Clinical heterogeneity in respiratory chain complex III deficiency in childhood


Abstract

Six children are presented with an isolated complex III deficiency in muscle tissue. More specifically, oxidation rates and ATP+CrP production rates from both pyruvate and succinate as substrates and/or the activity of decylubiquinolcytochrome c oxidoreductase were all markedly reduced. Complex III deficiency was also present in liver of two patients tested, but could not be demonstrated in cultured fibroblasts of four patients tested. Mitochondrial DNA, extracted from muscle, was analyzed; no deletions or common point mutations were found. Four patients presented with a multi-organ disorder. Among these patients three presented at neonatal age with neurological signs and lactate elevation in blood and CSF, of whom two had severe neonatal Fanconi syndrome. One child, aged seven years, had encephalomyopathy, ophthalmoplegia, retinopathy and Wolff-Parkinson-White syndrome. The remaining two patients exhibited myopathy only, within the first year of life. Thus, like in other respiratory chain disorders, patients with complex III deficiency may present at any age and show variable symptoms and outcome, ranging from neonatal death to failure to thrive only. Apparently there are no clinical findings which are specific for complex III deficiency. © 1997 Elsevier Science B.V.

Keywords: Mitochondrial encephalomyopathy; Complex III; Decylubiquinolcytochrome c oxidoreductase; Fanconi syndrome; Respiratory chain

1. Introduction

Disorders of oxidative phosphorylation are degenerative diseases with a wide spectrum of clinical manifestation. In most cases the term mitochondrial encephalomyopathy is appropriate, because it refers to the tissues that appear to be affected most frequently by one of the biochemical defects involved. However, every organ can be affected and tissue-specific expression of oxidative phosphorylation defects appear to be frequent (Munnich et al., 1992; Robinson, 1993; Shoffner and Wallace, 1995). In the group of mitochondrial disorders some circumscript clinical presentations are recognized which are associated with mitochondrial DNA (mtDNA) point mutations or deletions (Shoffner and Wallace, 1995). In most pediatric patients suffering from mitochondrial encephalopathy the clinical picture does not fit in one of these distinct presentations and no mutation in the mtDNA is found (Tulinius et al., 1991a,b). In all these patients, demonstrating abnormal biochemistry of oxidative phosphorylation in skeletal muscle is the most important measure in the diagnostic process (Trijbels et al., 1988).

Oxidative phosphorylation is mediated by five mitochondrial enzyme complexes, which are named complex I (NADH:ubiquinone oxidoreductase), complex II (succinate:ubiquinone oxidoreductase), complex III (ubiquinol:cytochrome c oxidoreductase), complex IV (cytochrome c
oxidase) and complex V (ATP synthase). Complexes I and II accept electrons from various sources and transfer them to ubiquinone. The electrons are further transmitted through complex III, cytochrome c and complex IV and finally react with oxygen, the terminal electron acceptor. Complex V mediates ATP synthesis. Each enzyme complex consists of a number of subunits which are encoded by nuclear DNA and partly by mtDNA, except for complex II, which is entirely encoded by nuclear DNA. Complex III is composed of eleven subunits among which only one, cytochrome b, is encoded by mitochondrial DNA. The ten remaining subunits, among them the non-heme iron-sulfur (von Rieske) protein, two large core proteins and cytochrome c$_{1}$, are encoded by nuclear DNA.

Deficiencies of complex I and complex IV, either isolated or combined, occur frequently, whereas complex III deficiencies are rare. Due to a variety of descriptions of large groups of patients with complex I and/or complex IV deficiencies (DiMauro et al., 1990; Korenke et al., 1990; Robinson, 1993), the wide spectrum of biochemical data and clinical presentations in these oxidative phosphorylation disorders is well known. However, only little is known about complex III deficiency in childhood. Here we report upon different clinical presentations of the disease in pediatric patients with isolated complex III deficiency of the respiratory chain.

2. Materials and methods

2.1. Patients

In this study only subjects are included in whom biochemical investigation of skeletal muscle revealed a defect in the respiratory chain localized at the level of complex III. Since such a block ought to impair utilisation of both NAD$^{+}$-linked and FAD-linked substrates, the following criteria had to be fulfilled: (1) Decreased oxidation rates measured in fresh skeletal muscle supernatant with pyruvate, malate and succinate as substrates as well as decreased ATP and creatine phosphate (CrP) production rates using pyruvate and succinate as substrates. (2) Reduced activities of decylubiquinol:cytochrome c oxidoreductase (reflecting complex III capacity), and succinate:cytochrome c oxidoreductase (reflecting complexes I+III capacity, CoQ content included); this in combination with normal activities of NADH:ubiquinone oxidoreductase (reflecting complex I capacity), cytochrome c oxidase (reflecting complex IV capacity) and pyruvate dehydrogenase. The biochemical investigations in muscle and liver tissue and in skin fibroblasts were performed at the University Hospital of Nijmegen. Details of the patients' clinical features, clinical-biochemical data and muscle morphology were obtained retrospectively from the case notes. Data from patient 1 and 2 have been reported previously (Sperl et al., 1988; Wendel et al., 1995).

2.2. Methods

Material for biochemical and morphological investigations was obtained by a surgical biopsy of the quadriceps muscle and by needle biopsy of the liver (patient 1 and 2). Skin fibroblasts were grown in M199 medium, according to standard procedures.

Substrate oxidation rates and ATP+CrP production rates from different substrates were measured in 600 g supernatants derived from homogenates of fresh skeletal muscle tissue (stored at 0 °C for less than 1 h). Mitochondrial enzyme activities were measured in 600 g supernatants of fresh or frozen (stored at -70 °C) muscle tissue. The test procedures and selection criteria for control muscle specimens have been described previously (Fischer et al., 1986). Complex III activity was measured using decylubiquinol as substrate, according to the method of Zheng (Zheng et al., 1990). However, Tween 20 [0.04% (v/v)] was added to the assay mixture in order to reduce the non-enzymatic reaction. Enzyme activities were also measured in mitochondria enriched fractions from cultured fibroblasts which were prepared by homogenisation and subsequent differential centrifugation (600 g and 14 000 g) of approximately 20×10$^{6}$ cells.

Substrate oxidation rates, ATP+CrP production rates and enzyme activities were normalized to citrate synthase to correct for differences in mitochondrial content and recovery. In studies with frozen muscle specimens and cultured fibroblasts cytochrome c oxidase was used as the reference enzyme. In all samples studied activities of both reference enzymes were within the control ranges. Because recovery of mitochondria from liver tissue is invariably high, results in studies with frozen liver specimens were not normalized to a reference enzyme. Mitochondrial DNA was screened for large deletions and common point mutations as reported earlier (De Vries et al., 1993).

3. Results

Biochemical investigations in skeletal muscle tissue revealed an isolated complex III deficiency in six children. The data are specified in Table 1. From four patients fresh muscle tissue was available, whereas in two patients only frozen muscle specimens could be investigated. In supernatants derived from fresh muscle tissue the oxidation and ATP+CrP production rates from both pyruvate and succinate as substrates were found to be severely diminished in each patient (<40% of the lowest control values), as were the activities of complex III (<40% of the lowest control value). In frozen muscle specimens the complex III activity was diminished to 40% (Patient 1) and 80% (Patient 6) of the lowest control value. In all patients the activity of succinate:cytochrome c oxidoreductase, reflecting complex II+III capacity, was also diminished, except for Patient 6, in whom activity was low normal. Activities of complex I,
complex IV and pyruvate dehydrogenase complex (data not shown) were all within the control range.

Liver tissue was investigated in two patients (Patients 1 and 2, Table 2). In both specimens decylubiquinol:cytochrome c oxidoreductase and succinate:cytochrome c oxidoreductase activities were clearly diminished, whereas the other respiratory chain enzyme activities were within the control range. This demonstrates that in both patients complex III deficiency was also expressed in liver. Cultured fibroblasts from four patients (Patients 1-4) were studied. In none of the cell lines could complex III deficiency be demonstrated (data not shown).

Mitochondrial DNA, extracted from the muscle specimens, was screened for large deletions and the point mutations on nucleotide positions 3243 (MELAS) and 8993 (NARP/Leigh). In no patient was a deletion or point mutation detected.

Clinical and laboratory data of the complex III deficient patients are listed in Table 3. Among the six children, three presented in the neonatal period with hyperlactic acidemia, two with myopathic features in infancy and one with encephalomyopathy only at the age of 7 years. Family history was negative with respect to neuromuscular diseases in all patients. Two siblings of Patient 1 had died of an unknown cause in the neonatal period (in Turkey). Patients 2 and 5 had consanguineous parents. In all patients muscle biopsy showed nonspecific morphological changes and frequently ultrastructural abnormalities of mitochondria, but no ragged-red fibres.

### 3.1. Patients with multiorgan involvement

Four patients (Patients 1-4) had a complex multiorgan disorder. Three of them (Patients 1-3) presented in infancy with a combination of neurological disease, hyperlactic acidemia and an increased lactate/pyruvate ratio in blood. They showed pre- and postnatal growth retardation and the course of disease was progressive in three of them. Among them two developed a severe neonatal renal tubulopathy (De Toni-Fanconi-Debré syndrome). Patient 1 had severe lactic acidosis and renal tubulopathy immediately after birth. Although the acidosis could be controlled the child showed failure to thrive and severe psychomotor retardation. Despite therapy with biotin, thiamine and carnitine she deteriorated with respiratory distress. At the age of 8 months she died of sudden respiratory arrest. Patient 2 was referred at the age of 2 months because of failure to thrive. On admission he showed severe hypotonia, irritability, rickets and signs of proximal renal tubulopathy. There was only mild hyperlactic acidemia. However, in urine, amounts of lactate and 3-hydroxybutyrate together with a complex pattern of organic acids were increased.

### Table 1

<table>
<thead>
<tr>
<th>Substrate oxidation, ATP+CrP production rates and enzyme activities of the respiratory chain complexes (600 g supernatant derived from muscle specimens)</th>
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<tbody>
<tr>
<td><strong>Fresh muscle</strong></td>
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<tr>
<td>Control range</td>
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<td>Patient 2</td>
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<td>Patient 3</td>
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<td>Patient 4</td>
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<td>Patient 5</td>
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### Table 2

| Enzyme activities (mU/mg protein) in liver tissue (600 g supernatant) |
|----------------|------------------|------------------|------------------|------------------|
|                  | NADH:Q<sup>2</sup> (Complex I) | SCC<sup>2</sup> (Complex II+III) | QCC<sup>2</sup> (Complex III) | COX<sup>2</sup> (Complex IV) |
| Control range    | 4.1-10 (n = 10) | 6.0-50 (n = 14) | 69-107 (n = 4) | 14-108 (n = 11) |
| Patient 1        | 6.3 | 4.4 | 23 | 74 |
| Patient 2        | 3.1 | 41 | 71 | 71 |
Table 3

Clinical and laboratory features in six children with complex III deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at onset</th>
<th>Clinical features</th>
<th>Course</th>
<th>Brain imaging</th>
<th>Blood lactate (mmol/l)</th>
<th>Lact/pyr ratio</th>
<th>CSF lactate (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Sperl et al., 1988</td>
<td>f</td>
<td>Birth</td>
<td>Intrauterine growth retardation, Hypertonic acidemia, Fanconi syndrome, Developmental delay, Hypotonia, Retinopathy</td>
<td>Progressive (died at 8 months)</td>
<td>CT-scan: Normal</td>
<td>5.6-18.0</td>
<td>41</td>
<td>n.d.</td>
</tr>
<tr>
<td>2 Wendel et al., 1995</td>
<td>m</td>
<td>Birth</td>
<td>Intrauterine growth retardation, Hypertonic acidemia, Retinopathy, Failure to thrive</td>
<td>Progressive (died at 7 months)</td>
<td>Ultrasonography: Normal</td>
<td>1.4-3.5</td>
<td>41</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>Birth</td>
<td>Intrauterine growth retardation, Hypertonic acidemia, Failure to thrive, Hypotonia</td>
<td>Stable</td>
<td>Ultrasonography and MRI-scan: Normal</td>
<td>3.0-10.0</td>
<td>35-56</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>m</td>
<td>7 years</td>
<td>Pyramidal/extrapyramidal symptoms, Mental regression, Myopathy, Ophthalmoplegia, Tapetoretinal degeneration, Exercise intolerance</td>
<td>Progressive</td>
<td>MRI-scan: Cortical atrophy</td>
<td>3.0</td>
<td>n.d.</td>
<td>7.7</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>15 months</td>
<td>Proximal muscle weakness, Hypotonia</td>
<td>Slowly progressive</td>
<td>MRI-scan: Normal</td>
<td>2.6</td>
<td>n.d.</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>9 months</td>
<td>Hypotonia, Exercise intolerance</td>
<td>Stable</td>
<td>CT-scan: Normal</td>
<td>&lt;2.2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d.: not determined.

were markedly increased, suggestive of a respiratory chain defect. Despite symptomatic treatment in combination with menadione and riboflavin he deteriorated gradually. Eventually he died of respiratory distress at the age of 7 months. Patient 3 presented at the age of 3 months with muscular hypotonia and failure to thrive. Lactate concentrations were clearly elevated in blood, cerebrospinal fluid and urine. Treatment with a ketogenic diet, vitamin E and β-carotene did not show any effect on lactate concentrations. At the age of 3 years she was still severely retarded in growth and showed mildly impaired psychomotor development. In Patient 4 psychomotor development was normal until the age of 7 years, then he developed a progressive loss of concentration and learning abilities and exercise intolerance. At 14 years he was referred because of deterioration with episodes of apathy and diminished consciousness, due to epileptic insults. He developed muscular hypertonia, with contractures in all four extremities, hyperrigidity and dyspraxia. There was only mild hyperlactic acidemia, while lactate was distinctly elevated in cerebrospinal fluid. An MRI scan showed a hypoplastic cerebellar vermis inferior with wide ventricles and cisternae. Neurophysiological tests revealed brainstem dysfunction. One year later he suffered from mental regression, tapetoretinal degeneration, polyneuropathy and worsening of the hypokinetic hyperrigidity.

3.2. Patients with isolated myopathy

Patients 5 and 6 presented with myopathic features within the first years of life. Both did not have distinctly elevated blood lactate concentrations. Patient 5 had a normal psychomotor development in the first year of life and was able to walk at 14 months. A few months later he was referred because of walking difficulties and dystrophy. He had a general muscular hypotonia, and suffered from loss of muscular strength and exercise intolerance. He developed joint contractures. Intellectual development was completely normal, but his motor abilities have gradually declined. At the age of 5 years he suffered from severe hypotonia and was unable to walk. Patient 6 presented at the age of 9 months with hypotonia and mild psychomotor developmental delay. No other organs were involved, and height growth was normal. Exercise intolerance and muscle weakness appeared not to be progressive at the age of 5 years.

4. Discussion

Deficiency of complex III of the respiratory chain is rare and so far no more than about 25 patients with an isolated defect of complex III have been reported (Morgan-Hughes et al., 1982; Hayes et al., 1984; Kennaway et al., 1984; Papadimitriou et al., 1984; Morgan-Hughes et al., 1985; Darley-Usmar et al., 1986; Reichmann et al., 1986; Morgan-Hughes et al., 1987; Birch-Machin et al., 1989; Bouzidi et al., 1993; Marin-Garcia et al., 1995). The six children with isolated complex III deficiency reported here represent approximately 2% of subjects in whom a respira-
Complex III deficiency has been identified in our laboratory. That our figure is much lower than that of Rustin et al. (Rustin et al., 1994), who found complex III deficiency in 15% of identified respiratory chain defects, can be attributed to the fact that in our study only patients with an isolated and markedly reduced complex III activity were taken into consideration. Kennaway (1988) summarized clinical and biochemical findings of 14 preponderantly adult subjects with isolated complex III deficiency. In the patients worked up in that review a variety of indirect methods were applied to pinpoint the site of the defect in the respiratory chain. In fresh muscle specimens the diagnosis was based on markedly reduced oxidation rates of NADH-linked substrates and succinate with normal ascorbate+tetramethylphenylenediamine (TMPA) oxidation rates. By that approach it is not possible to differentiate between a defect in the region of coenzyme Q and a defect in complex III. In other patients an isolated defect of complex III was concluded indirectly from a reduced activity from both rotenone-sensitive NADH:cytochrome c oxidoreductase and succinate:cytochrome c oxidoreductase and normal activities for succinate dehydrogenase and cytochrome c oxidase. In some cases low levels of cytochrome b and also of cytochrome c_{1}, in combination with normal levels of cytochrome aa_{3} were used to confirm the defect of complex III. However, by all the indirect methods applied, it is not possible to localize the defect unequivocally to complex III and to exclude definitely coenzyme Q deficiency or a combination of complex III deficiency with defects of complex I and/or complex IV. Combinations of these respiratory chain defects occur frequently (Kennaway, 1988; Ruitenbeek et al., 1989; Robinson, 1993) and among our patients we found at least ten.

In the present study complex III deficiency was demonstrated in each patient by severely reduced activity of decylubiquinol:cytochrome c oxidoreductase in muscle tissue, whereas activities of complex I and IV were found to be normal. In this way we could localize the defect in the respiratory chain precisely and exclusively to complex III. The residual activity varied between 13 and 45% of the lowest control value, except for Patient 6, whose muscle showed an 80% residual activity.

Among our patients only one presented with encephalomyopathy of later onset type (presentation of that phenotype in childhood or adult life), which is the most frequently reported phenotype of complex III deficiency, characterized by various combinations of muscle weakness, intellectual impairment and cerebral motor disorders (Morgan-Hughes et al., 1982; Kennaway et al., 1984; Morgan-Hughes et al., 1985; Darley-Usmar et al., 1986; Reichmann et al., 1986; Slipetz et al., 1991). The rare occurrence of this phenotype in our series can be explained by the fact that in our laboratory biochemical studies on muscle specimens are performed predominantly from a pediatric population.

Three patients presented with a neonatal multiorgan disorder, characterized by a combination of neurological disease, lactate elevation in blood and cerebrospinal fluid, and in two cases renal dysfunction. In common, these children presented with intrauterine growth retardation and postnatal failure to thrive. Since intrauterine energy demands are low compared to postnatal life and fetal energy metabolism is provided with ample glucose, with lactic acid being carried off by placental circulation, intrauterine growth retardation may be directly attributed to impairment of oxidative phosphorylation.

Multiorgan disorder was biochemically verified in two patients by demonstrating complex III deficiency in skeletal muscle and liver. In the patients in whom multiorgan involvement was present according to clinical symptoms, proximal tubular abnormality, indicating kidney involvement, was relatively frequently encountered. Renal tubular dysfunction, frequently reported in lethal infantile disease due to cytochrome c oxidase deficiency (DiMauro et al., 1985, 1990), appears to occur particularly frequently in complex III deficiency (Rötig et al., 1990; Jackson et al., 1995; Morris et al., 1995). In two infants myopathic features dominated the clinical picture. In both patients hyperlactic acidemia was absent. This observation suggests that in these patients the disease was restricted to skeletal muscle. Muscle biopsies of all patients showed nonspecific morphological changes, but no ragged red fibres, in contrast to reports on older patients (Morgan-Hughes et al., 1985).

Investigations of fibroblasts did not reveal clearly reduced activities of complex III as measured by decylubiquinol:cytochrome c oxidoreductase and succinate:cytochrome c oxidoreductase assays. This indicates that the biochemical defect was likely not expressed in cultured skin fibroblasts of the four patients studied. To our knowledge, there is no paper in which diagnosis of complex III deficiency has been based unequivocally on reduced enzyme activities in fibroblasts, implying that using cultured fibroblasts is not appropriate in order to arrive at the diagnosis of complex III deficiency (Morris et al., 1995).

Heterogenous tissue involvement of oxidative phosphorylation defects is common and has been reported particularly in patients with cytochrome c oxidase deficiency. In our patients with complex III deficiency tissue-specific expression of the defect could be explained either by a mutation in one of the nuclear genes encoding for the different subunits or by mutations in the mitochondrial genome under the assumption that different levels of heteroplasmy occur in different tissues. With respect to mtDNA, mutations affecting the cytochrome b gene or one of the 22 tRNAs can cause complex III deficiency. Mutations within one of the tRNAs are less likely to have occurred, because they usually give rise to combined defects of respiratory chain complexes, such as in MELAS and MERRF (Tulinius et al., 1991a,b; Bentlage et al., 1995). Moreover, no deletions or common point mutations were found in our patients. However, in the presented set
of patients a mutation in the cytochrome b gene might be responsible for isolated complex III deficiency. Taking the clinical features and the studies on the mitochondrial genome into consideration we favour the idea of mutations in nuclear genes. Although no isoforms of subunits of complex III have been reported in humans so far (Vázquez-Acevedo et al., 1993), most probably genes encoding for polypeptides being involved in the tissue-specific expression or assembly of complex III are concerned. Such nuclear genes encoding for factors involved in the assembly of complex III have been identified in yeast (Rosing et al., 1985; Wu and Tzagoloff, 1989). In humans tissue-specific regulation of complex V is reported (Akiyama et al., 1994). Analogous genes involving complex III might exist.

In conclusion, we demonstrated that the clinical presentation of complex III deficiency in childhood is very heterogeneous. Obviously, there are no clinical findings which are specific for complex III deficiency, although neonatal Fanconi syndrome appears to be frequent.

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