New Familial Mitochondrial Encephalopathy with Macrocephaly, Cardiomyopathy, and Complex I Deficiency

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Two siblings presented with a new phenotype consisting of fatal progressive macrocephaly and hypertrophic cardiomyopathy. Onset of symptoms started in both patients at the end of the first month of life with massive brain swelling causing macrocephaly and evolving to extensive brain destruction. Light microscopy of the lesions showed extensive small-vessel proliferation and gliosis. A distinct deficiency of complex I of mitochondrial respiratory chain was established in cultured fibroblasts, skeletal muscle, and heart muscle. Specific lack of complex I protein was demonstrated by two-dimensional gel electrophoresis.


Pure complex I deficiency of the mitochondrial respiratory chain is associated with either a myopathic or a multisystem disorder [1]. The latter has been further classified into different clinical groups including a fatal infantile phenotype, a second phenotype with milder encephalopathy and multiorgan dysfunction, and a third phenotype in some cases with maternally inherited mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. In some kindreds there was consanguinity suggesting an autosomal...
normal recessive inheritance. However, most cases appear to be sporadic [1].

We describe a new phenotype in 2 siblings with fatal infantile progressive macrocephaly, hypertrophic cardiomyopathy, lactic acidosis, and complex I deficiency.

**Patients and Methods**

**Patient 1**

The proband, a boy, was the second child of healthy unrelated Italian parents. The mother was gravida 2, para 2. He was born by cesarean section after an uncomplicated full-term pregnancy. Birth weight was 2,570 g (third percentile), length 48 cm (third percentile), and head circumference 33.5 cm (third percentile). The neonatal period was uneventful.

At the age of 2 months he was hospitalized because of developmental delay and progressive irritability. He was macrocephalic with an enlarged and bossing anterior fontanel, head circumference was 45 cm (above the 97th percentile). Limbs were hypertonic and axial tone was reduced, tendon reflexes were brisk. Funduscopy and electroencephalography were normal.

Persistent elevation of blood lactate (range, 8–13 mmol/L; normal, 0.6–2 mmol/L), metabolic acidosis (pH 7.27; base excess, −10.7 mmol/L), and elevated plasma alanine (650 μmol/L; normal, 250–450 μmol/L) were detected. Cerebrospinal fluid lactate (17 mmol/L; normal, 0.6–2 mmol/L) was elevated. Serum levels of creatine kinase, transaminases, glucose, and ammonia were normal. Urinary organic acids showed increased excretion of lactate and Krebs cycle intermediates.

Brain magnetic resonance imaging (MRI) showed diffuse hyperintensity in T2-weighted sections involving the white and gray matter indicating severe brain swelling (Fig 1A). Echocardiography disclosed hypertrophic cardiomyopathy. Abdominal ultrasonography was normal.

Despite steroid and mannitol therapy, the clinical course deteriorated with the appearance of frequent tonic and tonic-clonic seizures, swallowing difficulties, and recurrent pulmonary infections. A second brain MRI, at 5 months of age, disclosed a generalized brain destruction with wide cavitations (see Fig 1B). He died 1 month later of cardiac failure.

At autopsy, there was a multicystic degeneration of all the supratentorial structures. There were no malformations or thromboembolism of the principal brain arteries. Light microscopy (see Fig 1C–F) showed diffuse small-vessel proliferation and gliosis of the brain; focal and symmetrical lesions were detected in the tegmentum and basis peduncles of mesencephalon, and randomly in the cerebellum. Heart showed hypertrophic cardiomyopathy with dilatation of the right ventricle. Light and electron microscopy of the heart showed pronounced mitochondrial proliferation. Biopsy of the left quadriceps muscle showed mild mitochondrial proliferation with succinate dehydrogenase (SDH) histochemistry and no ragged-red fibers, using modified Gomori's trichrome stain. There was no evidence of lipid and glycogen accumulation. Polymerase chain reaction analysis of mtDNA for the most well-known point mutations and deletions were negative and the sequence of whole tRNA<sup>Lys</sup> was normal.

**Patient 2**

The oldest sibling of Patient 1 was evaluated at another hospital. He was born at term after an uneventful pregnancy. Birth weight was 2,800 g (10th percentile), length 49 cm (10th percentile), and head circumference 33 cm (10th percentile). At birth, muscle tone was normal, but sucking was weak. At 2 months of age, hydrocephalus was suspected because of progressive increase of head circumference up to 43.5 cm (above the 97th percentile) with bulking of the anterior fontanel and patent sagittal fissure. He was extremely irritable and hypertonic. Brain computed tomographic scanning showed ventriculomegaly and diffuse supratentorial cortical atrophy. Electrocardiography showed signs of biventricular hypertrophy. Routine laboratory examinations were normal; lactate was not investigated. The child died at the age of 3 months. Autopsy showed diffuse brain destruction and hypertrophic cardiomyopathy.

**Biochemical Studies**

Lactate and pyruvate production and mitochondrial enzyme activities in fibroblasts [2, 3], enzymatic activities of the respiratory chain, pyruvate dehydrogenase complex, and citrate synthase from skeletal and heart muscle were measured as described previously [4, 5]. Heart tissue was obtained within 3 hours after death and was frozen immediately in liquid nitrogen.

The protein composition of oxidative phosphorylation complexes was studied by Blue Native electrophoresis and sodium dodecyl sulfate (SDS) electrophoresis in the second dimension [6].

**Results**

The lactate and pyruvate production in fibroblasts after incubation with glucose showed an increased lactate/pyruvate ratio (68; controls, 18 ± 10). This increased ratio, which reflects the cytosolic reduced/oxidized nicotinamide–adenine dinucleotide phosphate (NADH/NAD) redox state, suggested that the patient, indeed, suffered from a defect in the mitochondrial respiratory chain.

Enzyme activities, summarized in the Table, revealed a complex I deficiency in skeletal muscle, myocardium, and fibroblasts. The residual NADH:Q<sub>d</sub> reductase activity was as low as 10% of the control mean in skeletal muscle.
Table. Enzyme Activities of the Skeletal Muscle, Heart Muscle, and Fibroblasts from Patient 1

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Skeletal Muscle</th>
<th>Heart Muscle</th>
<th>Fibroblasts</th>
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<tr>
<td>NADH-Q&lt;sub&gt;1&lt;/sub&gt; oxidoreductase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.019 (0.200 ± 0.099)</td>
<td>0.024 (0.220 ± 0.198)</td>
<td>0.026 (0.19 ± 0.05)</td>
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<tr>
<td>Succinate:cytochrome c oxidoreductase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.208 (0.234 ± 0.062)</td>
<td>0.200 (0.214 ± 0.085)</td>
<td>0.21 (0.31 ± 0.06)</td>
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<tr>
<td>Decylubiquinol:cytochrome c oxidoreductase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.62 (1.82 ± 0.32)</td>
<td>1.30 (1.56 ± 0.56)</td>
<td>1.70 (2.70 ± 0.41)</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase complex&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.033 (0.040 ± 0.018)</td>
<td>0.014 (0.034 ± 0.015)</td>
<td>ND</td>
</tr>
<tr>
<td>Cytochrome c oxidase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 (94 ± 51)</td>
<td>122 (168 ± 143)</td>
<td>162 (182 ± 27)</td>
</tr>
<tr>
<td>Citrate synthase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168 (95 ± 34)</td>
<td>492 (302 ± 112)</td>
<td>149 (197 ± 42)</td>
</tr>
</tbody>
</table>

All activities were measured in a 600-g supernatant of the muscle homogenate, except for pyruvate dehydrogenase complex and citrate synthase, which were measured in total homogenate. Control mean ± SD values are in parentheses.

<sup>a</sup>Expressed as milliunits per milligram of cytochrome c oxidase.

<sup>b</sup>Expressed as milliunits per milligram of protein.

ND = not determined.

and heart muscle, and 14% in cultured fibroblasts. The other enzymes showed normal activities, except for the pyruvate dehydrogenase complex in heart, which was in the low-normal range (42%). This reduction probably has been caused by the severe condition of the myocardium, pyruvate dehydrogenase complex being a sensitive, regulated enzyme complex.

A specific reduction of the amount of complex I was demonstrated by two-dimensional (Native/SDS) electrophoresis in skeletal muscle and in heart muscle (Fig 2). Amounts and subunit compositions of complexes II to V were normal.

Discussion

We have reported a new fatal phenotype with lactic acidosis, macrocephaly, hypertrophic cardiomyopathy, and a defect of complex I of the mitochondrial respiratory chain. The prominent feature of this encephalopathy was massive brain swelling causing macrocephaly and evolving to extensive brain destruction. It is noteworthy that this condition mostly involved the supratentorial brain structures. There were no inducing events, such as a febrile illness, and in both cases progressive macrocephaly began at the end of the first month of life.

![Fig 2. Two-dimensional resolution of oxidative phosphorylation complexes from control skeletal muscle and control heart muscle, respectively (A and C), and skeletal muscle and heart muscle, respectively (B and D), from Patient 1 with complex I deficiency. The characteristic complex I protein subunits in the 25- to 75-kd range (marked by arrows) were not detectable in patient tissues. Oxidative phosphorylation complexes from 10 mg (wet weight) of skeletal muscle and 5 mg of heart muscle, respectively, were solubilized and separated in the native state by Blue Native electrophoresis. The subunits were resolved subsequently by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension and silver stained (A and B) or stained with Coomassie Blue dye (C and D).](image-url)
Infantile macrocephaly is a characteristic symptom of some metabolic diseases, such as Canavan's disease, glutaric aciduria type 1, 3-hydroxy-3-methylglutaric aciduria, and 1-2-hydroxyglutaric aciduria [7, 8]. However, all these disorders do not lead to massive brain destruction.

Severe cerebral edema is a typical finding of hyperammonemic coma in urea cycle defects [9]. In these disorders, brain edema is sustained by astrocyte swelling due to the osmotic effect of accumulated glutamine [9]. Moreover, edema confined to cerebral and cerebellar white matter, and basal ganglia is a distinctive feature of maple syrup urine disease [10].

Brain swelling and macrocephaly is exceptional in primary lactic acidosis. Neonatal macrocephaly and dysmorphism were observed in a patient with a deficiency of the mitochondrial transport protein voltage-dependent anion channel [11]. Two siblings with fumarase deficiency showed a severe neonatal encephalopathy with macrocephaly and ventriculomegaly [12].

In pyruvate dehydrogenase deficiency, brain-destructive changes appear to occur in the prenatal period and patients may present at birth with severe microcephaly, multiple cystic lesions, hydranencephaly, as well as developmental malformations [13].

The pathogenesis of generalized brain swelling in our patient with complex I deficiency remains to be clarified. Focal brain swelling with midline shift has been established in MELAS patients [14]. Because the endothelium of brain capillaries is rich in mitochondria, impairment of oxidative phosphorylation in cerebral microvessels has been suggested to explain focal lesions in MELAS [15] and, based on neuropathological findings, can be assumed to be causal in our patients, although with a more wide distribution.

We conclude that a mitochondrial respiratory chain defect should be suspected in infants with generalized brain swelling progressing rapidly to massive destruction.

References