Neurol 2002; 52: 750–4.

Visapaa I, Fellman V, Vesa J, Dasvarma A, Hutton JL, Kumar V, et al. GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in BCS1L. Am J Hum Genet 2002; 71: 863–76.

Wallace DC. Mitochondrial diseases in man and mouse. Science 1999; 283: 1482–7.

Wallace DC, Zheng XX, Lott MT, Shoffner JM, Hodge JA, Kelley RI, et al. Familial mitochondrial encephalomyopathy (MERRF): genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. Cell 1988a; 55: 601–10.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 1988b; 242: 1427–30.

Wang J, Wilhelmsson H, Graff C, Li H, Oldfors A, Rustin P, et al. Dilated cardiomyopathy and atrioventricular conduction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. Nat Genet 1999; 21: 133–7.

Wong A, Cavelier L, Collins-Schramm HE, Seldin MF, McGrogan M, Savontaus ML, et al. Differentiation-specific effects of LHON mutations introduced into neuronal NT2 cells. Hum Mol Genet 2002; 11: 431–8.

Yang MY, Bowmaker M, Reyes A, Vergani L, Angeli P, Gringeri E, et al. Biased incorporation of ribonucleotides on the mitochondrial L-strand accounts for apparent strand-asymmetric DNA replication. Cell 2002; 111: 495–505.

Yao J, Shoubridge EA. Expression and functional analysis of SURF1 in Leigh syndrome patients with cytochrome c oxidase deficiency. Hum Mol Genet 1999; 8: 2541–9.

Zeviani M, Carelli V. Mitochondrial disorders. Curr Opin Neurol 2003; 16: 585–94.

Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, et al. Deletions of mitochondrial DNA in Kearns–Sayre syndrome. Neurology 1988; 38: 1339–46.

Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S, Di Donato S. An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. Nature 1989; 339: 309–11.

Zeviani M, Muntoni F, Savarese N, Serra G, Tiranti V, Carrara F, et al. A MERRF/MELAS overlap syndrome associated with a new point mutation in the mitochondrial DNA $tRNA^{Lys}$ gene. Eur J Hum Genet 1993; 1: 80–7.

Zeviani M, Spinazzola A, Carelli V. Nuclear genes in mitochondrial disorders. Curr Opin Genet Dev 2003; 13: 262–70.

Zhao H, Li R, Wang Q, Yan Q, Deng JH, Han D, et al. Maternally inherited aminoglycoside-induced and nonsyndromic deafness is associated with the novel C1494T mutation in the mitochondrial 12S rRNA gene in a large Chinese family. Am J Hum Genet 2004; 74: 139–52.

Züchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, et al. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot–Marie–Tooth neuropathy type 2A. Nat Genet 2004; 36: 449–51.