Barth syndrome presenting with acute metabolic decompensation in the neonatal period.

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SHORT REPORT

Barth syndrome presenting with acute metabolic decompensation in the neonatal period

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Summary We describe two patients affected by Barth syndrome. Their symptoms became manifest on respectively the third and first day of their lives. Clinical presentation included poor sucking, lethargy, hypotonia, hypothermia and cardiomyopathy. Laboratory findings such as hypoglycaemia, metabolic acidosis, elevated transaminases, hyperlactacidaemia and mild hyperammonaemia pointed to an inborn error of energy metabolism with possible mitochondrial involvement. Molecular analysis of the *TAZ* (*G4.5*) gene showed the c.877G > A mutation leading to the G197R amino acid substitution in patient 1, and the new splice donor c.829 + 1G > A genetic lesion in patient 2.

Introduction

Barth syndrome (OMIM 302060) is an X-linked inherited disorder characterized by cardiac and skeletal myopathy, growth retardation, neutropenia and 3-methylglutaconic aciduria (Barth et al 1999). The disease is caused by mutations in the *TAZ* gene (D'Adamo et al 1997), which maps to Xq28, resulting in a defect of cardiolipin, a component of inner mitochondrial membrane. The observed cardiomyopathy and skeletal myopathy could be due to a loss of tetralinoleoyl-cardiolipin, causing an alteration in the mitochondrial electron transfer chain as speculated by Barth and colleagues (2004) and supported by experimental studies in *Saccharomyces cerevisiae* by Brander and colleagues (2005).

The cardiac pathology sometimes shows evidence of endocardial fibroelastosis. Mutations in the *TAZ* gene are also responsible for isolated left ventricular noncompaction (Chen et al 2002; Kenton et al 2004). In the past, many patients died of cardiac failure or sepsis early in life; those who survived often showed improving or stable heart disease (Hodgson et al 1987; Kelley et al 1991). With the better recognition of the disease and correct treatment of the heart and bone marrow problems, death in infancy is now less common for Barth syndrome.

Cardiac transplantation in some patients appears to increase life expectancy (Adwani et al 1997), although one patient developed a fatal Epstein–Barr-negative T cell non-Hodgkins lymphoma 5 years after transplantation (Ronghe et al 2001).

We report two patients affected by Barth syndrome with neonatal onset (at 3 days and 1 day, respectively), with poor feeding, lethargy, hypotonia, hypothermia and cardiomyopathy. Laboratory investigations showed acute metabolic decompensation, suggesting an inborn error of metabolism.

Materials and methods

β-Oxidation and carnitine metabolism studies were performed in cultured fibroblasts using radiochemical protocols

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(DiDonato et al 1989). Respiratory chain determination was performed according to Bugiani and colleagues (2004).

Mutation analysis was performed after the parents' informed consent had been obtained. Genomic DNA was isolated from whole blood following standard procedures. Analysis of the *TAZ* gene was performed as described by Bione and colleagues (1996).

Case reports

Patient 1

The patient was born at term to unrelated Italian parents after an uneventful pregnancy and delivery with a birth weight of 2780 g and Apgar score of 9^{I}, 10^{V}. Family history was relevant for three maternal great-uncles and two maternal second cousins who died in the first years of life (Fig. 1). At 3 days he presented poor feeding, lethargy, hypotonia and hypothermia; there was no hepatomegaly. Chest radiography revealed cardiomegaly. Echocardiography showed a slightly dilated left ventricle with a reduced ejection fraction (25%, normal 65–84%).

Metabolic investigations showed hypoglycaemia (2.2 mmol/L, normal>2.8 mmol/L), elevated transaminases (ALT 244 U/L, normal<40; AST 253 U/L, normal<40), CK 196 U/L (normal<170), severe metabolic acidosis (pH 6.99; BE −26.9 mmol/L; HCO_3^- 4.8 mmol/L; P_{CO_2} 20.9 mmHg), and elevated blood lactate (10.8 mmol/L, normal <2.4) and pyruvate (329 μmol/L, normal <190); ammonaemia was 58 μmol/L (normal <95), free carnitine 19.48 μmol/L (normal 28–50), and total carnitine 26.23 μmol/L (normal 35–60). Ketonuria was absent. Urinary organic acid analysis (GC-MS) showed a high excretion of lactic acid (70 mmol/mol creatinine, normal <25), 3-methylglutaric acid (9.1 mmol/mol creatinine, normal <7) and 3-methylglutaconic acid (36.8 mmol/mol creatinine, normal <9). There was no evidence of neutropenia. Intravenous infusions of glucose, bicarbonate, and antibiotics, and therapy with digitalis led to clinical and biochemical improvement. In the following days cardiomyopathy improved until cardiac function normalized at 40 days, when the patient was discharged. In the following months the patient failed to thrive (weight and length <3rd centile) and presented mild motor delay. At about 14 months of age he presented with a high fever for three days and poor feeding and was admitted to our hospital because he was hypotonic, lethargic and tachycardic. Laboratory investigations revealed neutropenia with an absolute neutrophil count of 1018/mm^3 (8.7%), WBC 11 700/mm^3, and glucose 2.94 mmol/L. Chest radiography showed cardiomegaly and echocardiography revealed severe left ventricular dilated cardiomyopathy with hypokinesia. The child died suddenly after a few hours.

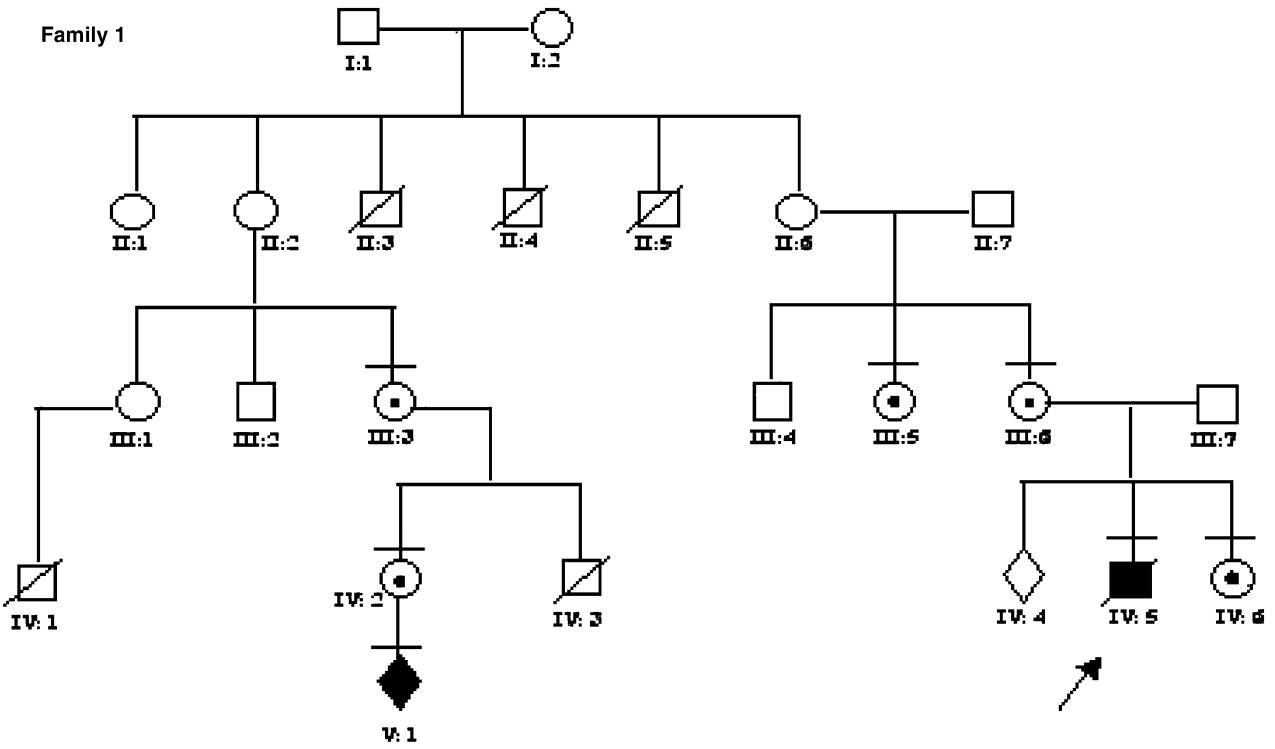

Fig. 1 Family pedigree of patient 1. II:3; II:4, II:5, IV:1, IV:3 are the three of his grandmother's brothers and the two maternal cousins who died in the first years of life. The members of his family who were analysed by genetic sequencing are shown with an upper dash

Biochemical studies performed in cultured fibroblasts from perimortem skin biopsy showed normal oxidation of fatty acids (data not shown) and no reduction in the activity of the respiratory chain enzymes (Table 1). The patient's autopsy showed endocardial fibroelastosis and fatty liver.

Molecular analysis of patient 1's *TAZ* gene, previously reported by D'Adamo and colleagues (1997), showed the c.877G > A genetic lesion in exon 8 leading to the G197R amino acid substitution. This mutation was found in his mother and his maternal aunt and in other females in his family (Fig. 1). A prenatal diagnosis was performed in the patient's mother and revealed a carrier status (III:6). Recently, a prenatal diagnosis performed in a patient's relative (IV:2) showed an affected male fetus (V:1).

Patient 2

The patient was born at term to unrelated Italian parents after an uneventful pregnancy by Caesarean delivery due to breech presentation. Birth weight was 2830 g, Apgar score 9^{I}, 9^{V}. Family history was unremarkable. At the 12th hour of life the patient exhibited hypothermia, hypotonia, and poor sucking. Laboratory findings showed hypoglycaemia (1.9 mmol/L) with normal serum ketone bodies, mild hyperammonaemia (129 μmo/L), elevated lactate with a range of 2.67–9.56 mmol/L, AST 100 U/L, ALT 36 U/L, CK 1200 U/L, and metabolic acidosis (pH 7.33; BE −11.6 mmol/L; HCO_3^- 12.5 mmol/L; P_{CO_2} 23.4 mmHg); there was no neutropenia. Urinary organic acid analysis showed a high excretion of lactic acid (no 3-methylglutaconic acid). Chest radiography demonstrated cardiomegaly and echocardiography showed left hypokinetic dilated cardiomyopathy and hypertrophic right ventricle. β-Oxidation (data not shown) and respiratory chain studies performed in cultured fibroblasts were normal in this patient also (Table 1).

The patient began therapy with carnitine, furosemide, and digoxin which was continued for 4 months, when cardiac evaluation showed normal cardiac function despite the presence of generalized muscular weakness, motor retardation, and diminished growth. Blood cell count showed neutropenia (WBC 10 600/mm^3; neutrophils 784/mm^3, 7.4%) At 7 months he showed respiratory distress, tachycardia and oedema. He had grossly dilated left ventricle with a severely reduced ventricular ejection fraction (40%, normal 65–84%); mechanical ventilation was initiated but the patient died 2 days later. Urinary organic acid analysis showed excretion of 3-methylglutaconic acid. There was no neutropenia. Liver biopsy showed widespread steatosis with large lipid droplets. At autopsy, endocardial fibroelastosis and fatty liver were present.

Molecular analysis of the *TAZ* gene in patient 2 led to the identification of a new genetic lesion, c.829 + 1G>A, affecting the donor splice site of intron 6 (IVS6 + 1G > A).

Table 1 Mitochondrial respiratory chain activities[a] in cultured fibroblasts

	Patient 1	Patient 2	Controls
Complex I	21	12	19 ± 9
Complex II	22	12	15 ± 7
SDH	17	10	15 ± 10
Complex III	216	89	120 ± 60
Complex IV (COX)	105	71	96 ± 35
Complex V	82	99	102 ± 40
Citrate synthase (CS)[b]	82	98	128 ± 70

[a] Values are expressed as ratio relative to CS activity
[b] Activity is expressed as nmol substrate/min per mg protein

Discussion

Both of these patients presented acute neonatal onset with metabolic decompensation. Clinical presentation at 3 days and 12 h of life, respectively, with nonspecific symptoms such as poor sucking, lethargy, hypotonia, hypothermia, but with cardiomyopathy and hypoglycaemia, metabolic acidosis, elevated transaminases, hyperlactacidaemia and mild hyperammonaemia indicated an inborn error of metabolism. Other patients affected by Barth syndrome with acute neonatal cardiac failure and lactic acidosis have been reported (Barth et al 1981, 1983; Rugolotto et al 2002), but a clinical and biochemical presentation with hypoglycaemia, mild hyperammonaemia, hypoketosis and elevated transaminases, to our knowledge, has never been described. Some hypoglycaemic episodes either spontaneously or after fasting have occurred in other reported patients (Barth et al 1983; Kelley et al 1991), whereas in one patient reported by Barth and colleagues (1981) fasting for 11 h caused no hypoglycaemia, and in two patients reported by Chistodoulou and colleagues (1994) blood glucose levels were maintained at >3 mmol/L after provocative fasting. Our findings, together with those of Barth and colleagues (1983) and Kelley and colleagues (1991) should alert one to the possible occurrence of unexpected hypoglycaemic incidents in Barth syndrome, both in the neonatal period and subsequently. Even though hypoglycaemia has not been fully explained, disturbed gluconeogenesis, together with several other causes, will be responsible for the hypoglycaemia (Kelley et al 1991). A single patient with a neonatal clinical and biochemical presentation similar to our patients was reported by Ibel and colleagues (1993). However, this patient is not considered to have been suffering from Barth syndrome but from a defect of the mitochondrial respiratory chain (Barth et al 1999; Johnston et al 1997).

In our patients, we suspected a β-oxidation defect or a defect in carnitine metabolism primarily because of the

hypoglycaemia, the absence of ketosis, elevated liver enzymes and mild hyperammonaemia in addition to their clinical courses. Patient 1's fatty liver and his sudden death at 14 months of age during a period of fever and fasting, and patient 2's fatty liver and cardiac failure at 7 months support this hypothesis. However the biochemical studies of β-oxidation and carnitine metabolism performed in cultured fibroblasts of both patients failed to provide confirmation. The family history of the first patient (five related male infants had died in the first months or years of life: Fig. 1) suggested X-linked inheritance. The presence of cardiomyopathy in the neonatal period together with elevated levels of urinary 3-methylglutaconic acid in patient 1 strongly suggests Barth syndrome. In both patients diagnosis was confirmed by mutational analysis. Patient 1 was hemizygous for the G197R amino acid substitution (D'Adamo et al 1997). The residue G197 is highly conserved between species. So far about 80 mutations have been reported in the *TAZ* gene. Although they are heterogeneous and distributed widely over the entire length of the gene, some such as the G197R and the G216R are the most common amino acid substitutions reported in the *TAZ* gene (Bleyl et al 1997; Cantlay et al 1999; Johnston et al 1997). Mutations in splice sites of the *TAZ* gene, with the exception of introns 4 and 5, have also been reported and most of them affect introns 1, 2 and 10. A new splicing defect at the donor site of intron 6 was identified in patient 2 (IVS6 + G > A). At the same donor splice site, a different nucleotide change (IVS6 + 1G > T) was also identified in another Barth syndrome patient (direct submission to the Barth Foundation database by Gonzalez IL, A.I. duPont Hospital for Children, Wilmington, Delaware 19899, USA). The identification of two different nucleotide changes at the same splice site in two unrelated patients suggests the pathogenicity of such splice site mutations.

All mutations in the *TAZ* gene so far described are listed on the web site www.barthsyndrome.org. Johnston and colleagues (1997) found no correlation between genotype and severity or age at onset of the disease. In some families important phenotypic variations were also present, although family members were affected by the same mutations. Moreover, mutation analyses in patients with neonatal onset (when it has been possible to obtain these data from literature) did not show a correlation between onset and specific mutations or clustering of mutations. Other genetic or environmental factors could modify the Barth phenotype (Johnston et al 1997).

In the two patients, WBC and neutrophil cell counts were repeatedly normal in the neonatal period and during the phase of metabolic decompensation, but neutropenia was present in patient 1 during his terminally ill phase and in patient 2 at a follow-up when he was 4 months old, confirming that neutropenia can be present in cyclic phases (Kelley et al 1991; Barth et al 1983). Furthermore, in both patients, 3-methylglutaconic aciduria, like neutropenia, was present only occasionally; its absence, therefore, cannot exclude the hypothesis of Barth syndrome and repeated urinary organic acid analysis in the course of a patient's illness is the only way to ensure the detection of 3-methylglutaconic aciduria (Kelley et al 1991).

Barth syndrome is a very rare disorder, and the coincidence of two patients at our hospital during a short period has no epidemiological significance; nevertheless this disease, like other rare syndromes, may be underdiagnosed. Therefore, it is important to stress that in all male patients with unexplained severe infantile dilated cardiomyopathy and biochemical abnormalities suggestive of a Reye-like syndrome presentation, Barth syndrome should be suspected and mutation analysis of the *TAZ* gene should be carried out to confirm or exclude this diagnosis.

In conclusion, the biochemical findings reported in our two patients are very similar to those observed in other inborn errors of metabolism such as organic acidurias. These biochemical alterations can be due to a mitochondrial dysfunction involving several metabolic pathways. Alterations in the inner mitochondrial membrane due to cardiolipin deficiency could cause a secondary defect of the respiratory chain, of which most elements are integral proteins of the inner mitochondrial membrane. In our two patients, respiratory chain enzyme activity was normal in cultured fibroblasts but no data are available in other tissues such as heart or liver. Moreover, this defect is not always evident in Barth patients.

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