The “new” mitochondrial disorders

A H V Schapira

The “new” mitochondrial disorders

It is now clear that mitochondria serve two fundamental biological roles: the provision of ATP by aerobic respiration, and the mediation of cell death by apoptosis. Production of ATP is achieved primarily by oxidative phosphorylation and also through the tricarboxylic acid cycle and β-oxidation of fatty acids. The oxidative phosphorylation system comprises the respiratory chain and ATP synthase and consists of five multisubunit proteins, termed complexes I to V (table 1), embedded in the inner mitochondrial membrane. Electrons are passed down the chain and proteins are pumped from the matrix side to the space between the inner and outer mitochondrial membranes. This creates an electrochemical gradient (membrane potential) which is used to drive ATP production via ATPase (complex V). The operation of the mitochondrial respiratory chain and oxidative phosphorylation (MITOX) system is responsible for the production of more than 95% of a cell’s superoxide ions.

Mitochondria contain their own DNA, which is present in multiple copies. Human mitochondrial DNA (mtDNA) is a circular double stranded molecule 16 493 bases long encoding two ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA), and 13 proteins. These proteins are all part of the oxidative phosphorylation system. mtDNA remains dependent on the nucleus for the proteins that undertake and regulate its transcription, translation, replication, and repair. Indeed the nucleic acids more than 98% of mitochondrial proteins, including 70 of the 83 proteins of complexes I to V.

The mtDNA encoded proteins are translated on cytosolic ribosomes and incorporated directly into complexes I, II, IV, and V on the inner membrane. By contrast, nuclear encoded proteins are translated on cytosolic ribosomes and must be imported into the mitochondrion. This import system is highly complex and includes, for most proteins, a targeting sequence at the N-terminus that interacts with membrane receptors before being cleaved. The protein is transported through the membrane and then folded and sorted to its correct intramitochondrial location.

There is now ample evidence that mitochondria play an important part in determining apoptotic cell death. Apoptosis is important in embryological development, immune cell regulation, and several other physiological functions. Numerous factors may induce apoptosis, many of which seem to act through mitochondria by opening of the mega-pore thereby decreasing the electrochemical gradient (permeability transition) and releasing apoptosis initiating factors. The megapore is located at inner/outer membrane junctions and comprises several components including the peripheral benzodiazepine receptor, voltage dependent anion channel, and cyclophilin D. The pore is opened by proapoptotic molecules such as Bax and Bak, and by free radicals and respiratory chain defects, and is closed by antiapoptotic agents—for example, Bcl-x and cyclosporine. The voltage dependent anion channel seems to play an important part in mediating the modulatory effects of Bcl-x, proteins Bax and Bak bind to the voltage dependent anion channel and accelerate opening of its channel, thereby allowing the release of cytochrome c. Bcl-x binds to and closes the voltage dependent anion channel, preventing cytochrome c release. Cytochrome c is an integral part of the respiratory chain and is released from the mitochondrial intermembranous space during the preapoptotic phase. Cytochrome c binds to Apaf-1 and activates procaspase 9 to initiate the caspase cascade, which in turn induces the changes that constitute the final events in apoptosis. The critical role of mitochondria in the pathway to apoptotic cell death adds an additional dimension to the possible mechanisms that may be involved in the pathogenesis of mitochondrial diseases.

Numerous disorders have now been described in which mutations of mtDNA, or nuclear genes encoding respiratory chain subunits, have been identified. These have been the subject of several recent reports and reviews1–4 and will not be discussed further. This review focuses on a separate group of “new” mitochondrial diseases in which defective oxidative phosphorylation is due to mutations of nuclear gene that encode non-respiratory chain proteins that are involved in mitochondrial biogenesis and, directly or indirectly, cause defective mitochondrial energy metabolism and neurological disease.

DEFECTS OF MITOCHONDRIAL DNA BIogenesis

Multiple deletions of mtDNA

Several families have been described with autosomal dominant or recessive inheritance of mitochondrial myopathy. The families with the dominantly inherited disorder usually have chronic progressive external ophthalmoplegia and proximal limb myopathy with ragged red fibres on biopsy, deficiency of oxidative phosphorylation on biochemical analysis, and multiple mtDNA deletions on Southern blotting. These mtDNA mutations are secondary to a nuclear gene defect and contrast with the single deletions that characterise primary mitochondrial diseases such as the Kearns-Sayre syndrome.

Table 1 The respiratory chain and oxidative phosphorylation system

<table>
<thead>
<tr>
<th>Complex</th>
<th>Enzyme activity</th>
<th>No of subunits</th>
<th>mtDNA encoded subunits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>NADH ubiquinone reductase</td>
<td>41</td>
<td>7</td>
</tr>
<tr>
<td>Complex II</td>
<td>Succinate ubiquinone reductase</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>Complex III</td>
<td>Ubiquinol cytochrome c reductase</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Complex IV</td>
<td>Cytochrome c oxidase</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Complex V</td>
<td>ATP synthase</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations: MITOX, mitochondrial oxidative phosphorylation system; mtDNA, mitochondrial DNA; rRNA, ribosomal RNA; tRNA, transfer RNA; ANT-1, gene for the heart/skeletal muscle form of the adenine nucleotide translocator; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; GAMA, guanine adenine adenine; YTH1, yeast frataxin homologue 1; CAD, cytochrome c-adenine-ribosyltransferase; OMCQ, OmcQ, B hydrorxide-2-deoxogummine; 31P-MRS, 31-phosphorus magnetic resonance spectroscopy
There are at least three chromosomal loci linked to chromosome 22 with the nucleus from a normal cell, indicating that mtDNA depletion in patients Friedreich's ataxia is caused by a mutation of the thymidine phosphorylase gene. Patients with MNGIE typically present with chronic progressive external ophthalmoplegia, sensorimotor peripheral neuropathy, recurrent nausea, vomiting, and diarrhoea. A leukodystrophy may be seen on MRI. Thymidine phosphorylase activity is severely decreased and blood thymidine concentrations are high.

**Depletion of mtDNA**

Many patients with depletion of mtDNA present in the first few days or weeks of life with hypotonia and lactic acidosis or progressive hepatorenal failure. Most die before the age of 12 months, although occasional less severely affected infants have been reported. In affected tissues, the concentrations of mtDNA are usually less than 30% but can be as low as less than 1% of age matched normal subjects. In some cases, the depletion is expressed in culture. The mtDNA depleted cells are capable of growing and dividing in specially supplemented medium. Genomic transplantation studies have shown that the mtDNA remaining in these patients' cells can be restored to normal by replacement of the nucleus with the nucleus from a normal cell, indicating that mtDNA depletion in these patients is caused by a nuclear gene defect.

**DISEASES DUE TO NUCLEAR GENE MUTATIONS IN NON-MITOCHONDRIAL PROTEINS**

Friedreich's ataxia

Friedreich's ataxia is an autosomal recessive disorder with an incidence of 1 in 30 000 to 50 000 live births. The disorder usually becomes evident in childhood or adolescence, with ataxia, skeletal deformities, hyporeflexia, pyramidal tract deficits, and a hypertrophic cardiomyopathy. Most patients become wheelchair bound 10 to 15 years after diagnosis and die 5 to 10 years later from progressive cardiac failure. In 98% of patients Friedreich's ataxia is caused by an expanded guanine-adenine-adenine (GAA) repeat in intron I of the gene on chromosome 9 which encodes frataxin and results in an abnormality of RNA processing. Frataxin is a widely expressed 18 kDa protein of unknown function. The frataxin gene has close homology to a yeast gene (now termed yeast frataxin homologue I, YFH1) which when deleted results in defective energy metabolism, intramitochondrial iron accumulation, induction of the high affinity iron transport system, and low mtDNA concentrations. Frataxin was subsequently found to have a mitochondrial targeting sequence and to be a mitochondrial protein. Mitochondrial function studies have disclosed severe deficiencies in the activities of complexes I, II, and III, and aconitase in cardiac and skeletal muscle. Furthermore, there is histological evidence for iron accumulation in these tissues. Thus, there is a striking parallel between the yeast knock-out model and the human disease. Of particular interest is the demonstration in vivo in patients with Friedreich's ataxia of a defect of oxidative phosphorylation in skeletal muscle by 31P magnetic resonance spectroscopy (31P-MRS) correlating with the length of the patient's GAA repeat.

Complexes I, II, and III and aconitase are all iron-sulphur containing enzymes, and their combined deficiency in Friedreich's ataxia, coupled with tissue iron accumulation, suggests that frataxin deficiency may result in a defect of non-sulphur protein construction. Iron is a pro-oxidant, and the pattern of enzyme defect in patients with Friedreich's ataxia is identical to that seen in the manganese superoxide dismutase knockout mouse model of oxidative stress supporting an important role for free radicals in the pathogenesis of Friedreich's ataxia. This raises the possibility that effective antioxidants may have a disease modifying role in this neurodegenerative disorder.

Two recent studies have shown objective improvement in patients with Friedreich's ataxia given antioxidants. In one, 10 patients took high dose ubiquinone (coenzyme Q10) and vitamin E and significant improvements in their skeletal and cardiac muscle biochemical defects, as determined by 31P-MRS, were noted at 3 and 6 months and 24 months (R. Iodice et al, unpublished results). In another study, three patients were given idebenone with improvements in cardiac hypertrophy over 3 to 6 months. Whether antioxidant therapy in Friedreich's ataxia will modify the course of the neurological dysfunction is not yet known, but this issue is currently the subject of a long term study.

**Leigh syndrome**

Leigh syndrome usually presents in infancy or childhood with failure to thrive, hypotonia, recurrent vomiting, lactic acidosis, psychomotor retardation, and evidence of brain stem or basal ganglia dysfunction. Magnetic resonance spectroscopy shows bilateral and symmetric high signal in the thalamus and brain stem on T2 weighted images. Leigh syndrome is heterogeneous at the biochemical and molecular levels; defects of pyruvate dehydrogenase and respiratory chain complexes have been described, and mutations have been identified in pyruvate dehydrogenase or mtDNA genes in some patients.

Interest has recently focused on a group of patients with Leigh syndrome with non-maternally inherited cytochrome oxidase (complex IV) deficiency. Somatic cell genetics demonstrated the nuclear basis of complex IV deficient Leigh syndrome, and mutations in the SURF-1 gene were subsequently identified in a high proportion of these patients. SURF-1 is a nuclear encoded mitochondrial protein involved in the maintenance of complex IV activity and mitochondrial respiration in yeast and is probably important in homologous assembly. SURF-1 mutations in complex IV-deficient Leigh syndrome result in a severe decrease in enzyme activity. Mutations in other nuclear encoded genes encoding proteins involved in COX assembly have been identified including SCO1, SCO2, and COX 10. These mutations were associated with infantile onset of Leigh syndrome, and in some cases led to iron deficiency, cardiomyopathy and lactic acido-
of a nuclear gene mutation causing apparently widespread mitochondrial abnormalities.

Dystonia

Movement disorders, and dystonia in particular, are known to occur more often than expected in patients with primary mitochondrial diseases. Several families with maternally inherited Leber's hereditary optic neuropathy with dystonia and mutations in mtDNA genes encoding complex I subunits have been described. A specific and reproducible deficiency of complex I activity in patient muscle. This has been identified in not only by nuclear dystonia linked or not to the DNA gene.

A nuclear encoded mitochondrial protein defect has now been identified in one particular type of dystonia. Deafness dystonia (Mohr-Tranebjaerg syndrome) is an X linked disorder characterised by progressive sensorineural deafness, cortical blindness, dystonia, dysphagia, and paranoia. Deafness dystonia syndrome is due to deletion or truncation of a gene (DFN1) encoding a 11 kDa protein termed DDF1. This protein has recently been identified, through homology studies, as a component of the mitochondrial import system. It is not yet known which proteins are affected by this import defect, nor whether oxidative phosphorylation is compromised—although the clinical phenotype is reminiscent of other mitochondrial encephalopathies.

DISEASES DUE TO NUCLEAR GENE DEFECTS IN NON-MITOCHONDRIAL PROTEINS

Huntington's disease

Huntington's disease is an autosomal dominant neurodegenerative disorder characterised clinically by chorea, ataxia, and dementia and pathologically by the loss of neurons of the striatum containing γ-aminobutyric acid and enkephalin. It usually presents in early to middle adult life, although both juvenile and late onset forms are recognised. The mutation responsible is an abnormally expanded (>56) cytosine-adenine-guanine (CAG) repeat in the huntingtin gene on chromosome 4. Knockout of the huntingtin gene results in early fetal death in mice, implying a critical role in embryogenesis. The gene product, huntingtin, is a widely expressed 349 kDa protein of unknown function. Cultured cells expressing mutant huntingtin molecules have intranuclear inclusions, which are also seen in the brains of patients with Huntington's disease and in Huntington transgenic mice. Motor toxicity has been suggested to play an important part in neuronal cell death in Huntington's disease.

The development of such severe defects of mitochondrial respiratory chain and aconitase function must be secondary to the primary huntingtin gene defect. The question arises as to whether this is a causal relation or the consequence of alternative biochemical and pharmacological events. Huntingtonin is expressed in muscle and, at a low level, in fibroblasts and lymphoblasts. Mitochondrial respiratory chain and aconitase activities were normal in fibroblasts and platelets from patients with Huntington's disease. Magnetic resonance spectroscopy in muscle of patients with Huntington's disease has been reported to be abnormal. This finding has recently been confirmed and also decreased rates of ATP synthesis (which correlated with the length of the CAG repeat—that is, the longer the repeat, the worse the defect) were found in the muscle of both symptomatic and asymptomatic patients.

The presence of mitochondrial defects in Huntington's disease brain and muscle, in particular the correlation of the latter with the length of the CAG repeats, suggests that mutant huntingtin may have a direct role in inducing mitochondrial abnormalities. These findings are supported by the protective effects of creatine in the R6/2 transgenic mouse and have been the basis for a trial in the United States of ubiquinone therapy in Huntington's disease.

Wilson's disease

Wilson's disease is an autosomal recessive disorder which results in liver disease in 40% of affected patients, neurological dysfunction (dystonia, rigidity, parkinsonism) in 40%, and psychiatric disease in 20% of patients. The Wilson's disease gene on 14q13.4 codes for a protein that functions as a P-type ATPase localised in both the Golgi network and possibly in mitochondria. We have recently identified severe defects of MITOX function, particularly complex I, II, and III, and aconitase deficiency in liver samples from patients with Wilson's disease. This pattern of enzyme defect, identical to that seen in Friedreich's ataxia and to that found in the superoxide dismutase 2 knockout mouse, suggests that free radical formation and oxidative damage may contribute to the pathogenesis of cirrhosis in Wilson's disease. This might be expected as copper, which accumulates in the liver in Wilson's disease, may substitute for iron in free radical generating reactions. The results suggest that effective antioxidant therapy may be helpful in patients with Wilson's disease.

DISEASES WITH UNDEFINED MITOCHONDRIAL CONTRIBUTION TO AETIOLOGY AND PATHOGENESIS

There are a group of neurodegenerative diseases where there is clear evidence of mitochondrial dysfunction. However, the role of mitochondrial dysfunction and pathogenesis as yet remains undefined—and may be primary or secondary. Indeed it is increasingly likely that in Parkinson's disease in particular, the mitochondrial abnormality may be primary in some patients, secondary in others, but still plays an important contributory part in pathogenesis in many. The position with respect to Alzheimer's disease and motor neuron disease is less clear.

Parkinson's disease

The cause of dopaminergic cell death in Parkinson's disease is not known. However, during the past 2 years several mutations have been identified in both autosomal dominant and recessive Parkinson's disease. Environmental agents—for example, 1-methyl-4-phenyl 1,2,3,6 tetrahydropyridine, manganese, and carbon monoxide—have also been associated with the development of parkinsonism. A rat model of parkinsonism...
The mitochondrial abnormalities in motor neuron disease are most easily explained as a secondary phenomenon to free radical mediated damage. The pattern of respiratory chain deficiency is consistent with both sporadic and familial forms of motor neuron disease. **Whether the mitochondrial dysfunction is merely an epiphenomenon, or whether it also contributes to disease pathogenesis, is not clear.** However, the recent demonstration that the SOD-1 transgenic model suggests that improving mitochondrial function may be of benefit to patients with motor neuron disease.** This may be achieved for instance with either carnitine or ubiquinone—or both—and could be used in conjunction with other potentially disease modifying therapies including NMDA antagonists. A double blind placebo controlled trial to address these issues is currently being initiated.

**CONCLUSIONS**

The mitochondrion plays a central part in cell biology in maintaining life through ATP generation and in determining cell death through its role in apoptosis. It is hardly surprising, therefore, that mitochondrial dysfunction, both primary and secondary, is associated with disease. The dependence of nervous tissue, in particular, on intact mitochondrial activity means that neurological dysfunction will be a frequent consequence of both primary and secondary respiratory chain defects. **Evidence is emerging that there may be some pathogenic features in several of the neurodegenerative diseases. These may include defects of intracellular protein handling, protein damage with consequent aggregation and accumulation, and mitochondrial dysfunction. The development of therapeutic strategies to intervene in the cascade of events that leads to disease pathogenesis, is not clear.** However, the recent demonstration that the SOD-1 transgenic model suggests that improving mitochondrial function may be of benefit to patients with motor neuron disease. This may be achieved for instance with either carnitine or ubiquinone—or both—and could be used in conjunction with other potentially disease modifying therapies including NMDA antagonists. A double blind placebo controlled trial to address these issues is currently being initiated.

**REFERENCES**


**Author’s affiliation**

A H V Schapira, University Department of Neurology, University of Cambridge, Cambridge, UK.

Correspondence to: A H V Schapira; Schapira@rfc.ucl.ac.uk

**www.jnnp.com**

---

**Alzheimer’s disease**

Evidence for mitochondrial dysfunction in Alzheimer’s disease is derived from several different but complementary studies. A specific deficiency in the activity of complex IV (cytochrome oxidase) has been described in Alzheimer’s disease in postmortem tissue. Mitochondrial morphological changes have been found in anterior horn cells from patients with Alzheimer’s disease.** Implying that an mtDNA defect is the cause of the complex IV deficiency. Indeed a group of mtDNA “mutations” were identified, but subsequently found to be amplifications of nuclear pseudogenes.** Down regulation of COX gene expression is known to occur in association with decreased neuronal activity or degeneration.** Complex IV deficiency has also been associated with the pathologies of Friedrich’s ataxia and spinocerebellar ataxia-1 brains** and so is not specific to Alzheimer’s disease— unlike the complex I deficiency in Parkinson’s disease. At present, therefore, there remains uncertainty about the relevance of complex IV deficiency in Alzheimer’s disease and whether it may represent a primary phenomenon in some, or a secondary epiphenomenon in all.

**Motor neuron disease**

The evidence for mitochondrial dysfunction in motor neuron disease (amyotrophic lateral sclerosis) is compelling and fits well with our current understanding of the pathogenesis of this disorder. Mutations in the gene for superoxide dismutase 1 (SOD-1) are found in about 20% of patients with familial forms of motor neuron disease* and may induce neuronal damage by enhanced peroxidation.** There have been several reports of free radical induced damage to proteins, lipid, and DNA in postmortem tissue from patients with motor neuron disease.** Increased concentrations of 8-hydroxy-2-deoxyguanosine (8OHdG)—an indicator of oxidatively damaged DNA—have also been found in plasma, urine, and CSF from patients with motor neuron disease, and with increase in progression of the disease.** Changes have been seen in both sporadic and familial forms of the disease, as well as in a transgenic mouse model with a SOD-1 mutation (G93A).** Furthermore, fibroblasts from patients with sporadic and familial motor neuron disease are more susceptible to damage by free radicals.** Increased activities of respiratory chain complexes I-III were seen in postmortem brain tissue from families but not sporadic patients. Complex I activity was increased in the forebrain of the G93A transgenic SOD-1 mouse model but complex I and IV activities were decreased in the spinal cord.** Complex I activity has been found to decrease in skeletal muscle from patients with motor neuron disease* and respiratory defects were associated with the presence of mtDNA deletions** which in turn were correlated with negative staining for complex IV in single fibres.** Mitochondrial morphological changes have been found in anterior horn cells from patients and the G37R transgenic mouse model.** Complex IV was decreased in motor neuron disease motor neurons* and an excess of the 4977 “common” mtDNA deletion was found in motor neuron disease motor cortex.**

**EDITORIAL**

has been produced by parental infusion of rotenone—a naturally occurring pesticide and complex I inhibitor.** It seems increasingly likely that idiopathic, sporadic Parkinson’s disease may be the clinical, pathological, and possibly biochemical end point common to various different pathological agents.** There is evidence of a mitochondrial complex I defect in the substantia nigra of patients with Parkinson’s disease (see Schapira et al.** for review), and this defect is also detectable in platelets in some patients with sporadic Parkinson’s disease.** Genomic transplantation studies have demonstrated that the complex I defect in this subgroup is determined by mtDNA.** Whether this defect is caused by mtDNA mutations or a series of polymorphisms that render cells susceptible to damage by, for instance, an environmental agent, is not yet known, nor whether such mtDNA abnormalities are somatic or inherited. Any mtDNA mutation may be systematically distributed but, as with primary mtDNA mutations, may be heteroplasmic (present in varying proportions of wild-type and mutant genomes) and therefore, for instance, be present in higher concentration in certain tissues—for example, the substantia nigra. In addition, the biochemical (oxidative stress, increased iron) and pharmacological (dopamine oxidation, excitotoxicity) characteristics of the substantia nigra may exaggerate the deleterious effects of all of these processes may influence complex I function and respiratory (protein pumping) activity such as to decrease membrane potential, thereby lowering the cell’s threshold to undergo apoptosis. These events and their effects may vary from one cell type to another, and could particularly affect neurones in view of their dependence on oxidative phosphorylation. These findings suggest that, in some patients with Parkinson’s disease, mtDNA defects may be primary, whereas in others the mitochondrial abnormalities may be secondary to, for instance, free radical general or possibly exogenous toxins.

**REFERENCES**


**Author’s affiliation**

A H V Schapira, University Department of Neurology, University of Cambridge, Cambridge, UK.

Correspondence to: A H V Schapira; Schapira@rfc.ucl.ac.uk

**www.jnnp.com**
Mitochondrial respiratory chain disorders II: third locus predisposing to multiple deletions
and mtDNA depletion.

Neurodegenerative disorders and nuclear respiratory chain disorders II: 
éntéphalomyopathy syndrome maps to 3p.

MNGIE: a human mitochondrial disorder.

Molecular mechanisms in mitochondrial iron-sulphur proteins. Annu Rev Biochem

Transcript, L, Schwartz, C, Eriksen H, Jacobsen S. A new X linked recessive deafness syndrome 
with blindness, dysarthria, ataxia, and mental deficiency is linked to Xq22. J Med Genet


Davies SW, Turner M, Puccetti PT, et al. Mitochondrial respiratory chain deficiencies 
are associated with mutations in the human heme Aconitase and mitochondrial iron-sulphur 

Kwatchko E, Pamer EJ, Manolopoulou P, et al. Neurodegeneration with autoantibodies 
to nuclear respiratory factor 1 (NRF1) is a distinct clinical entity. J Biol Chem 1998; 273:15196–201.

Karteszi E, Butorics Z, Kohl C, et al. Mitochondrial respiratory chain dysfunction related 


Transcript, L, Schwartz, C, Eriksen H, Jacobsen S. A new X linked recessive deafness syndrome 
with blindness, dysarthria, ataxia, and mental deficiency is linked to Xq22. J Med Genet


Davies SW, Turner M, Puccetti PT, et al. Mitochondrial respiratory chain deficiencies 
are associated with mutations in the human heme Aconitase and mitochondrial iron-sulphur 

Kwatchko E, Pamer EJ, Manolopoulou P, et al. Neurodegeneration with autoantibodies 
to nuclear respiratory factor 1 (NRF1) is a distinct clinical entity. J Biol Chem 1998; 273:15196–201.

Karteszi E, Butorics Z, Kohl C, et al. Mitochondrial respiratory chain dysfunction related 
EDITORIAL