Mitochondrial disorders

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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 neuro-gastro-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.

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 multi-heteromeric 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

![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 phosphate; 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).](https://academic.oup.com/brain/article-abstract/127/10/2153/404539)
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 F0 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 heterogeneous. 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

<table>
<thead>
<tr>
<th>Neurological manifestations</th>
<th>Systemic manifestations</th>
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<tr>
<td>Neuromuscular</td>
<td>Heart</td>
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<td>Ophthalmoplegia</td>
<td>Cardiomyopathy</td>
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<td>Myopathy</td>
<td>Cardiac conduction defects</td>
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<td>Exercise intolerance</td>
<td>Endocrine system</td>
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<td>Peripheral sensory–motor neuropathy</td>
<td>Diabetes</td>
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<td>CNS</td>
<td>Exocrine pancreas dysfunction</td>
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<td>Myelopathy</td>
<td>Hypoparathyroidism</td>
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<td>Headache</td>
<td>Multiple endocrinopathy</td>
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<td>Stroke</td>
<td>Short stature</td>
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<td>Seizures</td>
<td>Blood</td>
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<td>Dementia</td>
<td>Pancytopenia</td>
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<td>Sideroblastic anaemia</td>
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<td>Movement disorders</td>
<td>Mesenchymal organs</td>
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<td>Ataxia</td>
<td>Hepatopathy</td>
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<td>Dystonia</td>
<td>Nephropathy</td>
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<td>Parkinsonism</td>
<td>Intestinal pseudo-obstruction</td>
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<td>Myoclonus</td>
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<td>Eye</td>
<td>Metabolism</td>
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<td>Blindness</td>
<td>Metabolic acidosis</td>
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<tr>
<td>Optic neuropathy</td>
<td>Nausea and vomiting</td>
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<tr>
<td>Pigmentary retinopathy</td>
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<tr>
<td>Cataract</td>
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<td>Ear</td>
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<td>Sensorineural deafness</td>
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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 E1\alpha 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

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).
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 mitochondrial-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 ATP synthase (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 polyplloid. 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 polyplody, 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 heteroplastic mtDNA, and increases the remarkable variability in the phenotypic presentations of mitochondrial disorders. Because of mitotic mtDNA segregation and polyplody, a threshold effect dictates the phenotypic expression of a mtDNA-associated character (Jenuth et al., 1997). For a given heteroplastic 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 (Thorburn 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 rho0, 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...
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 neuro-radiological 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 ophthalmoplegia, 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 recurrence 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.

### Table 2 Mitochondrial OXPHOS diseases due to mtDNA mutations

<table>
<thead>
<tr>
<th>Large-scale rearrangements of mtDNA</th>
<th>Phenotype</th>
<th>mtDNA mutation</th>
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</thead>
<tbody>
<tr>
<td>KSS</td>
<td>Ataxia, neuropathy, PEO, pigmentary retinal degeneration, cardiomyopathy and conduction block, short stature, high CSF protein</td>
<td>Single deletions or duplications (mostly sporadic)</td>
</tr>
<tr>
<td>Pearson’s syndrome</td>
<td>Frequent death in infancy, Refractory sideroblastic anaemia with vacuolization of marrow precursors.</td>
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<tr>
<td>PEO</td>
<td>Late-onset bilateral ptosis and ophthalmoplegia, proximal muscle weakness and wasting, and exercise intolerance</td>
<td></td>
</tr>
<tr>
<td>Point mutations of mtDNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MELAS</td>
<td>Stroke-like episodes due to focal brain lesions in the parieto-occipital lobes, lactic acidosis and/or RRFs</td>
<td>Heteroplasmic point mutations (maternally inherited)</td>
</tr>
<tr>
<td>MERRF</td>
<td>Myoclonus, epilepsy, muscle weakness and wasting with RRFs, cerebellar ataxia, deafness and dementia</td>
<td></td>
</tr>
<tr>
<td>NARP</td>
<td>Ataxia, pigmentary retinopathy, peripheral neuropathy and distal neurogenic weakness</td>
<td></td>
</tr>
<tr>
<td>Hearing loss–ataxia–myoclonus</td>
<td>Syndromic hearing loss, myoclonus epilepsy, ataxia, myopathy</td>
<td></td>
</tr>
<tr>
<td>LHON</td>
<td>Loss of central vision, large centro-caecal absolute scotoma, circumpapillary telangiectatic microangiopathy</td>
<td>Homoplasmic point mutations (maternally inherited)</td>
</tr>
<tr>
<td>SNHL</td>
<td>Non-syndromic and aminoglycoside-induced hearing loss</td>
<td></td>
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Transmitochondrial cybrids, obtained by introducing deleted mtDNAs into mtDNA-less rho<sup>0</sup> 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<sup>0</sup>-mutants, which cannot synthesize functional OXPHOS enzymes. However, partial correction of the rho<sup>0</sup>-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<sup>syn</sup> 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 <i>mit</i> 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 feedback 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, pigmented retinopathy and deafness. Ataxia can be observed in some patients. Diabetes, intestinal pseudo-obstructions and cardiomypathy 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<sup>Glu(UUR)</sup> gene, 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, 2000a; 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., 1988). 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 tRNALeu(UUR) gene (Wallace et al., 1988a). 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 (Corona et al., 2001). However, even though the genotype-phenotype correlation between MERRF syndrome and the A8344G mutation is tighter than that of other mutations (Hammons 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 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., 2002b).

**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
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 unknown.

LHON

This was the first maternally inherited disease to be associated with a mtDNA point mutation (Wallace et al., 1988b). 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., 2002a).

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., 1988b; 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., 2002a).

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 that binds aminoglycosides, and increases the similarity of the human 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 that binds aminoglycosides, and increases the similarity of the human 12S rRNA to its bacterial counterpart. The growth rate that binds aminoglycosides, and increases the similarity of the human 12S rRNA to its bacterial counterpart.

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<sup>Val</sup> 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 (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).

A prevalent mutation, the Y955C, has later been identified in a family composed of a healthy mother and three affected daughters. Both parents were never exposed to aminoglycosides (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 expression of the cochlea are very energy dependent and local gene expression may also play a relevant role in the strict expression of the cochlea are very energy dependent and local gene expression may also play a relevant role in the strict expression of the cochlea.

A nuclear gene mutations
A clinical–genetic classification can now be proposed for these defects, as follows (Leonard and Schapira, 2000b; 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 (Lamantia 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

<table>
<thead>
<tr>
<th>Genes controlling the stability of mtDNA</th>
<th>Phenotype</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANT1</td>
<td>Multiple deletions mtDNA, PEO, muscle weakness, ataxia, depression, hypogonadism, hearing loss, peripheral neuropathy</td>
<td>adPEO</td>
</tr>
<tr>
<td>Twinkle</td>
<td>Multiple deletion/depletion mtDNA, ophthalmoplegiasis, peripheral neuropathy, leucoencephalopathy, and gastrointestinal symptoms with intestinal dismotility</td>
<td>MNGIE</td>
</tr>
<tr>
<td>POLG1 (autosomal dominant or recessive)</td>
<td>Fatal infantile congenital myopathy with or without a DeToni–Fanconi renal syndrome</td>
<td>MDS</td>
</tr>
<tr>
<td>TK2</td>
<td>Fatal infantile hepatopathy leading to rapidly progressive liver failure</td>
<td></td>
</tr>
<tr>
<td>DGUOK</td>
<td>Congenital microcephaly of Amish</td>
<td></td>
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<table>
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<tr>
<th>Genes encoding protein respiratory chain components</th>
<th>Phenotype</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I NDUFS1</td>
<td>Leigh syndrome, complex I deficiency</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>Complex I NDUFS2</td>
<td>Cardiomyopathy—encephalomyopathy</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>Complex I NDUFS4</td>
<td>Leigh-like syndrome</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>Complex I NDUFS7</td>
<td>Leigh syndrome</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>Complex II SDHA</td>
<td>Leigh syndrome</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>Complex II SDHB</td>
<td>Leigh syndrome, leucodystrophy, myoclonus</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>Complex II SDHC and SDHD</td>
<td>Hereditary paraganglioma</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>Complex III UQCRB gene subunit VII</td>
<td>Hypokalaemia and lactic acidosis</td>
<td>Autosomal recessive homozgyous deletion</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Defects of non-protein respiratory chain constituents</th>
<th>Phenotype</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coenzyme Q deficiency</td>
<td>Ataxia, seizures, myopathy</td>
<td>(?)</td>
</tr>
<tr>
<td>Tafazzin (cardiolipin acyltransferase?)</td>
<td>Barth syndrome</td>
<td>X-linked recessive</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Genes encoding respiratory chain assembly components</th>
<th>Phenotype</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>SURF1</td>
<td>COX− Leigh syndrome</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>SCO1</td>
<td>COX− hepathopathy and ketoacidotic coma</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>SCO2</td>
<td>COX− infantile cardiomyopathy</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>COX10</td>
<td>COX− leucodystrophy and renal tubulopathy</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>COX15</td>
<td>COX− hypertrophic cardiomyopathy</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>BCS1L</td>
<td>Complex III-deficient encephalopathy, liver failure, renal tubulopathy</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>LRPPRC (mRNA-binding protein)</td>
<td>COX− Leigh syndrome</td>
<td>Autosomal recessive mutations</td>
</tr>
<tr>
<td>ATP12</td>
<td>Complex V deficiency—encephalopathy</td>
<td>Autosomal recessive mutations</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Phenotype</th>
<th>Nuclear DNA mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freidreich’s ataxia (FRDA1 gene)</td>
<td>Ataxia, loss of DTR, sensory neuropathy, Babinski sign, cardiomyopathy, diabetes</td>
<td>Autosomal recessive mutation in the frataxin gene (iron handler iron–sulfur cluster assembly)</td>
</tr>
<tr>
<td>X-linked ataxia and sideroblastic anaemia</td>
<td>Ataxia, sideroblastic anaemia</td>
<td>Autosomal recessive mutation in the ABC7 iron exporter</td>
</tr>
<tr>
<td>Hereditary spastic paraplegia</td>
<td>Spastic paraplegia</td>
<td>Autosomal recessive mutation in the SPG7 gene encoding a metalloprotease</td>
</tr>
<tr>
<td>X-linked deafness–dystonia syndrome</td>
<td>Deafness and dystonia</td>
<td>X-linked recessive mutation in the DDP1 gene encoding protein mitochondrial transporter</td>
</tr>
<tr>
<td>Autosomal dominant optic atrophy (OPA1 gene)</td>
<td>Optic atrophy and visual failure</td>
<td>Autosomal dominant mutations in the OPA1 gene encoding a dynamin-related protein</td>
</tr>
</tbody>
</table>
Another disease in this series, mitochondrial neuro-gastro-intestinal encephalomyopathy (MNGIE), is a devastating disorder of juvenile onset, characterized by ophthalmalmparesis, 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 De Toni–Fancconi 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 hepatoneuropathic 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., 2002a).

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 deoxyribonucleotide carrier is responsible for a rare form of congenital microcephaly, found in interrelated Old Order Amish. These data indicate that mitochondrial deoxyribonucleotide 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 (Shoupbridge, 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 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., 2000a), mutations in SCO2 are more frequent (Papadopoulou et al., 1999). The usual clinical presentation is that of an early-onset, fatal cardioencephalomyopathy with COX deficiency, but clinical variants have been reported resembling early-onset (type 1) spinal muscular atrophy (Salviati et al., 2002b). 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., 2002c).

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., 2000b).

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 BCSIL 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 fatigueability 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, et al., 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 maintenance (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 establishment 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 pathogenesis 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.

Acknowledgements

We are gratefully indebted to Dr Loredana Lamaneta, Division of Molecular Neurogenetics, for the artwork of Fig. 1, and to Dr Marina Mora, Laboratory of Muscle Biology, Istituto Nazionale Neurologico Besta, for the serial transverse sections of a muscle biopsy in Fig. 2. This work was supported by Fondazione Telethon-Italy (grant no. GGP030039), Fondazione Pierfranco e Luisa Mariani (Ricerca 2000), Ricerca Finalizzata Ministero della Salute RF-2002/158, Ricerca Finalizzata Ministero della Salute RF 2002-03 and MitEuro network grant from the European Union Framework Program 5 (M.Z.), and Ricerca Finalizzata Ministero della Salute RF 2002/157 (S.D.D.).

References

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