INTRODUCTION

For stealing fire from the gods, Prometheus was bound to a rock on Caucasus, a vulture forever to consume his liver. As an additional punishment, the mortals were sent, by the gods, a beautiful and gifted woman, Pandora, whose only weakness was curiosity. Pandora was entrusted with a box, which she was sworn never to open. Alas, she did, and all evils flew out of the box and around the world. Thus, to compare mitochondrial DNA (mtDNA) to Pandora’s box seems fitting not only because of the myriad diseases that are potentially harbored within the mtDNA circle, but also because Pandora’s box was brought by a woman, just as mtDNA is given to us by our mothers. Lest this mythological parable exposes me to the accusation of sexism, let me hasten to point out the positive side of the story. Prometheus, a male giant, had given humans the wonderful gift of external fire, a source of heat and light, but Pandora, a god-given woman, gave humans their internal fire, their source of intracellular energy.

Although mitochondrial DNA (mtDNA) was discovered in 1963 (1), pathogenic mutations in the mitochondrial genome were not reported until 1988, when Holt et al. described large-scale deletions in patients with mitochondrial myopathy (2), and Wallace et al. described a point mutation in the gene encoding subunit 4 of complex I (ND4) in a family with Leber’s hereditary optic neuropathy (LHON) (3). These two papers marked the opening of the genetic Pandora’s box to which I referred: in the past 12 years, the “morbidity map” of mtDNA has grown from one point mutation (1988) to the 115 point mutations listed in January 2001 (4) (Fig. 1, see over). To this, of course, must be added the innumerable mtDNA rearrangements (deletions, duplications, or both together) first identified by the group of the late Anita Harding (2), and quickly associated with various forms of progressive external ophthalmoplegia by our group (5,6).

The aim of this lecture is not to review the variety of clinical presentations associated with mutations in mtDNA, but rather to ask the question, “Is Pandora’s box empty?” – have we ex-
hausted the clinical research potential of mtDNA? The answer to this, as I trust I shall make clear, is a resounding “no”. However, before discussing the reasons that led me to this conclusion, a brief review of mitochondrial genetics is in order.

GENETIC CLASSIFICATION OF MITOCHONDRIAL DISORDERS

Although mitochondria contain multiple metabolic pathways, including the pyruvate dehydrogenase complex (PDHC), the β-oxidation spic-
and the Krebs cycle, by convention the term “mitochondrial disorders” has come to indicate diseases due to defects of the mitochondrial respiratory chain. Because the respiratory chain is under the control of both the nuclear and the mitochondrial genomes, these disorders can be due to mutations in nuclear DNA (and be transmitted by autosomal inheritance) or to mutations in mtDNA (and be transmitted, as a rule, by maternal inheritance). To further complicate matters, mtDNA is a slave of nDNA, in that it depends on a multitude of nuclear factors for its transcription, translation, and replication. Thus, an interesting group of mitochondrial disorders, the defects of intergenomic signalling, are mendelian diseases affecting directly the integrity or the abundance of the mitochondrial genome (multiple mtDNA deletions, mtDNA depletion). Table I proposes a simple genetic classification of the mitochondrial disorders: this lecture will focus on defects of mtDNA.

MITOCHONDRIAL GENETICS

Human mtDNA is a 16,569 bp circle of double-stranded DNA (7). It is highly compact, and contains only 37 genes (Fig. 1): 2 genes encode ribosomal RNAs (rRNAs), 22 encode transfer RNAs (tRNAs), and 13 encode polypeptides. All 13 polypeptides are components of the respiratory chain, including 7 subunits of complex I (NADH dehydrogenase-ubiquinone oxidoreductase), 1 subunit of complex III (ubiquinone-cytochrome c oxidoreductase), 3 subunits of complex IV (cytochrome c oxidase), and 2 subunits of complex V (ATP synthetase). The respiratory complexes also contain nuclear DNA (nDNA)-encoded subunits, which are imported into the mitochondria from the cytosol and assembled, together with their mtDNA-encoded counterparts, within the respective holoenzymes in the mitochondrial inner membrane. Complex II (succinate dehydrogenase-ubiquinone oxidoreductase) is encoded entirely by nDNA.

Mitochondrial genetics differs from mendelian genetics in three major aspects.

1. Maternal inheritance. At fertilization, the oocyte gives the zygote all its mitochondria (and all its mtDNAs). Therefore, a mother carrying an mtDNA mutation will pass it on to all her children, but only her daughters will transmit it to their progeny.

Table I - Genetic classification of respiratory chain defects

1. Defects of mtDNA
   A. Mutations in genes affecting mitochondrial protein synthesis (tRNA, rRNA, rearrangements)
   B. Mutations in protein-coding genes:
      – Multisystemic (LHON; NARP/MILS)
      – Tissue-specific (exercise intolerance/myoglobinuria)

2. Defects of nDNA
   A. Mutations in genes encoding respiratory chain subunits
      Complex I; Complex II
   B. Mutations in genes encoding ancillary proteins
      Complex IV (SURF1; SCO2; COX10; SCO1)
   C. Defects of intergenomic signalling
      mtDNA depletion
      AR-PEO with multiple mtDNA deletions (ARCO; MNGIE)
      AD-PEO with multiple mtDNA deletions

Abbreviations: LHON = Leber’s hereditary optic neuropathy; NARP = neuropathy, ataxia, retinitis pigmentosa; MILS = maternally inherited Leigh syndrome; AR-PEO = autosomal recessive progressive external ophthalmoplegia; ARCO = autosomal recessive cardiomyopathy and ophthalmoplegia; MNGIE = mitochondrial neurogastrointestinal encephalomyopathy
2. **Heteroplasm/homoplasm effect.** In contrast to nuclear genes, which each consist of one maternal and one paternal allele, mtDNA molecules are present in hundreds or thousands of copies in each cell. Deleterious mutations of mtDNA usually affect some but not all genomes. As a result, cells, tissues, and whole individuals in fact, will harbor two populations of mtDNA: normal (wild-type) and mutant. This situation is known as **heteroplasm.** In normal subjects, in whom all mtDNAs are identical, we have what is known as **homoplasm.** Non-deleterious mutations of mtDNA (neutral polymorphisms) are homoplasmic, whereas pathogenic mutations are usually, but not invariably, heteroplasmic.

Not surprisingly, a critical number of mutant mtDNAs must be present before tissue dysfunction and clinical signs become apparent. This **threshold effect** will manifest itself at lower concentrations of mutant mtDNAs in tissues that are highly dependent on oxidative metabolism than in tissues that can rely on anaerobic glycolysis.

3. **Mitotic segregation.** At cell division, the proportion of mutant mtDNAs in daughter cells can shift: if and when the pathogenic threshold for that tissue is surpassed, the phenotype can also change. This explains the time-related variability of clinical features frequently observed in mtDNA-related disorders.

Mitochondria and mtDNA are ubiquitous, which explains why every tissue in the body can be affected by mtDNA mutations. This is illustrated by Table II, a compilation of all the symptoms and signs reported in patients with three different types of mtDNA mutation, including single deletions, point mutations in two distinct tRNA genes, and point mutations in a protein-coding gene. As shown by the “boxes”, certain constellations of symptoms and signs are so characteristic as to make the diagnosis in typical patients relatively easy. On the other hand, due to heteroplasm and the threshold effect, different tissues harboring the same mtDNA mutation may be affected to different degrees or not at all, which explains the sometimes puzzling variety of syndromes associated with mtDNA mutations, even within a single family. It is often stated that any patient having multiple organ involvement and evidence of maternal inheritance should be suspected of harboring a pathogenic mtDNA mutation until proven otherwise. While this generalization has some practical value, there are exceptions, some of which are discussed below.

**IS PANDORA’S BOX EMPTY?**

The answer to this question is most certainly no, and for several reasons. First, although the mtDNA circle is crowded with pathogenic mutations, there is room for more, and more are being described, especially in protein-coding genes. Second, some of the “dogmas” of mitochondrial genetics are being questioned. For example, how often are homoplasmic mutations pathogenic? And, is there a role for mtDNA haplotypes in human pathology? Third, while we have learnt a lot about the molecular etiology of mtDNA disorders, much less is known about biochemical pathogenesis, and very little about pathophysiology. Fourth, newly obtained animal models promise to help us understand the physiopathology of mtDNA mutations. Fifth, the epidemiology of mtDNA-related disorders is only just beginning to be studied systematically. Finally, therapy of these conditions is still woefully inadequate, but some interesting approaches are being considered and actually implemented. I will consider these six points one by one.

1. **Mutations in protein-coding genes**

Generalizations regarding mutations in mtDNA protein-coding genes were based on the only two disorders of this type that were known until recently, Leber’s hereditary optic neuropathy (LHON) and neuropathy, ataxia, retinitis pigmentosa/maternally inherited Leigh syndrome (NARP/MILS). LHON is usually associated with
Table II - Clinical features of mitochondrial diseases associated with mtDNA mutations

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>SYMPTOM/SIGN</th>
<th>Δ-mtDNA</th>
<th>tRNA</th>
<th>ATPase</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>KSS</td>
<td>PEARSON</td>
<td>MERRF</td>
</tr>
<tr>
<td>CNS</td>
<td>Seizures</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td></td>
<td>Ataxia</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td></td>
<td>Myoclonus</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td>Psychomotor retardation</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Psychomotor regression</td>
<td>+</td>
<td>+</td>
<td>±</td>
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<tr>
<td></td>
<td>Hemiparesis/hemianopia</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>Cortical blindness</td>
<td>–</td>
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<tr>
<td></td>
<td>Migraine-like headaches</td>
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<tr>
<td></td>
<td>Dystonia</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>PNS</td>
<td>Peripheral neuropathy</td>
<td>±</td>
<td>–</td>
<td>±</td>
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<tr>
<td>Muscle</td>
<td>Weakness/exercise intolerance</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td>Ophthalmoplegia</td>
<td>+</td>
<td>±</td>
<td>–</td>
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<td></td>
<td>Ptosis</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Eye</td>
<td>Pigmentary retinopathy</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>Optic atrophy</td>
<td>–</td>
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<td>–</td>
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<td></td>
<td>Cataracts</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Blood</td>
<td>Sideroblastic anemia</td>
<td>±</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Endocrine</td>
<td>Diabetes mellitus</td>
<td>±</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>Short stature</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td>Hypoparathyroidism</td>
<td>±</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Heart</td>
<td>Conduction block</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>Cardiomyopathy</td>
<td>±</td>
<td>–</td>
<td>–</td>
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<tr>
<td>GI</td>
<td>Exocrine pancreatic dysfunction</td>
<td>±</td>
<td>+</td>
<td>–</td>
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<td></td>
<td>Intestinal pseudo-obstruction</td>
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<td>–</td>
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<tr>
<td>ENT</td>
<td>Sensorineural hearing loss</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Kidney</td>
<td>Fanconi syndrome</td>
<td>±</td>
<td>±</td>
<td>–</td>
</tr>
<tr>
<td>Laboratory</td>
<td>Lactic acidosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Muscle biopsy: RRF</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Inheritance</td>
<td>Maternal</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td>Sporadic</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Boxes are used to highlight the typical features of the various syndromes. Maternally inherited Leigh syndrome instead is defined on the basis of neuroradiologic or neuropathologic criteria.

Symbols and abbreviations: Δ-mtDNA = deleted mtDNA; RNA = ribonucleic acid; KSS = Kearns-Sayre syndrome; MERRF = myoclonic epilepsy and ragged-red fibers; MELAS = mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; NARP, neuropathy, ataxia, retinitis pigmentosa; MILS = maternally inherited Leigh syndrome; + = present; – = absent.

Modified from DiMauro and Bonilla (70).

Mutations in complex I (NADH dehydrogenase, or ND) genes (3,8,9). The NARP/MILS syndrome is associated with mutations in the ATPase 6 gene (10-13). Because both conditions are multisystemic, maternally inherited, inconsistently accompanied by lactic acidosis, and never associated with ragged-red fibers (RRF) in muscle, the syllogistic conclusion was drawn that all mtDNA
mutations in protein-coding genes would have the same characteristics. In fact, recent experience of patients with exercise intolerance has taught us that all three generalizations were incorrect.

Exercise intolerance is a common complaint in mitochondrial encephalomyopathies, but it is often overshadowed by other symptoms and signs. It is only recently that we have come to appreciate that exercise intolerance, myalgia, and myoglobinuria can be the sole presentation of respiratory chain defects. These can affect complex I, complex III, or complex IV, although they seem to be more commonly associated with complex III deficiency (14).

Exercise intolerance (without myoglobinuria) was the predominant clinical feature in two sporadic patients with complex I deficiency and COX-positive RRF in their muscle biopsies. One had a nonsense mutation (G11832A) in the ND4 gene (15), the other (16) had an intragenic inversion of seven nucleotides within the ND1 gene, resulting in the alteration of three amino acids (17).

Nine patients with isolated complex III deficiency in muscle complained of exercise intolerance, but only two had myoglobinuria (18). All patients in whom muscle histochemistry was performed showed COX-positive RRF. The nine mutations in the cytochrome b gene were different from one another although, except for a single deletion, they were all G-to-A transitions. Most patients had no detectable mutant mtDNA in blood or fibroblasts, but one patient had low levels (0.7%) of the mutation in non-muscle tissues, suggesting skewed heteroplasmy (19). Two other patients had pathogenic mutations in the cytochrome b gene. One was a 20-year-old man with signs of Parkinsonism and mitochondrial encephalopathy (MELAS) and an apparently ex novo microdeletion (20). The other was an infant girl with a missense mutation (21), who died of histiocytoid cardiomyopathy and in whose muscle we had documented complex III deficiency and decreased concentration of reducible cytochrome b (22).

The first mtDNA molecular defect identified in a patient with complex IV (cytochrome c oxidase, COX) deficiency was a 15-bp microdeletion in the COX III gene. The patient was a 16-year-old girl with recurrent myoglobinuria triggered by prolonged exercise or viral illness (23). Between attacks, both physical and neurological examinations were normal, as were routine laboratory tests, including serum creatine kinase (CK) and lactate. No tissue other than muscle was affected, and the family history was entirely negative. Muscle biopsy showed many SDH-positive, COX-negative RRF and marked isolated COX deficiency. We have identified a nonsense mutation (G5920A) in the COX I gene of muscle mtDNA in a 34-year-old man with life-long exercise intolerance and recurrent myoglobinuria induced by intense or repetitive exercise (24). His muscle biopsy showed scattered COX-negative RRF and numerous COX-negative non-RRF, and isolated COX deficiency. The mutation was not present in blood or fibroblasts from the patient nor in blood from his asymptomatic mother and sister.

2. Homoplasmic mutations and haplotypes

Although the very first mtDNA point mutation associated with a human disease, LHON, was homoplasmic (3), it has become common knowledge that most pathogenic mutations are heteroplasmic. In fact, heteroplasmia is one of the canonical criteria supporting the pathogenicity of a novel mutation, especially when there is rough correspondence between mutational load and clinical severity in different maternal relatives of the same family. A refinement of this concept was the introduction of the single-fiber polymerase chain reaction (PCR). This technique allows mutational load to be correlated with functional abnormality by physically harvesting from thick cross sections of muscle histologically normal and abnormal fibers and measuring the abundance of the mutation in each fiber by PCR (25). Abnormal fibers are
chosen on the basis of excessive mitochondrial proliferation (RRF) or oxidative impairment, such as histochemical COX-negativity.

However, we may have underestimated the pathogenic role of homoplasmic mutations. For example, about 95% of all patients with LHON harbor homoplasmic levels of one of three ND mutations: G11778A in ND4 (3), G3460A in ND1 (8), or T14484C in ND6 (9). Non-syndromic neurosensory hearing loss has been associated with two homoplasmic mutations in the tRNA\textsuperscript{Ser(UCN)} gene: A7455G (26), and T7511C (27). A homoplasmic mutation in the 12S rRNA gene (A1555G) has been described in patients with deafness (28) or cardiomyopathy (29). In addition, a homoplasmic mutation (G12192A) in the tRNA\textsuperscript{His} gene was found in 5 of 181 Japanese patients with cardiomyopathy but in none of 168 controls (30). As all five patients shared evolution-related D-loop sequences, it was concluded that this mutation derived from a common ancestor and was a risk factor for cardiomyopathy. It is interesting, and apparently counterintuitive, that all of these mutations are associated with tissue-specific syndromes, involving selectively the optic nerve, the cochlea, or the myocardium.

Naturally, proving pathogenicity for homoplasmic mutations is more difficult than for heteroplasmic mutations, as mutational loads cannot be compared to clinical or biochemical phenotypes, and single-fiber PCR is inapplicable. However, biochemical studies in platelets from LHON patients documented that each of the three “primary” mutations impaired complex I function, as manifested either by decreased enzyme activity or by altered sensitivity to specific inhibitors (31,32).

To explain the variable penetrance and tissue specificity of homoplasmic mtDNA mutations, three factors have been proposed: environment, mtDNA haplotype, and nuclear background (33). The prime example of environmental factors is exposure to aminoglycosides, which is required to induce deafness in some, but not all, carriers of the A1555G mutation. The influence of the rest of the mitochondrial genome is illustrated by the different penetrance of the A7455G mutation in a Scottish and a New Zealand pedigree. The higher frequency of deafness in the New Zealand family was attributed to the coexistence of three “secondary” LHON mutations in ND genes, which were not present in the Scottish pedigree (33,34).

The influence of the nuclear background has been more difficult to document, but some evidence has come from studies of cell cultures harboring the A1555G deafness mutation or the A3460G LHON mutation. Lymphoblastoid cell lines from members of a family with maternally inherited non-syndromic deafness and the A1555G mutation showed several features of impaired mitochondrial function compared to control cell lines, but the degree of impairment was greater in cells derived from symptomatic individuals than in those derived from asymptomatic family members (35). A genome-wide search led to the conclusion that not one but multiple nuclear loci may be involved (33).

Skin fibroblasts cultured from patients with LHON and the A3460G mutation in the ND1 gene showed decreased complex I activity. When homoplasmic cybrid cell lines were obtained by fusing enucleated patient fibroblasts with two different mtDNA-less (rho\textsuperscript{0}) cell lines, one derived from osteosarcoma and the other from the lung, the severity of the enzyme defect was greater in one than in the other, presumably due to the different nuclear backgrounds (36).

The tissue-specific manifestations of most homoplasmic mtDNA mutations remain a puzzle. Possible explanations include interaction of the mutant gene with nuclear-encoded tissue-specific mitochondrial proteins, an unknown tissue-specific function of the gene product, or a local environmental factor increasing the vulnerability of a given tissue to the gene defect, such as a relatively poor defense system against oxidative stress.
I have already introduced the concept of mtDNA haplotype as a risk factor for cardiomyopathy (30) and as an enhancer of the clinical penetrance of the A7455G mutation (33). The general idea is that certain constellations of polymorphisms in mtDNA may somehow “weaken” respiratory chain function, thus predisposing people who share the same haplotype to different disorders (37). For example, specific mtDNA haplogroups have been associated with reduced spermatozoa motility (38), with susceptibility to Alzheimer’s disease and dementia with Lewy bodies (39), and with susceptibility to multiple sclerosis in Caucasians (40).

Although many questions remain unanswered, it is amply clear that homoplasmic mtDNA mutations are not always neutral. It is also intriguing that “all mtDNA lineages are not equal” (37), and some may predispose individuals to disease.

3. Problems in pathogenesis

It is not readily apparent why defects of one and the same metabolic pathway, all presumably resulting in “energy crisis”, should cause the bewildering variety of symptoms and signs that can accompany mtDNA mutations.

Some answers are provided by the specific rules of mitochondrial genetics, especially heteroplasmy and the threshold effect. For example, the involvement of different tissues in patients with the same molecular defect can be attributed to differences in mutational loads, which surpass the pathogenic threshold in some tissues but not in others. This is probably true of the A3243G “MELAS” mutation, which causes typical MELAS in one member of a family and a variety of soft signs in oligosymptomatic maternal relatives. Different mutational loads, which are however more evenly distributed among tissues, can explain the different severity of two encephalomyopathies, NARP and MILS, both due to the T8993G mutation (41).

Encephalopathies pose special questions. For instance, it is not apparent why epilepsy, which is almost invariably part of the MELAS and myoclonic epilepsy and ragged-red fiber (MERRF) syndromes, is rarely seen in patients with Kearns-Sayre syndrome (KSS) (42). Nor is it clear why myoclonus is so common in MERRF. The pathogenesis of the strokes in MELAS remains a puzzle, although both metabolic tissue impairment and microvascular dysfunction probably play a role. Given the variety of cells and the organizational complexity of the brain, one could postulate a variation on the heteroplasmy/threshold effect idea, explained above, to explain the involvement of different tissues. Thus, it can be imagined that the specific mutations associated with MELAS, MERRF, and KSS may accumulate to different extents in different areas of the brain, reaching pathogenic thresholds only in certain structures, such as the choroid plexus in KSS (43), or in discrete cortical areas. Comparative immunohistochemical studies using antibodies against mtDNA-encoded versus nDNA-encoded subunits of the respiratory chain provide indirect evidence that there may well be something in this concept (44).

However, even if we do prove able to draw “mutational maps” of the brain giving us spatial frequencies for different mutations, this will still not explain why specific mutations should favor certain areas of the brain. In general, the rules of mitochondrial genetics are not very helpful in addressing the issue of “tissue specificity”. As discussed above, this is especially true for homoplasmic mtDNA mutations, but it applies to heteroplasmic mutations as well. For example, it is perplexing that mutations in certain tRNA genes are predominantly associated with cardiomyopathy (45). There may be more to tRNA genes than their role in mtDNA translation; think, for example, of tRNA^{Leu(UUR)}, which contains a transcription termination site for rRNA synthesis (46). An interesting observation is that certain mutation sites in the cloverleaf structure of the tRNA molecule are preferentially associated with certain clinical phenotypes, such as
cardiomyopathy, progressive external ophthalmoplegia, and encephalopathy (47).

Until very recently (see below), the lack of animal models of mtDNA mutations prevented detailed pathogenetic studies. As an alternative, the biochemical and functional consequences of mtDNA point mutations and deletions were studied in cybrid cell cultures, that is, in established human cell lines first depleted of their mtDNA then repopulated with various percentages of mutated or deleted genomes (48). This ingenious system has been of great value, but caution has to be used in extrapolating data to patients. For example, the high threshold shown by cybrid cells harboring the A3243G MELAS mutation appears to be much lower in vivo. In one study, maximal ATP production measured by magnetic resonance spectroscopy in the calf muscle from an oligosymptomatic patient was markedly decreased although the mutational load was very low in muscle (49). In a second study, proton magnetic resonance spectroscopy showed that brain lactate in carriers of the A3243G mutation was increased and linearly related to the proportion of mutant mtDNAs (50). These data agree with our own observation that lactate levels were increased in the CSF of the lateral ventricles in oligosymptomatic or asymptomatic relatives of MELAS patients (51).

4. “Mito-mice”: animal models for mtDNA-related diseases

Since the first description of pathogenic mutations in human mtDNA, one of the goals of clinical scientists has been the generation of animal models. These animals would greatly facilitate our understanding of pathogenetic mechanisms and would allow us to try out different methods of treatment. A formidable obstacle to the generation of “mito-mice” was our inability to introduce mutant (or, for that matter, even wild-type) mtDNA into the mitochondria of a mammalian cell. To solve this problem, Inoue et al. (52) used a brilliant stratagem. They first prepared synaptosomes from aging mice brains, which contained a certain percentage of deleted mtDNAs. They then fused the synaptosomes with mtDNA-less rho0 cells, thus obtaining cytoplasmic hybrid (cybrid) cell lines. One such cell line harboring mtDNA deletions was enucleated, fused with donor embryos, and implanted in pseudopregnant females. Heteroplasmic founder females were bred and mtDNA deletions were transmitted through three generations. Although there are significant differences between these mito-mice and human patients with mtDNA deletions, the fact remains that the animals show mitochondrial dysfunction in various organs (53). In fact, the differences between mito-mice and patients (including the maternal transmission of deleted mtDNA in mice, which rarely occurs in humans) render this model even more interesting.

A similar but slightly more complicated strategy was employed by Sligh et al. (54) to generate mice harboring a point mutation for chloramphenicol resistance (CAPR). The mutation in homoplasmic or heteroplasmic CAPR mutants was severe enough to cause death in utero or within 11 days of birth, and affected animals showed dilated cardiomyopathy and abnormal mitochondria in both cardiac and skeletal muscle, features often observed in human mtDNA diseases. As pointed out by Hirano in a thoughtful editorial to Sligh’s article (55), the papers by Inoue et al. and Sligh et al. prove the principle that heteroplasmic mtDNA mutations can be transmitted through germlines and will produce phenotypic abnormalities. Hirano also correctly predicted that these pioneering studies would prompt a new wave of research into the pathogenesis and therapy of human mitochondrial diseases.

5. Epidemiology

One of the questions I am most frequently asked when I speak about mitochondrial diseases – in particular mtDNA diseases – is how common (or rare) these disorders are. It took a while
for epidemiological studies to appear, but three such studies have now been published, all three from northern European countries. The reason for this geographical “bias” is that epidemiological studies are more easily conducted in countries with homogeneous health care systems and with relatively low migration of the population, and both these conditions are present in the north of Europe. Although each of these studies addresses a different question, the results are remarkably consistent.

The first study, from northern Finland, investigated the frequency of the A3243G MELAS mutation in the adult population of Northern Ostrobothnia using a wide array of clinical criteria as a screening “funnel”: diabetes mellitus, sensorineural hearing loss, epilepsy, occipital brain infarct, ophthalmoplegia, ataxia, hypertrophic cardiomyopathy, and basal ganglia calcifications (56). These authors came up with a frequency of at least 16.3 per 100,000 adult people.

A second study, this time from north-eastern England and centered on Newcastle upon Tyne, sought to ascertain the frequency of patients with diseases due to mtDNA mutations in general (57). The authors concluded that the minimum prevalence of pathogenic mtDNA mutations in the general population was 12.48 per 100,000.

The third study, from western Sweden, focused on pre-school children and spread the net even wider. These authors set out to establish the incidence of mitochondrial diseases as a whole, using clinical, histochemical, biochemical, and molecular diagnostic criteria (58). They found an incidence of 1:11,000 pre-school children.

Although the extreme clinical heterogeneity of mitochondrial disorders makes epidemiological studies difficult, and although each of the three studies used different selection criteria, the clear conclusion that emerges is that mitochondrial diseases, including those due to mtDNA mutations, are far from rare. In fact, they may be among the most common metabolic disorders.

Related to epidemiology is the question of prenatal diagnosis. This poses special problems in mtDNA-related disorders because of two main concerns: 1) that the mutant load in amniocytes or chorionic villi will not correspond to that of other fetal tissues; 2) that the mutant load in prenatal samples may shift in utero or after birth due to mitotic segregation. These concerns still impede prenatal diagnosis of tRNA mutations, including the common causes of MELAS and MERRF. However, there is good evidence that mutations in the ATPase 6 gene (T8993G and T8993C), commonly associated with MILS, do not show tissue- or age-related variations (59), thus making prenatal diagnosis an option for families affected by this devastating condition (60).

6. Approaches to therapy

Therapy of mtDNA-related diseases is still woefully inadequate. Besides pharmacological and surgical interventions directed at alleviating symptoms (palliative therapy), approaches to therapy include: (i) removing toxic products, in primis lactic acid; (ii) administration of artificial electron acceptors, such as vitamin K3 and vitamin C; (iii) administration of metabolites and cofactors, such as L-carnitine and coenzyme Q10 (CoQ10); (iv) administration of oxygen radical scavengers, such as CoQ10 (61).

Gene therapy is daunting in these conditions for much the same reasons that made the creation of animal models such a challenge. However, if we could cause even a small shift in the relative proportion of mutant and wild-type mtDNAs, thus lowering the mutant load below the pathogenic threshold, we might improve the clinical expression dramatically. Various strategies are being considered, including the use of peptide nucleic acids (PNAs) to inhibit the replication of complementary mutant mtDNAs (62), or pharmacologic approaches directed at the same end (63). The observation that myoblasts often contain lesser amounts of pathogenic mtDNA mutations than mature muscle fibers (64-66), has suggested the use of myotoxic agents (67) or isomet-
ric exercise (68) to cause limited muscle damage, a step that would be followed by regeneration of muscle fibers harboring lower mutational loads.

CONCLUDING REMARKS

Despite its large cargo of evils, Pandora’s box must have been a beautiful vessel: likewise, we should not forget that our mtDNA is, as Grivell (69) aptly defined it, “small, beautiful and essential”. The very abundance of diseases that are due to mutations in mtDNA proves the essential nature of this small genome.

In the past thirteen years we have been able to give molecular labels to most (but not all) the evils that escaped from Pandora’s box, but we have been much less successful in understanding how exactly these molecular defects impair cell function, and we still do not know how to correct their deleterious effects. The recent arrival of mito-mice holds great promise for a better understanding of the pathogenesis of mitochondrial diseases and for the development of effective therapy. It is abundantly clear that Pandora’s box still harbors a host of evils waiting to be discovered. In this process, however, we must continue to appreciate the beauty of this small genome and enjoy deciphering the language that keeps it attuned to the much larger nuclear genome.

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