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ORIGINAL ARTICLE

Hypertrophic cardiomyopathy, cataract, developmental delay, lactic acidosis: A novel subtype of 3-methylglutaconic aciduria

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Summary 3-Methylglutaconic aciduria is the biochemical marker of several inherited metabolic diseases. Four types of 3-methylglutaconic aciduria can be distinguished. In the type I form, accumulation of 3-methylglutaconate is due to deficient activity of 3-methylglutaconyl-CoA hydratase, an enzyme of the leucine degradation pathway. In the other forms, 3-methylglutaconic acid is not derived from leucine but is of unidentified origin, possibly derived from other metabolic pathways, such as mevalonate metabolism. We report five patients, all presenting a severe early-onset phenotype characterized by 3-methylglutaconic aciduria, hypertrophic cardiomyopathy, cataract, hypotonia/developmental delay, lactic acidosis, and normal 3-methylglutaconyl-CoA hydratase activity. This peculiar phenotype, for which a primary mitochondrial disorder is hypothesized, identifies a novel subtype of 3-methylglutaconic aciduria.

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Introduction

3-Methyglutaconic aciduria (3-MGA) is a common biochemical marker in a variety of syndromes with different phenotypes. So far, four subtypes of 3-MGA have been distinguished based on mode of inheritance, clinical manifestations, and enzymatic and genetic defects (Gunay-Aygun 2005). 3-MGA type I (McKusick 250950) is an autosomal recessive disorder of leucine degradation due to deficiency of 3-methylglutaconyl-CoA hydratase (3-MGH) and is mainly characterized by mental retardation with impaired speech development (Narisawa et al 1986). Barth syndrome, or 3-MGA type II (McKusick 302060), is an X-linked disorder characterized by dilated cardiomyopathy (CMP), neutropenia, skeletal myopathy, and normal cognitive development (Barth et al 2004) and an impairment in cardiolipin metabolism due to mutations in the tafazzin gene (Bione et al 1996). 3-MGA type III, also known as Costeff optic atrophy syndrome (McKusick 258501), is an autosomal recessive disorder described in Iraqi-Jews due to mutation in the *OPA3* gene (Anikster et al 2001). Besides these well-defined forms of 3-MGA, an 'unclassified' group of disorders with variable clinical presentation and normal 3-MGH activity is collected under the term 3-MGA type IV (Gunay-Aygun 2005). Furthermore, 3-MGA has also been reported in patients with proven mitochondrial disorders, such as Pearson (Jackobs et al 1991) and MELAS (De Kremer et al 2001) syndromes as well as in patients with various enzymatic defects of the respiratory chain

(Gibson et al 1993). Some patients with Smith–Lemli–Opitz syndrome, a defect of cholesterol biosynthesis, also excrete large amounts of 3-MGA because of abnormal isoprenoid/cholesterol biosynthesis (Kelley and Kratz 1995).

Here we describe five patients, presenting a severe early-onset phenotype characterized by 3-MGA, hypertrophic CMP, cataract, hypotonia/developmental delay, lactic acidosis, and normal 3-MGH activity. This peculiar phenotype identifies a novel subtype of 3-MGA.

Patients and methods

The five patients originated from five unrelated families (Fig. 1). The relevant clinical and biochemical findings are summarized in Table 1. Urine organic acids were analysed by gas-chromatography–mass spectrometry and blood acylcarnitines by tandem mass spectrometry according to previously described methods (Rizzo 2000, 2003). Muscle biopsy was performed in all patients and samples were processed for histochemical and biochemical studies. Mitochondrial respiratory chain enzyme activities in muscle were determined by standard spectrophotometric methods in patients 1, 2, 4 and 5.

The enzyme activity of 3-MGH was measured in all subjects, as described elsewhere (Loupatty et al 2004). Cardiolipin levels were measured in cultured human skin fibroblasts using tandem mass spectrometry (Valianpour et al 2005).

Mutation analysis of nuclear-encoded *ATP11* and *ATP12* genes, and of mitochondrially encoded *ATP6* and *ATP8* genes was performed by direct sequencing in patients 2 to 5.

Results

Clinical findings

All patients were born at term after normal pregnancy and delivery. Their weights and lengths were normal at birth. Three patients had congenital microcephaly, two had respiratory distress with severe metabolic acidosis at birth, and the other presented status epilepticus and metabolic acidosis at 1 month of age. The other two patients were observed for the first time at 8 months either because of congenital cataract or for mild developmental delay. By the age of 8 months, hypertrophic CMP, cataract and mild developmental delay were noticed in all cases.

Brain MRI was performed in four patients, revealing severe cerebellar hypoplasia, marked simplified cortical gyral pattern and hypoplastic corpus callosum only in patient 4. Three patients died due to cardiorespiratory failure, two at birth and the other at the age of 22 months. Autopsy was performed in only one patient, confirming hypertrophic CMP and bilateral cataract. Patient 2, at 9 years, had an acute episode of severe metabolic acidosis with coma. At follow-up, the two surviving patients, now aged 9 and 3 years, have both moderate mental retardation and stable CMP under propranolol therapy.

As shown in Fig. 1, two siblings of patient 1 (one male and one female) died within the first year of life. Both had hypertrophic CMP and developmental delay, and the girl died soon after birth with documented lactic acidosis and micropolygyria of the left temporal lobe. Information on the presence of 3-MGA and cataract was not available from the clinical records. Two male siblings of patient 2 also died at birth. One had documented neonatal unresponsive lactic acidosis and 3-MGA (1001 mmol/mol creatinine). In addition, he presented with hypospadias, club foot and facial dysmorphisms. Cataract was not searched for and autopsy did not reveal CMP. The elder male sibling of patient 3 died at the age of 14 months, presenting developmental delay, cataract, hypertrophic CMP and lactic acidosis. Urine organic acid analysis was not performed.

Biochemical findings

All patients presented with severe lactic acidosis. Urinary organic acid analysis showed increased excretion of 3-methylglutaconic acid in all cases, and 3-methylglutaric acid in four out five patients. In addition, variable increased excretion of lactate, pyruvate, and Krebs cycle intermediates was observed in most cases. There was no elevation of 3-hydroxyisovaleric acid. Blood acylcarnitine analysis showed increased levels of 3-methylglutaconylcarnitine and 3-methylglutarylcarnitine in only one patient. In the other three patients studied the profile of blood acylcarnitines was normal.

Fibroblast 3-MGH activity and cardiolipin levels were within normal limits in all patients.

Histochemical studies of muscle samples revealed diffuse reduction of cytochrome-*c* oxidase staining with signs of mitochondrial proliferation in patient 1, and similarly reduced cytochrome-*c* oxidase staining was detected in patient 3. Mitochondrial respiratory chain enzyme activities were examined in four patients, revealing a partial reduction of complex IV activity in patient 1, and reduced complex III and citrate synthase activities in patient 5. The other two patients did not show any enzymatic abnormalities. However, the heterogeneous results of the enzymatic studies among patients seem to indicate that these partial abnormalities are secondary changes and do not represent primary defects of mitochondrial respiratory chain enzymes.

Table 1 Clinical and biochemical characteristics of the five patients with 3-MGA. The activity of 3-methylglutaconyl-CoA hydratase (3-MGH) in fibroblasts is expressed as nmol/min per mg protein. Muscle respiratory chain enzymes are expressed as % residual activity

	Patient				
	1	2	3	4	5
Ethnicity	Family 1, II:2 Italy (Sardinia)	Family 2, II:2 Gipsy (Yugoslavia)	Family 3, II:2 Italy (Sardinia)	Family 4, II:1 Italy/Rumania	Family 5, II:2 Egypt
Affected siblings	+	+	+	−	+
Sex	M	F	F	F	F
Age at diagnosis	8 months	1 month	8 months	Birth	Birth
Outcome — age (†died)	†22 months	9 years	3 years	†8 days	†7 days
Clinical signs					
Hypertrophic cardiomyopathy	+	+	+	+	+
Cataract	+	+	+	+	+
Microphthalmia	−	+	−	+	−
Hypotonia/developmental delay	+	+	+	+	+
Mental retardation	+	+	+		
Microcephaly	−	+	−	+	+
Abnormal brain MRI	−	−	−	+	ND
Biochemical findings					
Blood lactate (normal <2.0 mmol/L)	6.0−8.0	3.5−14.0	4.0	25.0	7.0−8.0
Urine organic acids					
3-Methylglutaconic (normal <6 mmol/mol creatinine)	131	375−488	20−40	76−270	205−278
3-Methylglutaric	+	+	−	+	+
Lactate	−	−	+	+	+
Pyruvate	−	−	+	+	+
Krebs cycle intermediates	+	+	+	+	+
Blood acylcarnitines					
3-Methylglutaconylcarnitine	ND	−	−	−	+
Enzyme activities					
Fibroblast 3-MGH (normal 2.39 ± 1.13)	2.09	1.07	2.15	0.98	1.43
Muscle respiratory chain enzymes	C-IV 37%	Normal	ND	Normal	C-III 40%

ND, not done

Genetic studies

Mutation analysis of the mitochondrial subunits genes *ATP6* and *ATP8*, and mutations of the nuclear genes *ATP11* and *ATP12* did not reveal abnormalities in the four patients studied.

Discussion

We report on five patients with a very similar constellation of clinical and biochemical features, consisting of 3-methylglutaconic aciduria, hypertrophic CMP, cataract, hypotonia/developmental delay and lactic acidosis. Additional

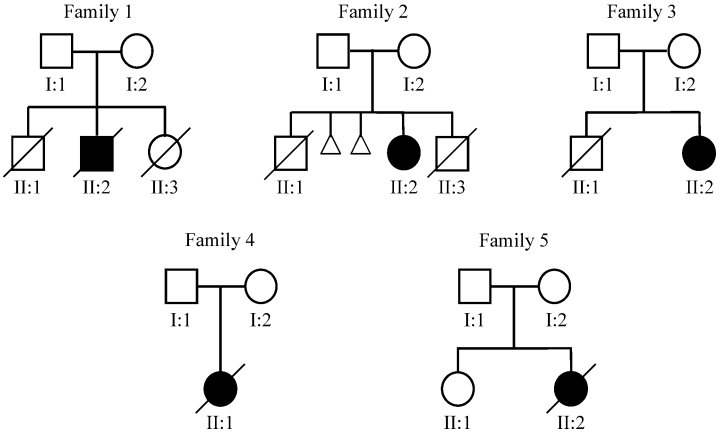

Fig. 1 Familial pedigrees of the five patients (in black) with 3-methylglutaconic aciduria, hypertrophic cardiomyopathy, cataract, developmental delay and lactic acidosis

features include congenital microcephaly, facial dysmorphisms, microphthalmia, genital anomalies and brain dysplasia. Mental retardation became evident in the three patients who survived after the first year of life. These clinical and biochemical findings strongly suggest the existence of a primary mitochondrial disorder that identifies a novel subtype of 3-MGA. Biochemical studies in fibroblasts excluded both 3-MGA type I and Barth syndrome. Furthermore, optic atrophy, the hallmark of 3-MGA type III, was not observed in our series of patients.

Differing from Barth syndrome, our patients presented with hypertrophic and not with dilated CMP, and they also lacked neutropenia. In Barth syndrome, the defect of cardiolipin, one of the major phospholipid components of the inner mitochondrial membrane, may be responsible for the impaired mitochondrial transport of 3-methylglutaconic acid, given the known regulatory function of cardiolipin on several mitochondrial membrane-bound carrier systems (Barth et al 2004). Since it is known that 3-MGA may also occur in many disorders of mitochondrial energy metabolism (De Kremer et al 2001; Gibson et al 1993; Jackobs et al 1991), we can hypothesize that in our patients, all showing severe lactic acidosis, a still unknown mitochondrial defect might underlie impaired 3-methylglutaconic acid metabolism.

Interestingly, hypertrophic CMP, cataract and exercise-induced lactic acidosis are the characteristic features of Sengers syndrome (McKusick 212350), a mitochondrial disorder without 3-MGA, whose underlying biochemical and genetic defects remain unknown. Sengers syndrome patients usually present muscular hypotonia, delayed motor development, easy fatigability, relatively long life expectancy and normal cognitive outcome (Sengers et al 1975). A reduced activity of the mitochondrial carrier protein ANT1 (adenine nucleoside translocator I) was reported in two patients, although with no alterations of the ANT1 gene (Jordens et al 2002). Our patients share some of the clinical characteristics of Sengers syndrome, but the presence of 3-MGA combined with a more severe phenotype in our patients seems to exclude this diagnostic option.

A girl with hypertrophic CMP, developmental delay, hypoplastic corpus callosum, lactic acidosis and 3-MGA was reported by Holme and colleagues. The clinical course was characterized by nonprogressive cardiological and neurological signs and cataract was not recorded. Biochemical studies showed a severe defect of muscle mitochondrial ATP synthase (Holme et al 1992). More recently, Morava and colleagues reported on two siblings with hypertrophic CMP, mild developmental delay, cataract, mitochondrial myopathy, and lactic acidosis (Morava et al 2004). Muscle morphology revealed variable structural alterations, but no ragged-red fibres. Biochemically, both patients showed decreased activities of respiratory chain complexes I, III and IV, and reduced ATP production in muscle but not in fibroblasts. 3-MGA was determined only in one sibling, whereas it is not reported in the other. In contrast to our series of patients, these two siblings had a milder clinical course with nonprogressive CMP and normal developmental outcome. Furthermore, two unrelated patients with severe encephalopathy, CNS malformations, dysmorphic features, lactic acidosis, 3-MGA and early fatal outcome but without CMP were recently reported (De Meirleir 2004). A reduction of ATPase activity was detected in muscle and/or in the liver, and genetic studies revealed a mutation of nuclear-encoded gene *ATP12*. Unlike in that report, extensive molecular analysis of both nuclear and mitochondrial ATPase related genes were performed in our patients without revealing any abnormalities.

In conclusion, the peculiar clinical and biochemical characteristics of our patients, along with 3-MGA and marked lactic acidosis, strongly suggest a well recognizable primary mitochondrial syndrome whose molecular and genetic defects remain to be defined.

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