Mitochondrial myopathies: clinical defects

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The mitochondrial myopathies are a clinically heterogeneous group of diseases characterized by abnormal mitochondrial proliferation in skeletal muscle and in other affected cells [1,2]. Studies of mitochondrial metabolism in vitro in over 100 published cases have identified a number of different functional defects which have mostly involved the oligomeric complexes of the mitochondrial respiratory chain. In the majority of these patients, the defect was localized to complex I [3,4], complex III [5] or complex IV [6], but multiple enzyme defects [7-9], as well as isolated deficiencies of complex II [10], and of the mobile carrier coenzyme Q10 [11], have also been described. More recent strategies aimed at characterizing these diseases at a molecular and genetic level are beginning to provide substance for the contention that defects in this pathway may be caused by mutations of nuclear or mitochondrial genes [3, 12-19]. The fact that only about one in five patients has an affected relative [20] would suggest that the defect in some cases may be acquired. Viruses [21] and autoimmune mechanisms [22] have been implicated, and the observation that 1-methyl-4-phenylpyridium (MPP+) is a powerful inhibitor of complex I [23], and that complex I activity may be selectively impaired in patients with idiopathic Parkinson’s disease [15, 24], suggests that environmental factors may also be involved.

This paper summarizes clinical and biochemical data from 57 patients with mitochondrial myopathies, 46 of whom had defects in the respiratory chain. Muscle mitochondria freshly isolated from each case were investigated biochemically according to previously described methods [14, 25]. Muscle mitochondria from six patients with no clinical, morphological or biochemical evidence of mitochondrial dysfunction served as controls. Restriction endonuclease digests of mtDNA from blood and from muscle were analysed as described by Holt et al. [12, 14]. The site of the respiratory chain defect in each case was determined exclusively on the basis of the polarographic results according to the criteria summarized in Table 1. Cases have been separated into four clinical categories according to the major presenting features, as shown in Table 2. All procedures were carried out with the informed consent of the patient and approval of the local ethics committee.

There were six males and nine females aged 13-51 years (median age 24 years). Symptoms began in childhood or adolescence, except in one man who first developed weakness in the fifth decade. All cases had mild diffuse fatigable weakness of the limb and trunk muscles and all but two experienced exercise-related muscle aches. The extra ocular muscles were unaffected in 13 cases, but one patient had mild ptosis and another had slight limitation of lateral eye movements without ptosis. Identical twin brothers had pigmentary retinopathy which was asymptomatic and a 21-year-old female developed congestive heart failure at the age of 11 years which resolved after 6 months and has not recurred. Seven patients had isolated or infrequent attacks of increased weakness or paralysis lasting from a few hours to several days which when severe were accompanied by headache, nausea and vomiting. In three cases, the attacks were shown to be associated with a severe metabolic acidosis [26, 27]. Precipitating factors included intercurrent infection, unaccustomed exercise and small quantities of alcohol. The family history was negative in eight cases. The remaining seven cases included identical twin brothers, an affected mother and daughter and two female patients each with an affected sister. The brother and sister of another female patient died from a similar disorder in adult life. None of the cases in this group had a detectable deletion of mitochondrial DNA.

The defect was localized to complex I in seven cases, complex I-III in four, and complex I-IV in the remaining four cases (Table 2). The clinical features were similar irrespective of the site of the defect. Five of the seven cases with complex I deficiency showed additional biochemical abnormalities.

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Table 1. Polarographic criteria for determining the site of respiratory chain defect

<table>
<thead>
<tr>
<th>Site of defect</th>
<th>Polarographic criteria</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>Low O2, uptake rates with pyruvate and glutamate; normal succinate oxidation</td>
<td>23</td>
</tr>
<tr>
<td>Complex I-III</td>
<td>Low O2, uptake rates with pyruvate, glutamate and succinate; normal respiratory rates with ascorbate + TMPD</td>
<td>12</td>
</tr>
<tr>
<td>Complex I-IV</td>
<td>Low O2, uptake rates with all the above substrates</td>
<td>11</td>
</tr>
<tr>
<td>No defect</td>
<td>Normal polarography</td>
<td>11</td>
</tr>
</tbody>
</table>

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Table 2. The number of patients and the site of the defect in each clinical category

Abbreviations: PEO, progression external ophthalmoplegia; CNS, central nervous system.

<table>
<thead>
<tr>
<th>Site of defect</th>
<th>Complex I</th>
<th>Complex I-III</th>
<th>Complex I-IV</th>
<th>Normal</th>
<th>Total no. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Limb weakness and exercise intolerance</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>B. PEO + pigmentary retinopathy</td>
<td>5(4*)</td>
<td>4(4*)</td>
<td>2(2*)</td>
<td>4(4*)</td>
<td>15(14*)</td>
</tr>
<tr>
<td>C. PEO + CNS disease</td>
<td>2(2*)</td>
<td>2</td>
<td>2(1*)</td>
<td>5(3*)</td>
<td>11(6*)</td>
</tr>
<tr>
<td>D. Major CNS disease</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>

*Cases with mtDNA deletions.
Cytochrome $aa$, was low in three cases and was below 25% of the mean control value in two cases. Of the four cases with complex I–II defects, cytochrome $aa$, and $b$ were low in two cases, $aa$, was low in the third and $b$ was low in the fourth. Cytochrome $aa$, was low in all four cases with complex I–IV defects and in three of these, reducible cytochrome $b$ was also low.

Category B: cases with progressive external ophthalmoplegia ± pigmentary retinopathy

This group consisted of six males and nine females with an age range of 22–57 years (median age 33 years). Onset was in childhood or adolescence except in two cases who first developed ptosis in the third decade. All cases had mild fatigable limb weakness and three had dysphagia. Pigmentary retinopathy was present in eight cases and one of these had severe nerve deafness.

All but one of these patients had a partially deleted population of muscle mtDNAs. Two patients, both harbouring a deletion had affected relatives. One female patient had an affected daughter and a male patient had an affected niece, the daughter of his unaffected sister [13].

The defect involved complex I in five cases, complex I–III in four and complex I–IV in two, but polarography was normal in the remaining four (Table 2). Cytochrome $aa$, measured less than 25% of the control mean in two patients with complex I deficiency and in one of these cytochrome $b$ was also low (56% of the control mean). Cytochrome $aa$, was low in one patient with a defect of complex I–III and $aa$, and $b$ were low in a patient with a defect of complex I–IV. Cytochrome $aa$, was also low in three out of four patients with normal polarography.

Category C: cases with progressive external ophthalmoplegia + central nervous system disease

There were six males and five females aged 12–45 years (median age 29 years). Onset was in childhood or adolescence except in one patient who developed symptoms in the third decade. Four cases had dysphagia and all but two had fatigable limb weakness. Two patients had a combination of pigmentary retinopathy, nerve deafness, cerebellar ataxia, dementia and a cardiac conduction defect. Three others had retinopathy, deafness and ataxia and one of these had a cardiac conduction defect. Further two cases had retinopathy and cerebellar ataxia which in one was associated with mild deafness. Of the remaining four cases, one had cerebellar ataxia and a peripheral sensory neuropathy and two had isolated stroke-like episodes in the 4th and 5th decades, respectively. Neither of these cases had evidence of cerebrovascular or cardiac disease. One patient had been partially deaf and ataxic for several years before the onset of a left-sided stroke, which recovered, but followed by persistent myoclonus in the affected arm. The second patient had a transient left hemiparesis in his mid 40s which recovered completely.

Six out of the 11 cases in this group had a deletion of muscle mitochondrial DNA. They included the two patients with retinopathy, deafness, ataxia, dementia and a cardiac conduction defect, the two patients with retinopathy, deafness and ataxia and two with retinopathy and ataxia, one of whom had mild dementia. None of the cases in this group had affected relatives.

The defect involved complex I in two cases, complex I–III in two and complex I–IV in two. Polarography was normal in five. Cytochrome $aa$, was low in both patients with complex I deficiency and both cytochromes $aa$, and $b$ were low in one of the patients with a defect of complex I–III. Of the four patients with complex I–IV defects, cytochrome $aa$, was low in one and in the other cytochrome $b$ was low, but $aa$, was not detected. Cytochrome $aa$, was also low in two out of the four cases with normal polarography.

Category D: cases with major central nervous system disease

The 16 cases in this group included nine males and seven females, aged 13–63 years (median age 40 years). Onset was in the first decade in five cases, the second in four and the third or fourth in six. One patient first developed symptoms at the age of 60 years. Dementia was present in all cases and was severe in 11. Fifteen patients had retinopathy, deafness and ataxia and 11 of these had nerve deafness. Two patients, one with and one without cerebellar ataxia, had pyramidal signs with mild spastic weakness in the legs. Nine patients had seizures which were generalised in three, focal in five and myoclonic in one. Three of the cases with focal seizures also had myoclonus. Five patients had recurrent stroke-like episodes which in three sometimes followed seizure activity. Three of these patients had pigmentary retinopathy and a fourth had optic atrophy without retinal changes. Three other patients had pigmentary retinopathy, one of whom also had focal seizures and myoclonus. Two cases, one with seizures and recurrent strokes, developed severe axial and limb dystonia and another patient with infrequent generalised seizures had choreaathetoid movements. Two patients both with seizures and recurrent strokes had a cardiomyopathy. Fourteen of the 16 cases had proximal limb weakness and four had mild extracranial involvement. Two patients had a peripheral sensory neuropathy.

The family history was negative in ten cases. The remaining six cases included an affected mother and son and two females, whose mothers probably had a similar disease. The brother and sister of one of these cases were also affected and died in early childhood. Another female patient was said to have several affected siblings and children, but they were not examined. A male patient had a brother who had died from a progressive encephalopathy in the third decade. None of the patients in this group had a detectable deletion of mtDNA.

The defect was localized to complex I in nine cases, complex I–III in two cases and complex I–IV in three. Polarography was normal in two cases. There was no correlation between the clinical features and the site of the lesion. Cytochrome $aa$, was low in eight cases with complex I deficiency and was less than 50% of the control mean in three. Cytochrome $b$ was also slightly low in one of these cases. Cytochromes $aa$, and $b$ were low in two cases which had cerebellar signs and a peripheral sensory neuropathy and two had isolated stroke-like episodes in the 4th and 5th decades, respectively. Neither of these cases had evidence of cerebrovascular or cardiac disease. One patient had been partially deaf and ataxic for several years before the onset of a left-sided stroke, which recovered, but was followed by persistent myoclonus in the affected arm. The second patient had a transient left hemiparesis in his mid 40s which recovered completely.

Discussion

This study highlights a number of interesting points concerning the biochemistry and clinical expression of these diseases. First, it has confirmed previous observations that no single clinical phenotype appears to be consistent with or exclusive to any one type of respiratory chain abnormality. All four clinical categories as defined here were represented in each of the three types of respiratory chain defect and in some patients belonging to three of these clinical categories respiratory chain activity, as determined in muscle mitochondria, was unimpaired. This was also true for each of the different organs affected in multisystem cases (see Table 3). These findings would suggest that the respiratory chain may function as a single unit and that the effects of any given defect on the metabolism and functional integrity of an
Table 3. The frequency of the different clinical features in the four biochemical groups

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Complex I</th>
<th>Complex I-III</th>
<th>Complex I-IV</th>
<th>Normal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellar ataxia</td>
<td>11(2*)</td>
<td>3</td>
<td>5(1*)</td>
<td>7(3*)</td>
<td>26</td>
</tr>
<tr>
<td>Pigmentary retinopathy</td>
<td>11(5*)</td>
<td>1(1*)</td>
<td>3(2*)</td>
<td>8(6*)</td>
<td>23</td>
</tr>
<tr>
<td>Dementia</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>6(3*)</td>
<td>20</td>
</tr>
<tr>
<td>Deafness</td>
<td>8(2*)</td>
<td>2</td>
<td>4</td>
<td>3(2*)</td>
<td>17</td>
</tr>
<tr>
<td>Seizures</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2(1*)</td>
<td>8</td>
</tr>
<tr>
<td>Cardiopathy</td>
<td>3</td>
<td>0</td>
<td>2(1*)</td>
<td>3(2*)</td>
<td>8</td>
</tr>
<tr>
<td>Strokes</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>+Family history</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>17</td>
</tr>
</tbody>
</table>

*No. of cases with deletions of mtDNA.

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Hypoxia–reoxygenation induced damage in the myocardium: the role of mitochondria

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Introduction

The energy demands within an actively respiring tissue such as cardiac muscle must be satisfied, for the most part, by mitochondrial ATP synthesis. One of the principal mechanisms for the matching of ATP synthesis with demand is the control of the mitochondrial iso-citrate, oxoglutarate and pyruvate dehydrogenases by the mitochondrial matrix Ca2+ concentration which is itself dependent upon cytosolic Ca2+ levels [1, 2]. Fig. 1 shows the principal elements which form the Ca2+ homoeostasis system in heart mitochondria. The steady-state matrix Ca2+ concentration is the net sum of both the principal influx (Ca2+ uniporter) and efflux pathways (Na+/Ca2+ exchange). From Fig. 1 it is evident that cytosolic Na+ and Ca2+ levels will be important in keeping matrix Ca2+ levels within the physiological norm. In experiments with isolated mitochondria, the response of the organelle to increasing Ca2+ concentration has been clearly established and has arbitrarily been categorized as consisting of a ‘physiologic’ uptake at low Ca2+ concentrations followed by a ‘pathologic’ response at higher levels [3]. Whether this behaviour of isolated mitochondria occurs in the intact cell has yet to be established. We have been studying the response of mitochondria to hypoxia–reoxygenation where homeostatic control of cytosolic Na+ and K+ is perturbed. This arises early in ischaemia when insufficient energy from ATP hydrolysis results in inhibition of the Na+/K+-ATPase and causes an increase in intracellular Na+ [4]. Under these conditions the lack of oxygen also inhibits mitochondrial function.

We have already shown in perfused rat hearts that on reoxygenation, after a period of hypoxia, the oxygen-dependent cell lysis and Ca2+ uptake are dependent for their manifestation on mitochondrial electron transport [5]. In the present study, we will explore this aberrant effect of mitochondrial electron transport using both perfused hearts and isolated myocytes.

Response of hypoxic mitochondria to reoxygenation in isolated cardiac myocytes

If isolated myocytes are made hypoxic for a period of 40 min then reoxygenation results in an approximately 2-fold increase in total cell Ca2+ [5]. This oxygen-dependent process can be inhibited completely by inhibitors of mitochondrial electron transport such as antimycin [5]. Since this agent inhibits both ATP synthesis and the formation of the mitochondrial electron transport such as antimycin [5]. Since this agent inhibits both ATP synthesis and the formation of the mitochondrial electron transport such as antimycin [5]. Since this agent inhibits both ATP synthesis and the formation of the mitochondrial electron transport.

It has been assumed by some investigators that the mitochondrial Ca2+ uptake brought about by reoxygenation of hypoxic tissues is secondary to an increased permeability of the sarcolemmal membrane [6]. In perfused hearts, for example, reoxygenation is accompanied by extensive cell lysis and must therefore involve a profound perturbation of the integrity of the sarcolemma [7]. This would necessarily expose any functional mitochondria in these tissues to high concentrations of Ca2+ and phosphate and cause Ca2+ sequestration by the organelle [3]. In the isolated hypoxic myocytes, however, reoxygenation does not result in overt disruption of the sarcolemmal membrane [5]. Despite this, we have direct evidence for an uptake of Ca2+ by the mitochondria on reoxygenation. There have been relatively few measurements of cytosolic Ca2+ levels in hypoxic myocytes or cells subjected to metabolic blockade. The increase in

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