Review Article

Mitochondrial DNA Mutations, Pathogenicity and Inheritance

Massoud Houshmand

National Research Center for Genetic Engineering and Biotechnology, P.O. Box: 14155-6343, Tehran, Iran.

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*

Table of contents:

Correspondence to: **Massoud Houshmand, Ph.D**
Tel: +98 21 6415143; Fax: +98 21 6419834;
E-mail: massoudh@nrcgeb.ac.ir

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.

References

Aguilera I, Garcia-Lozano JR, Munoz A, Arenas J, Campos Y, Chinchon I, Roldan AN, Bautista J. (2001) Mitochondrial DNA point mutation in the COI gene in a patient with McArdle's disease. *J Neurol Sci*. 15; 192(1-2): 81-4.

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. (1981) Sequence and organization of the human mitochondrial genome. *Nature* 290: 457-465.

Ashley MV, Laipis PJ, Hauswirth WW. (1989) Rapid segregation of heteroplasmic bovine mitochondria. *Nucleic Acids Res* 17: 7325-7331.

Austin SA, Vriesendorp FJ, Thandroyen FT, Hetcht JT, Jones OT, Johns DR. (1998) Expanding the phenotype of the 8344 transfer RNA Lysine mitochondrial DNA mutation. *Neurology* 51: 1447-1450

Ballinger SW, Shoffner JM, Hedaya EV, Trounce I, Polak MA, Koontz DA, Wallace DC. (1992) Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nat Genet* 1: 11-15.

Ballinger SW, Shoffner JM, Gebhart S, Koontz DA, Wallace DC. (1994) Mitochondrial diabetes revisited. *Nat Genet* 7: 458-459.

Bernes SM, Bacino C, Prezant TR, Pearson MA, Wood TS, Fournier P, Fischel-Ghodsian N. (1993) Identical mitochondrial DNA deletion in mother with progressive external ophthalmoplegia and son with Pearson marrow-pancreas syndrome. *J Pediatr* 123: 598-602.

Bindoff LA, Howell N, Poulton J, McCullough DA, Morten KJ, Lightowlers RN, Turnbull DM, Weber K. (1993) Abnormal RNA processing associated with a novel tRNA mutation in mitochondrial DNA. A potential disease mechanism. *J Biol Chem* 268: 19559-19564.

Bindoff LA, Turnbull DM. (1990) Defects of the respiratory chain. *Baillieres Clin Endocrinol Metab* 4: 583-619.

Blok RB, Gook DA, Thorburn DR, Dahl H-Hm. (1997) Skewed segregation of the mtDNA nt 8993 (T->G) mutation in human oocytes. *Am J Hum Genet* 60: 1495-1501.

Boulet L, Karpati G, Shoubridge EA (1992) Distribution and threshold expression of the tRNA (Lys) mutation in skeletal muscle of patients with myoclonic epilepsy and ragged-red fibers (MERRF). *Am J Hum Genet* 51: 1187-1200.

Brennan WAJ, Bird ED, Aprille JR (1985) Regional mitochondrial respiratory activity in Huntington's disease. *J Neurochem* 44: 1948-1950.

Brown MD, Sun F, Wallace DC. (1997) Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 11778 or 14484 mutations on an mtDNA lineage. *Am J Hum Genet* 60: 381-387.

Brown MD, Starikovskaya E, Derbeneva O, Hosseini S, Allen JC, Mikhailovskaya IE, Sukernik RI, Wallace DC. (2002) The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup J. *Hum Genet*. 110(2): 130-8.

Casali C, Santorelli FM, D'Amati G, Bernucci P, DeBiase L, DiMauro S. (1995) A novel mtDNA point mutation in maternally inherited cardiomyopathy. *Biochem Biophys Res Commun* 213: 588-593.

Chomyn A, Martinuzzi A, Yoneda M, Daga A, Hurko O, Johns D, Lai ST, Nonaka I, Angelini C, Attardi G. (1992) MELAS mutation in mtDNA binding site for transcription termination factor causes defects in protein synthesis and in respiration but no change in levels of upstream and downstream mature transcripts. *Proc Natl Acad Sci USA* 89: 4221-4225.

Chomyn A, Meola G, Bresolin N, Lai ST, Scarlato G, Attardi G. (1991) In vitro genetic transfer of protein synthesis and respiration defects to mitochondrial DNA-less cells with myopathy-patient mitochondria.

Mol Cell Biol 11: 2236-2244.

Ciafaloni E, Ricci E, Shanske S, Moraes CT, Silvestri G, Hirano M, Simonetti S, Angelini C, Donati MA, Garcia C, Martinuzi A, Mosewich R, Servidei S, Zammarchi E, Bonilla E, DeVivo DC, Rowland LP, Schon EA, DiMauro S. (1992) MELAS: clinical features, biochemistry, and molecular genetics. *Ann Neurol* 31: 391-398.

Clayton DA. (1991) Mammalian Mitochondrial Transcription. In: Sato T, Dimauro S (eds) Mitochondrial Encephalomyopathies, vol 7. Raven Press, 1185 Ave of the Americas, New York, NY 10036, pp 47-55.

Clayton DA. (1992) Transcription and replication of animal mitochondrial DNAs. *Int Rev Cytol* 141: 217-232.

Clayton DA. (1998) Nuclear-mitochondrial intergenomic communication. *BioFactors* 7: 203-205.

Cormier V, Rötig A, Tardieu M, Colonna M, Saudubray JM, Munnich A. (1991) Autosomal dominant deletions of the mitochondrial genome in a case of progressive encephalomyopathy. *Am J Hum Genet* 48: 643-648.

Cormier-Daire V, Bonnefont JP, Rustin P, Maurage C, Ogier H, Schmitz J, Ricour C, Saudubray J-M, Munnich A, Rötig A. (1994) Mitochondrial DNA rearrangements with onset as chronic diarrhea with villous atrophy. *J Pediatr* 124: 63-70.

Cummins JM. (1996) Inheritance of mitochondrial DNA and its implications. *Modern art in the 2000s* 131-137.

De Meirleir L, Seneca S, Lissens W, Schoentjes E, Desprechins B. (1995) Bilateral striatal necrosis with a novel point mutation in the mitochondrial ATPase 6 gene. *Pediatr Neurol* 13: 242-246.

De Vries DD, Van Engelen BGM, Gabreëls FJM, Ruitenbeek W, Van Oost BA. (1993) A second missense mutation in the mitochondrial ATPase 6 gene in Leigh's syndrome. *Ann Neurol* 34: 410-412.

Degli Esposti M, Carelli V, Ghelli A, Ratta M, Crimi M, Sangiorgi S, Montagna P, Lenaz G, Lugaresi E, Cortelli P. (1994) Functional alterations of the mitochondrially encoded ND4 subunit associated with Leber's hereditary optic neuropathy. *FEBS Letters* 352: 375-379.

Degoul F, François D, Diry M, Ponsot G, Desguerre I, Héron B, Marsac C, Moutard Ml. (1997) A near homoplasmic T8993G mtDNA mutation in a patient with atypic Leigh syndrome not present in the mother's tissues. *J Inher Metab Dis* 20: 49-53.

DiMauro S. (1992) Mitochondrial encephalomyopathies. *Brain Pathol* 2: 111-112.

DiMauro S. (1993) Mitochondrial involvement in Parkinson's disease: the controversy continues. *Neurology* 43: 2170-2172.

DiMauro S. (1998) Mitochondrial diseases: Clinical considerations. *BioFactors* 7: 277-285.

DiMauro S, Bonilla E. (1996) Mitochondrial encephalomyopathis. *The molecular and genetic basis of neurological disease* second edition: 201-235.

DiMauro S, Bonilla E, Davidson M, Hirano M, Schon EA.. (1998) Mitochondria in neuromuscular disorders. *Biochim Biophys Acta* 1366: 199-210.

DiMauro S, Bonilla E, Lombes A, Shanske S, Minetti C, Moraes CT. (1990) Mitochondrial encephalo-myopathies. *Neurol Clin* 8: 483-506.

DiMauro S, Bonilla E, Zeviani M, Nakagawa M, DeVivo DC. (1985) Mitochondrial myopathies. *Ann Neurol* 17: 521-538.

Doriguzzi C, Palmucci L, Pollo B, Mongini M, Mainscalco M, Chiado-Piat L, Schiffer D. (1990) Cytochrome c oxidase and coenzyme Q in neuromuscular diseases: a histochemical study. *Acta Neuropathol* 81: 25-29.

Easteal S (1991) The relative rate of DNA evolution in primates. *Mol Biol Evol* 8: 115-127.

Enriquez JA, Chomyn A, Attardi G (1995) MtDNA mutation in MERRF syndrome causes defective aminoacylation of tRNA (Lys) and premature translation termination. *Nat Genet* 10: 47-55.

Ephrussi B (1950) The interplay of heredity and invironment in the synthesis of respiratory enzymes in yeast. *Harvy Lect* 46: 45-67.

Fukuhara N, Tokiguchi S, Shirakawa K, Tsubaki T. (1980) Myoclonus epilepsy associated with ragged-red fibres (mitochondrial abnormalities): disease entity or a syndrome? *J Neurol Sci* 47: 117-133.

Gelfand R, Attardi G. (1981) Synthesis and turnover of mitochondrial ribonucleic acid in HeLa cells: the mature ribosomal and messenger ribonucleic acid species are metabolically unstable. *Mol Cell Biol* 1: 497-511

Giles RE, Blanc H, Cann HM, Wallace DC (1980). Maternal inheritance of human mitochondrial DNA. *Proc Natl Acad Sci USA* 77: 6715-6719

Goto Y, Nonaka I, Horai S (1990). A mutation in the tRNA (Leu) (UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 348: 651-653.

Goto Y. (1995). Clinical features of MELAS and mitochondrial DNA mutations. *Muscle and Nerve* 3: S107-112.

Goto Y, Nishino I, Horai S, Nonaka I. (1996). Detection of DNA fragments encompassing the deletion junction of mitochondrial genome. *Biochem Biophys Res Commun* 222: 215-219.

Goto Y, Nonaka I, Horai S. (1990). A mutation in the tRNA (Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 348: 651-653.

Goto Y, Nonaka I, Horai S. (1991) A new mtDNA mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). *Biochim Biophys Acta* 1097: 238-40.

Goto Y, Tojo M, Tohyama J, Horai S, Nonaka I. (1992) A

novel point mutation in the mitochondrial tRNA (Leu)(UUR) gene in a family with mitochondrial myopathy. *Ann Neurol* 31: 672-675.

Goto Y, Tsugane K, Tanabe Y, Nonaka I, Horai S. (1994) A new point mutation at nucleotide pair 3291 of the mitochondrial tRNA (Leu (UUR)) gene in a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). *Biochem Biophys Res Commun* 202: 1624-1630.

Grasso M, Diegoli M, Brega A, Campana C, Tavazzi L, Arbustini E. (2001) The mitochondrial DNA mutation T12297C affects a highly conserved nucleotide of tRNA (Leu (CUN)) and is associated with dilated cardiomyopathy. *Eur J Hum Genet*. 9 (4): 311-5.

Carrozzo R, Tessa A, Vazquez-Memije ME, Piemonte F, Patrono C, Malandrini A, Dionisi-Vici C, Vilarinho L, Villanova M, Schagger H, Federico A, Bertini E, Santorelli FM. (2001) The T9176G mtDNA mutation severely affects ATP production and results in Leigh syndrome. *Neurology*. 13; 56(5): 687-90.

Hao H, Bonilla E, Manfredi G, DiMauro S, Moraes CT. (1995) Segregation patterns of a novel mutation in the mitochondrial tRNA glutamic acid gene associated with myopathy and diabetes mellitus. *Am J Hum Genet* 56: 1017-1025.

Hattori Y, Goto Y, Sakuta R, Nonaka I, Mizuno Y, Horai S. (1994) Point mutations in mitochondrial tRNA genes: sequence analysis of chronic progressive external ophthalmoplegia (CPEO). *J Neurol Sci* 125: 50-55.

Hauswirth W, Laipis P. (1985) Transmission genetics of mammalian mitochondria: a molecular model and experimental evidence. *In: Quagliariello E (ed) Achivments and perspectives of mitochondrial research.* Elsevier, Amesterdam: 49-59.

Hayashi J, Ohta S, Kagawa Y, Takai D, Miyabayashi S, Tada K, Fukushima H, Inui K, Okada S, Goto Y, Nonaka I. (1994) Functional and morphological abnormalities of mitochondria in human cells containing mitochondrial DNA with pathogenic point mutations in tRNA genes. *J Biol Chem* 269: 19060-19066.

Hayashi J-I, Ohta S, Takai D, Miyabayashi S, Sakuta R, Goto Y, Nonaka I. (1993) Accumulation of mtDNA with a mutation at position 3271 in tRNA (Leu)(UUR) gene introduced from a MELAS patient to HeLa cells lacking mtDNA results in progressive inhibition of mitochondrial respiratory function. *Biochem Biophys Res Commun* 197: 1049-1055.

Hess JF, Parisi MA, Bennett JL, Clayton DA. (1991) Impairment of mitochondrial transcription termination by a point mutation associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 351: 236-239.

Holme E, Larsson NG, Oldfors A, Tulinius M, Sahlin P, Stenman G. (1993). Multiple symmetric lipomas with high levels of mtDNA with the tRNA (Lys) A—>G(8344) mutation as the only manifestation of disease in a carrier of myoclonus epilepsy and ragged-red fibers (MERRF) syndrome. *Am J Hum Genet* 52: 551-556.

Holme E, Tulinius MH, Larsson N-G, Oldfors A. (1995) Inheritance and expression of mitochondrial DNA point mutations. *Biochim Biophys Acta* 1271: 249-252.

Holt IJ, Harding AE, Morgan-Hughes JA. (1988) Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 331: 717-719.

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

Houshmand M, Larsson N-G, Holme E, Oldfors A, Tulinius MH, Andersen O. (1994) Automatic sequencing of mitochondrial tRNA genes in patients with mitochondrial encephalomyopathy. *Biochim Biophys Acta* 1226: 49-55.

Houshmand M, Larsson N-G, Oldfors A, Tulinius MH, Holme E. (1996) Fatal mitochondrial myopathy lactic acidosis, and complex I deficiency associated with a heteroplasmic A->G mutation at nt3251 in the mitochondrial $tRNA^{Leu(UUR)}$ gene. *Hum Genet* 97: 269-273.

Houshmand M, Holme E, Hanson C, Wennerholm UB, Hamberger L. (1997) Is paternal mitochondrial DNA transferred to the offspring following intracytoplasmic sperm injection? J *Assist Reprod Genet*. 14 (4): 223-7.

Houshmand M, Lindberg C, Moslemi A-R, Oldfors A, Holme E. (1999) A novel heteroplasmic point mutation in the mitochondrial tRNALys gene in a sporadic case of mitochondrial encephalomyopathy: De novo mutation and no transmission to the offspring. *Hum mutat* 13: 203-209.

Howell N, Halvorson S, Kubacka I, McCullough DA, Bindoff LA, Turnbull DM. (1992) Mitochondrial gene segregation in mammals: is the bottleneck always narrow? *Hum Genet* 90: 117-120.

Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA, Mackey D. (1995) Phylogenetic analysis of the mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. *Genetics* 140: 285-302.

Howell N, Kubacka I, Mackey DA. (1996) How rapidly does the human mitochondrial genome evolve? *Am J Hum Genet* 59: 501-9.

Howell N, Kubacka M, Xu M, McCullough DA. (1991) Leber hereditary optic neuropathy: Involvment of the mitochondrial ND I gene and evidence for an intragenic suppressor mutation. *Am J Hum Genet* 48: 935-942.

Huoponen K, Vilkki J, Aula P, Nikoskelainen EK, Savontaus ML. (1991) A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. *Am J Hum Genet* 48: 1147-53.

Hutchin TP, Navarro-Coy NC, Van Camp G, Tiranti V,

Zeviani M, Schuelke M, Jaksch M, Newton V, Mueller RF. (2001) Multiple origins of the mtDNA 7472insC mutation associated with hearing loss and neurological dysfunction. *Eur J Hum Genet*. 9 (5): 385-7.

Inui K, Tsukamoto H, Fukushima H, Taniike M, Tanaka J, Nishigaki T, Okada S. (1992) Detection of the A-Mutation to G(3243)-Mutation of Mitochondrial DNA in Japanese Families with Mitochondrial Encephalomyopathies. *J Inher Metab Dis* 15: 311-314.

Jenuth JP, Peterson AC, Shoubridge EA. (1997) Tissue-specific selection for different mtDNA genotypes in heteroplasmic mice. *Nat Genet* 16: 93-95.

Johns DR, Neufeld MJ, Park RD. (1992) An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. *Biochem Biophys Res Commun* 187: 1551-1557.

Jun AS, Brown MD, Wallace DC. (1994) A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. *Proc Natl Acad Sci USA* 91: 6206-6210.

Jun AS, Trounce IA, Brown MD, Shoffner JM, Wallace DC. (1996) Use of transmitochondrial cybrids to assign a complex I defect to the mitochondrial DNA-encoded NADH dehydrogenase subunit 6 gene mutation at nucleotide pair 14459 that causes Leber hereditary optic neuropathy and dystonia. *Mol Cell Biol* 16: 771-777.

Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto Y, Hayakawa T, Matsuoka K, Kawamori R, Kamada T, Horai S, Nonaka I, Hagura R, Akanuma Y, Yazaki Y. (1994) A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N Engl J Med* 330: 962-968.

Kameoka K, Isotani H, Tanaka K, Azukari K, Fujimura Y, Shiota Y, Sasaki E, Majima M, Furukawa K, Haginomoei S, kitaoka H, Ohsawa N. (1998) Novel mitochondrial DNA mutation in $tRNA^{Lys}$ (8296 A->G) associated with diabetes. *Biochem Biophys Res Commun* 245: 523-527.

King MP, Koga Y, Davidson M, Schon EA. (1992) Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the tRNA (Leu (UUR)) mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes. *Mol Cell Biol* 12: 480-490.

Koga Y, Davidson M, Schon EA, King MP. (1995) Analysis of cybrids harboring MELAS mutations in the mitochondrial tRNA (Leu (UUR)) gene. *Muscle Nerve* 3: S119-123.

Kruse B, Narasimhan N, Attardi G. (1989) Termination of transcription in human mitochondria: identification and purification of a DNA binding protein factor that promotes termination. *Cell* 58: 391-397.

Lander E, Lodish H. (1990). Mitochondrial disease: gene mapping and gene therapy. *cell* 61: 925-926.

Larsson N-G, Andersen O, Holme E, Oldfors A, Wahlström J (1991) Leber's hereditary optic neuropathy and complex I deficiency in muscle. *Ann Neurol* 30: 701-708.

Larsson N-G, Clayton DA. (1995) Molecular genetic aspects of human mitochondrial disorders. *Annu Rev Genet* 29: 151-178.

Larsson N-G, Oldfors A, Holme E, Clayton DA. (1994) Low levels of mitochondrial transcription factor A in mitochondrial DNA depletion. *Biochem Biophys Res Commun* 200: 1374-1381.

Larsson N-G, Tulinius MH, Holme E, Oldfors A, Andersen O, Wahlström J, Aasly J. (1992) Segregation and manifestations of the mtDNA tRNA (Lys) A—>G (8344) mutation of myoclonus epilepsy and ragged-red fibers (MERRF) syndrome. *Am J Hum Genet* 51: 1201-1212.

Lauber J, Marsac C, Kadenbach B, Seibel P. (1991) Mutations in mitochondrial tRNA genes: a frequent cause of neuromuscular diseases. *Nucleic Acids Res* 19: 1393-1397.

Lestienne P, Ponsot G. (1988) Kearns-Sayre syndrome with muscle mitochondrial DNA deletion. *Lancet* 1: 885.

Lightowlers RN, Chinnery PF, Turnbull DM, Howell N. (1997) Mammalian mitochondrial genetics: Heredity, heteroplasmy and disease. *Trends Genet* 3: 450-455.

Lott MT, Voljavec AS, Wallace DC. (1990) Variable genotype of Leber's hereditary optic neuropathy patients. *Am J Ophthalmol* 109: 625-631.

Luft r, Ikkos D, Palmieri G, Ernster L, Afzelius B. (1962) A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: A correlated clinical, biochemical, and morphological study. *J Clin Invest* 41: 1776-1804.

Ma L, Wang H, Chen J, Jin W, Liu L, Ban B, Shen J, Hua Z, Chai J. (2000) Mitochondrial gene variation in type 2 diabetes mellitus: detection of a novel mutation associated with maternally inherited diabetes in a Chinese family. *Chin Med J (Engl).* 113 (2): 111-6.

Majander A, Finel M, Savontaus ML, Nikoskelainen E, Wikström M. (1996) Catalytic activity of complex I in cell lines that possess replacement mutations in the ND genes in Leber's hereditary optic neuropathy. *Eur J Biochem* 239: 201-207.

Majander A, Suomalainen A, Vettenranta K, Sariola H, Perkkio M, Holmberg C, Pihko H. (1991) Congenital Hypoplastic Anemia, Diabetes, and Severe Renal Tubular Dysfunction Associated with a Mitochondrial DNA Deletion. *Pediatr Res* 30: 327-330.

Manfredi G, Schon EA, Bonilla E, Moraes CT, Shanske S, DiMauro S. (1996) Identification of a mutation in the mitochondrial tRNA (Cys) gene associated with mitochondrial encephalopathy. *Hum Mutat* 7: 158-163.

Manfredi G, Schon EA, Moraes CT, Bonilla E, Berry GT, Sladky JT, DiMauro S. (1995a) A new mutation asso-

ciated with MELAS is located in a mitochondrial DNA polypeptide-coding gene. *Neuromusc Disord* 5: 391-398.

Manfredi G, Servidei S, Bonilla E, Shanske S, Schon EA, DiMauro S, Moraes CT. (1995b) High levels of mitochondrial DNA with an unstable 260-bp duplication in a patient with a mitochondrial myopathy. *Neurology* 45: 762-768.

Manfredi G, Vu T, Bonilla E, Schon EA, DiMauro S, Arnaudo E, Zhang L, Rowland LP, Hirano M. (1997) Association of Myopathy with Large-Scale Mitochondrial DNA Duplications and Deletions: Which is Pathogenic? *Ann Neurol* 42: 180-188.

Marchington DR, Hartshorne GM, Barlow D, Poulton J. (1997) Homopolymeric tract heteroplasmy in mtDNA from tissues and single oocytes: support for a genetic bottleneck. *Am J Hum Genet* 60: 408-416.

Marchington DR, Macaulay V, Hartshorne GM, Barlow D, Poulton J. (1998) Evidence from human oocytes for a genetic bottleneck in an mtDNA disease. *Am J Hum Genet* 63: 769-775.

Margulis L (1970) Origin of eucaryotic celles. *Ysle University press, New Haven.*

Marie SK, Goto Y, Passos-Bueno MR, Zatz M, Carvalho AA, Carvalho M, Levy JA, Palou VB, Campiotto S, Horai S. (1994) A Caucasian family with the 3271 mutation in mitochondrial DNA. *Biochem Med Metab Biol* 52: 136-139.

Marzuki S, Sudoyo H, Lertrit P. (1995) Update in molecular genetics: mitochondrial energy transduction disorders. *Southeast Asian J Trop Med Public Health* 26: S155-161.

Masucci JP, Davidson M, Koga Y, Schon EA, King MP. (1995). In vitro analysis of mutations causing myoclonus epilepsy with ragged-red fibers in the mitochondrial tRNA(Lys)gene: two genotypes produce similar phenotypes. *Mol Cell Biol* 15: 2872-2881.

Matthews PM, Hopkin J, Brown RM, Stephenson JB, Hilton-Jones D, Brown GK. (1994) Comparison of the relative levels of the 3243 (A->G) mtDNA mutation in heteroplasmic adult and fetal tissue. *J Med Genet* 31: 41-44.

McLean JR, Cohn GL, Brandt IK, Simpson MV. (1958) Incorporation of labeled amino acids into the protein of muscle and liver mitochondria. *J Biol Chem* 233: 657-663.

McShane MA, Hammans SR, Sweeney M, Holt IJ, Beattie TJ, Brett EM, Harding AE. (1991) Pearson syndrome and mitochondrial encephalomyopathy in a patient with a deletion of mtDNA. *Am J Hum Genet* 48: 39-42.

Meirelles FV, Smith LC. (1997) Mitochondrial genotype segregation in a mouse heteroplasmic lineage produced by embryonic karyoplast transplantation. *Genetics* 145: 445-451.

Merante F, Tein I, Benson L, Robinson BH. (1994) Maternally inherited hypertrophic cardiomyopathy due to a novel T-to-C transition at nucleotide 9997 in the mitochondrial tRNA (glycine) gene. *Am J Hum Genet* 55: 437-446.

Merriwether DA, Clark AG, Ballinger SW, Schurr TG, Soodyall H, Jenkins T, Sherry ST, Wallace DC. (1991) The Structure of Human Mitochondrial DNA Variation. *J Mol Evol* 33: 543-555

Michaels GS, Hauswirth WW, Laipis PJ. (1982) Mitochondrial DNA copy number in bovine oocytes and somatic cells. *Dev Biol* 94: 246-251.

Mita S, Schmidt B, Schon EA, DiMauro S, Bonilla E. (1989) Detection of ”deleted” mitochondrial genomes in cytochrome-c oxidase-deficient muscle fibers of a patient with Kearns-Sayre syndrome. *Proc Natl Acad Sci USA* 86: 9509-9513.

Mizuno Y, Ikebe S, Hattori N, Kondo T, Tanaka M, Ozawa T. (1993) Mitochondrial energy crisis in Parkinson’s disease. *Adv Neurol* 60: 282-287.

Mizuno Y, Ikebe S, Hattori N, Nakagawa-Hattori Y, Mochizuki H, Tanaka M, Ozawa T. (1995) Role of mitochondria in the etiology and pathogenesis of Parkinson’s disease. *Biochim Biophys Acta* 1271: 265-274.

Moraes CT, Ciacci F, Bonilla E, Ionasescu V, Schon EA, DiMauro S. (1993a) A mitochondrial tRNA anticodon swap associated with a muscle disease. *Nat Genet* 4: 284-288.

Moraes CT, Ciacci F, Bonilla E, Jansen C, Hirano M, Rao N, Lovelace RE, Rowland LP, Schon EA, DiMauro S. (1993b) Two novel pathogenic mitochondrial DNA mutations affecting organelle number and protein synthesis. Is the tRNA(Leu(UUR)) gene an etiologic hot spot? *J Clin Invest* 92: 2906-2915

Moraes CT, Ciacci F, Silvestri G, Shanske S, Sciacco M, Hirano M, Schon EA, Bonilla E, DiMauro S. (1993c) Atypical clinical presentations associated with the MELAS mutation at position 3243 of human mitochondrial DNA. *Neuromuscul Disord* 3: 43-50.

Moraes CT, DiMauro S, Zeviani M, Lombes A, Shanske S, Miranda AF, Nakase H, Bonilla E, Werneck LC, Servidei S. (1989) Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome: see comments: *N Engl J Med* 320: 1293-1299.

Moraes CT, Ricci E, Bonilla E, DiMauro S, Schon EA. (1992) The mitochondrial tRNA(Leu(UUR)) mutation in mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS): genetic, biochemical, and morphological correlations in skeletal muscle. *Am J Hum Genet* 50: 934-949.

Moraes CT, Sciacco M, Ricci E, Tengan CH, Hao H, Bonilla E, Schon EA, DiMauro S. (1995) Phenotype-genotype correlations in skeletal muscle of patients with mtDNA deletions. *Muscle Nerve* 3: S150-153.

Moraes CT, Shanske S, Tritschler HJ, Aprille JR, Andreetta F, Bonilla E, Schon EA, DiMauro S. (1991).

mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet* 48: 492-501.

Morten KJ, Cooper JM, Brown GK, Lake BD, Pike D, Poulton J. (1993) A new point mutation associated with mitochondrial encephalomyopathy. *Hum Mol Genet* 2: 2081-2087.

Nakamura M, Nakano S, Goto Y, Ozawa M, Nagahama Y, Fukuyama H, Akiguchi I, Kaji R, Kimura J. (1995). A novel point mutation in the mitochondrial tRNASer (UCN) gene detected in a family with MERRF/MELAS overlap syndrome. *Biochem Biophys Res Commun* 214: 86-93.

Nakase H, Moraes CT, Rizzuto R, Lombes A, DiMauro S, Schon EA. (1990) Transcription and translation of deleted mitochondrial genomes in Kearns-Sayre syndrome: implications for pathogenesis. *Am J Hum Genet* 46: 418-427.

Nass S, Nass MMK. (1963) Intramitochondrial fibers with DNA characteristics. *J Cell Biol* 19: 613-629.

Nelson I, Hanna MG, Alsanjari N, Scaravilli F, Morgan-Hughes JA, Harding AE. (1995) A new mitochondrial DNA mutation associated with progressive dementia and chorea: a clinical, pathological, and molecular genetic study. *Ann Neurol* 37: 400-403.

Nikoskelainen EK, Savontaus ML, Wanne OP, Katila MJ, Nummelin KU. (1987) Leber's hereditary optic neuroretinopathy, a maternally inherited disease. A genealogic study in four pedigrees. *Arch Ophthalmol* 105: 665-71.

Nishino I, Seki A, Maegaki Y, Takeshita K, Horai S, Nonaka I, Goto Y. (1996) A novel mutation in the mitochondrial tRNA (Thr) gene associated with a mitochondrial encephalomyopathy. *Biochem Biophys Res Commun* 225: 180-185.

Noer AS, Sudoyo H, Lertrit P, Thyagarajan D, Utthanaphol P, Kapsa R, Byrne E, Marzuki S. (1991) A tRNA (Lys) mutation in the mtDNA is the causal genetic lesion underlying myoclonic epilepsy and ragged-red fiber (MERRF) syndrome. *Am J Hum Genet* 49: 715-722.

Ozawa M, Goto Y, Sakuta R, Tanno Y, Tsuji S, Nonaka I. (1995) The 8,344 mutation in mitochondrial DNA: a comparison between the proportion of mutant DNA and clinico-pathologic findings. *Neuromuscul Disord* 5: 483-8.

Parisi MA, Clayton DA. (1991) Similarity of human mitochondrial transcription factor 1 to high mobility group proteins. *Science* 252: 965-969.

Parker WDJ, Boyson SJ, Luder AS, Parks JK. (1990a) Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease. *Neurology* 40: 1231-1234

Parker WDJ, Filley CM, Parks JK. (1990b) Cytochrome oxidase deficiency in Alzheimer's disease. *Neurology* 40: 1302-1303.

Pavlakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. (1984). Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: a distinctive clinical syndrome. *Ann Neurol* 16: 481-488.

Pearson HA, Lobel JS, Kocoshis SA, Naiman JL, Windmiller J, Lammi AT, Hoffman R, Marsh JC. (1979) A new syndrome of refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic function. *J Pediatr* 95: 976.

Peterson C, Goldman JE. (1986) Alterations in calcium content and biochemical processes in cultured skin fibroblasts from aged and Alzheimer donors. *Pro Natl Acad Sci USA* 83: 2758-2762.

Petty RK, Harding AE, Morgan-Hughes JA. (1986) The clinical features of mitochondrial myopathy. *Brain* 109: 915-938.

Poulton J, Deadman ME, Turnbull DM, Lake B, Gardiner RM. (1991) Detection of mitochondrial DNA deletions in blood using the polymerase chain reaction: non-invasive diagnosis of mitochondrial myopathy. *Clin Genet* 39: 33-38.

Poulton J, Morten K, Freeman-Emmerson C, Potter C, Sewry C, Dubowitz V, Kidd H, Stephenson J, Whitehouse W, Hansen FJ. (1994a) Deficiency of the human mitochondrial transcription factor h-mtTFA in infantile mitochondrial myopathy is associated with mtDNA depletion. *Hum Mol Genet* 3: 1763-1769.

Poulton J, Morten KJ, Marchington D, Weber K, Brown GK, Rötig A, Bindoff L. (1995a) Duplications of mitochondrial DNA in Kearns-Sayre syndrome. *Muscle Nerve* 3: S154-158.

Poulton J, Morten KJ, Weber K, Brown GK, Bindoff L. (1994b) Are duplications of mitochondrial DNA characteristic of Kearns-Sayre syndrome? *Hum Mol Genet* 3: 947-951.

Poulton J, O'Rahilly S, Morten KJ, Clark A. (1995b) Mitochondrial DNA, diabetes and pancreatic pathology in Kearns-Sayre syndrome. *Diabetologia* 38: 868-871.

Preiss T, Lowerson SA, Weber K, Lightowlers RN. (1995) Human mitochondria: distinct organelles or dynamic network? *Trends Genet* 11: 211-212.

Prezant TR, Agapian JV, Bohlman MC, Bu X, Öztas S, Qiu W-Q, Arnos KS, Cortopassi GA, Jaber L, Rotter JI, Shohat M, Fischel-Ghodsian N. (1993) Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat Genet* 4: 289-294.

Reid FM, Vernham GA, Jacobs HT. (1994) Complete mtDNA sequence of a patient in a maternal pedigree with sensorineural deafness. *Hum Mol Genet* 3: 1435-1436.

Reynier P, Chrétien M-F, Savagner F, Larcher G, Rohmer V, Barrière P, Malthièry Y. (1998). Long PCR analysis of human gamete mtDNA suggestsdefective mitochon-

drial maintenance in spermatozoa and supports the bottleneck theory for oocytes. *Biochem Biophys Res Common* 252: 373-377.

Richter C, Park J-W, Ames BN. (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci USA* 85: 6465-6467.

Rosing HS, Hopkins LC, Wallace DC, Epstein CM, Weidenheim K. (1985) Maternally inherited mitochondrial myopathy and myoclonic epilepsy. *Ann Neurol* 17: 228-237.

Rötig A, Bourgeron T, Chretien D, Rustin P, Munnich A. (1995) Spectrum of mitochondrial DNA rearrangements in the Pearson marrow-pancreas syndrome. *Hum Mol Genet* 4: 1327-1330.

Rötig A, Colonna M, Blanche S, Fischer A, Le Deist F, Frezal J, Saudubray JM, Munnich A. (1988) Deletion of blood mitochondrial DNA in pancytopenia. *Lancet* 2: 567-568.

Rötig A, Colonna M, Bonnefont JP, Blanche S, Fischer A, Saudubray JM, Munnich A. (1989) Mitochondrial DNA deletion in Pearson's marrow/pancreas syndrome. *Lancet* 1: 902-903.

Rötig A, Cormier V, Blanche S, Bonnefont JP, Ledeist F, Romero N, Schmitz J, Rustin P, Fischer A, Saudubray JM. (1990) Pearson's marrow-pancreas syndrome. A multisystem mitochondrial disorder in infancy. *J Clin Invest* 86: 1601-1608.

Rötig A, Cormier V, Chatelain P, Francois R, Saudubray J-M, Rustin P, Munnich A. (1993) Deletion of mitochondrial DNA in a case of early-onset diabetes-mellitus, optic atrophy, and deafness (Wolfram syndrome, MIM 222300). *J Clin Invest* 91: 1095-1098.

Rovio AT, Marchington DR, Donat S, Schuppe HC, Abel J, Fritsche E, Elliott DJ, Laippala P, Ahola AL, McNay D, Harrison RF, Hughes B, Barrett T, Bailey DM, Mehmet D, Jequier AM, Hargreave TB, Kao SH, Cummins JM, Barton DE, Cooke HJ, Wei YH, Wichmann L, Poulton J, Jacobs HT. (2001) Mutations at the mitochondrial DNA polymerase (POLG) locus associated with male infertility. *Nat Genet*. 29 (3): 261-2.

Sacconi S, Salviati L, Gooch C, Bonilla E, Bonilla E, Shanske S, DiMauro S. (2002). Complex neurologic syndrome associated with the G1606A mutation of mitochondrial DNA. *Arch Neurol*. 59(6): 1013-5.

Sakuta R, Goto Y, Horai S, Nonaka I. (1993) Mitochondrial DNA mutations at nucleotide positions 3243 and 3271 in mitochondrial myopathy, encephalopathy, lactic acidosis, and Stroke-Like episodes - a comparative study. *J Neurol Sci* 115: 158-160.

Sancho S, Moraes CT, Tanji K, Miranda AF. (1992) Structural and functional mitochondrial abnormalities associated with high levels of partially deleted mitochondrial DNAs in somatic cell hybrids. *Somat Cell Mol Genet* 18: 431-442.

Santorelli FM, Barmada MA, Pons R, Zhang LL, DiMauro S. (1996a) Leigh-type neuropathology in Pearson syndrome associated with impaired ATP production and a novel mtDNA deletion. *Neurology* 47: 1320-3.

Santorelli FM, Mak S-C, El-Schahawi M, Casali C, Shanske S, Baram TZ, Madrid RE, DiMauro S. (1996b) Maternally inherited cardiomyopathy and hearing loss associated with a novel mutation in the mitochondrial tRNA (Lys) gene (G8363A). *Am J Hum Genet* 58: 933-939.

Santorelli FM, Mak SC, Vàzquez-Acevedo M, Gonzàlez-Astiazaràn A, Ridaura-Sanz C, Gonz: alez-Halphen D, DiMauro S. (1995) A novel mitochondrial DNA point mutation associated with mitochondrial encephalocardiomyopathy. *Biochem Biophys Res Commun* 216: 835-840.

Santorelli FM, Schlessel JS, Slonim AE, DiMauro S. (1996c) Novel mutation in the mitochondrial DNA tRNA glycine gene associated with sudden unexpected death. *Pediatr Neurol* 15: 145-149.

Santorelli FM, Shanske S, Jain KD, Tick D, Schon EA, DiMauro S. (1994) A T—>C mutation at nt 8993 of mitochondrial DNA in a child with Leigh syndrome. *Neurology* 44: 972-974.

Satoh M, Kuroiwa T. (1991) Organization of multiple nucleotodes and DNA molecules in mitochondrial of a human cell. *Exp Cell Res* 196: 137-140.

Schapira AH, Mann VM, Cooper JM, Krige D, Jenner PJ, Marsden CD. (1992) Mitochondrial function in Parkinson's disease. The Royal Kings and Queens Parkinson's Disease Research Group. *Ann Neurol* 32: S116-124.

Sciacco M, Bonilla E, Schon EA, DiMauro S, Moraes CT. (1994) Distribution of wild-type and common deletion forms of mtDNA in normal and respiration-deficient muscle fibers from patients with mitochondrial myopathy. *Hum Mol Genet* 3: 13-19.

Seibel P, Degoul F, Bonne G, Romero N, François D, Paturneau-Jouas M, Ziegler F, Eymard B, Fardeau M, Marsac C, Kadenbach B. (1991) Genetic biochemical and pathophysiological characterization of a familial mitochondrial encephalomyopathy (MERRF). *J Neurol Sci* 105: 217-224.

Seibel P, Lauber J, Klopstock T, Marsac C, Kadenbach B, Reichmann H. (1994) Chronic progressive external ophthalmoplegia is associated with a novel mutation in the mitochondrial tRNA (Asn) gene. *Biochem Biophys Res Commun* 204: 482-489.

Shaag A, Saada A, Steinberg A, Navon P, Elpeleg O. (1997) Mitochondrial encephalomyopathy associated with a novel mutation in the mitochondrial tRNA (Leu)(UUR) gene (A3243T). *Biochem Biophys Res Commun* 233: 637-639.

Shay JW, Pierce DJ, Werbin H. (1990) Mitochondrial DNA copy number is proportional to total cell DNA

under a variety of growth conditions. *J Biol Chem* 265: 14802-14807.

Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. (1990) Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA (Lys) mutation. *Cell* 61: 931-937.

Shoffner JM, Wallace DC. (1991) A Mitochondrial Transfer RNALYS Mutation Causes Myoclonic Epilepsy and Ragged-Red Fiber Disease. In: Sato T, Dimauro S (eds) Mitochondrial Encephalomyopathies, vol 7. Raven Press, 1185 Ave of the Americas, New York, NY 10036, pp 161-167.

Shoffner JMt, Wallace DC. (1990) Oxidative phosphorylation diseases. Disorders of two genomes. *Adv Hum Genet* 19: 267-330.

Shoubridge EA, Karpati G, Hastings KEM. (1990) Deletion mutants are functionally dominant over wild-type mitochondrial genomes in skeletal muscle fiber segments in mitochondrial disease. *Cell* 62: 43-49.

Silvestri G, Moraes CT, Shanske S, Oh SJ, DiMauro S. (1992) A new mtDNA mutation in the tRNA (Lys) gene associated with myoclonic epilepsy and ragged-red fibers (MERRF). *Am J Hum Genet* 51: 1213-1217.

Silvestri G, Santorelli FM, Shanske S, Whitley CB, Schimmenti LA, Smith SA, DiMauro S. (1994) A new mtDNA mutation in the tRNA (Leu (UUR)) gene associated with maternally inherited cardiomyopathy. *Hum Mutat* 3: 37-43.

Silvestri G, Servidei S, Rana M, Ricci E, Spinazzola A, Paris E, Tonali P. (1996) A novel mitochondrial DNA point mutation in the tRNA (Ile) gene is associated with progressive external ophtalmoplegia. *Biochem Biophys Res Commun* 220: 623-627.

Sims NR, Finegan JM, Blass JP, Bowen DM, Nearly D. (1987) Mitochondrial function in brain tissue in primary degenerative dementia. *Brain Res* 436: 30-38.

Singh G, Neckelmann N, Wallace DC. (1987) Conformational mutations in human mitochondrial DNA. *Nature* 329: 270-272.

Smith PR, Cooper JM, Govan GG, Harding AE, Schapira AH. (1994) Platelet mitochondrial function in Leber's hereditary optic neuropathy. *J Neurol Sci* 122: 80-83.

Stoneking M. (1994) Mitochondrial DNA and human evolution. *J Bioenerg Biomembr* 26: 251-259.

Stoneking M, Soodyall H. (1996) Human evolution and the mitochondrial genome. *Curr Opin Genet Dev* 6: 731-736.

Suomalainen A, Majander A, Pihko H, Peltonen L, Syvànen AC. (1993) Quantification of tRNA3243 (Leu) point mutation of mitochondrial DNA in MELAS patients and its effects on mitochondrial transcription. *Hum Mol Genet* 2: 525-534.

Superti-Furga A, Schoenle E, Tuchschmid P, Caduff R, Sabato V, DeMattia D, Gitzelmann R, Steinmann B. (1993) Pearson bone marrow-pancreas syndrome with insulin-dependent diabetes, progressive renal tubulopathy, organic aciduria and elevated fetal haemoglobin caused by deletion and duplication of mitochondrial DNA. *Eur J Pediatr* 152: 44-50.

Sweeney MG, Bundey S, Brockington M, Poulton KR, Winer JB, Harding AE. (1993) Mitochondrial myopathy associated with sudden death in young adults and a novel mutation in the mitochondrial DNA leucine transfer RNA (UUR) gene. *Q J Med* 86: 709-713.

Tanaka M, Ino H, Ohno K, Hattori K, Sato W, Ozawa T, Tanaka T, Itoyama S. (1990) Mitochondrial mutation in fatal infantile cardiomyopathy. *Lancet* 336: 1452.

Taniike M, Fukushima H, Yanagihara I, Tsukamoto H, Tanaka J, Fujimura H, Nagai T, Sano T, Yamaoka K, Inui K, Okada S. (1992) Mitochondrial tRNA (Ile) mutation in fatal cardiomyopathy. *Biochem Biophys Res Commun* 186: 47-53.

Tanno Y, Yoneda M, Tanaka K, Kondo R, Hozumi I, Wakabayashi K, Yamada M, Fukuhara N, Ikuta F, Tsuji S. (1993) Uniform tissue distribution of transfer RNA (Lys) mutation in mitochondrial DNA in MERRF patients. *Neurology* 43: 1198-1200.

Tatuch Y, Christodoulou J, Feigenbaum A, Clarke JTR, Wherret J, Smith C, Rudd N, Petrova-Benedict R, Robinson BH. (1992) Heteroplasmic mtDNA mutation (T->G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. *Am J Hum Genet* 50: 852-858.

Tessa A, Giannotti A, Tieri L, Vilarinho L, Marotta G, Santorelli FM. (2001) Maternally inherited deafness associated with a T1095C mutation in the mDNA. *Eur J Hum Genet*. 9 (2): 147-9.

Thyagarajan D, Shanske S, Vazquez-Memije M, De Vivo D, DiMauro S (1995) A novel mitochondrial ATPase 6 point mutation in familial bilateral striatal necrosis. *Ann Neurol* 38: 468-472.

Torroni A, Schurr TG, Yang CC, Szathmary EJ, Williams RC, Schanfield MS, Troup GA, Knowler WC, Lawrence DN, Weiss KM. (1992) Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. *Genetics* 130: 153-162.

Tritschler HJ, Andreetta F, Moraes CT, Bonilla E, Arnaudo E, Danon MJ, Glass S, Zelaya BM, Vamos E, Telerman-Toppet N, Shanske S, Kadenbach B, DiMauro S, Schon EA. (1992) Mitochondrial myopathy of childhood associated with depletion of mitochondrial DNA. *Neurology* 42: 209-217.

Trounce I, Neill S, Wallace DC (1994) Cytoplasmic transfer of the mtDNA nt 8993 T—>G (ATP6) point mutation associated with Leigh syndrome into mtDNA-less cells demonstrates cosegregation with a decrease in state III respiration and ADP/O ratio. *Proc Natl Acad Sci USA* 91: 8334-8338.

Träff J, Holme E, Ekbom K, Nilsson BY. (1995) Ekbom's syndrome of photomyoclonus, cerebellar ataxia and

cervical lipoma is associated with the tRNA (Lys) A8344G mutation in mitochondrial DNA. *Acta Neurol Scand* 92: 394-397.

Tulinius MH, Oldfors A, Holme E, Larsson NG, Houshmand M, Fahleson P, Sigstr:om L, Kristiansson B. (1995) Atypical presentation of multisystem disorders in two girls with mitochondrial DNA deletions. *Eur J Pediatr* 154: 35-42.

Tulinius MH, Houshmand M, Larsson NG, Holme E, Oldfors A, Holmberg E, Wahlström J. (1996) De novo mutation in the mitochondrial ATP synthase subunit 6 gene (T8993G) with rapid segregation resulting in Leigh syndrome in the offspring. *Hum Genet* 96: 290-294.

Van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, Sandkuijl LA, Devijlder MF, Struyvenberg PAA, Vandekamp JJP, Maassen JA. (1992) Mutation in Mitochondrial transfer RNA (Leu(UUR)) Gene in a Large Pedigree with Maternally Transmitted Type-II Diabetes-Mellitus and Deafness. *Nat Genet* 1: 368-371.

Van den Ouweland JMW, Lemkes HHPJ, Trembath R, Ross R, Velho G, Cohen D, Froguel P, Maassen JA. (1994) Maternally inherited diabetes and deafness is a distinct subtype of diabetes and associates with a single point mutation in the mitochondrial tRNALeu (UUR) gene. *Diabetes* 43: 746-751.

Veltri KL, Espiritu M, Singh G (1990) Distinct genomic copy number in mitochondria of different mammalian organs. *J Cell Physiol* 143: 160-164.

Verma A, Piccoli DA, Bonilla E, Berry GT, DiMauro S, Moraes CT. (1997). A novel mitochondrial G8313A mutation associated with Prominent Initial Gastrointestinal Symptoms and Progressive Encephaloneuropathy. *Pediatr Res* 42: 448-454.

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC. (1991) African populations and the evolution of human mitochondrial DNA. *Science* 253: 1503-1507.

Vigilant L, Stoneking M, Wilson AC (1988) Conformational mutation in human mtDNA detected by direct sequencing of enzymatically amplified DNA. *Nucleic Acids Res* 16: 5945-55.

Wallace DC. (1989) Mitochondrial DNA mutations and neuromuscular disease. *Trends Genet* 5: 9-13.

Wallace DC. (1992) Diseases of the Mitochondrial DNA. *Annu Rev Biochem* 61: 1175-1212.

Wallace DC. (1993) Mitochondrial diseases - genotype versus phenotype. *Trends Genet* 9: 128-133.

Wallace DC. (1996) Mitochondrial DNA mutations and bioenergetic defects in aging and degenerative diseases. *The molecular and genetic basis of neurological disease* second edition: 237-269.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJd, Nikoskelainen EK. (1988a) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 242: 1427-1430.

Wallace DC, Ye JH, Neckelmann SN, Singh G, Webster KA, Greenberg BD. (1987) Sequence analysis of cDNAs for the human and bovine ATP synthase b-subunit: mitochondrial DNA genes sustain seventeen times more mutations. *Curr Genet* 12: 81-90.

Wallace DC, Zheng X, Lott MT, Shoffner JM, Hodge JA, Kelley RI, Epstein CM, Hopkins LC. (1988b) .Familial mitochondrial encephalomyopathy (MERRF): genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. *Cell* 55: 601-610.

Yang T, Lam CW, Tsang MW, Tong SF, Kam GY, Chan LY, Poon PM, Wu X, Pang CP. (2002) Novel mitochondrial 16S rRNA mutation, 3200T—>C, associated with adult-onset type 2 diabetes. *Chin Med J (Engl)*; 115(5): 753-8.

Yoneda M, Tanno Y, Horai S, Ozawa T, Miyatake T, Tsuji S. (1990) A common mitochondrial DNA mutation in the t-RNA (Lys) of patients with myoclonus epilepsy associated with ragged-red fibers. *Biochem Int* 21: 789-796.

Yoon KL, Aprille JR, Ernst SG. (1991) Mitochondrial tRNA (thr) mutation in fatal infantile respiratory enzyme deficiency. *Biochem Biophys Res Commun* 176: 1112-1115.

Zeviani M, Bresolin N, Gellera C, Bordoni A, Pannacci M, Amati P, Moggio M, Servidei S, Scarlato G, DiDonato S. (1990) Nucleus-driven multiple large-scale deletions of the human mitochondrial genome: a new autosomal dominant disease. *Am J Hum Genet* 47: 904-914.

Zeviani M, Gellera C, Antozzi C, Rimoldi M, Morandi L, Villani F, Tiranti V, DiDonato S. (1991) .Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA tRNALeu (UUR). *Lancet* 338: 143-147.

Zeviani M, Moraes CT, DiMauro S, Nakase H, Bonilla E, Schon EA, Rowland LP. (1988) Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 38: 1339-1346.

Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S, DiDonato S. (1989) An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. *Nature* 339: 309-311.