Association of 3-methylglutaconic aciduria with sensori-neural deafness, encephalopathy, and Leigh-like syndrome (MEGDEL association) in four patients with a disorder of the oxidative phosphorylation

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Abstract

In this paper, we describe a distinct clinical subtype of 3-methylglutaconic aciduria. 3-Methylglutaconic aciduria is a group of different metabolic disorders biochemically characterized by increased urinary excretion of 3-methylglutaconic acid. We performed biochemical and genetic investigations, including urine organic acid analysis, NMR spectroscopy, measurement of 3-methylglutaconyl-CoA hydratase activity, cardiolipin levels, OPA3 gene analysis and measurement of the oxidative phosphorylation in four female patients with 3-methylglutaconic aciduria. 3-Methylglutaconic aciduria type I, Barth syndrome, and Coste syndrome were excluded as the activity of 3-methylglutaconyl-CoA hydratase, the cardiolipin levels, and molecular analysis of the OPA3 gene, respectively, showed no abnormalities. The children presented with characteristic association of hearing loss and the neuro-radiological evidence of Leigh disease. They also had neonatal hypotonia, recurrent lactic acidemia, episodes with hypoglycemia and severe recurrent infections, feeding difficulties, failure to thrive, developmental delay, and progressive spasticity with extrapyramidal symptoms. Our patients were further biochemically characterized by a mitochondrial dysfunction and persistent urinary excretion of 3-methylglutaconic acid.

Keywords: Leigh-like syndrome; Sensori-neural deafness; Lactic acidemia; 3MGA; Hypoglycemia; Extrapyramidal; Mitochondrial

Introduction

3-Methylglutaconic aciduria is a group of metabolic disorders biochemically characterized by increased urinary excretion of 3-methylglutaconic acid (3MGA) and 3-methylglutaric acid. At the present time, four distinct forms have been recognized. 3-Methylglutaconic aciduria type I (OMIM 250950) is an inborn error of leucine catabolism and is caused by the isolated deficiency of 3-methylglutaconyl-CoA hydratase (3MGH; EC 4.2.1.18) [1–3]. These pediatric patients display a range of clinical manifestations varying from progressive neurologic deterioration to mild speech delay. Three additional forms of 3-methylglutaconic aciduria have been documented—type II (Barth syndrome, OMIM 302060); type III (Coste syndrome, OMIM 258501); type IV (“unspecified,” OMIM 250951)—all characterized by normal hydratase activities and mildly elevated urinary levels of 3MGA. The precise etiology of the increased 3MGA excretion in these latter subtypes has not been elucidated yet. Among the four types, patients with 3-methylglutaconic aciduria type I excrete the highest levels of 3MGA.
Barth syndrome is a X-linked disorder presenting in males with cardiomyopathy, cyclic neutropenia, muscle hypotonia, and a normal cognitive function [4,5]. The disorder is caused by mutations of the Tafazzin gene (TAZ) [6]. Patients with Barth syndrome demonstrate decreased levels of total cardiolipins and cardiolipin subclasses, especially tetraineoyl-cardiolipin [7,8]. A skewed X-inactivation has been reported in obligate carriers, and no female patients have been reported yet [6,9].

Costeff syndrome is a progressive, late onset disease with bilateral optic atrophy, extrapyramidal symptoms with choreiform movements, ataxia, and spasticity [10,11]. Patients carry an autosomal dominant mutation in the OPA3 gene underlying the neuro-ophthalmic presentation [12]. No mitochondrial dysfunction has been reported in Costeff syndrome patients so far.

The diagnosis of 3-methylglutaconic aciduria type IV is based on the exclusion of the other, well-defined clinical subtypes [6,12–15]. This type of mild, non-syndromic 3-methylglutaconic aciduria is frequently associated with progressive neurological impairment and variable organ dysfunction [16]. Symptoms often present during the first year of life. In some patients clinical and biochemical features of a dysfunctional oxidative phosphorylation have been observed [17,18]. It is very likely, that this heterogeneous group of patients can be further subdivided into different genetic disorders [15].

We performed biochemical and genetic investigations, including urine organic acid analysis, NMR spectroscopy, measurement of 3-methylglutaconyl-CoA hydratase activity, cardiolipin levels, OPA3 gene analysis, and measurement of the oxidative phosphorylation, in four children with 3-methylglutaconic aciduria, presenting with neuro-radiological evidence of Leigh disease, hearing loss, recurrent lactic acidemia and hypoglycemia, and other clinical features comparable with a mitochondrial disorder.

Patients and methods

Patients

As part of the regular work-up for a suspected disorder in the oxidative phosphorylation we applied the diagnostic criteria for mitochondrial disease [19] based on clinical symptoms, metabolic alterations, and abnormal neuro-imaging findings. Serum lactic acid (multiple measurements), pyruvic acid levels, blood gas, serum acylcarnitine, amino acid, and urine organic acid profiles were analyzed in all children. Cerebral spinal fluid investigations have been successfully performed in three patients (patients 1, 2, and 4). The children also underwent a diagnostic protocol of multiple investigations including ECG, chest X-ray, EEG, visual evoked potentials (VEP), acoustic evoked potentials (BAEP), sensory evoked potentials (SEP), and a cranial MRI. Based on the diagnostic score [19], an open muscle biopsy was performed under general anaesthesia in all four children. Molecular genetic analysis for Rett syndrome was requested in patients 3 and 4. The clinical features of the patients are described in Table 1.

All patients were born from an uneventful pregnancy by spontaneous delivery. In two cases decreased child movements were reported. Three couples of the parents were consanguineous and of Turkish origin (Table 1). One child had non-consanguineous Dutch parents. All children presented with severe infections in the neonatal period: two with CMV-infection, one with GBS-sepsis and one without a known causative agent. Except for patient 3 the children had neonatal hypoglycemia, patients 2 and 3 had later recurrent episodes of hypoglycemia, and all four patients had recurrent lactic acidemia from the first weeks of life. No lactic acid elevations were detected in patient 2 and 4 in blood after puberty, however lactate levels remained repeatedly, significantly increased in the CSF.

### Table 1

<table>
<thead>
<tr>
<th>Patients</th>
<th>Patient 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Patient 2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Patient 3&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Patient 4&lt;sup&gt;bc&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consanguinity</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bilateral sensory hearing loss</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>MRI: bilateral hyperdensity of basal ganglia</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MRI: bilateral atrophy of the cerebrum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MRI: bilateral atrophy of the cerebellum</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neonatal features of “sepsis”</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Recurrent infections</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Feeding problems</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Delayed motor development</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Loss of motor skills</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mental retardation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Muscle hypotonia, progressive spasticity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Extrapyramidal symptoms</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Abnormal behaviour</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>3-MGA-uria (mean level)</td>
<td>53.5 (31–76)</td>
<td>47.2 (16–68)</td>
<td>132.5 (102–196)</td>
<td>125.0 (97–141)</td>
</tr>
<tr>
<td>Excretion range (µmol/mmol creat. control&lt;20)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactic aciduria</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactic acidemia (controls&lt;2.1 mmol/L)</td>
<td>2.9–3.8</td>
<td>1.8–26</td>
<td>2.0–8.2</td>
<td>0.95–2.3</td>
</tr>
<tr>
<td>Clinical diagnostic score&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Constant laughing.
<sup>b</sup> Constant laughing, auto-mutilation.
<sup>c</sup> Score 8–12: definite mitochondrial disorder.
Feeding problems were present from the neonatal period, making tube feeding necessary in two cases, leading to failure to thrive. The children suffered from recurrent upper respiratory infections without proven alterations of the humoral or cellular immune system. All children (except for patient 4) had a delayed motor development and muscle hypotonia. In patient 1 there was no development at all, patient 4 had a developmental regression from the age of 6 years and in two of the cases further motor skills were lost during the early childhood (patients 2 and 3). The patients developed severe spasticity, combined with extrapyramidal symptoms. Mental retardation was present in all children. Two of the patients showed behavioural problems with constant laughing, and/or auto-mutilation (Table 1). Patient 1 died at the age of 3 and patient 2 at the age of 16 years.

The EEG showed no signs of epilepsy in any of the patients, except for a multifocal epilepsy in patient 2. No cardiologic alteration was noted. The MRI of the brain revealed characteristic bilateral hyper-dense lesions of the basal ganglia in all children. Diffuse cerebellar and cerebro atrophy was also observed in three out of the four patients. Visual evoked potential analysis was bilaterally delayed in two patients (patients 1 and 4), however, no optic atrophy was noted. The BAEP studies showed severe sensori-neural hearing loss in three patients, making hearing devices necessary. A severity assessment (Table 1) was applied based on the clinical, metabolic, and neurological (neuro-imaging) alterations. Using the mitochondrial diagnostic score [19] all children scored above 8 points (mitochondrial score 8–12; definite mitochondrial disorder) comparable with the clinical diagnosis of a respiratory chain disorder.

**Mutation analysis**

PCR amplification of both exons and their flanking intronic DNA sequences of the *OPA3* gene (GenBank Accession No. BC047316) was performed by using the oligonucleotide primers for exon 1—F1 (5'-CGTACATACGTACTGACGCA-3'), R1 (5'-TAAGGACACCATCTGACGAGG-3'), and for exon 2—F2 (5'-TCCCCAGGGCCAGCTTGGAC-3'), R2 (5'-GCCAAGTTGTCAATAGATCTT-3') [12]. The PCR products were electrophoresed on a 1% agarose gel, extracted from the gel (Qiagen, Valencia, CA) and directly sequenced. Automated sequencing was performed on a Beckman CEQ 2000, by the CEQ Dye Terminator Cycle Sequencing kit, according to the manufacturer's protocol (Beckman Coulter).

Screening for the common mitochondrial point mutations was carried out using Pyro-sequencing™ according to the protocol of the manufacturer. Deletions were analyzed by long template PCR. Additional sequence analysis of the mitochondrial ND genes and sequencing of the nuclear coded structural complex I genes was performed (in patients 1 and 3) on an ABI 3730 DNA analyzer using BigDye terminator chemistry (Applied biosystems, Lekkerkerk a/d IJssel, The Netherlands).

**In vitro NMR spectroscopy**

Body fluid NMR spectroscopy and quantification of the cis and trans forms of 3MGA were performed essentially as described elsewhere [20,25].

**Sample preparation**

The urine samples were centrifuged before analysis. An aliquot (70 µL for urine) of 20.2 mM trimethylsilyl-2,2,3,3-tetradeuteropropionic acid (TSP, sodium salt; Aldrich) in D2O was added to 700 µL of the urine, providing a chemical shift reference (δ = 0.00), a concentration reference and a deuterium lock signal. The pH of the urine was adjusted to 2.50 ± 0.05 with concentrated HCl. Finally, 650 µL of the sample was placed in a 5 mm NMR tube (Wilmad Royal Imperial).

**One-dimensional 1H NMR spectroscopy**

Urine samples were measured at 500 MHz on a Bruker DRX 500 spectrometer equipped with a triple-resonance inverse (TXI) 1H(15N, 13C) probehead and equipped with x, y, z gradient coils. 1H spectra were acquired as 128 transients in 32K data points with a spectral width of 6002 Hz. Sample temperature was 298 K and the H2O resonance was presaturated by single-frequency irradiation during a relaxation delay of 10 s, and a 90° excitation pulse was used. Shimming of the sample was performed automatically on the deuterium signal. To improve the spectral resolution, the samples were spun (7 Hz) during the measurements. The resonance line-widths for TSP and metabolites were <1 Hz. A π/2-shifted sine bell window function was applied to the FID. Fourier transformation was performed after zero-filling to 64K data points. The phase and the baseline were corrected manually. The cis 3MGA and trans 3MGA resonances and the TSP singlet were fitted semi-automatically with Lorentzian line shapes. The concentration of cis 3MGA and trans 3MGA were calculated from the relative integrals of the fitted line-shapes using the known concentration of TSP [20].

**Cardiolipin analysis**

Cardiolipin concentrations were measured in blood samples or in cultured skin fibroblasts. High-performance liquid chromatography–electrospray mass spectrometry was applied to quantify total cardiolipin and subclasses of cardiolipin molecular species, foremost tetratetraerynoic-cardiolipin [8].

**Biochemical analysis in muscle and fibroblast samples**

Parallel with routine immune-histological analysis and electron-microscopy, the ATP production from pyruvate oxidation and the activity of the mitochondrial complexes I–V in our patients were measured in a fresh muscle biopsy sample and in cultured fibroblasts as described elsewhere [21,22].

**Results**

As shown in Table 1, urine organic acid analysis and in vitro NMR spectroscopy showed an increased urinary excretion of 3-methylglutaconic acid, with a strict 1:1 ratio of the cis and trans isoforms of 3MGA. The excretion of 3MGA in the children was consecutively the following: patient 1—62, 45, 76, and 31 (mean 53.5) µmol/mmol creatinine; patient 2—31, 68, 54, and 16 (mean 47.2) µmol/mmol creatinine; patient 3—107, 196, 115, and 102 (mean 132.5) µmol/mmol creatinine; patient 4—141, 97, 106, and 116 (mean 125.0) µmol/mmol creatinine, respectively (controls: <20 µmol/mmol creatinine). The excretion of 3-hydroxyisovaleric acid in the most recent samples were 46 µmol/mmol creatinine, 10 µmol/mmol creatinine, 16 µmol/mmol creatinine, and 33 µmol/mmol creatinine, respectively; controls: <42 µmol/mmol creatinine. Hence, we conclude that our patients suffer from 3-methylglutaconic aciduria. 3-Methylglutarconic aciduria type I, Barth syndrome, and Costeef syndrome were excluded as the activity of 3-methylglutaconyl-CoA hydratase, the cardiolipin levels, and molecular analysis of the *OPA3* gene, respectively, showed no abnormalities.

The clinical symptoms of our patients are demonstrated in Table 1. As all our patients scored above 10 points on the clinical diagnostic scoring system for mitochondrial disorders a respiratory chain defect was suspected. Indeed, the biochemical analysis of mitochondrial function in the fresh muscle biopsy and/or that of fibroblasts showed reduced oxidative phosphorylation in all four patients. Patient 1 had a slight decrease in the ATP production from pyruvate oxidation with a decreased activity of the mitochondrial
enzyme complex I, but no alterations in fibroblasts. Patients 2 and 4 had a more pronounced decrease in the ATP production, but no alteration in the enzyme complex activities. Patient 3 had normal ATP production, however the activity of complex I in muscle and that of complex I and III in fibroblasts were decreased (Table 2).

Mutation analysis of the MELAS 3243A>G, MERRF 8344A>G and Leigh/NARP 8993T>C point mutations and long template PCR analysis for mitochondrial deletions were normal in all children. Sequence analysis of the mtDNA for mutations in the genes ND1, ND2, ND3, ND4, tRNA Leu (uur), tRNA Lys (uag), and sequencing the mRNA of the nuclear coded NDUF51, NDUF52, NDUF54, NDUF57, and NDUF58 genes detected no mutations in patients 1 and 3.

Discussion

Until now, four types of 3-methylglutaconic aciduria have been described, three of which have a distinct genetic background. Patients diagnosed with unclassified 3-methylglutaconic aciduria, or type IV, present with mild increased urinary levels of 3MGA. Although this type of non-syndromic 3-methylglutaconic aciduria is frequently associated with progressive neurological impairment and variable organ dysfunction [15], some patients have been reported with variable features of mitochondrial dysfunction, or the biochemical finding of disturbed oxidative phosphorylation [17,18,23,24]. Therefore it is very likely that this heterogenous group of patients could be further subdivided into different genetic disorders [15].

Indeed, here we describe a distinct clinical subtype of 3-methylglutaconic aciduria. All of our patients are biochemically characterized by a mitochondrial dysfunction and a recurrent mild urinary elevation of 3MGA. The level of 3MGA acid excretion, confirmed by the in vitro NMR analysis, was comparable to that previously described in type IV 3MGC-aciduria (Table 1). It is also important to note the equal presence of cis and trans isoforms (“no stereospecificity”) of 3MGC acid in the urine of the children.

Engelke et al. [25] described recently a 2:1 cis/trans 3MGC acid isoform ratio in 3-MG CoA hydratase deficiency (3MGC-aciduria type I), and in a second disorder of leucine metabolism; 3-OH-3-methylglutaryl-CoA lyase deficiency, as well. Stereo-specificity has been already observed for 3-methylcrotonyl CoA carboxylase, another enzyme in leucine metabolism. Non-enzymatic isomerization of cis and trans 3-methylglutaconyl CoA has been also described under alkaline conditions. Further investigations are required to find the background of the different cis/trans ratios in the different types of 3MGC-acidurias. The lack of stereospecificity in these four patients with mitochondrial dysfunction and 3MGC-aciduria further supports the biochemical homogeneity of our clinical group.

3-Methylglutaconyl CoA hydratase deficiency and Barth syndrome were excluded in our patients. Although the children have many features in common with that of Coste syndrome, except for the optic atrophy, recent studies reported the presence of OPA3 mutations in patients without the development of optic atrophy [26]. However, sequence analysis of the OPA3 gene revealed no mutations in our cases. Moreover, no mitochondrial dysfunction has been reported in patients with Coste syndrome.

The finding of the neuro-radiological evidence of Leigh(-like) disease is rather specific for oxidative phosphorylation disorders. The combination of Leigh syndrome and hearing loss has been previously described in association with both mitochondrial and nuclear coded mutations. Hypotonia, lactic acidemia, hypoglycemia, feeding difficulties, failure to thrive, developmental delay, progressive spasticity, and extrapyramidal symptoms are less specific, but common features of mitochondrial dysfunction. 3MGA-uria, however is not a common finding in respiratory chain defects.
Biochemical evaluation confirmed a deficient oxidative phosphorylation in the muscle biopsy and/or in fibroblasts of the four children. Previous reports on mitochondrial dysfunction in association with 3MGA-uria, however showed a very different clinical presentation in patients [17,18,23,24], suggesting genetic heterogeneity.

There are only a few reports on clinically comparable patients with 3-methylglutaconic aciduria. One patient described by Broide et al. [27] showed clinical signs of sepsis at the age of three days, without a proven underlying infective agent. Hepato-splenomegaly and hepatic dysfunction, later on severe feeding difficulties and failure to thrive occurred. Hypotonia of the trunk and hypertonia of the extremities, neurosensory hearing loss and the neuro-radiological picture of Leigh syndrome with bilateral symmetric changes in basal nuclei region have been noted. The mitochondrial respiratory chain enzyme activity in muscle biopsy was reduced by 50% [27].

In addition Al Aqeel et al. [28] described patients with 3-methylglutaconic aciduria with neonatal acidosis and hypoglycemia. In one case the clinical features were very similar to that of our patients, including a possible sepsis, myoclonus epilepsy, and deafness. Brain imaging showed global cerebral/cerebellar atrophy, loss of the cerebral white matter, and bilateral putaminal necrosis, suggestive for Leigh-like syndrome. One of the patients died at 3 years of age. Unfortunately, no data concerning oxidative phosphorylation were given.

So far no underlying mutation has been discovered in our patients. The presence of consanguinity in three out of four cases could be comparable with an autosomal recessive inheritance. We have not found the etiology of the mitochondrial dysfunction associated increased 3MG excretion either. In the lack of a proven inborn error in the leucine metabolism one might hypothesize that the metabolic abnormalities occur due to an interaction between different epigenetic factors and the primary disease causing gene defect.

Based on the association of the clinical features and biochemical abnormalities we suggest that our patients form a genetic factors and the primary disease causing gene defect. Maladies occur due to an interaction between di metabolism one might hypothesize that the metabolic abnormality associated increased 3MGC excretion in patients. The presence of consanguinity in three out of four patients. The presence of consanguinity in three out of four patients.

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Based on the association of the clinical features and biochemical abnormalities we suggest that our patients form a distinct clinical subgroup of deficient oxidative phosphorylation and 3-methylglutaconic aciduria; with deafness, encephalopathy, and neuro-radiological evidence of Leigh-like disease (MEGDEL association). Further genetic mapping studies might be helpful for the elucidation of the etiology within the heterogeneous group of 3MG-aciduria type IV.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymgme.2006.01.013.

References


