Mitochondrial DNA defects: a widening clinical spectrum of disorders

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1. Mitochondrial DNA has a number of interesting properties including maternal transmission, the ability to replicate in post-mitotic cells, a high mutation rate and an extremely compact molecular architecture with no introns and no large non-coding sequences.

2. Point mutations, deletions and duplications of mitochondrial DNA may occur. Mitochondrial DNA defects may co-exist with wild-type sequence within a cell (heteroplasmy). The level of heteroplasmy may vary in different tissues within the same individual (segregative replication).

3. A number of neurological disorders are characterized by morphological and biochemical mitochondrial defects. It is now clear that mitochondrial DNA mutations underlie these conditions although there is not always a clear correlation between a particular mutation and clinical presentation.

4. Mitochondrial DNA defects, particularly deletions, accumulate in senescent tissue and studies have been performed with the aim of linking such somatic mutations with degenerative disorders.

5. Recently mitochondrial DNA mutations have been implicated in a wider range of clinical disorders including diabetes and nerve deafness.

6. Nuclear gene defects may result in mitochondrial disorders by predisposing to multiple mitochondrial DNA deletions or quantitative depletions of mitochondrial DNA content.

INTRODUCTION

Recent years have seen a rapid increase in the understanding of how genetic factors influence human disease. This has been largely accomplished by the introduction of powerful laboratory methods which allow detailed analysis of the human genome. Previously, genetic variability could only be assessed indirectly by observing the resulting changes in protein structure; now the goal of mapping and sequencing all human genes is considered an achievable target. The majority of human DNA lies within the nucleus; however, a vital length of DNA is also present outside the nucleus in the mitochondria. The relatively small size of mitochondrial DNA (mtDNA) and the presence of specific human diseases linked to mitochondrial dysfunction made it an attractive target for genetic analysis. The entire human mtDNA sequence was published in 1981 [1] and subsequent studies have revolutionized the classification and diagnosis of mitochondrial disorders. This review outlines the major developments in the field and the many questions that remain unanswered.

MITOCHONDRIA AND MITOCHONDRIAL DNA

The mitochondrion is an intracellular organelle of pivotal importance to cellular energy production. By light microscopy mitochondria appear as long thin threads (mitochondrion = thread-like body). The number of these organelles in a particular cell is variable and tends to reflect the requirement for oxidative phosphorylation; in hepatocytes there may be several thousand mitochondria whereas in resting lymphocytes there may be only a few and in mature erythrocytes there are no mitochondria at all. Each mitochondrion consists of an outer membrane and a highly folded inner membrane separated by the inter-membrane space. The mitochondrial matrix is bounded by the inner membrane. The outer membrane is freely permeable to low-molecular-mass species and ions. Transmembrane proteins (porins) allow the passage of larger proteins through the outer membrane. The inner mitochondrial membrane is relatively impermeable, especially to ions, and this contributes to the development of an ionic (proton) gradient across the membrane. The passage of electrons down this gradient is linked to
ATP production in the oxidative phosphorylation pathway. Mitochondria are not static structures and by phase-contrast microscopy they may be observed to be constantly changing shape and positions within a cell. They have been observed to move linearly, perhaps along specific cytoskeletal elements, and often accumulate in regions of the cell where energy requirements are high, e.g. near cilia [2]. Within the mitochondrial matrix there is a variety of enzymes and fibre-like structures can be seen by electron microscopy. In 1963 Nass and Nass [3] demonstrated that these structures could be digested by DNase, the first demonstration of the presence of DNA within the mitochondrial matrix.

Within the mitochondrial matrix there are between 2 and 10 molecules of double-stranded circular mtDNA (Fig. 1). The entire sequence of the 16 569 bp of human mtDNA is known [1]. Extraction of mtDNA on a caesium chloride gradient results in two bands, the heavy strand (H-strand) and light strand (L-strand), corresponding to the complementary chains of mtDNA. The differential buoyancy in caesium chloride is a reflection of the high levels of pyrimidine (T and C) residues in the L-strand and the high levels of purine (A and G) residues in the H-strand. The two strands of mtDNA are replicated from distinct origins in a manner similar to that of many prokaryotic extrachromosomal DNAs. The origin of H-strand replication (OH) is located in a short non-coding region and replication extends away from the rRNA genes. When the daughter H-strand is approximately two-thirds of its final length the origin of L-strand replication (OL) is exposed and L-strand synthesis begins. Replication of the two strands continues asymmetrically until the two daughter strands are complete.

**MITOCHONDRIAL DNA REPLICATION AND TRANSCRIPTION**

OH is located in the non-coding mitochondrial D-loop. Commencement of mtDNA replication in this area produces a third strand of nascent DNA which displaces the H-strand and interferes with the regular circular structure of mtDNA. Although this D-strand may be considered as an intermediate of replication, the majority of these molecules do not go on to form new complete H-strands but separate away from the mtDNA [1]. Whether replication of the H-strand proceeds by elongation of D-strands or by initiation of a separate strand is not known. The result of this phenomenon is that the copy number of mtDNA may depend not on the rate of replication initiation, but on the rate at which synthesis proceeds beyond the D-loop.

mtDNA transcription is under the control of heavy- and light-strand promoter sequences which lie around 50 bp apart and close to OH. The precise mechanisms involved in mtDNA transcription are unknown but there appears to be a requirement for the binding of a nuclear-encoded transcription factor termed mitochondrial transcription factor A [4]. The entire H-strand is transcribed as a single polycistronic molecule containing the mRNAs for 12 subunits of oxidative phosphorylation, 14 tRNAs and the 2 rRNAs. The L-strand transcript is also polycistronic and codes for the remaining eight tRNAs and single mRNA. The transcribed mitochondrial RNA is processed by nucleases which release the rRNAs, tRNAs and structural mRNAs. There is a requirement for the synthesis of sufficient rRNAs to allow for the production of all 13 mRNAs and this would not be possible if all H-strand transcripts were polycistronic. Therefore, a shorter transcript extending from the heavy-strand promoter to beyond the 16S rRNA is synthesized at about 25 times the rate of the full length transcript [5]. Synthesis of this short transcript is terminated by the binding of a terminator protein to a tridecamer sequence within the tRNAleuc(UUR) gene which stalls the progress of further transcription [6]. Only when the terminator protein is not bound at this site can transcription continue to produce the full polycistronic RNA.

![Fig. 1. Organization of the human mtDNA. The mitochondrial genome consists of 16 569 bp forming a circle of double-stranded DNA. The origin of heavy-stranded DNA replication (OH) is located in the D (displacement) loop, close to the heavy-strand promoter site (HSP) and the light-strand promoter site (LSP). The origin of light-strand replication (OL) is located within a cluster of five mitochondrial tRNA genes, represented by the letter codes for their specific amino acid: ND1, ND2, ND3, ND4, ND6. ND1 and ND6 represent subunits of NADH dehydrogenase; COI, COII and COIII, cytochrome c-oxidase subunits; Cyt b, the apocytochrome b subunit; and ATPase 6 and 8, ATPase subunits. 16S and 12S denote the large and small rRNAs, respectively.](image-url)
Mitochondrial DNA defects

SPECIFIC BIOLOGICAL PROPERTIES OF MITOCHONDRIAL DNA

A number of features of mtDNA biology are of particular clinical importance; firstly, mtDNA is essentially maternally inherited [1]. At fertilization, the vast majority of the cytoplasm, mitochondria and mtDNA are derived from the ovum – only the spermatozoal head enters the ovum and almost all the paternal mitochondria are located in the neck or tail of the spermatozoan. Repeated back-crossing of mice has suggested that a few paternal mtDNA molecules may pass to the zygote [7] but this has not been demonstrated in man. Interestingly, bipaternal transmission of mtDNA does occur in a few species, for example in the blue mussel *Mytilus*. In this case the female has one type of mtDNA which she passes to both sons and daughters but males have two populations of mtDNA; an F type which they receive from their mother and is not transmitted and an M type which they receive from their father and transmit to their sons [8]. Nevertheless, for all practical purposes, human mtDNA may be considered as totally maternally inherited.

Secondly, mtDNA transcription is not closely restricted by the cell cycle. Thus, in each cell cycle the nuclear DNA divides only once and is divided equally between daughter nuclei and cells. mtDNA may replicate several times during a cell cycle, or not at all; furthermore, mtDNA may replicate even when a cell is not dividing, e.g. within post-mitotic neurons. The precise mechanisms which govern the extent of mtDNA replication are poorly understood.

Thirdly, mtDNA is far more susceptible to mutation than nuclear DNA [9]. This is due to the fact that mtDNA is not protected by histones and lies in the mitochondrial matrix where it is exposed to high levels of free radicals produced during oxidative phosphorylation. Of the five eukaryotic DNA polymerases known, only one (DNA polymerase γ) is present in mitochondria. This polymerase is several-fold less efficient at dealing with mismatches than the corresponding nuclear enzymes [10]. Since mtDNA is present in high copy number within a cell, if a mutation occurs, the situation will arise that a given cell will have two populations of mtDNA (wild-type and mutant). This phenomenon is termed heteroplasmy. Since, unlike nuclear DNA, mtDNA may segregate unevenly to daughter cells during cell division, heteroplasmy ratios may vary widely between daughter cells. If the new mutation confers a replicative advantage and is not deleterious to the cell, it may quickly become the dominant form of mtDNA within a cell – perhaps replacing the original wild-type sequence completely (homoplasy). If this occurs in female germ cells, the new mtDNA sequence will be passed on to offspring. This mechanism is probably responsible for establishing the mtDNA polymorphisms observed in different racial groups [11]. If the novel mtDNA mutant is highly deleterious, 100% homoplasy is considered unlikely to arise since these cells would be non-viable.

While highly deleterious mtDNA mutations are likely to be lost from cells, some mild or moderately deleterious mtDNA defects may persist in a state of heteroplasmy. This is often the case in mutations associated with human disease. The mechanisms underlying such persistent heteroplasmy are poorly understood and the ratio of mutant to wild-type mtDNA often varies between different tissues in the same individual (replicative segregation) and between the different offspring of a mother with a mutation. Rapidly dividing cells such as leucocytes often have low levels of heteroplasmy while mutant mtDNA molecules may accumulate in post-mitotic myocytes and neuronal tissue. The familial clustering of different disease phenotypes associated with the same mtDNA mutation suggests that the replicative segregation of mutated mtDNA is influenced by as yet unidentified genetic factors [12]. Pedigree analysis of a (presumably neutral) heteroplasmic mitochondrial DNA sequence in Holstein cows has shown a return to homoplasy within two generations [13]; to account for the phenomena of rapid segregation of mtDNA sequences and the marked differences in levels of heteroplasmy among offspring of the same mother, a genetic 'bottleneck' for mtDNA has been postulated in the female germline or early embryo. Under this hypothesis, only a small subset of mtDNA molecules are selected (perhaps only a single mtDNA) and the adult complement of mtDNA is derived from only these molecules. This would act as a barrier to the inheritance of mitochondrial disorders. Recently Jenuth et al. [14] have lent further support to this hypothesis by demonstrating that random genetic drift of mtDNA in the female germline can explain the rapid segregation of mtDNA mutations.

mtDNA defects can take the form of point mutations, deletions and duplications. Many of the deletions are flanked by direct repeats, suggesting that homologous recombination or slippage mispairing may be responsible for their generation. Deleted mtDNA molecules appear to be transcribed but not translated [15]. Duplications may be transient recombination intermediates since they have been observed to occur transiently in sequential muscle biopsies and cell cultures where deletions eventually predominate [16].

The mitochondrial genome is extremely compact with no introns and no large non-coding sequences. Only a region of around 380 bp in the D-loop is non-coding, although it does contain the O-H, and light- and heavy-strand promoters. This area is highly polymorphic with many single-base substitutions having been described. These are usually homoplasmic in individuals, but a G to C substitution is associated with heteroplasmic length variation in the D-loop [17]. The significance of these polymorphisms is unclear although it has been suggested that they may be associated with some mito-
mitochondrial disorders [18]. Unlike nuclear DNA, there appears to be little crossover of information between individual mtDNA molecules. Together with the high mutation rate and maternal transmission, this makes the study of mtDNA sequences particularly valuable for assessing ancestral lineages. Parsimony analysis depends on the construction of 'trees' which may be rooted with an ancient mtDNA sequence. Using these techniques it is possible to map the successive accumulation of mtDNA polymorphisms with time and derive information regarding the relationships between different racial groups [19, 20].

mtDNA encodes 13 polypeptides which are all subunits of the enzymes responsible for oxidative phosphorylation (Table 1), together with the 22 tRNAs and 2 rRNAs required for their intra-mitochondrial synthesis. All the other subunits of oxidative phosphorylation (i.e. the majority) are coded for by nuclear DNA. The nucleus must also encode a variety of (currently poorly characterized) enzymes and proteins which are important for the control of mtDNA replication, transcription and processing. These proteins are targeted to the mitochondrion by the presence of specific signal peptides at the 3' end and are carried in the cytoplasm in association with molecular chaperones which keep them in an unfolded state [21]. Proteins enter the mitochondrion by means of porins in the outer mitochondrial membrane. One such protein is mitochondrial transcription factor A which binds immediately upstream of the heavy- and light-strand promoters and is essential for normal electron transport chain activity and ATP production. Mitochondria are present to excess in cells and clinical features of oxidative phosphorylation deficiency will only arise if the levels of mtDNA-encoded proteins fall below a threshold level. Thus, if a specific cell has a particular population of mutant mtDNA, disordered function may not arise until the level of heteroplasmy is sufficient to interfere with normal function. Some cells such as muscle, neurons and endocrine cells are particularly energy dependent and this may explain why mitochondrial dysfunction is often characterized by clinical disorders affecting these tissues.

**CLASSICAL MITOCHONDRIAL DISORDERS**

The first mitochondrial disorder was described by Luft et al. in 1956 [23]. He described a patient with excessive hypermetabolism but normal thyroid function. Further studies revealed that this patient had an uncoupling of oxidative phosphorylation within the mitochondria. A similar case was subsequently reported [24] but no recent cases have appeared in the literature. However, a number of disorders of muscle were presumed to have a mitochondrial aetiology since muscle biopsies stained by the modified Gomori-Trichrome method revealed collections of many abnormal mitochondria (red-ragged fibres). Electron microscopy confirmed bizarre mitochondrial morphology with paracrystalline inclusions within the matrix. Some of these disorders were sporadic but others showed maternal transmission. These disorders tended to have heterogeneous clinical features and marked variation in severity was seen both between individuals and within families. On clinical grounds a number of patterns were recognized.

**Chronic progressive external ophthalmoplegia (CPEO)**

This is a common clinical feature of patients with mitochondrial myopathy. It usually presents in young adults but has been reported in the elderly. Typically there is ptosis and variable limitation of eye movements in all directions of gaze. The signs may be asymmetrical with disconjugate eye movements and diplopia. Patients may develop progressive signs and later develop other features of mitochondrial disorders (e.g. encephalopathy). Neurological examination often reveals limb weakness with exercise intolerance in patients with CPEO leading to a clinical picture similar to myasthenia gravis. The majority of patients with CPEO

<table>
<thead>
<tr>
<th>Table 1. Mitochondrial-encoded polypeptides</th>
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<tbody>
<tr>
<td>Complex I (NADH CoQ reductase) – 7 of the at least 25 known subunits:</td>
</tr>
<tr>
<td>ND1 35 kDa</td>
</tr>
<tr>
<td>ND2 39 kDa</td>
</tr>
<tr>
<td>ND3 13 kDa</td>
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<tr>
<td>ND4 52 kDa</td>
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<tr>
<td>ND4L 11 kDa</td>
</tr>
<tr>
<td>ND5 68 kDa</td>
</tr>
<tr>
<td>ND6 19 kDa</td>
</tr>
<tr>
<td>Complex II (Succinate CoQ reductase) – all 4 subunits are encoded by nuclear DNA</td>
</tr>
<tr>
<td>Complex III (Ubiquinol cytochrome c reductase) – 1 of the 11 known subunits:</td>
</tr>
<tr>
<td>Subunit III – cytochrome B 43 kDa</td>
</tr>
<tr>
<td>Complex IV (Cytochrome c oxidase) – 3 of the 13 subunits:</td>
</tr>
<tr>
<td>Cytochrome c-oxidase I (COXII) 57 kDa</td>
</tr>
<tr>
<td>Cytochrome c-oxidase II (COXIII) 26 kDa</td>
</tr>
<tr>
<td>Cytochrome c-oxidase III (COXIV) 30 kDa</td>
</tr>
<tr>
<td>Complex V (ATP synthase) – 2 of the 14 subunits:</td>
</tr>
<tr>
<td>ATPase subunit 6</td>
</tr>
<tr>
<td>ATPase subunit A6L</td>
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</tbody>
</table>
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Kearns Sayre (KS) Syndrome

This eponymous term is used to describe patients who present with CPEO and pigmentary retinopathy before 20 years of age, together with one or more of the following: ataxia, complete heart block and cerebrospinal fluid protein concentration of >1 g/l. As with CPEO, the majority of patients with KS syndrome have a mtDNA deletion detectable on muscle biopsy. Pearson's syndrome [29] is a condition of neonatal pancytopenia associated with deletions of mtDNA. If the child survives, KS syndrome may develop in later life. It is postulated that the initial clinical presentation is due to high levels of heteroplasy as an acquired somatic mtDNA defect in senescent tissue (see later).

Myoclonic epilepsy and red-ragged fibre disease (MERRF)

This disorder may present at any age with progressive myoclonus, ataxia and seizures. Patients may also have limb weakness, optic atrophy, deafness and dementia. Red-ragged fibres are present in muscle biopsies. The disorder is maternally inherited, but individual family members may be variably affected. Some may be asymptomatic with well-controlled myoclonus or the presence of synergistic defects in the Y chromosome. Patients present with sudden deterioration of central vision in one eye and then the other (usually within months). Fundoscopy reveals capillary microangiopathy progressing to optic atrophy. LHON was the first mitochondrial disorder to be associated with a mtDNA point mutation (at bp 11778 in the ND4 gene) [33, 34]. Several other point mutations have subsequently been described in this condition (Table 2). Some mutations appear to be primary in that their presence alone is sufficient to cause disease; others are synergistic, interacting

Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS)

The acronym MELAS is used to describe a mitochondrial disorder characterized by stroke-like episodes which may result in hemiparesis, hemianopia or cortical blindness [31]. Patients may present with focal seizures and the neurological deficit becomes apparent on cessation of ictal activity. CT scanning reveals low-density areas affecting both the grey and white matter and not necessarily correlating with the clinical features. Resolution of the neurological deficit may occur or the patient may have recurrent attacks leading to progressive encephalopathy, disability and death. MELAS, like MERRF, is associated with mtDNA point mutations. The most common mutation found is an A to G substitution at bp 3243 in the tRNA^leu(UUR) gene [32].

Leber's hereditary optic neuropathy (LHON)

This distressing disorder results in acute or subacute bilateral visual loss. Although maternally inherited, 70% of patients present under the age of 30 years and affected women tend to present at a slightly later age than men. The mechanism underlying this gender difference is unclear; possible explanations include the modulating role of sex hormones or the presence of synergistic defects in the Y chromosome. Patients present with sudden deterioration of central vision in one eye and then the other (usually within months). Fundoscopy reveals capillary microangiopathy progressing to optic atrophy. LHON was the first mitochondrial disorder to be associated with a mtDNA point mutation (at bp 11778 in the ND4 gene) [33, 34]. Several other point mutations have subsequently been described in this condition (Table 2). Some mutations appear to be primary in that their presence alone is sufficient to cause disease; others are synergistic, interacting

Table 2. Mutations associated with LHON

<table>
<thead>
<tr>
<th>Site</th>
<th>Gene</th>
<th>High risk (primary) mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>11778</td>
<td>ND1</td>
<td>50–70% of families with LHON</td>
</tr>
<tr>
<td>3460</td>
<td>ND2</td>
<td>Several Finnish, British, American and Australian pedigrees</td>
</tr>
<tr>
<td>4160</td>
<td>COX1</td>
<td>Single Australian pedigree</td>
</tr>
<tr>
<td>5244</td>
<td>ND5</td>
<td>Single pedigree</td>
</tr>
<tr>
<td>14484</td>
<td>ND1</td>
<td>Several Australian and British pedigrees</td>
</tr>
<tr>
<td>15257</td>
<td>ND5</td>
<td>Four pedigrees</td>
</tr>
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</table>

Synergistic mtDNA mutations in LHON

<table>
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<tr>
<th>Site</th>
<th>Gene</th>
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<tbody>
<tr>
<td>4216</td>
<td>ND1</td>
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<tr>
<td>4917</td>
<td>ND2</td>
</tr>
<tr>
<td>7444</td>
<td>COX1</td>
</tr>
<tr>
<td>13708</td>
<td>ND5</td>
</tr>
<tr>
<td>15812</td>
<td>Cytochrome B</td>
</tr>
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with primary mutations to modulate disease expression.

OVERLAP SYNDROMES

Although it is often possible to classify patients into one of the recognized mitochondrial disorders, many patients do not fulfil all the diagnostic criteria at presentation or have features of more than one syndrome. Some propose further clinical entities such as CPEO, mitochondrial myopathy and cardiomyopathy (MiMyCa) [35] and neuropathy, ataxia and retinitis pigmentosa (NARP) [36]. A few of these divisions are justified on molecular grounds (e.g. NARP is associated with a T to G transversion at np 8893 of the ATPase 6 gene). Others prefer not to place patients in arbitrary clinical classifications when it is possible that the clinical features may progress with time and reclassification will be required.

MOLECULAR GENETICS OF MITOCHONDRIAL DNA DISORDERS

As outlined above, the classical mitochondrial myopathies have been associated with a variety of mtDNA defects. Single mtDNA deletions are often found in the muscle of patients with CPEO and KS syndrome. Point mutations have been detected in patients with MERRF, MELAS and LHON. However, there is not always a clear correlation between the clinical syndrome and the molecular defect (Table 3). Thus some patients with CPEO have been found to have the 3243 bp mutation characteristic of MELAS, patients with MELAS may have mutations other than at 3243 bp and a large number of mutations have been associated with LHON. Hammans et al. [12] have described familial clustering of different disease phenotypes associated with the 3243 mutation and this has been interpreted as evidence for hitherto undefined genetic factors in modifying disease expression.

One of the issues raised by genetic studies is the difficulty in determining whether a described mtDNA variation is truly pathogenic or merely reflects a mtDNA polymorphism. It is considered that pathogenic mutations should segregate with the disease in a family and that the same mutation be found in at least two independent families with the same clinical features. However, the variability in clinical expression of these disorders and the possibility of asymptomatic carriers of the mutation makes such criteria open to debate. Pathogenic mtDNA mutations are usually heteroplasmic and a correlation between the level of heteroplasmy in affected tissue (e.g. muscle) and clinical phenotype is further support for a causative role of a particular mtDNA mutation in disease.

It has not been easy to extrapolate clinical features from known molecular defects. The 3243 bp mutation occurs in the tRNA_{\text{Lys(UUR)}} gene and it is difficult to explain pathogenicity solely on this basis. The mutation occurs within the region that binds the TERM factor which modulates termination of transcription for synthesis of the short-form of H-strand transcripts and this might be of importance; others have suggested that defective splicing of the rRNA/tRNA_{\text{Lys(UUR)}}/ND1 polycistronic transcript might play a role in the pathogenesis of MELAS [37].

SOMATIC MUTATIONS: AGEING AND DEGENERATIVE DISORDERS

Studies on a number of senescent and degenerative tissues have documented decreased oxidative phosphorylation activity. For example, there is an age-related increase in the number of cytochrome c-oxidase-deficient fibres in skeletal muscle, and human liver mitochondria show a marked decrease in oxygen utilization with age [38, 39]. Since mtDNA has a high mutation rate, somatic mtDNA defects tend to accumulate with age and this has led to the hypothesis that acquired mtDNA mutations may be important in a number of disorders other than the classical germ-line mitochondrial diseases. Within this hypothesis it is postulated that the progressive decline in oxidative phosphorylation and the accumulation of mtDNA mutations may result from damage by oxygen radicals and this might be accentuated in conditions characterized by recurrent episodes of ischaemia and subsequent reperfusion (e.g. ischaemic heart disease).

A number of investigators have documented the accumulation of a 4997 bp mtDNA deletion in ageing human tissues (the ‘common 5 kb deletion’) [40]. More recently a 7.4 kb deletion has also been reported with increased frequency in senescent human myocardium [41]. Autopsy studies reveal that deleted mtDNA molecules are most common in tissues which divide slowly, such as brain and muscle. However, it is important to note that the level of deleted mtDNA remains low and is probably insufficient to account for the age-related decline in

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Gene</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>A→G at 3243</td>
<td>tRNA_{\text{Lys(UUR)}}</td>
<td>MELAS, CPEO, diabetes</td>
</tr>
<tr>
<td>T→C at 3271</td>
<td>tRNA_{\text{Lys(UUR)}}</td>
<td>MELAS</td>
</tr>
<tr>
<td>T→C at 3250</td>
<td>tRNA_{\text{Lys(UUR)}}</td>
<td>Myopathy</td>
</tr>
<tr>
<td>A→G at 8344</td>
<td>tRNA_{\text{Lys(UUR)}}</td>
<td>MERRF, MERRF+CPEO, cervical lipomatosis</td>
</tr>
<tr>
<td>T→G at 8993</td>
<td>ATPase 6</td>
<td>NARP</td>
</tr>
<tr>
<td>G→A at 1178</td>
<td>ND1</td>
<td>LHON</td>
</tr>
<tr>
<td>G→A at 3460</td>
<td>ND1</td>
<td>LHON, diabetes</td>
</tr>
<tr>
<td>T→C at 4160</td>
<td>ND1</td>
<td>LHON</td>
</tr>
<tr>
<td>G→A at 5246</td>
<td>ND2</td>
<td>LHON</td>
</tr>
<tr>
<td>G→A at 14709</td>
<td>tRNA_{\text{Lys(UUR)}}</td>
<td>Diabetes</td>
</tr>
<tr>
<td>A→G at 1555</td>
<td>tS-rRNA</td>
<td>Sensorineural deafness</td>
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oxidative phosphorylation function. Nevertheless, there is evidence that mitochondrial RNA levels also fall sharply with age, probably related to a reduction in the mtDNA transcription rate rather than a fall in total mtDNA levels [42].

ALZHEIMER'S DISEASE

This is by far the commonest cause of pre-senile dementia. Pathological changes are confined to the brain and consist of neuronal loss, neurofibrillary tangles and amyloid deposition. Gene expression of the mtDNA-encoded ND4 subunit is decreased in the temporal cortex of patients with Alzheimer's disease [43], and occasional abnormal mitochondria containing paracrystalline inclusions have been reported. A point mutation at 4336 bp in the mitochondrial tRNA^\beta^ gene has been reported in patients with Alzheimer's disease although the association of this mutation with the disease is controversial [44, 45]. Further mutations, including a unique transition in the 12S rRNA gene and a transition at 3397 bp in the ND1 gene, have been detected in patients with Alzheimer's disease [46].

PARKINSON'S DISEASE

Parkinson's disease is characterized by the degeneration of dopaminergic neurons in the substantia nigra and clinically by tremor, rigidity and bradykinesia. Positron emission tomography studies indicate that the disease may be more prevalent among identical twins, suggesting that genetic factors may play a role [47]. Family studies do not suggest maternal transmission but this does not exclude a role for acquired mtDNA defects in disease pathogenesis. The dopaminergic system is a potential site for oxygen radical-mediated mtDNA damage since the oxidation of catecholamines increases with age and this is a source of reactive oxygen species. Decreased complex I activity has been detected in the substantia nigra (and skeletal muscle and platelets) of patients with Parkinson's disease [48–50]. Levels of mtDNA-encoded subunits of complex I were found to be reduced in the nigrostriatal region of a patient with Parkinson's disease [49]. Although the common 5 kb deletion is found in the brains of elderly patients with Parkinson's disease, it was not detected in 15 patients with younger-onset disease, suggesting the phenomenon is an age-related change rather than specific for the disease [51, 52]. The significance of a reported heteroplasmic mtDNA mutation at 4336 kb in the tRNA^\beta^ remains unclear [46, 53]; one study also found a heteroplasmic G to A transition at 5460 kb in the ND2 gene in patients with Parkinson's disease [53].

ISCHAEMIC HEART DISEASE, HYPERTROPHIC AND DILATED CARDIOMYOPATHY

A number of studies have found increased levels of deleted mtDNA molecules in cardiomyocytes. Deletions seem to be most common in ischaemic myocardium but even then they probably represent <1% of the total cellular mtDNA [54]. It is unclear whether these observations merely reflect bystander damage to mtDNA or whether they directly contribute to myocardial dysfunction in these diseases.

MITOCHONDRIAL DIABETES MELLITUS

Diabetes mellitus represents a heterogeneous group of conditions characterized by hyperglycaemia. The pathological hallmark of type 1 diabetes is insulinitis, and autoimmune phenomena centred on cells play an important part in disease progression. Type 2 diabetes is associated with amyloid deposition around pancreatic islets and hyperglycaemia supervenes as a result of both deficient insulin secretion and insulin resistance. Genetic factors play a role in both types of diabetes; in general terms HLA haplotypes play a role in susceptibility to type 1 but not type 2 diabetes.

The observation that patients with type 2 diabetes more frequently report an affected mother than an affected father suggested a possible role for mitochondrial gene defects in the pathogenesis of the disease [55–58]. Alternative explanations include the effects of intra-uterine environment, sex-chromosome-linked genes or other imprinted genetic elements. However, a number of other lines of evidence point to the potential role of mitochondrial disorders in diabetes. Mitochondrial toxins such as streptozotocin result in diabetes in experimental rodents. Streptozotocin reduces the transcript of mitochondrial but not nuclear genes in the islets of these diabetic rats [59]. Furthermore, diabetes mellitus is a feature of a number of well-characterized mitochondrial disorders such as MELAS and KS syndromes [60].

In 1992 Ballinger et al. [61] reported a single large pedigree with maternally inherited diabetes and nerve deafness associated with a 10.4 kb deletion of mtDNA. Further studies on this pedigree have shown the presence of a variety of mtDNA rearrangements and it is likely that the primary defect is of mtDNA duplication [62]. Subsequently Van den Ouweland et al. [63] described a pedigree with maternally inherited diabetes harbouring the heteroplasmic A to G mutation at np 3243 that had previously been described in families with the MELAS syndrome. As with the earlier report of Ballinger [61], affected members of the pedigree suffered from both diabetes and nerve deafness. The mutation was detectable in both muscle and peripheral blood samples. A large number of other reports of the 3243 bp mutation in association with diabetes and nerve deafness followed [64–66]. It
should be noted that the association of diabetes with MELAS had been known for many years; the importance of the recent observations lay in the fact that a pathogenic mtDNA mutation was being found in patients with a common condition (i.e., diabetes) but no other clinical features of mitochondrial myopathy.

Several studies have examined the prevalence of the 3243 bp mutation among unselected patients attending general diabetic clinics [65]. Currently it is estimated that around 1.5% of patients have the mutation [67]. Although this percentage is small, since diabetes is a common disorder, it represents around 20,000 patients in the U.K. The prevalence of the mutation may reach 10% in cohorts selected for maternal history. Clinically these patients often have associated nerve deafness and it is interesting to note that deafness is more common in patients with diabetes in any case. Controversy has surrounded whether the 3243 bp mutation results in type 1 or type 2 diabetes and much of this has arisen out of problems with nomenclature. Clinically most patients present with type 2 diabetes but some do develop absolute insulinopenia and ketosis and require insulin therapy. A recent study suggests that, at least in Japan, the 3243 bp mutation may be associated with the presence of glutamic acid dehydrogenase antibodies, a marker seen more commonly in type 1 than type 2 diabetes [68]. However, it is not clear whether these antibodies represent a primary pathological process or merely reflect an immune phenomenon triggered by pre-existing β-cell damage. Patients are generally not obese but are prone to the same diabetic complications (i.e., retinopathy, nephropathy, macrovascular disease) as other patients with diabetes. A variety of studies confirm that this form of diabetes is associated primarily with impaired insulin secretion rather than insulin resistance [69, 70].

The prevalence of other mtDNA mutations in diabetes is unknown. A search for potentially pathogenic mutations using PCR-heteroduplex and PCR-single-stranded conformational polymorphism analysis revealed a small number of defects which might be important [71]. A mutation in the ND1 gene at np 3394, previously reported in patients with LHON, has been found by several groups in occasional patients with diabetes; however, this mutation has also been reported in normal 'control' populations [72, 73]. A heteroplasmic point mutation in the tRNA\(^{\text{Gln}}\) gene has recently been reported in two pedigrees with diabetes [74, 75]. Complete sequencing of mtDNA in patients with the 3243 bp mutation and diabetes failed to reveal any other mtDNA mutations which may have contributed to the disease phenotype [63].

Current data suggest that germ cell line mtDNA mutations are unlikely to contribute to more than 2% of all cases of diabetes. This is insufficient to explain the maternal excess observed in studies of type 2 diabetes. It is not possible to exclude the additive role of various mtDNA variants in concert with the nuclear gene environment. Furthermore, the role of acquired somatic mtDNA mutations in the islets of patients with diabetes remains to be investigated. While it has been possible to study brain tissue from patients with Parkinson's and Alzheimer's disease, rapid pancreatic autolysis limits the study of β-cells in patients with diabetes.

**DIABETES INSIPIDUS, DIABETES MELLITUS, OPTIC ATROPHY AND DEAFNESS (DIDMOAD)**

A number of diabetes/deafness syndromes have been described. Classical DIDMOAD is an autosomal recessive condition and recently linkage has been found to a marker on chromosome 4 in at least some families [76]. One subject with DIDMOAD has been reported to show morphologically abnormal mitochondria and reduced glutamate dehydrogenase (complex I) activity [77]. Another patient was found to have a mtDNA rearrangement; however, this case was not typical of DIDMOAD in that the optic atrophy may have been due to retinitis pigmentosa and diabetes insipidus was not present [78]. Given that deafness is frequently seen in patients with diabetes mellitus and the 3243 bp mutation, it seems likely that other mtDNA defects will be found in families with diabetes and deafness.

**SUSCEPTIBILITY TO AMINOGLYCOSIDE-INDUCED NERVE DEAFNESS**

The aminoglycoside antibiotics (e.g., gentamycin) are broad-spectrum antibiotics in wide clinical use. Their activity is related to their ability to bind to ribosomes and stall protein synthesis. Nerve deafness is an adverse reaction to aminoglycosides and reports have suggested that the susceptibility to this complication is maternally inherited, implicating a mtDNA defect [79]. Recently a homoplasmic mutation at np 1555 in the 12 S rRNA gene has been found in some cases of aminoglycoside-induced and maternally inherited sensorineural deafness, although not all individuals with the mutation in the pedigrees studied had deafness [80, 81].

**AUTOSOMAL DOMINANT MULTIPLE MITOCHONDRIAL DNA DELETIONS**

Apart from the sporadic or maternally inherited single heteroplasmic mtDNA deletions outlined above, several families have been described in which mtDNA analysis reveals multiple deletions [82-84]. Clinical features include CPEO, ataxia, tubulopathy, encephalopathy, recurrent myoglobinuria and also recurrent ketoacidotic comas. These disorders appear to be autosomal dominant in character, suggesting a defect in a nuclear-encoded protein responsible for maintaining mtDNA integrity. Thus,
defects in either the nuclear or mitochondrial genomes may result in a similar clinical picture.

**AUTOSOMAL RECESSIVE DEPLETION OF MITOCHONDRIAL DNA**

A number of pedigrees have been described in which mitochondrial disorders are not due to qualitative mtDNA mutations but rather a quantitative depletion of mtDNA within specific tissues [85, 86]. They appear to be inherited as Mendelian traits suggesting that the mtDNA depletion is due to a defect in nuclear DNA – presumably a defect in one of the nuclear genes responsible for mtDNA replication. Further support for this view comes from the fact that mtDNA levels may be restored in vitro by fusing enucleated cultured cells obtained from patients with normal myoblasts previously rendered mtDNA-deplete [87]. Reduced levels of human mitochondrial transcription factor A have been detected in muscle fibres from patients with mtDNA depletion but this may be a secondary effect [88, 89].

Clinically, patients with mtDNA depletion fall into early- and late-onset categories. Infants with the early-onset form exhibit symptoms at birth (floppy, neuropathies, hepatic failure) and death occurs within a few months. mtDNA depletion may be severe (>98%). Later onset forms become manifest in the second year of life with myopathy and slow motor development.

**AZIDOTHYMIDINE (AZT)-ASSOCIATED MITOCHONDRIAL DNA DEPLETION**

Patients with AIDS were noted to develop a myopathy with red-ragged fibres on muscle biopsy and depletion of mtDNA by molecular study [90]. AZT was implicated since the disorder is only found in patients taking this agent for prolonged periods [91]. AZT is incorporated (as a dideoxynucleoside analogue) into DNA from which it cannot be removed by exonucleolytic cleavage, causing termination of replication. Mitochondrial polymerase-γ can insert AZT into mtDNA inhibiting daughter strand synthesis [92]. The depletion of mtDNA appears reversible since analysis of muscle from a patient who had ceased AZT treatment for 4 months showed that the number of red-ragged fibres had decreased while mtDNA levels increased.

**CLINICAL ASSESSMENT OF SUSPECTED MITOCHONDRIAL DISORDERS**

The increasing spectrum of mitochondrial disorders has expanded the number of clinical symptoms which should alert the astute physician to the possible diagnosis. To the classical symptoms described above should be added mild ptosis, limb weakness or fatigue, retinopathy, hearing loss, diabetes, and unexplained stroke-like episodes. Lack of a maternal history should not deter investigation since many mitochondrial myopathies are sporadic and some show autosomal dominant or recessive modes of inheritance. Family members may have been so mildly affected that they never came to medical attention or were incorrectly diagnosed. Many patients attending routine diabetic clinics have been found to harbour the 3243 mutation without previous clinical suspicion of a mitochondrial disorder although, in retrospect, deafness and insulinopenia are characteristic features.

In patients with mitochondrial myopathy, serum creatine kinase is usually normal or marginally increased and is unhelpful in diagnosis [93]. An increased lactate concentration or lactate/pyruvate ratio is seen in more than 50% of cases and this is accentuated after exercise. Electromyography is neither specific nor sensitive, showing a variety of features including myopathic changes and denervation. CT scanning or magnetic resonance imaging may reveal low-density lesions in the encephalomyopathies, particularly in MELAS. Magnetic resonance spectroscopy of muscle is a useful investigation since the inorganic phosphate/phosphocreatine ratio is increased in most patients with mitochondrial myopathies. A modified Gomori-Trichrome stain may demonstrate red-ragged fibres on muscle biopsy but these are not always present and a few may be seen in non-mitochondrial myopathies. Molecular genetic analysis of both peripheral blood leucocytes and muscle biopsy tissue may pinpoint a pathological mutation. Heteroplasmy levels may be very low in peripheral blood samples and therefore a muscle biopsy is preferable. Muscle biopsies may also be used for ultrastructural and biochemical studies.

**CONCLUDING REMARKS**

The clinical spectrum of mitochondrial disorders has expanded from the relatively rare classical mitochondrial myopathies to common disorders as diverse as diabetes mellitus and aminoglycoside-induced nerve deafness. MtDNA has also become of interest in forensic pathology and trans-racial phylogeny. Many complexities remain to be unravelled including the mechanisms underlying nuclear/mitochondrial DNA interactions and the precise phenotype/genotype relationships of the various disorders. The continued development of mtDNA expression systems will yield further advances in the field of mitochondrial medicine.

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REFERENCES

Mitochondrial DNA defects


