Review Article

Mitochondrial DNA Mutations, Pathogenicity and Inheritance

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Abstract

Mitochondria contain their own DNA (mtDNA), which codes for 13 proteins (all subunits of the respiratory chain complexes), 22 tRNAs and 2 rRNAs. Several mtDNA point mutations as well as deletions have been shown to be causative in well-defined mitochondrial disorders. A mixture of mutated and wild type mtDNA (heteroplasmy) is found in most of these disorders. Inheritance of mtDNA is maternal, and mothers with heteroplasmic mtDNA transmit different proportions of normal and mutated mtDNA to the children. Mitochondrial tRNA genes have a central role in mitochondrial gene expression at the level of transcription, RNA processing and protein synthesis and they appear to be the mitochondrial genes most frequently affected by mutations causing diseases in man.

Keywords: *Mitochondria, mtDNA, Inheritance and Pathogenecity*

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Mitochondria

The earliest reports on intracellular structures that probably represented mitochondria go back to 150 years ago. The name mitochondrion was used for the first time 100 years ago. It originates from the Greek "mitos" (thread) and "chondros" (granule). The main function of mitochondria is oxidative phosphorylation, i.e. the oxidation of substrates (mainly pyruvate and fatty acids) to H2O and CO2, providing the cells most widely used high-energy compound, ATP. ATP is the universal "currency" of chemical energy. Thus, this organelle has rightly been called the "power plant" of the cell (Margulis 1970).

Mitochondria are spherical or rod-shaped organelles that are present in there thousands in each human cell. The mitochondrion contains an outer membrane and an inner membrane that define two internal compartments: the internal matrix space and a much narrower intermembrane species. The inner membrane is folded into numerous cristae, which greatly increase its total surface area. The respiratory chain is located in the inner membrane of the mitochondrion. It consists of five enzyme complexes: Complex I (NADH dehydrogenase or NADH: ubiquinone oxidoreductase), Complex II (succinate dehydrogenase or succinate: ubiquinon oxidoreductase), Complex III (cytochrome c reductase or ubiquinol: cytochrome c reductase), Complex IV (cytochrome c oxidase or ferrocytochrome c: oxygen oxidoreductase), Complex V (ATP synthase). The matrix also contains several identical copies of the mitochondrial DNA (mtDNA) genome, special mitochondrial ribosomes, tRNAs, and various enzymes required for expression of the mitochondrial genes.

The human mitochondrial genome

The presence of DNA in mitochondria was demonstrated by electron microscopy by Nass and Nass in 1963 (Nass and Nass 1963). Human mtDNA is a double-stranded 16569-nucleotide pair closed circular molecule. The two strands have an unusual asymmetry in the composition of their bases: the "heavy" or H strand is rich in purines (i.e. A+G) while the "light" or L strand is correspondingly rich in pyrmidines (i.e. C+T). "Heavy" and "light" refer to the differential mobility of the separated strands in alkaline cesium chloride gradients.

The human mtDNA is one of the most compact pieces of genetic information. There is no intron in mtDNA and some of its genes are even overlapping. MtDNA has a 1000 bp non-coding sequence only in its short regulatory region (displacement loop, D-loop). Human mtDNA contains the genes for 13 proteins, all of which are subunits of the respiratory chain enzyme complexes, 22 tRNAs and 2 rRNAs (Anderson *et al*. 1981). The rest of the protein subunits of respiratory chain complexes are encoded by the nuclear genome and transported to the mitochondria (Table 1).

There are between a hundred to a thousand mitochondria in each cell and as each mitochondrion has 2-10 copies of mtDNA (Shay *et al*. 1990; Satoh and Kuroiwa 1991). Mitochondria are unique among the cell's organelles in that they are under the control of two genetic systems: nuclear DNA and mtDNA. The inheritance of mtDNA differs from the Mendelian inheritance of nuclear genes, being maternal in humans (Giles *et al*. 1980) . Paternal transmission of mtDNA has not been demonstrated in man (Cummins 1996) even by the intracytoplasmic sperm injection (ICSI) method (Houshmand *et al* 1997). The mtDNA nucleotide sequence evolves 6 to 17 times faster than comparable nuclear DNA gene sequences (Wallace *et al*. 1987; Easteal 1991); several possible explanations for this exist. Mitochondria lack DNA repair systems present in the nucleus that may make mitochondria less efficient in repairing DNA damage. Histones are not present in mitochondria. Mitochondria consume >90% of the oxygen that enters the cell, and free oxygen radicals may thus preferentially cause damage to mtDNA (Richter *et al*. 1988) .

High mutation rates of mtDNA resulted in multiple restriction fragment length polymorphisms, in the control region and coding region nucleotide variants, conformational variants (Singh *et al*. 1987; Vigilant *et al*. 1988), and length variants. Polymorphic variants correlate with the ethnic and geographic origin of the samples, presumably because mtDNA mutations have accumulated along radiating maternal lineages as women migrated out of Africa and into different continents (Merriwether *et al*. 1991; Vigilant *et al*. 1991; Torroni *et al*. 1992; Stoneking 1994; Stoneking and Soodyall 1996) .

Replication of mtDNA

Human mtDNA has a single origin of replication. The mtDNA origin has been physically separated into two "halves", each controlling synthesis of one of the daughter DNA strands. The transcription of mtDNA is important for replication because it is needed for synthesis of the RNA primer required for replication at the origin of heavy strand replication (OH). OH is located at the top of the circle, around map position 200 within the 1123 bp "control region" between the $tRNA^{Pro}$ gene (at position 16023) and

Table 1: Genetic origin of the oxidative phosphorylation system protein subunits

Complex	Total No of subunits	mtDNA-encoded subunits
I	43	7: ND1, ND2, ND3, ND4, ND4L, ND5, ND6
II	4	0
III	11	1: Cyt b
IV	13	3: COX I, COX II, COX III
V	14	2: ATPase 6, ATPase 8

ND: NADH dehydrogenase subunits, Cyt b: Cytochrome b, Cyt c: Cytochrome c oxidase subunits, ATPase: ATP synthase (Anderson *et al*. 1981).

the $tRNA^{Phe}$ gene (at position 577). Synthesis of one strand begins at OH and proceeds in a clockwise direction. Synthesis of the other strand begins at the origin of light strand replication (OL), which is located at about "8 o'clock" on the circle (near position 5750), and proceeds in a counter-clockwise direction (Clayton 1982; Clayton 1998).

Transcription of mtDNA

All of the 37 genes encoded by human mtDNA are initially synthesised on two huge polycistronic precursor transcripts, one encoded by the L-strand and the other by the H-strand. Of the 37 genes, 28 are encoded by the H-strand; only 8 tRNAs and 1 mRNA (ND6) are encoded by the L-strand. Human mtDNA contains only two promoters for RNA transcription, both located within a 150-bp region in the D-loop containing conserved sequence blocks. One promoter controls transcription of the H-strand, whereas the other controls L-strand transcription (Clayton 1991; Parisi and Clayton 1991; Clayton 1992; Larsson and Clayton 1995; Clayton 1998).

Besides the long 16.6-kb polycistronic transcript generated off the H-strand promoter and encompassing all the H-strand genes, a shorter 3-kb transcript is also synthesised. This transcript, which encompasses only the two rRNA genes and their flanking tRNAs, is synthesised at approximately 25 times the abundance of the long transcript (Gelfand and Attardi 1981), thereby enabling a sufficient amount of 12S and 16S rRNA to be made for all the ribosomes that the organelle needs for translation.

Translation of mtDNA

The genetic code directing translation of mtDNA differs from the universal genetic code (Anderson *et al*. 1981). In mammals mtDNA, UGA encodes tryptophan instead of being a termination codon. AUA encodes methionine instead of isoleucine, and AGA and AGG are termination codons, instead of encoding arginine. Only 22 tRNAs are enough for translation of the protein coding sequences of the human mitochondrial genome, due to a more simplified codon-anticodon pairing than that required for reading the universal genetic code. In humans, eight mitochondrial tRNAs recognise eight codon families with four-fold degeneracy, and 14 recognise the remaining codon pairs. A single $tRNA^{Met}$ occurs in human mtDNA, specifying both methionine and n-formyl methionine. As in prokaryotes, the latter replaces methionine as the initial aminoacid. Moreover, AUA or AUU are sometimes used as initiation codons instead of AUG. While the RNA components of the translation apparatus are mtDNA-encoded, the genes encoding the protein factors involved in translation are encoded in the nucleus. These include the aminoacyle-tRNA syntheases, the ribosomal proteins, elongation and termination factors etc. (Anderson *et al*. 1981).

Mitochondrial DNA mutations and diseases

Mitochondrial defects occur in a wide variety of degenerative diseases, aging and cancer. The concept of oxidative phosphorylation disorders introduced by Luft *et al*. (Luft *et al*. 1962). Besides the clinical and biochemical investigations, they investigated patients' skeletal muscle by light and electron microscopy. The term mitochondrial disorder (mitochondrial cytopathy, myopathy, and encephalomyopathy) mainly refers to disorders with abnormal morphological aspect of mitochondria in muscle (DiMauro *et al*. 1985). Muscle fibres with an abnormal proliferation of mitochondria can be detected histochemically with modified Gomori trichrome stain as ragged-red fibres (RRF), a hallmark of mitochondrial encephalomyopathies (Bindoff and Turnbull 1990). These fibres are filled with mitochondria that have responded to their functional defect in the ATP production by increasing their number. Ultrastructurally, the mitochondria are abnormal: they are enlarged, with distorted cristae, and often contain different kinds of inclusions (DiMauro *et al*. 1985). Staining for cytochrome c oxidase (COX) activity provides information about the terminal portion of the respiratory chain and reflects mitochondrial function (Doriguzzi *et al*. 1990).

The first mitochondrial diseases to be understood at the molecular level were reported in a patient with chronic progressive external ophthalmoplegia (CPEO) and Kearns-Sayre Syndrome (KSS) (Holt *et al*. 1988) . In the same year, Wallace (Wallace *et al*. 1988a) reported a point mutation in the ND6 gene, which was associated with LHON (Leber's hereditary optic neuropathy). In 1990, two new mutations, an A8344G in the $tRNA^{Lys}$ gene (Shoffner *et al*.

1990) in MERRF syndrome (Myoclonus epilepsy and ragged-red fibres) and an A3243G in the $tRNA^{Leu(UUR)}$ gene (Goto *et al*. 1990) in MELAS syndrome (Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes), were reported. The spectrum of phenotypes of mitochondrial diseases has thereafter expanded from rare myophathies to multiple diseases probably representing all branches of medicine.

In normal situations the mtDNA molecules of one individual are identical (homoplasmy). If two different mtDNA populations exist in the ovum (heteroplasmy) because of a mutation, these two mtDNA populations segregate randomly to the different stem cells of the offspring. In the case of a disease causing mutation, different tissues may finally have various amounts of mutant and wild type mtDNA (Wallace 1992). The concepts of maternal inheritance and heteroplasmy have important implications in human pathology. Because there are multiple copies of mtDNA in each cell, the phenotypic expression of a mtDNA mutation will depend on the relative numbers of mutant and wild type genomes, a minimum critical number of mutant genomes being necessary for expression (threshold effect). Several well-defined multisystemic syndromes have been associated with mtDNA deletion or point mutations. The following mtDNA genetic defects cause the mitochondrial diseases:

A) Single mtDNA deletions, duplications or duplications and deletions (Table 2)

Kearns-Sayre syndrome (KSS) is a sporadic condition defined by the triad of early onset (before age 20). KSS is a progressive multisystem disease with external ophthalmoplegia, ptosis, retinopathy, myopathy cardic conduction defects, ataxia, deafness and elevated CSF protein among its commonest features (Petty *et al*. 1986) .

Chronic progressive external ophthalmoplegia (CPEO), including KSS (Zeviani *et al*. 1988), may be a mild form of KSS: it presents later, weakness is usually confined to extracular and proximal limb muscles and other systems are not involved.

Histological analysis of CPEO and KSS muscle revealed that the deleted mtDNAs become regionally enriched within the muscle fibres and accumulation of abnormal mitochondria, which contribute to ragged-red fibres (Mita *et al*. 1989; Shoubridge *et al*. 1990; Moraes *et al*. 1995) .

Pearson syndrome, (Pearson *et al*. 1979; Rötig *et al*. 1989) is characterised by a sideroblastic anaemia with vacuolisation of marrow precursors, accompanied by neutropenia, thrombocytopenia, exocrine pancreatic dysfunction and abnormal liver function, but neurological symptoms. Pearson's Syndrome can result from either deletion or combined duplication/deletion mutations, and some Pearson's patients spontaneously recover from their childhood sideroblastic anaemia, and ultimately progress to a KSS-like phenotype (McShane *et al*. 1991; Poulton *et al*. 1995a; Rötig *et al*. 1995).

Sporadic rearrangements in the mtDNA have been associated with ocular myopathies including CPEO, KSS (Moraes *et al*. 1989), Pearson's Marrow /Pancreas Syndrome (Rötig *et al*. 1988; Rötig *et al*. 1989), and maternally inherited adult-onset diabetes and deafness (Ballinger *et al*. 1992; Ballinger *et al*. 1994).

Table 2: Large-scale rearrangements of mtDNA and associated phenotypes

Phenotypes	Mutation	Inheritance	Reference
KSS	LS del	S	(Zeviani *et al*. 1988)
CPEO	LS del	S	(Holt *et al*. 1988)
PS	LS del	S	Rötig *et al*. 1990)
PS	Del/Dup	S	(Superti-Furga *et al*. 1993)
Diabetes, Deafness	LS dup	M	(Dunbar *et al*. 1993)
Diabetes, Deafness, optic atrophy	LS del	S	(Rötig *et al*. 1993)
Chronic diarrhoea, villous atrophy	Del/Dup	S	(Cormier-Daire *et al*. 1994)
Myopathy	260bp dup	S	(Manfredi *et al*. 1995b)

KSS: Kearns-Sayre syndrome, CPEO: Chronic progressive external ophthalmoplegia, PS: Pearson's syndrome, LS del: Large single deletion, LS dup: Large single duplication, S: Sporadic, M: Maternal.

High levels of a rearranged mtDNA molecule containing both a partial duplication and a deletion have been reported in children with infantile onset of a multisystem disorder (Cormier-Daire *et al*. 1994) . The theory that the large-scale deletions can be pathogenic in human has been extensively supported both in vivo (Mita *et al*. 1989; Shoubridge *et al*. 1990; Sciacco *et al*. 1994; Manfredi *et al*. 1997) and in in vitro hybrids (Sancho *et al*. 1992; Hayashi *et al*. 1994). It has been postulated that mtDNA deletions impair mitochondrial protein synthesis due to the loss of tRNA genes (Nakase *et al*. 1990). In contrast, the pathogenic significance of mtDNA duplications is still uncertain, and the tRNA hypothesis does not apply to duplications, as there are no fewer mtDNA genes and probably no mutation of tRNA sequences (Lander and Lodish 1990).

B) Point mutations of protein genes (Table 3)

Neuropathy, ataxia and retinitis pigmentosa (NARP) is a maternally transmitted multisystem disorder of young adult life comprising, in various combinations, sensory neuropathy, ataxia, seizures, dementia and retinitis pigmentosa (Holt *et al*. 1990). It is associated with a T8993G mutation (mutation resulting in the replacement of a leucine by arginine) in the ATPase 6 gene (Holt *et al*. 1990; Tatuch *et al*. 1992). The mutation is heteroplasmic, the clinical severity of the disease being dependent on the proportion of mutant mtDNA. A T8993C mutation (De Vries *et al*. 1993; Santorelli *et al*. 1994; Santorelli *et al*. 1996a) (mutation resulting in the replacement of leucine by proline) and T9176C mutation (Thyagarajan *et al*. 1995) alter the ATPase 6 gene were associated with a less severe clinical course of NARP. These mutations are invariably heteroplasmic and result in a broad range of clinical manifestations from mild peripheral retinitis pigmentosa to severe neurological disease, depending on the percentage of mutant mtDNAs.

Leigh syndrome is a more severe clinical expression of NARP, where the proportion of mutated DNA is very high, i.e. greater than 90-95%. Leigh syndrome is characterised by subacute infantile necrotising encephalomyelopathy. Children who inherit close to 100% mutant mtDNAs can present with Leigh Syndrome, a frequently lethal disease associated with basal ganglia degeneration (Tatuch et al. 1992; De Vries et al. 1993). The T8993G mutation has been linked to the inhibition of proton translocation of ATP synthase through cybrid transfer experiments (Trounce *et al*. 1994). *Leber hereditary optic neuropathy (LHON)* causes acute loss of vision in young adults, predominantly males (Nikoskelainen *et al*. 1987). It was the first mitochondrial disease where a point mutation was shown to be the underlying cause (Wallace *et al*. 1988a).

Four mutations in mtDNA electron transport genes are generally thought play a significant role in the

Table 3: Phenotypes associated with mitochondrial missense mutations

Mutation	Gene	Phenotypes	Reference
T1095C	12SRNA	Deafness	(Tessa *et al*. 2001)
G3460A	ND1	LHON	(Huoponen *et al*. 1991)
A7444G	COXI	McArdle's disease	(Aguilera *et al* 2001)
T8851C	ATPase6	Bilateral striatal necrosis	(De Meirleir *et al*. 1995)
T8993G	ATPase6	Leigh's or NARP	(Holt *et al*. 1990)
T8993C	ATPase6	Leigh's or NARP	(De Vries *et al*. 1993)
T9176C	ATPase6	Bilateral striatal necrosis	(Thyagarajan *et al*. 1995)
T9176C	ATPase6	Leigh syndrome	(Carrozzo *et al*. 2001)
T9957C	COXIII	MELAS	(Manfredi *et al*. 1995a)
T10662C	ND4	LHON	(Brown *et al*. 2002)
G11778A	ND4	LHON	(Wallace *et al*. 1988a)
G14459A	ND6	LHON	(Jun *et al*. 1994)
T14484C	ND6	LHON	(Johns *et al*. 1992)

ND: NADH dehydrogenase subunits, ATPase: ATP synthase, Cyt c: Cytochrome c oxidase subunits, LHON: Leber's hereditary optic neuropathy, NARP: Neuropathy, ataxia and retinitis pigmentosa, MELAS: Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes.

etiology of LHON. In order of decreasing severity, these four LHON mutations are ND6 G14459A (Jun *et al*. 1994) , ND4 G11778A (Wallace *et al*. 1988a) , ND1 G3460A (Huoponen *et al*. 1991) and ND6 T14484C (Johns *et al*. 1992) . Fifteen other LHON mutations have been identified, but their pathogenicity is unclear (Howell *et al*. 1995; Brown *et al*. 1997). Using transmitochondrial cybrids (Jun *et al*. 1996) showed that ND6 G14459A mutation causes LHON and dystonia.

A complex I defect has been reported in a patient with ND4 G11778A (Larsson *et al*. 1991). The G14459A mutation causes a 50% reduction in Complex I specific activity as well as coenzyme Q substrate-product inhibition (Jun *et al*. 1996). G11778A, G3460A and T14484C are associated with a reduction in respiration of NADH-linked substrates (Majander *et al*. 1996) and a partial reduction in Complex I activity (Howell *et al*. 1991; Larsson *et al*. 1991; Majander *et al*. 1991; Degli Esposti *et al*. 1994; Smith *et al*. 1994).

C) Point mutations of tRNA and rRNA genes (Table 4)

Myoclonus epilepsy and ragged-red fibres (MERRF) (Fukuhara *et al*. 1980) are a maternally inherited disorders (Rosing *et al*. 1985) characterised by myoclonus, generalised seizures, cerebral ataxia and myopathy. The most commonly observed mutation in MERRF is an A8344G in the $tRNA^{Lys}$ gene (Shoffner *et al*. 1990; Yoneda *et al*. 1990). The mutation is always heteroplasmic and the fraction of mutated mtDNA varies widely between different individuals and even between different tissues of the same individual (Wallace *et al*. 1988b; Shoffner *et al*. 1990; Shoffner and Wallace 1991; Larsson *et al*. 1992; Wallace 1993).

Multiple symmetric lipomas of the neck have been described in several patients with the A8344G mutation (Holme *et al*. 1993). A second, less frequent, heteroplasmic T8356C mutation of the tRNALys gene has been described in patients with MERRF (Silvestri *et al*. 1992). A number of studies suggested that there is no close correlation between the amount of mutant mtDNA and the degree of dysfunction of different organs (Ozawa *et al*. 1995). Therefore, not only may thresholds of expression differ between different organs (Tanno *et al*. 1993) but differences are also possible concerning the dependence of different cell types on individual subunits of the respiratory chain (Chomyn *et al*. 1991; Noer *et al*. 1991; Seibel *et al*. 1991; Boulet *et al*. 1992; Marzuki *et al*. 1995). For the A8344G mutation, this defect has been correlated with a 50-60% reduction in $tRNA^{Lys}$ aminoacylation (Enriquez *et al*. 1995).

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) (Pavlakis *et al*. 1984) is characterised by stroke-like episodes with hemiparesis or hemianopia, almost invariably occurring before the age of 40, and often in childhood. The tRNA mutation gives several distinctive clinical presentations, the most notable of which is caused by the A3243G mutation in $tRNA^{Leu(UUR)}$ (Goto *et al*. 1990). The A3243G mutation changes a highly conserved base pair in different species. High levels of A3243G mutation have been found in muscle (Ciafaloni *et al*. 1992; Inui *et al*. 1992). PCR analysis of single muscle fibres has demonstrated that there is an uneven distribution of normal and mutated mtDNA in muscle. The ragged red fibres always contain high levels of mtDNA with the A3243G mutation (Moraes *et al*. 1992).

The A3243G mutation has been observed in patients with MELAS and KSS (Goto *et al*. 1990b; Goto 1995) when present at a high percentage of mutants. However, patients with a low percentage of mutant mtDNAs can present with adult-onset diabetes mellitus (Type I diabetes) with or without deafness (Van den Ouweland *et al*. 1992; Van den Ouweland *et al*. 1994). In addition, a T3271C mutation was found in some MELAS patients (Goto *et al*. 1991; Hayashi *et al*. 1993; Sakuta *et al*. 1993; Marie *et al*. 1994). The A3243G and T3271C mutations as well as a third, rare mutation, T3291C (Goto *et al*. 1994), all lie inside the $tRNA^{Leu(UUR)}$ gene. These findings suggest that mutations of the mitochondrial $tRNA^{Leu(UUR)}$ gene are the most common cause of the MELAS syndrome. Other mutations suggested to play a pathogenic role in MELAS include a T7512C mutation in the $tRNA^{Ser(UCN)}$ gene detected in a family with MERRF/MELAS overlap syndrome (Nakamura *et al*. 1995), and even a T9957C mutation located in a cytochrome c oxidase subunit III gene (Manfredi *et al*. 1995a).

Eleven point mutations in the $tRNA^{Leu(UUR)}$

Table 4: Phenotypes associated with mitochondrial tRNA and rRNA mutations.

Mutations	Gene	Phenotypes	Reference
A1555G	12S rRNA	Deafness	(Prezant *et al*. 1993)
G1606A	12S rRNA	Complex Neurology	(Sacconi *et al*. 2002)
T3200C	12S rRNA	Diabetes type II	(Yang *et al*. 2002)
A3243G	tRNALeu(UUR)	MELAS, PEO	(Goto *et al*. 1990)
A3243T	tRNALeu(UUR)	MM	(Goto *et al*. 1992)
A3251G	tRNALeu(UUR)	Encephalomyopathy	(Morten *et al*. 1993)
A3251G	tRNALeu(UUR)	Cardiomyopathy	(Houshmand *et al*. 1996)
C3256T	tRNALeu(UUR)	Multisystem disorder	(Moraes *et al*. 1993b)
A3260G	tRNALeu(UUR)	Cardiomyopathy	(Zeviani *et al*. 1991)
T3271C	tRNALeu(UUR)	MELAS	(Goto *et al*. 1991)
T3285C	tRNALeu(UUR)	Diabetes Type II	(Ma *et al*. 2000)
T3291C	tRNALeu(UUR)	MELAS	(Goto *et al*. 1994)
A3302G	tRNALeu(UUR)	MM	(Bindoff *et al*. 1993)
C3303T	tRNALeu(UUR)	Cardiomyopathy	(Silvestri *et al*. 1994)
A4269G	tRNAIle	Multisystem disorder	(Taniike *et al*. 1992)
T4285C	tRNAIle	PEO	(Silvestri *et al*. 1996)
A4300G	tRNAIle	Cardiomyopathy	(Casali *et al*. 1995)
A4317G	tRNAIle	Cardiomyopathy	(Tanaka *et al*. 1990)
C4320T	tRNAIle	Encephalomyopathy	(Santorelli *et al*. 1995)
G5549A	tRNATrp	Dementia, Chorea	(Nelson *et al*. 1995)
T5692C	tRNAAsn	PEO	(Seibel *et al*. 1994)
G5703A	tRNAAsn	MM, PEO	(Moraes *et al*. 1993b)
T5814C	tRNACys	Encephalomyopathy	(Manfredi *et al*. 1996)
A7445G	tRNASer(UCN)	Deafness	(Reid *et al*. 1994)
T7512C	tRNASer(UCN)	MERRF/MELAS	(Nakamura *et al*. 1995)
+7472C	tRNASer(UCN)	Deafness	(Hutchin *et al*. 2001)
A8296G	tRNALys	Diabetes	(Kameoka *et al*. 1998)
G8313A	tRNALys	Encephalomyopathy	(Verma *et al*. 1997)
A8328A	tRNALys	Encephalomyopathy	(Houshmand *et al* 1999)
A8344G	tRNALys	MERRF, Lipomas	(Shoffner *et al*. 1990)
T8356T	tRNALys	MERRF	(Silvestri *et al*. 1992)
G8363A	tRNALys	Cardiomyopathy	(Santorelli *et al*. 1996b)
T9997C	tRNAGly	Cardiomyopathy	(Merante *et al*. 1994)
A10006G	tRNAGly	MM	(Lauber *et al*. 1991)
C12246A	tRNASer(GCU)	MM + PEO	(Lauber *et al*. 1991)
T12297C	tRNALeu(CUN)	Diabeted Cars. Myop.	(Grasso *et al*. 2001)
T12311C	tRNALeu(CUN)	CPEO	(Hattori *et al*. 1994)
T14709C	tRNAGlu	MM	(Hao *et al*. 1995)
G15915A	tRNAThr	Encephalomyopathy	(Nishino *et al*. 1996)
A15923G	tRNAThr	MM	(Yoon *et al*. 1991)
C15990T	tRNAPro	MM	(Moraes *et al*. 1993a)

MELAS: Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, MERRF: Myoclonus epilepsy and ragged-red fibres, MM: Mitochondrial myopathy, CPEO: Chronic progressive external ophthalmoplegia, PEO: Progressive external ophthalmoplegia.

Table 5: Nuclear and associated phenotypes

Phenotypes	Mutation	Inheritance	Reference
Familial CPEO	Mul del	AD	(Zeviani *et al*. 1989)
Encephalomyopathy	Mul del	AD	(Cormier *et al*. 1991)
Fatal infantile myopathy	Depletion	AR	(Moraes *et al*. 1991)
Myopathy of childhood	Depletion	AR	(Tritschler *et al*. 1992)
Infertility	Mutation In PLOG		(Rovio *et al*. 2001)

CPEO: Chronic progressive external ophthalmoplegia, Mul del: Multiple deletion, AD: Autosomal dominant, AR: Autosomal recessive.

gene have been reported to date (Table 4). The ribosomal DNA transcription unit, one of three polycistronic transcription units of human mtDNA, terminates at the 3´-end of the 16S rRNA gene just before the $tRNA^{Leu(UUR)}$ gene. This transcript, corresponding to the ribosomal gene, is processed to yield the mature rRNA and, due to its very high rate of synthesis, is responsible for the bulk of the rRNA formation. Transcription termination is mediated by a protein factor which binds specifically within the $tRNA^{Leu(UUR)}$ gene, and which promotes termination of transcription (Kruse *et al*. 1989; Hess *et al*. 1991). The nt 3243 mutation has been shown in vitro to impair the binding of this protein factor (Hess *et al*. 1991; Chomyn *et al*. 1992) and to affect the efficiency of transcription termination at the end of the 16S rRNA gene (Hess *et al*. 1991). Termination-competent extracts contain a factor capable of footprinting a tridecamer sequence and several of its flanking nucleotides (Kruse *et al*. 1989). Protein synthesis defects have been made to the A3243G and T3271C mutations (Chomyn *et al*. 1992; King *et al*. 1992; Koga *et al*. 1995).

Nuclear DNA mutations

Nuclear genes encode not only the majority of the respiratory chain subunits, but also all of the other proteins that comprise the organelle system and that are required for its biogenesis and maintenance. This includes all of the proteins required for replication and transcription of mtDNA, and processing and translation of mtDNA transcripts, as well as all proteins required for mitochondrial protein import. Because all the proteins involved in mitochondrial biogenesis and the maintenance of mtDNA are encoded by the nuclear DNA, defects in these genes may cause secondary mutations in mtDNA or may decrease the mtDNA copy number. Such genes include the mtDNA polymerase, mRNA polymerase, mtTFA, mitochondrial single-strand binding-protein andr putative mtDNA- or mRNA- processing enzymes.

Autosomal recessive myopathy with mtDNA depletion is characterised by tissue-specific loss of mtDNA molecules of up to 98% (Moraes *et al*. 1991; Tritschler *et al*. 1992). In this disease the levels of mtTFA are dramatically decreased in the tissues with low amounts of mtDNA (Larsson *et al*. 1994; Poulton *et al*. 1994a).

Oxidative phosphorylation defects have been reported in Parkinson's Disease tissues (Schapira *et al*. 1992; DiMauro 1993; Mizuno *et al*. 1993; Mizuno *et al*. 1995), Huntington's Disease (Brennan *et al*. 1985; Parker *et al*. 1990a) and Alzheimer's Disease (Peterson and Goldman 1986; Sims *et al*. 1987; Parker *et al*. 1990b).

Segregation and transmission of mtDNA

Mitochondrial diseases are characterised by extremely variable clinical phenotypes not only because of the genetics of mtDNA, but also due to different possible modes of inheritance. Inheritance may be: a) sporadic or spontaneously occurring, as in many cases of Kayre-Sayre syndrome, chronic progressive external ophthalmoplegia and Pearson's syndrome, b) maternal, as in the cases of point mutations seen in MERRF, MELAS, NARP and LHON, c) autosomal dominant or recessive, as in the case of progressive external ophthalmoplegia associated with variable deletions and in the case of generalised deficiency of cytochrome oxidase, respectively.

The mechanisms by which mtDNA mutations arise and become fixed in mammalian maternal lineages are not fully understood. A mother with a heteroplasmic mtDNA genome may transmit widely varying levels of mutated mtDNA to her children and the mtDNA genotype may change in a few generations (Larsson *et al*. 1992; Holme *et al*. 1995). This variation in proportion of mutant transmitted could arise from two sources: random segregation of a specific number of founder mtDNAs or nonrandom proliferation of a subpopulation because of some selective advantage that appears to be rare before birth (Suomalainen *et al*. 1993; Matthews *et al*. 1994). The rapid segregation of mutated mtDNA has been observed in human maternal pedigrees (Lott *et al*. 1990; Howell *et al*. 1991; Howell *et al*. 1996; Degoul et al. 1997). To explain the rapid segregation observed in vertebrate mitochondrial DNA, despite its high copy number and mutational rate, a model based on a "bottleneck" effect (Ashley *et al*. 1989) or a "sampling and amplification" mechanism (Lightowlers *et al*. 1997) has been proposed to occur during oogenesis and early embryogenesis. During

bovine germ-line development, the number of mitochondria increases 100-fold, from ~1000 per oogonium to ~100000 per oocyte, while the number of mtDNA molecules increases ~10-fold, from ~10000 to ~100000 (Michaels *et al*. 1982; Hauswirth and Laipis 1985). As a result, each organelle harbours approximately 1 mtDNA molecule per mitochondrion, instead of the usual 5-10 (Veltri *et al*. 1990). Mitochondria with a reduced mtDNA copy number will then segregate into the dividing cells of the embryo. As a consequence of this mitochondrial partitioning, a very limited number of mtDNA molecules serve to define the cytoplasmic genotype from one generation to the next. High mutation rate, maternal inheritance, mitotic segregation and absence of recombination co-operate to make mutations become fixed, after a transient period of heteroplasmy, as homoplasmic changes in a given maternal lineage.

A genetic bottleneck has been observed in human oocytes (Blok *et al*. 1997; Marchington *et al*. 1997; Reynier *et al*. 1998). Marchington has suggested that the bottleneck effect has occurred by the time that the oocyte has become mature (Marchington *et al*. 1997; Marchington *et al*. 1998).

The proportion of mutant mtDNA transmitted from mother to offspring is variable because of the genetic bottleneck, and the "dose" of mutant mtDNA received influences the severity of the phenotype. The possibility of prenatal diagnosis is critically dependent on the nature and timing of this bottleneck.

Slow segregation or stable heteroplasmy of mtDNA genotypes have been reported, which makes it difficult to explain by the "bottleneck" or "sampling and amplification" hypotheses (Howell *et al*. 1992; Larsson *et al*. 1992; Träff *et al*. 1995; Howell *et al*. 1996; Santorelli *et al*. 1996c). To explain this problem, some physical barrier must hold two different genotypes together. It is generally accepted that mtDNA does not exist in a naked form but is folded three-dimensionally to form a so-called mitochondrial nucleoid (mt-nucleoid). The mt-nucleoid or its equivalent is believed to be the segregation unit of mtDNA (Lightowlers *et al*. 1997). It has also been generally accepted that a mammalian mitochondrion harbours on average 2-10 mtDNA molecules. Therefore, any heteroplasmic organelle would rapidly tend towards homoplasmy by random drift during organelle and mtDNA turnover (Preiss *et al*. 1995). Segregation of heteroplasmic mtDNA genotypes will be slow if high proportions of nucleoids are heteroplasmic, but it will be more rapid as the proportion of homoplasmic nucleoid increases. Additional segregation may occur postembryonically (Meirelles and Smith 1997). Selection at the tissue level may occur because of postmutational conditions, such as the degree of tissue-dependence on respiratory chain function, different turnover rates for mitochondria containing one genotype, a replicative advantage conferred by sequence differences in the D-loop region (Jenuth *et al*. 1997).

Insertion-deletion mutations (Poulton *et al*. 1994b) can be spontaneous (Holt *et al*. 1988; Lestienne and Ponsot 1988; Zeviani *et al*. 1988). They can be maternally inherited (Poulton et al. 1991; Ballinger *et al*. 1992; Rötig *et al*. 1992; Bernes *et al*. 1993; Poulton *et al*. 1994b; Poulton *et al*. 1995b), or mendelianly inherited due to predisposing nuclear mutations (Zeviani *et al*. 1989; Zeviani *et al*. 1990). They are described by the size of the insertion-deletion, the nucleotides at the junction, the nature and size of any flanking repeat and the locations of the repeats.

Genetic counselling and prenatal diagnosis of mtDNA

Investigation of mitochondrial disease is further complicated by the high mutation fixation rate in the mt genome, which leads to the occurrence of many DNA polymorphisms. Whenever a new variation is identified in a particular patient, it is important to account for these factors. It is necessary to perform a database search for RFLPs and additional RFLP analysis should be performed on a large number of control specimens. Determining whether new mtDNA mutations contribute to the pathogenecity of disease is not a trivial matter. Individuals with the same mutation can present with very different clinical phenotypes, depending on genetic background.

There are many unanswered questions about the molecular bases of mitochondrial diseases. For example:

1) The same mutation can cause different problems.

Single deletions can cause Keayre-Sayre's syndrome, Pearson's syndrome or progressive external ophthalmoplegia. The T8993G can cause NARP, or Leigh's syndrome. The A3243G "MELAS mutation" can also cause maternally inherited progressive external ophthalmoplegia (Moraes *et al*. 1993c) or diabetes (Kadowaki *et al*. 1994), in the absence of strokes. The A8344G in tRNALys can cause MERRF syndrome (Shoffner *et al*. 1990; Larsson *et al*. 1992) or Ekbom disease (Holme *et al*. 1993; Träff *et al*. 1995; Austin *et al*. 1998).

2) Different mutations can cause the same syndrome. Two mutations, both in the $tRNA^{Lys}$ gene, have been seen in MERRF patients. Masucci and his colleagues demonstrated that both A8344G and T8356C mutations in the tRNALys gene were associated with the same mitochondrial disorders (Masucci *et al*. 1995). Several different point mutations, some in the $tRNA^{Leu(UUR)}$ gene, others in different tRNA genes, have been associated with typical MELAS. Progressive external ophthalmoplegia can be caused by a variety of mutations: single deletions, multiple deletions, the A3243G mutation or other tRNA point mutations.

3) Mutations in nuclear genes can also affect oxidative phosphorylations, often resulting in Mendelian diseases with phenotypes similar to those caused by mtDNA mutations.

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