Mitochondrial disease

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Defects of mitochondrial metabolism cause a wide range of human diseases that include examples from all medical subspecialties. This review updates the topic of mitochondrial diseases by reviewing the most important recent advances in this area. The factors influencing inheritance, maintenance and replication of mtDNA are reviewed and the genotype-phenotype of mtDNA disorders has been expanded, with new insights into epidemiology, pathogenesis and its role in ageing. Recently identified nuclear gene mutations of mitochondrial proteins include mutations of *frataxin* causing Friedreich's ataxia, *PINK1, DJ1* causing Parkinson's disease and *POLG* causing infantile mtDNA depletion syndrome, ophthalmoplegia, parkinsonism, male subfertility and, in a transgenic mouse model, premature senescence. Mitochondrial defects in neurodegenerative diseases include Parkinson's, Alzheimer's and Huntington's disease. Improved understanding of mtDNA inheritance and mutation penetrance patterns, and novel techniques for mtDNA modification offer significant prospects for more accurate genetic counselling and effective future therapies.

Mitochondria are ubiquitous in eukaryotes and are essential for survival. Their primary function is to support aerobic respiration and to provide energy substrates (such as ATP) for intracellular metabolic pathways. Mitochondria have also been shown to play an important role in cell signalling, particularly in signalling for apoptotic cell death. Mitochondria host several metabolic pathways, including the Krebs cycle, β-oxidation, and lipid and cholesterol synthesis. Given its fundamental role in the human body, defects of mitochondrial function can have disastrous consequences. The potential for mitochondrial dysfunction is increased by the presence of mitochondrial DNA (mtDNA) that, in humans, is responsible for the encoding of proteins critical for oxidative phosphorylation. Thus, human mitochondrial diseases encompass mutations of mtDNA and nuclear DNA, as well as toxin-induced defects. The 5 years since the last reviews in *The Lancet* on mitochondria[1,2] have seen major advances in our understanding of mitochondrial diseases, and in particular, the involvement of nuclear mutations in genes encoding mitochondrial proteins. This review aims to provide an update on the involvement of mitochondria in human disease. Not all mitochondrial clinical phenotypes will be discussed in detail, since these have been reviewed elsewhere.[3–5]

Mitochondrial biology

Structure

Mitochondria are intracellular double membrane-bound structures. Although traditionally considered as small isolated organelles within the cell, it is more likely that mitochondria form a complex branching network. The density of mitochondria varies from one tissue to another, and is related to that tissue's dependence upon oxidative phosphorylation for energy provision. Thus, neurones, and cardiac and skeletal muscle cells have a high density of mitochondria, which to some extent explains their sensitivity to energy-dependent defects resulting from mitochondrial abnormalities. Mitochondria contain their own DNA, which is thought to be a remnant from the time that they were free-living organisms before forming a symbiotic relationship with eukaryotes. Human mtDNA is a circular double stranded molecule about 16·6 Kb long. It is much smaller than most nuclear genes. MtDNA codes for 22 transfer and two ribosomal RNAs, and for 13 proteins. Human mtDNA is extremely compact and contains virtually no intronic (non-coding) regions. Although human mtDNA encodes the basic machinery for protein synthesis, it remains entirely dependent upon the nucleus for the provision of enzymes for replication, repair, transcription and translation. This dependency lies at the heart of several newly recognised human diseases that are characterised by secondary abnormalities of mtDNA.

Search strategy and selection criteria

References for this review were identified by searches of PubMed and MEDLINE using the search terms "mitochondrial DNA", "polymerase gamma" between 2000 and 2005. References were also identified from relevant articles and through searches of the author's files. Only papers reviewed in English were selected.

MtDNA replication and function

MtDNA is continuously replicating, and the timing of replication seems to proceed independently of that of the nucleus, and occurs in dividing as well as non-dividing cells.[6] MtDNA polymerase γ (POLG), thymidine kinase 2, and deoxyguanosine kinase are nuclear encoded enzymes responsible for replication. MtDNA is transcribed polycistronically and translated on mitochondrial ribosomes. MtDNA transcription is necessary for the initiation of replication. A novel D-loop replication origin (at position 57) has been identified and is thought to represent the major site of mtDNA replication under steady-state conditions.[7] There are important but subtle differences in the translation code that are specific for mtDNA and prevent the translation of nuclear DNA within mitochondria, and vice versa.

MtDNA is highly polymorphic, with several differences in sequence between individuals from the same ethnic group and more between those in different groups.

MtDNA haplotypes are based upon specific patterns of polymorphisms and seem to influence the ageing process, susceptibility to some diseases, and the expression of some mtDNA mutations.[8,9] MtDNA haplotypes have been used to track population movements across the globe and serve to provide a means to evaluate ethnic descent. There is evidence of intra-molecular recombination in mtDNA,[10,11] but only recently has evidence been obtained for intermolecular recombination.[12] All 13 proteins encoded by human mtDNA are subunits of the respiratory chain and oxidative phosphorylation system (OXPHOS) (figure 1 and figure 2). These proteins are essential for normal OXPHOS. Eradication of mtDNA from cells to produce ρ^0 cells results in the loss of OXPHOS and the provision of ATP by glycolysis alone. Furthermore, transfer of mtDNA to ρ^0 cells with another nuclear background results in OXPHOS activity that correlates with the donor activities.[13] Thus, the mtDNA-encoded subunits are not only essential for OXPHOS, but they also, at least in this model, influence the level of enzyme activity. The five OXPHOS complexes are located on the inner mitochondrial membrane, and comprise about 85 subunits. Substrates supply reducing equivalents to complexes I and II and electrons pass down the respiratory chain via ubiquinone and cytochrome c to complexes III and IV. An electrochemical gradient is established by the pumping of protons out of the mitochondrial inner membrane and this is used by complex V to synthesise ATP (figure 2).

MtDNA inheritance

MtDNA is inherited through the maternal line. Most researchers believed that no paternal mtDNA from sperm enters the fertilised ovum at the point of conception, the embryo thus developing with maternal mtDNA alone. However, one report of the paternal inheritance of a microdeletion mutation in a mtDNA complex I gene indicated that there may be rare exceptions to this.[14] Analysis of infants born after intracytoplasmic sperm injection failed to identify paternal mtDNA with methods capable of detecting levels as low as 0·001%.[15–17] This implied that paternal mtDNA replication was either suppressed, or diluted beyond the limits of detection, to levels at which its contribution would in any event be irrelevant. Indeed, there is some evidence for selective targeting of sperm mitochondria for degradation by the ovum.[18,19] Failure of an ovum to eliminate paternal mtDNA could result in loss of the embryo at the blastocyst stage.[20] Investigators have also failed to detect paternal mtDNA in patients with sporadic mitochondrial myopathies.[21,22] Thus, if paternal transmission does occur, it is rare and might depend on the presence of particular paternal mutations that allow the sperm's mtDNA to escape destruction as well as to permit replication. In any event, the dogma that human mtDNA is exclusively maternally inherited remains a reasonable basis for genetic counselling.

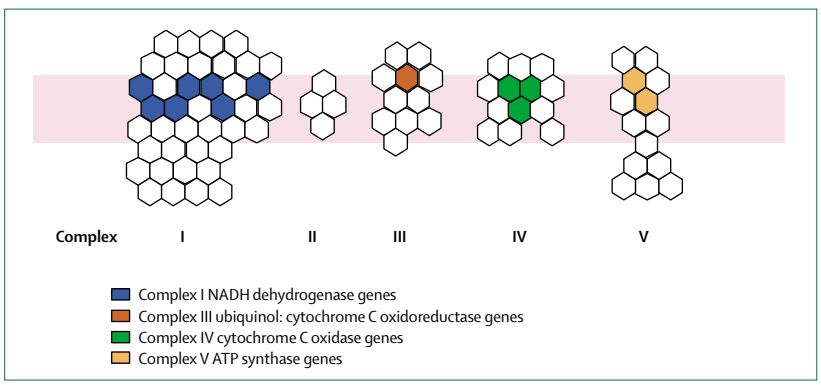

Figure 1: **Mitochondrial respiratory chain and oxidative phosphorylation system**
Each hexagon represents a polypeptide product of a single gene.

Homoplasmy and heteroplasmy

There are thousands of mtDNA molecules per cell, and millions per individual. For the most part, their sequence will be identical (homoplasmy). However, somatic mtDNA mutations arise and accumulate with ageing, and could have a role in the senescence of tissues. The most common source of somatic mutation of mtDNA is the free radicals generated by the respiratory chain itself. Mitochondrial diseases are characterised by the coexistence of wild type and mutant mtDNA in various proportions (heteroplasmy). The notion that the percentage of mutant form determines or contributes to disease expression is an important concept in mitochondrial disease.

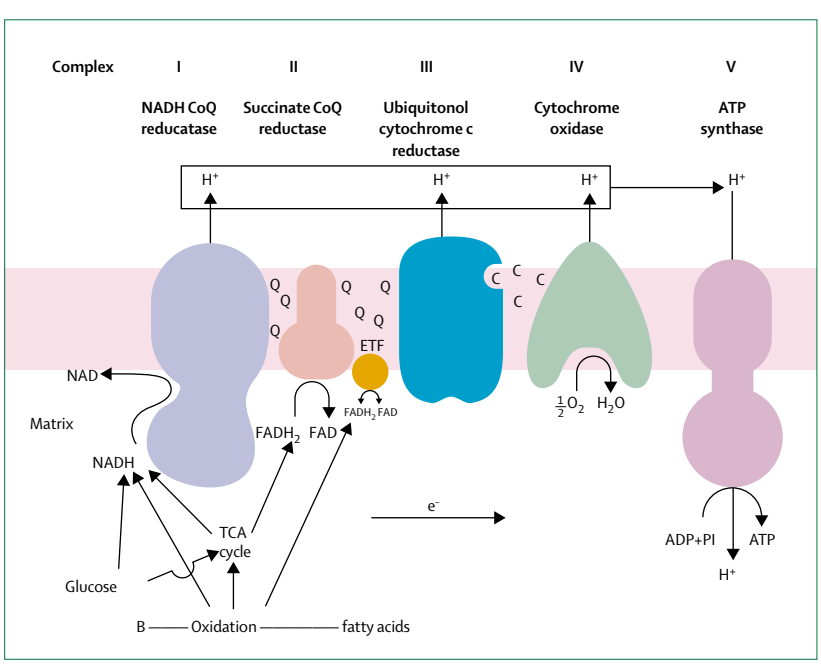

Figure 2: **The functional features of mitochondrial respiratory chain and oxidative phosphorylation system**
NADH=the reduced form of nicotinamide-adenine dinucleotide; CoQ=ubiquinone; ATP=adenosine triphosphate; NAD=nicotinamide adenine dinucleotide; TCA=tricarboxylic acid cycle; $FADH_2$=the reduced form of flavin adenine dinucleotide; FAD=flavin adenine dinucleotide; ETF=electron transfer flavoprotein; e^-=electron; ADP=adenosine diphosphate; Pi=inorganic phosphate.

Mitochondria are randomly segregated at cell division including oogenesis. It is during oogenesis that wild-type and mutant mtDNA molecules are randomly partitioned to oocytes creating a spectrum of heteroplasmy across the oocyte population. This random segregation occurs through tight "bottlenecks": of the 150 000 mtDNA molecules estimated to be present in human oocytes, only a small proportion of this mtDNA population is transmitted both during oogenesis and subsequently to the embryo. This has important implications for transmission of mutant mtDNA, as well as the tissue specific basis of disease expression. A high proportion of mutant load in the oocyte population could result in a high proportion of affected offspring—ie, a high transmission rate.[23]

Mitochondrial transport

Mitochondria have evolved a complex system to import nuclear-encoded proteins. Proteins might contain an N-terminal aminoacid sequence that targets them to outer mitochondrial membrane receptors, which unfold and import the protein before excision of the targeting sequence and chaperoning to the appropriate mitochondrial compartment. Identification of a targeting sequence is an important indicator of mitochondrial localisation, and has been useful in determining the distribution and function of proteins. Diseases caused by defects of the mitochondrial import system are rare, but mutations of a gene involved in mitochondrial import has been shown to be the cause of the Mohr-Tranebjaerg syndrome (sensorineural deafness, dystonia, dysphagia, cortical blindness, and paranoia).[24,25]

	mtDNA mutation
CPEO	3243 tRNALeu(UUR)
Cardiomyopathy with or without encephalopathy	4269 tRNAIle
	4317I tRNAIe
	3260 tRNALeu(UUR)
	3243 tRNALeu(UUR)
KSS	Deletion/duplication, 3243 tRNALeu(UUR)
	8344 tRNALys
MELAS	3243 tRNALeu(UUR)
	11084 ND4
	3271 tRNALeu(UUR)
MERRF	8344 tRNALys
	8356 tRNALys
NARP	8993 ATPase 6
Pearson's syndrome	
LHON	ND4 (11778)
	ND1 (3460)
	ND6 (14484)
Leigh's syndrome	8344 tRNALys
	8993 ATPase 6
Diabetes mellitus and deafness	3243 tRNALeu(UUR)

Table: **MtDNA mutations and associated diseases**

MtDNA mutations

Investigating the epidemiology of mtDNA genetics is complicated by the wide spectrum of clinical presentation, the diverse range of mutations and the high carrier rate, all of which will lead to underestimates of prevalence. A population-based study of the A3243G mutation in northern Finland estimated prevalence at 16·3 per 100 000.[26] The mutation was found in 14% of patients with hypertrophic cardiomyopathy, 13% of patients with ophthalmoplegia, 7·4% of maternally inherited deafness, and 6·9% of those with occipital stroke. These figures emphasise the multisystem nature of mitochondrial disease and in particular their relevance to all medical specialties. In Newcastle, UK, mitochondrial diseases or the risk of their development was estimated to have a prevalence of 12·48 per 100 000.[27] This study was based on the assessment of patients and relatives referred over 15 years with mtDNA mutations, and either had mitochondrial disease or were carriers. Two studies estimated the prevalence of respiratory chain diseases in children as 4·7 per 100 000 (8·9 per 100 000 in pre-school children)[28] or 5·0 per 100 000[29]—including mutations in nuclear-encoded genes as well as in mtDNA genes.

MtDNA deletions

MtDNA deletions were the first mutations to be described and associated with human disease.[30] The size of the deletion can vary from a single base to several kilobases and be located on any part of the molecule. The most common deletion is 5 Kb long, and spans the region between the genes for cytochrome b and cytochrome oxidase subunit II, thus encompassing tRNAs and protein-coding genes. Large-scale macrodeletions such as this have typically been associated with particular phenotypes including chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome, and Pearson's marrow-pancreas syndrome. However, the pathological expression of deletions is not restricted to these phenotypes and they have been described in association with virtually all mitochondrial encephalomyopathy syndromes.

The prevalence of single deletion disorders is estimated at 1·2 per 100 000. MtDNA deletions can occur as a single type or population, or as multiple deletions of different length. Deletions exist in heteroplasmic form, the proportion of deleted molecules varies between tissues, and the degree of heteroplasmy can shift over time. Single deletions arise as a primary mtDNA mutation, probably within the oocyte, and are transmitted to offspring who might then develop clinical features. It is highly uncommon for more than one of the offspring to be affected, and the risk of subsequent transmission from an affected woman has been estimated at 4%.[31]

Some patients have duplications of mtDNA, which although might not be pathogenic themselves, could be an intermediate step in the generation of deletions. Small microdeletions have been described in mtDNA genes,

including those for cytochrome oxidase, cytochrome b and complex I, that are associated with a variety of clinical presentations.

MtDNA point mutations

Over 100 point mutations associated with human disease have been described in protein coding genes, tRNAs, and rRNAs.[32] Their clinical expression is wide (table), and includes phenotypes such as MELAS (myopathy encephalopathy lactic acidosis and stroke-like episodes), MERRF (myoclonic epilepsy and ragged red fibres), NARP (neuropathy ataxia and retinitis pigmentosa), MILS (maternally inherited Leigh syndrome), and LHON (Leber hereditary optic neuropathy). In addition, oligosymptomatic syndromes arising from mtDNA point mutations can include diabetes mellitus, cardiomyopathy, sensorineural deafness, cardiomyopathy, and myoglobinuria. However, patients with the oligosymptomatic forms can also subsequently develop additional features. Rare and intriguing associations have been reported between mtDNA point mutations and Parkinson's disease, motor neurone disease (amyotrophic lateral sclerosis), palmoplantar keratoderma, multiple lipomas, and neurofibromatosis. Maternal inheritance of hypertension, hypercholesterolaemia, and hypomagnesaemia caused by a mtDNA mutation in the tRNA for isoleucine has been described.[33]

Pathogenesis of mtDNA mutations

The criteria for defining pathogenic mtDNA mutations have required revision as our understanding of mitochondrial biology and pathology has expanded. To a large degree, the complexity of this issue arises from the highly polymorphic nature of mtDNA. Traditionally, mutations have been defined as being absent in control populations, resulting in the substitution of a conserved aminoacid, segregating with the disease phenotype and with a relevant biochemical defect, and usually being heteroplasmic. However, there are several observations that have undermined these guidelines. Many individuals with LHON have homoplasmic mutations, and unaffected family members can have the same homoplasmic mtDNA mutation. A pathogenic tRNA mutation causing an encephalomyopathic phenotype present in heteroplasmic form in some affected members of a family, was also found in homoplasmic form in an asymptomatic 32-year-old family member.[34] Increasingly, researchers accept that mtDNA sequence changes can interact with each other, with nuclear genes, or with the environment to cause disease. Evidence also suggests that the nuclear background of a mtDNA mutation can affect its biochemical expression.[35]

The link between genotype and phenotype in mitochondrial diseases has always been recognised as complex. For instance, mtDNA deletions are not only associated with CPEO, Kearns-Sayre syndrome, and Pearson's syndrome, but have also been described in patients with MELAS, isolated diabetes, or cardiomyopathy. Similarly, the A3243G mutation is probably the commonest cause of MELAS, but has also been found in patients with CPEO, Kearns-Sayre syndrome, isolated diabetes, etc. Thus, the same mutation can result in multiple phenotypes, and the same phenotype can be caused by several different mutations, and this can apply to mutations in protein coding genes—eg, *MTND5*, just as much as tRNA genes. Furthermore, there is no helpful link between the site of an mtDNA mutation—ie, tRNA, rRNA, protein coding gene, and the resulting clinical phenotype. Multiple independent factors can influence the clinical expression of a mutation, including tissue distribution, levels of heteroplasmy, nuclear background, and the varying dependence of organs on OXPHOS for energy. Inevitably, these account for only some of the variation seen, and much remains to be discovered on the molecular pathogenesis of these mutations.

Leber hereditary optic neuropathy (LHON)

LHON is considered the most common disease caused by mtDNA mutations, with a prevalence of 11·82 per 100 000 population.[36] LHON is characterised clinically by bilateral sequential acute or sub-acute visual failure caused by degeneration of the retinal ganglion cells and their axons. Three mtDNA mutations G11778A, G3460A, and T14484C located in the *MTND4, MTND1,* and *MTND6* genes, respectively, account for about 95% of cases. The G11778A mutation is the most common, being present in 56% of cases, the G3460A in 31%, and the T14484C in 6·3%.[36] The disease occurs predominantly in young men, with usually little or no visual recovery, although the T14484C mutation is generally associated with a better prognosis. A range of secondary or intermediate polymorphisms can modify expression.[9] Additional mutations in *MTND1* and *MTND6* have been described,[37,38] with the C4171A mutation in *MTND1* being associated with significant visual recovery. All these mutations are usually present in homoplasmic or high mutant heteroplasmic proportions.

A mouse model of LHON with retinal degeneration was developed by expressing ribozymes in the eye that specifically cleaved the mRNA of a nuclear encoded complex I subunit (NDUFA1).[39] Suppressing the expression in the retina of mitochondrial Mn-superoxide dismutase (Mn-SOD), a free-radical scavenging enzyme, produced similar consequences.[40] Overexpression of Mn-SOD in the presence of inhibition of NDUFA1 expression ameliorated the deleterious effects of the latter.[41] These results imply that the complex I defect mediated damage through excess free-radical production. Additional insight into LHON pathogenesis was provided by the finding that the three primary causative mutations reduced expression of the glutamate transporter in cell culture and this effect correlated with complex I activity.[42] Mitochondrial free radical production inactivates the glutamate transporter and increases glutamate levels, indicating that oxidative stress is an important event in

LHON pathogenesis. The corollary of these observations is that effective antioxidant treatment early in the course of LHON might improve its prognosis.

Nuclear gene mutations

Although 72 of the 85 subunits of the OXPHOS system are encoded by nuclear DNA, translated on cytoribosomes and transported to the mitochondrion, mutations of these genes have only rarely been described. To some extent, this could be an indication of the deleterious nature of such mutations, with affected fetuses perhaps being aborted early in development. Mutations that have been described generally manifest in the neonatal period or early infancy, although occasional late-onset patients have been identified. Mutations in complex I subunits (*NDUFS 1,2,4,7*, and *8*; and *NDUFV1*) cause Leigh syndrome and leukodystrophy, and those of complex II subunits (I-IV) cause Leigh syndrome, ataxia, paraganglioma, and phaeochromocytoma. The association with paragangliomas and phaeochromocytomas is rare. The late onset cases have included progressive ataxia.

Several diseases have now been shown to be due to mutations in nuclear genes encoding mitochondrial proteins. These include disorders caused by mutations of proteins involved in the Krebs citric acid cycle, β-oxidation, and the urea cycle. Mutations of nuclear genes involved in the replication or maintenance of mtDNA or respiratory chain proteins can result in phenotypes identical to those associated with primary mtDNA mutations. Others cause distinct clinical presentations that might include progressive neurodegenerative diseases.

Mutations affecting MtDNA maintenance and replication

MtDNA remains dependent upon nuclear DNA for the production of a range of proteins involved in its replication, transcription, translation, repair, and maintenance. Mutations of these genes can induce multiple mtDNA deletions or depletion of mtDNA.

Adenine nucleotide translocator mutations

Adenine nucleotide translocator-1 (*ANT-1*) is an isoform specific to muscle, heart, and brain, and regulates the adenine nucleotide pool within mitochondria. *ANT1* mutations cause adult onset autosomal dominant CPEO with ragged red fibres and multiple mtDNA deletions in skeletal muscle.[43]

Twinkle mutations

Twinkle is a hexomeric 5′-3′ DNA helicase protein encoded by the *C10orf2* gene, which is responsible for unwinding the mtDNA replication fork.[44,45] Inhibition of twinkle in cell culture cells results in rapid mtDNA depletion, whereas over-expression of the gene leads to mtDNA accumulation, confirming its importance in regulating copy number.[46] Twinkle is highly expressed in human skeletal muscle and in a specific splice variant in testes, which is of interest since mtDNA replication is downregulated during spermatogenesis. Twinkle co-localises with mitochondrial transcription factor A and mitochondrial single-stranded DNA-binding protein, and together they are thought to stabilise mtDNA. Several mutations causing autosomal dominant progressive external ophthalmoplegia (PEO) are located at or near putative subunit interaction sites in the holoenzyme.

The clinical manifestations of *C10orf2* (twinkle) mutations typically include PEO. In some cases, this can be of late onset (>50 years of age) and be associated with myopathy and cardiomyopathy in addition to axonal neuropathy, diabetes, deafness, and osteoporosis.[47] Muscle biopsy shows changes of a mitochondrial myopathy with ragged red fibres. One patient with a twinkle mutation and the SANDO (sensory ataxia, neuropathy, dysarthria, and ophthalmoplegia) phenotype has been reported.[48]

MtDNA polymerase γ mutations

MtDNA polymerase γ (*POLG*) is a heterodimer comprising a 140kDa alpha subunit and a 41kDa beta subunit. It is located within the inner mitochondrial membrane and is essential for mtDNA replication. The alpha subunit is catalytic and contains both polymerase and exonuclease activities, the beta subunit facilitates DNA binding and promotes DNA synthesis.[49] Mutations of *POLG* have been associated with a range of clinical phenotypes including PEO (panel).

The human *POLG* gene contains a 10-CAG repeat length encoding a polyglutamine tract. A variation in this microsatellite—ie, section that has fewer or more than ten repeats has been associated with male subfertility.[50] This observation was confirmed in another study from Denmark, in which researchers found that the variation of one or both normal alleles was present at greater frequency in subfertile males compared to controls.[51] There was a positive association between the polymorphism and sperm concentration but not sperm motility or morphology. However, a study of Italian men failed to find any association between the *POLG* polymorphism and subfertility.[52]

POLG mutations have also been identified as a cause of Alpers syndrome, an autosomal recessive disorder characterised by epilepsy, cortical blindness, micronodular hepatic cirrhosis, and episodic psychomotor

***Panel:* Conditions associated with POLG mutations**

Autosomal dominant PEO, with or without parkinsonism
Autosomal recessive PEO, with or without parkinsonism
Alpers syndrome
SANDO—sensory ataxia, neuropathy, dysarthria, ophthalmoplegia
Male subfertility
Premature menopause
Cataracts

regression.[53,54] Onset is usually within the first few weeks or years of life, although early adult onset cases have also been described.[55,56] A homozygous stop mutation (*G2899T*) upstream of the polymerase domain was identified in affected children from unrelated families.[53] They were apparently normal until the ages of 11–19 months, after which they developed progressive encephalopathy. The children and their mothers also carried heterozygous mutations (G1681A) in the linker region of the polymerase protein that have been identified in patients with autosomal recessive PEO or SANDO.[57,58] The relevance of this co-existing heterozygous mutation is not known. POLG activity was less than 5% of normal in liver and muscle, and mtDNA levels were 25–30% of normal. A further eight patients with a variety of *POLG* mutations have been described and mtDNA depletion found in the liver and brain of two patients from whom samples were available.[54] One infant presented at 5 months with recurrent vomiting, feeding difficulty, growth arrest, hypotonia, areflexia, and liver dysfunction. She had a lactic acidosis and severe hypomyelinating neuropathy, and died at 6 months with respiratory failure. She was a compound heterozygote for mutations in the *POLG* gene. Both parents were carriers and suffered severe hypofertility.

POLG mutations have been described in patients with either autosomal dominant or recessive PEO. Many cases comprise a complex clinical phenotype which could be confined to PEO or include neuropathy, ataxia, mental retardation, psychiatric disorders, deafness and cataracts.[49,59] Muscle biopsy showed ragged red, COX negative fibres, multiple respiratory chain defects, and multiple mtDNA deletions on Southern blotting.

POLG mutations have also been identified in patients with PEO and parkinsonism. Autosomal dominant or recessive inheritance of PEO with age of onset ranging from 10 to 54 years was followed some years later (range 6–40 years) by the development of an asymmetric, levodopa-responsive bradykinetic-rigid syndrome together with resting tremor in some patients. Additional features included variable limb, pharyngeal, or facial weakness; cataracts; ataxia; peripheral neuropathy and premature ovarian failure.[60] Muscle biopsy showed ragged red, cytochrome oxidase negative fibres in all patients with multiple mtDNA deletions on Southern blotting. Symmetrically reduced striatal [18-F]β-CFT was seen in two patients. Brain histology was available on a further two patients, and both showed severe loss of substantia nigral dopaminergic neurones but without the development of Lewy bodies or other synuclein aggregates. Four families had the same *A2864G* mutation inherited autosomally in three and with a founder effect in the fourth. Mutations in the exonuclease or polymerase portions of the gene were identified in the autosomal recessive families. A patient with autosomal dominant PEO-parkinsonism and an A2492G mutation has also been reported.[61]

Deoxyguanosine kinase and thymidine kinase mutations

MtDNA depletion syndrome usually presents in the neonatal period or infancy with myopathy, liver failure, lactic acidosis and more rarely, de Toni Fanconi syndrome. Other reports describe patients with myopathy and progressive encephalomyopathy. Investigation shows defective respiratory chain function and a variable degree of mtDNA depletion. In some patients, only 1% of mtDNA might remain in a tissue, and such individuals usually die within a few weeks. A later onset form is associated with less severe depletion. The early onset hepatocerebral form is associated with mutations in the deoxyguanosine kinase gene,[62] and the late onset myopathic form to mutations in thymidine kinase 2.[63]

One of the intriguing features of mtDNA depletion syndrome is that although infants are born with severe mtDNA deficiency, their development to birth is apparently normal. A study that used cultured fibroblasts from depletion patients showed that the patients' mitochondria replicated only during the S-phase, in contrast with control cells in which mitochondria replicated independently of cell cycle.[6] Exponentially growing patient cells were able to maintain a normal level of mtDNA, but this fell during the resting phase. The explanation for these observations resides in the ability of mtDNA to use cytosolic deoxynucleotides generated during nuclear DNA replication. However, levels are insufficient to support mtDNA replication at other phases of the cell cycle, during which mtDNA in control cells would continue to replicate. Thus fetal development might be normal because the high rate of cell proliferation supports mtDNA replication but after birth, when cell proliferation declines, so too do mtDNA levels with the consequent biochemical decompensation. The implication is that nucleotide (dGMP and dAMP) supplementation after birth could prevent mtDNA depletion. Such an effect has been proven in vitro, but has not as yet been tried in patients.[6]

MtDNA depletion is clearly a genetically heterogeneous disorder and additional genes causing infantile fatal depletion syndrome have yet to be identified. Thymidine phosphorylase mutations causing mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) also result in mtDNA depletion but not as severe as in the infantile forms. MNGIE is associated with high levels of blood thymidine that somehow interfere with mitochondrial nucleotide pools. *ANT1* and *POLG* mutations can also cause mtDNA depletion, but again in more modest form than in the infantile depletion syndrome.

Mutations affecting respiratory chain protein assembly or stability

Cytochrome oxidase (COX) deficiency is a well-recognised biochemical and histochemical mitochondrial phenotype particularly in paediatric practice. It is probably the commonest respiratory chain defect and, genetically, a heterogenous group of disorders. Several mutations have

been described in the mtDNA COX genes and associated with a range of phenotypes including pure myopathy, MELAS, sideroblastic anaemia, encephalomyopathy, and a motor neurone disease-like presentation.[64] No mutation has yet been described in any of the nuclear-encoded COX subunits.

Several mutations have been identified in nuclear genes for mitochondrial proteins involved in the assembly and maintenance of COX including *SCO2*,[65] *SURF1*,[66,67] *COX 10*,[68] *COX15*,[69] and *LRPPRC*.[70] These result in autosomal recessive COX deficiency that usually presents in early life with Leigh syndrome, myopathy, and encephalopathy, lactic acidosis, and a progressive course with early death. Muscle biopsy shows severe, but not complete, COX deficiency on histochemistry, which can be confirmed by enzymatic assay.[71] The genes for these proteins have yeast homologues but a search for mutations of additional COX assembly genes (*COX16*, *COX19*, and *PET191*) was negative in 53 patients with isolated COX deficiency.[72]

Mutations in the *BSC1L* gene result in defective assembly of complex III. These are rare and have been associated with either Leigh syndrome,[73] or a phenotype comprising growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death (GRACILE).[74] One case of complex V deficiency has been attributed to a mutation of the gene for *ATP12* assembly protein.[75]

Coenzyme Q_{10} deficiency

Coenzyme Q_{10} is a lipophilic component of the respiratory chain that transfers electrons from complexes I and II, and from fatty acids and branched chain aminoacids via flavin-linked dehydrogenases, to complex III (figure 2). The first report of human disease associated with coenzyme Q_{10} deficiency was in a patient with encephalomyopathy and recurrent myoglobinuria with ragged red fibres and changes of lipid storage on muscle biopsy.[76] Severe coenzyme Q_{10} deficiency was then described in six patients with early-onset (age range 0–16 years) myopathy and ataxia.[77] Seizures, weakness, and mental retardation was described in some, and cerebellar atrophy in all. Genetic testing for Friedreich's ataxia and spinocerebellar ataxia was negative, and inheritance was consistent with an autosomal recessive pattern. Muscle biopsy in these patients showed non-specific abnormalities only and, in particular, no evidence of mitochondrial pathology. Residual muscle coenzyme Q_{10} levels were 26–35% of normal. Coenzyme Q_{10} administration (300–3000 mg/day) resulted in significant improvement in the ataxia.

Subsequent assay of muscle coenzyme Q_{10} levels in 135 patients with genetically undefined childhood onset ataxia identified significantly reduced levels in 10%.[78] All patients had cerebellar atrophy and some had seizures, developmental delay, and pyramidal features. Lactic acidosis in the ataxic patients is rare, and in contrast with the myopathic form, the muscle biopsy can seem normal in the ataxic form. The same researchers later described muscle coenzyme Q_{10} deficiency in two brothers with adult-onset (age 29 and 39 years) progressive cerebellar ataxia with cerebellar atrophy and hypergonadotrophic hypogonadism. Muscle morphology showed neurogenic changes only. Coenzyme Q_{10} 750–1200 mg/day led to improved ataxia, neurophysiology, and normal testosterone levels within 2 months. A pure myopathic form of coenzyme Q_{10} deficiency, without recurrent myoglobinuria but with mild mitochondrial changes on muscle biopsy, has been described.[79]

Mitochondria and neurodegenerative diseases

Abnormalities of mtDNA or OXPHOS activity have been identified in several different neurodegenerative diseases. An important issue is whether these represent primary abnormalities or defects because of other factors not directly related to pathogenesis. To some extent, this could be a circular argument, at least in relation to whether improving mitochondrial function can improve the outcome of these diseases. If mitochondrial dysfunction contributes to pathogenesis, ameliorating its effects could contribute to modifying the course of disease.

Parkinson's disease

The link between Parkinson's disease (PD) and mitochondria was first established with the identification of a deficiency in the activity of complex I in PD substantia nigra[80] and subsequently in the peripheral tissues of patients.[81–83] Complex I is the target of toxins known to produce parkinsonian features in people, such as MPTP and anonnacin, and also the target of toxins used to produce animal models of PD, such as rotenone and tetrahydroisoquinolines. Inhibition of complex I results in increased free radical generation and could contribute to the oxidative mediated damage seen in the PD nigra. This relationship is reciprocal in that free radicals can damage the respiratory chain and reduce activity, particularly of complexes I and IV. The pathogenesis of PD also includes protein aggregation (Lewy bodies). Mitochondrial dysfunction will contribute to dysfunction of the energy-dependent ubiquitin proteasomal system (UPS), and oxidative stress will add to the substrate load. This combination has been shown to enhance dopaminergic cell damage and death.[84]

Environmental and genetic factors important in PD interact with mitochondrial function. Environmental toxins that induce dopaminergic cell death and parkinsonism in people and in animal models, inhibit complex I. Genetic causes of familial PD affect mitochondrial function. For instance, over-expression of α-synuclein inhibits mitochondrial activity,[85,86] parkin-knockout mice have a striatal respiratory chain defect,[87] parkin-knockout flies have skeletal muscle mitochondrial abnormalities,[88] and parkin-positive PD patients have complex I deficiency.[89] Over-expression models of parkin

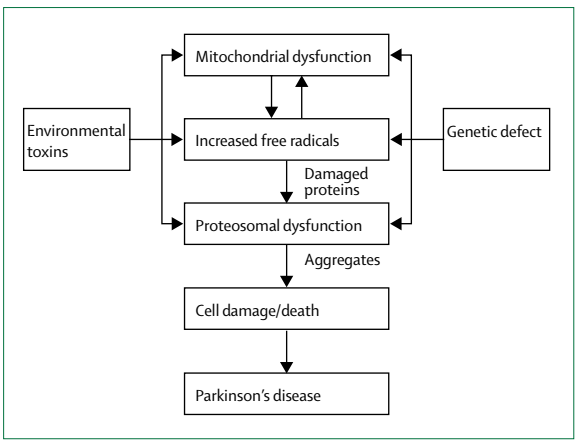

Figure 3: **Potential link between environmental and genetic factors, and Parkinson's disease**

have shown localisation of the protein to mitochondria.[90] Mutations of DJ1 cause PD and this protein localises to the outer mitochondrial membrane under conditions of oxidative stress and is thought to have a role in antioxidant defences.[91] Mutations in the *PINK1* gene that cause autosomal dominant PD have been described, and the protein product localises to the mitochondrion.[92,93] The function of *PINK1* is not known but it is a protein kinase and mutations enhance sensitivity to UPS inhibitors and lower the threshold to apoptotic cell death. Thus, the current pathogenetic model of PD reflects a complex network of interacting biochemical abnormalities that are in turn a consequence of genetic and environmental factors (figure 3).

The relation between mitochondrial dysfunction, mtDNA, and PD has been highlighted further by the recent description of mtDNA abnormalities in PD patients. Occasional mtDNA point mutations have been identified in PD but these have not been present in the general PD population. Thus, their association with PD might merely represent part of the wide clinical spectrum of mtDNA mutations and not necessarily imply a more common role in sporadic PD. *POLG* mutations have been described in patients with CPEO and PD with ragged red fibres and multiple mtDNA deletions, but mutations of this gene have not been found in sporadic PD.[94] A mutation in the mtDNA 12S RNA was identified in a patient with maternally inherited early onset PD, deafness, and neuropathy.[95] Several studies that have sequenced mtDNA in PD patients have not identified any consistent mutations.[96] However, none of the studies to date has focused on PD patients with complex I deficiency. Two studies have shown a link between mtDNA haplotypes and the risk for developing PD. The first showed a reduced risk for PD in individuals with haplotypes J and K,[97] and the second a 22% decrease in PD risk in those with the UKJT haplotype cluster.[98] In contrast, a smaller study reported an increased risk for PD with haplotypes J and T.[99]

The relation between mitochondrial dysfunction and PD has been exploited in an attempt to develop treatment that might improve disease progression. Coenzyme Q_{10} both enhances respiratory chain function and scavenges free radicals, and therefore might be predicted to have a beneficial effect upon PD pathogenesis. A pilot study with three different doses of coenzyme Q_{10} in early untreated PD patients showed that the highest dose (1200 mg/day) produced a significant improvement in clinical scores compared with placebo at 16 months.[100] As noted by the investigators, this result must be regarded as provisional, and coenzyme Q_{10} will be the subject of further study alone or in combination with other putative disease modifying therapies.

Alzheimer's disease

Mutations in the genes for amyloid precursor protein or presenilin 1 and 2 are associated with familial Alzheimer's disease (AD). Amyloid (Aβ) can inhibit OXPHOS in mitochondria.[101] Impaired OXPHOS has been shown in astrocytes cultured from Down's syndrome patients, and impaired OXPHOS causes accumulation of the toxic Aβ42 peptide.[102] Several studies have shown impaired COX activity, reduced immunoreactive protein, or decreased mRNA for mtDNA encoded proteins in the AD brain,[103–107] although these findings are not invariable.[108,109] COX deficiency present in platelets from AD patients[110] can be transferred to ρ^0 cells by the AD mtDNA.[111] However, no mtDNA mutation has consistently been identified in AD, and the COX deficiency could represent secondary damage from, for instance, free radical generation. This conclusion is supported by the finding that AD brains harbour a 15-fold increase in the 5 Kb "common" mtDNA deletion,[112] this particular mutation is known to arise in somatic form with increasing age. AD brains have also been shown to have a high proportion of mtDNA control region (including the L-strand and H-strand promoters) heteroplasmic mutations that increase with age.[113] These somatic mutations are associated with reductions in the L-strand complex I *MTND6* gene transcript and mtDNA copy number.

Perhaps the most interesting development in the relation of mitochondria to AD has come with the observation that Aβ interacts directly with Aβ-binding alcohol dehydrogenase (ABAD), a mitochondrial enzyme.[114] This protein-protein interaction seems very specific, occurring at nanomolar concentrations, and results in the inhibition of ABAD. ABAD is important in cell function; its inactivation results in a lethal phenotype.[115] It is upregulated in AD neurones[116] and its co-expression with amyloid precursor protein (APP) exacerbates A-β induced free radical mediated cell damage and death.[117] ABAD-Aβ complex was present in mitochondria isolated from transgenic mutant APP mice but only in trace amounts in age-matched control littermates. ABAD and Aβ extensively co-localise in the mitochondria of AD cortex and this interaction causes mitochondrial stress and apoptosis and

is associated with a learning deficit.[114] The consequences of the A-β-ABAD interaction could explain COX deficiency in AD on the basis of free radical mediated damage.

Other neurodegenerative diseases

This review cannot provide a comprehensive discussion of all the neurodegenerative diseases in which mitochondrial dysfunction has been identified or implicated in pathogenesis. However, reviews by other researchers can be helpful in this area[3,118]—a brief synopsis of recent developments is provided here.

Both structural and functional mitochondria abnormalities have been identified in motor neurone disease spinal cord. Rather like in AD, the predominant and most consistent defect is in COX activity. Superoxide dismutase (SOD)-1 is located both in the cytosol and the mitochondrial intermembraneous space, and the import of mutant SOD1 into mitochondria is impaired.[119] This observation provides a direct link between SOD1 mutations that cause familial motor neurone disease, and mitochondrial dysfunction.

In 97% of patients with Friedreich's ataxia, the condition is caused by abnormally expanded homozygous GAA repeats in intron 1 of the gene for frataxin. Frataxin is a mitochondrial protein that seems to be involved in haem biosynthesis and in the construction of iron-sulphur proteins, such as those that play a critical role in OXPHOS, as well as in aconitase, an enzyme involved in both the Krebs cycle and the regulation of iron homeostasis. Because a severe defect in OXPHOS activity has been shown in tissues of Friedreich's ataxia patients, researchers believe that this and the reduction in aconitase activity contribute directly to pathogenesis. An important step in this pathway to cell damage and death in Friedreich's ataxia is thought to be the generation of free radicals.

However, complete frataxin deficiency in a conditional knockout murine model of Friedreich's ataxia did not cause oxidative stress, and longevity was not prolonged by antioxidants.[120] In the Friedreich's ataxia mouse model, idebenone marginally delayed disease onset and prolonged survival,[121] and idebenone alone or high dose coenzyme Q_{10} with vitamin E have improved cardiac bioenergetics and cardiac function in FRDA patients,[122,123] with a sustained effect of coenzyme Q_{10} with vitamin E over 4 years.[124] Researchers have assumed that these compounds have been acting through an antioxidant effect as well as by stimulating OXPHOS, but the data described in the mouse model might need a reassessment of this.

Expansion of mitochondrial pathology

Mitochondria has long been thought to be involved in ageing. Two studies have highlighted the potential involvement of mitochondria in senescence.[125,126] The knock-in of a homozygous proof-reading deficient *POLG* genotype resulted in an accumulation of mtDNA point mutations and deletions and a phenotype that included shortened life-span, weight loss, osteoporosis, kyphosis, reduced subcutaneous fat, alopecia, reduced fertility, and cardiac hypertrophy.[125] These results support the proposition that the accumulation of mtDNA mutations that occurs with ageing can directly contribute to a senescent phenotype. The evidence that mitochondrial but not cytosolic targeting of catalase (an antioxidant enzyme) over-expression enhances lifespan and reduces age-related cardiac pathology and cataracts further emphasises the contribution of the mitochondrion to free-radical mediated cellular damage and dysfunction and the relation to ageing.[126]

Abnormalities of mtDNA or mitochondrial dysfunction, or both, have been described in several diseases. A critical approach is important in determining whether mitochondrial abnormalities play a direct part in pathogenesis. Some observations include the description of mtDNA mutations in various cancers including that of the colon[127] and prostate.[128] One report found that 11–12% of prostate cancer patients had mutations in the *MTCOX1* gene of mtDNA, compared with 7·8% of controls.[129] These mutations comprised both germ-line and somatic base changes that, in one particular case involving an ATPase gene mutation, resulted in increased free radical production and an increase in tumour size. Insulin-resistant offspring of patients with type 2 diabetes have been shown to have reduced insulin stimulated muscle glucose uptake, increased intramyocellular lipid and impaired OXPHOS in muscle as determined by phosphorous magnetic resonance spectroscopy, which might indicate an inherited or acquired mitochondrial defect.[130] Microarray analysis has shown reduced expression of OXPHOS genes in skeletal muscle from patients with type 2 diabetes mellitus.[131] These changes might be mediated by a transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha). Oestrogen-related receptor alpha (Erralpha) is also a transcriptional factor regulating OXPHOS and mediating the effects of PGC-1alpha.[132] On the basis of these findings, researchers propose that Erralpha agonists could be used to treat insulin resistance in patients with type 2 diabetes.

Treatment of mitochondrial diseases

Treatment for diseases caused by mutations of mtDNA remains unsatisfactory and mostly confined to supportive measures, such as eye props or ptosis surgery for patients with CPEO. Although coenzyme Q_{10} has shown some early promise in Parkinson's disease and Friedreich's ataxia, such results can only be regarded as provisional at this stage. There have been no large-scale studies to determine the effectiveness of coenzyme Q_{10} in primary mtDNA diseases. Since defects of the respiratory chain result in the increased production of free radicals, the use of antioxidants has some sound basis. N-acetylcysteine and coenzyme Q_{10}, both antioxidants, improved OXPHOS function and

reduced free radical production in cybrid (cytoplasmic hybrid) cells carrying the T8993G mutation that causes NARP or MILS.[133] However, the use of antioxidants in mtDNA disease has yet to be tested in a clinical trial.

Various strategies are being assessed to modify the mtDNA mutant load in cells and tissues in patients. An obvious target would be the preferential expansion of wild-type mtDNA or the suppression of mutant mtDNA expansion.[134] The recruitment of skeletal muscle satellite cell expansion seems to shift heteroplasmy in favour of wild type since mutant mtDNA is absent or present at a low level in these cells.[135] Satellite cells can be provoked to expand by vigorous exercise regimens or toxic damage, although, for obvious reasons, both have some practical limitation in patients. Manipulating mtDNA replication by the import into mitochondria of endonucleases that might selectively destroy a specific mutant sequence has been possible in vitro, but presents many challenges to transfer this to an in vivo application.[136] An alternative mechanism for salvaging OXPHOS function in cells with tRNA mutations of mtDNA is the import of normal tRNAs from the cytosol to mitochondria. The import of nuclear-encoded RNAs into the mitochondrial matrix has been done.[137] The import of normal $tRNA^{lys}$ from cytosol to mitochondria improved OXPHOS function in cybrid cells bearing the $tRNA^{lys}$ A8344G mutation that causes MERRF.[138]

The inheritance of primary mtDNA mutations will be maternal—ie, from the mother to all offspring, and subsequently transmitted by the daughters alone. Although there is one report of paternal inheritance,[14] males who carry a primary mtDNA mutation are highly unlikely to transmit the mutation. Mothers with CPEO and single deletions have a low risk of transmission (4%) and the risk of more than one sibling being affected is also low.[31] Mitochondrial diseases caused by nuclear gene mutations will be transmitted by Mendelian inheritance. Genetic counselling in mitochondrial disorders is a considerable challenge given the diversity of the clinical manifestations and the poor link between phenotype and genotype. Practical advice can include the possibility of in-vitro fertilisation with a donor egg, but another possibility for the future is of nuclear transfer from a maternal egg and fertilisation in a donor cytoplasm using paternal sperm. The recurrence risks for the relative of an individual with LHON are 30% for brothers, 8% for sisters, 46% for nephews, 10% for nieces, and 31% and 6% for male and female cousins, respectively.[139]

Conclusions

MtDNA mutations and mitochondrial dysfunction have been associated with, and implicated in, the aetiology and pathogenesis of a wide range of multi-system human diseases. The spectrum of mitochondrial diseases has been expanded by the recognition that mutations in the genes for nuclear-encoded mitochondrial proteins cause not only a number of neurodegenerative diseases but also haematological and ophthalmological disorders. Toxic influences of drugs such as the reverse transcriptase inhibitors used in HIV infection further emphasise the importance of mitochondria in human pathology. The field of mitochondrial diseases has progressed rapidly, but much remains to be learnt about molecular mechanisms in pathogenesis and about how we might treat patients with these disorders.

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Conflict of interest statement

I declare that I have no conflict of interest.

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