Exome sequencing identifies a new mutation in SERAC1 in a patient with 3-methylglutaconic aciduria

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3-Methylglutaconic aciduria

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1. Introduction

3-Methylglutaconic aciduria (3-MGA-uria) is a heterogeneous group of syndromes characterized by an increased excretion of 3-methylglutaconic and 3-methylglutaric acids. Five types of 3-MGA-uria (I to V) with different clinical presentations have been described [1]. The origin of 3-methylglutaconic acid accumulation is only understood in type I, which is due to a deficiency of 3-methylglutaconyl-CoA hydratase, an enzyme involved in the catabolism of leucine; this type is the least frequently described but presents the highest levels of 3-methylglutaconic acid in urine [2,3]. Type II, or Barth syndrome, is characterized by mutations in the TAZ gene, presenting with X-linked cardiomyopathy, neutropenia and skeletal myopathy [4,5]. The genetic defect in 3MGA-uria type III or Costeff syndrome is due to mutations in the OPA3 gene and patients showed bilateral optic atrophy and progressive neurological defects [6,7]. Type V was initially described in a cohort of Canadian Dariusleut Hutterite patients, with mutations in the DNAJ19 gene, presenting with cardiomyopathy and ataxia [8,9]. Finally, type IV comprises a heterogeneous group of patients with variable clinical presentation including neurological deterioration, central nervous system involvement, cardiomyopathy, retinitis pigmentosa, cataracts, hypotonia, microcephaly, lactic acidosis and defective mitochondrial respiratory chain activities, particularly complex V (ATP synthase) deficiency [10,11,12]. Three nuclear –ATP12, ATP5E and TMEM70–, and two mitochondrial –ATP6 and ATP7– genes are known to be involved in the pathogenesis of this latter deficiency [13]. In addition, in 2006 Wortmann and co-workers reported a subset of patients with 3-MGA-uria and a distinct clinical phenotype (MEGDEL syndrome) including sensorineural hearing loss, encephalopathy, dystonia and Leigh-like brain imaging [14]. Recently, MEGDEL syndrome has been associated to mutations in SERAC1 (serine active site containing 1) [15]. In the recent
years an important progress in the knowledge of the genetic and molecular bases of this heterogeneous group of disorders has been made and led some authors to classify the diseases into two groups primary (type I) and secondary (types II–V) 3-MGA-urias. The latter group was further subclassified according to the pathogenic mechanisms responsible of the disease [16].

Here we report the diagnostic steps to investigate a patient with 3-MGA-uria of unknown genetic origin using exome sequencing.

2. Material and methods

2.1. Case report

The patient was the only daughter of non-consanguineous healthy parents. Family history revealed a paternal cousin presenting with arthrogryposis at birth and a mother’s cousin with mental retardation and deafness. During the third month of pregnancy a threatened abortion occurred. The patient was born at term by a cesarean section because of meconium excretion into the amniotic fluid. Birth weight and height were 2.830 g and 49 cm, respectively. At four days of life she was admitted to the intensive neonatal care unit of another hospital, because of severe respiratory distress, refusal to feed and jaundice. Routine biochemical studies showed metabolic acidosis with respiratory alkalosis, ketonuria, hyperammonemia, increased liver transaminases and hyperbilirubinemia. After phototherapy and antibiotic treatment she showed progressive clinical improvement and was discharged at 16 days of age. At 6 months of age convergent strabismus with alternating occlusions was noted. At 8 months of age she showed oral dyskinesia, she was able to sit down but she could not take objects. Since that age refusal to feed and vomiting were usual features. At 16 months of age she showed hypotonia of the neck and truncal ataxia. She had visual contact and smile but she could not say any word. At 12 and 18 months of age she suffered two episodes of ketotic hypoglycemia with metabolic acidosis and coma, apparently triggered by an upper respiratory tract infection. During this period psychomotor deterioration was evident but sometimes she showed improvement in her developmental abilities (documented by consecutive videos with images and audios from the family). Brain MRI showed bilateral abnormalities of the basal ganglia, putamen, hyperintensities in T2 and hypointensities in T1, typical of Leigh syndrome.

At 20 months of age she was admitted to our hospital to be studied. Her weight and height were 9 kg and 79 cm, respectively. Cranial circumference was 45 cm (below 2 SD). From 4 to 7 years of age microcephaly was evident and cranial circumference stopped growing (46 cm below 3 SD). Clinical examinations showed axial hypotonia, esotropia of the left eye and she was not able to stand up or walk alone. Routine biochemical studies were normal including glucose, transaminases, cholesterol, ammonia, amino acids, plasma free- and bound-carnitine, plasma lactate and ketones. The urinary organic acid profile showed a slight increase of 3-methylglutarate and 3-methylglutaconate, but at that time it was not considered indicative of any particular disease except for a respiratory chain deficiency, but OXPHOS activities and histochemistry in muscle biopsy were normal.

Follow-up showed failure to thrive with weight and height being always under 3 SD. The patient developed sensorineural deafness, dystonia with axial hypotonia, tetraparesia and loss of manipulation skills. Although she kept social contact, language ability was severely impaired because of oral dyskinesia. Brain magnetic resonance imaging, at 15 years of age showed cerebral and cerebellar atrophy with bilateral symmetric lesions in basal ganglia (lenticular and thalamic). Brain spectroscopy did not show increase of lactate. In addition, at 16 years of age optic atrophy was diagnosed. Actually, at 19 years of age she is severely affected, she weights 21 kg, and shows bilateral strabismus, optic atrophy, severe hearing loss, poor communication skills and she is unable to hold up her head. She presents multiple joint problems and hip dislocations.

Biochemical analysis showed normal ammonia, cholesterol, amino acids and acylcarnitines but, occasionally, slight increases of lactate in urine. The urinary organic acid analysis showed persistently increased excretion of 3-methylglutaconic and 3-methylglutaric acids (Table 1).

The parents of the patient provided informed consent. The study was approved by the Ethics Committee of the Hospital Clinic-Barcelona, Spain. All samples were obtained in accordance with the revision of the Helsinki Declaration.

2.2. Methods

2.2.1. Organic acids

Organic acids in urine were analyzed by gas chromatography–mass spectrometry of their Trimethylsilyl (TMS) derivatives as previously described [17]. To quantitate 3-methylglutaconic acid the response factor of 3-methylglutaric acid was used.

2.2.2. Enzymatic determinations

PDHc and E1 activities were determined in cultured fibroblasts by measuring the 14CO2 production from [1-14C]-labeled pyruvate after activation with Ca++ and Mg++ as described [18]. Respiratory chain activities in skeletal muscle were determined spectrophotometrically [19].

2.2.3. Mutational studies

Exon and intron boundaries of the known genes causing 3-MGA-uria type IV (TMEM70, ATP12, ATP5E, OPA3 and DNAJC19) were analyzed by PCR followed by Sanger sequencing (available on request).

2.2.4. Whole genome analysis

Genomic DNA was isolated from blood following the manufacturer’s recommendations (QiAmp DNA Mini Kit, Qiagen, GmbH, Germany).

Table 1

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Patient</th>
<th>Reported patientsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first symptoms</td>
<td>4 d</td>
<td>1 d–6 y</td>
</tr>
<tr>
<td>Psychomotor delay</td>
<td>Yes</td>
<td>14/14</td>
</tr>
<tr>
<td>Neurosensory deafness</td>
<td>Yes</td>
<td>13/14</td>
</tr>
<tr>
<td>Dystonia</td>
<td>Yes</td>
<td>13/14</td>
</tr>
<tr>
<td>Leigh-like brain imaging</td>
<td>Yes</td>
<td>13/13</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>Yes</td>
<td>0/15</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>Yes</td>
<td>0/15</td>
</tr>
<tr>
<td>Alive</td>
<td>Yes</td>
<td>8/15</td>
</tr>
<tr>
<td>Present age</td>
<td>19 y</td>
<td>4 y–15 y</td>
</tr>
</tbody>
</table>

Biochemical data

| 3-methylglutacinate in urine, mmol/mol creatinine | 182–420 (C.V.<20) | 16–196 (C.V.<20) |
| 3-methylglutaric acid in urine, mmol/mol creatinine | 42–360 (C.V.<15) | NR (15/15) |
| Plasma lactate | Normal | Increased (14/15) |
| Plasma alanine | Normal | Increased (8/13) |
| Phosphatase in fibroblasts | Abnormal | Abnormal (3/3) |
| Plasma cholesterol | Normal | Low (4/11) |

Enzymatic activities

| 3-Methylglutaryl-CoA hydratase | Normal | NR (15/15) |
| PDH complex activity | Normal | NR (15/15) |
| Mitochondrial respiratory chain activities | Normal | Abnormal (9/11) |
| Muscle Fibroblasts | ND | Abnormal (3/8) |

a Ratios denote the number of patients showing a particular finding/total number of patients; d, days; w, weeks; y, years; C.V., control values; NR, not reported; ND, not done. In bold, clinical symptoms not previously associated to MEGDEL syndrome.
Homozgyosity mapping was performed in patient's DNA using the Genechip Human mapping 250K SNP array (Affymetrix, Santa Clara, CA, USA) as described [20]. Briefly, digestion with NspI, ligation of the adaptor, and amplification with a generic primer that recognizes the adaptor sequence were followed by fragmentation, end labeling, and hybridization to the chip in accordance with the manufacturer's instructions. Homozgyous regions > 1.0 Mb were manually detected.

Exome sequencing was performed in DNA of the patient and her healthy parents. Samples were subjected to exome enrichment with the Agilent SureSelect Human All Exon 50 Mb kit followed by sequencing using the Illumina HiSeq 2000 genome analyzer platform (CNAG). The analysis of the primary data (FASTQ files) was done using the pipeline of the BIER platform (CIBERER) as detailed in the Supplementary data.

2.2.5. Western blot

Fibroblasts from patients and control individuals were homogenized in SETH buffer (10 mM Tris–HCl pH 7.4, 0.25 M sucrose, 2 mM EDTA, 5 × 10⁻⁴ M U/l heparin). Cleared lysates were subjected to SDS-PAGE, electroblotted and proteins were visualized by immunostaining with specific antibodies followed by colorimetric detection (Opti-4CN™ Substrate Kit, Bio-Rad, U.S). Anti-SERAC1 (Sigma) and anti- GAPDH (Santa Cruz Biotechnology, Heidelberg, Germany) antibodies were used.

2.2.6. Filipin staining

The accumulation of free cholesterol was determined by cytochemical demonstration via filipin staining in cultured skin fibroblasts as described [21].

3. Results and discussion

3-MGA-uria is a heterogeneous group of syndromes characterized by an increased excretion of 3-methylglutaconic and 3-methylglutaric acids, but the origin of these acids is only understood in type I, which is due to a deficiency of 3-methylglutaconyl-CoA hydrolase in the metabolism of leucine. In our patient primary 3-MGA-uria was excluded as the activity of 3-methylglutaconyl-CoA hydrolase was normal (Table 1). The lack of evidence of X-linked cardiomyopathy in this family, and the mutational analysis of OPAL3, ATPI2, TMEM70, ATPISE and DNACJ19 genes excluded secondary 3-MGA-uria. The mtDNA substitutions frequently associated with Leigh disease (3243A>G, 8344A>G, 8993T>G, 8993T>C, 9176T>C) or with dystonia (14,459G>A) were also excluded. No mtDNA deletions or deletion were observed. Muscle biopsy analysis showed no histological alterations. Normal activities of the respiratory chain and of pyruvate dehydrogenase (PDH) complexes were found in muscle biopsy and fibroblasts, respectively.

In order to elucidate the genetic cause of the disease we searched for homozygous regions using the Genechip Human mapping 250K SNP array, but we could not detect a clear homozygous region. Therefore, we sequenced the cellular exome of the patient and her healthy parents. Using this approach we identified 68,750 variants in the patient sample. We have applied several filtering steps in order to exclude non-genic variants and selected for nucleotide changes predicted to have a damaging effect on the encoded proteins (Fig. 1A). On the other hand, as the different types of 3-MGA-uria, except Barth syndrome, are of autosomal recessive inheritance, we also expected this kind of inheritance in this family. Therefore, we further filtered the data according to this hypothesis and prioritized for genes containing variants found to be heterozygous in the healthy parents and homozygous in the index case as well as for genes with two heterozygous variants that segregate separately. This filtering step left nine genes. Because 3-methylglutaconic acid excretion is a biochemical marker of mitochondrial dysfunction we also filtered for genes encoding potential mitochondrial-targeted proteins using Mitopred and Psort predictors (Fig. 1A). This filter left only two candidate genes, DNACJ28 and SERAC1. Changes in the former were also found in other in house exomes of healthy individuals, but the homozygous variant found in SERAC1 (c.202C>T) had never been identified before and was predicted to be pathogenic as it generates a premature stop codon. Therefore, this mutation was considered the putative genetic cause of the disease. Sanger sequencing confirmed those findings (Fig. 1B).

Retrospectively, a closer look at the SNP array data showed a 2.9 Mb homozygous region spanning from chr6:156.9 Mb (rs1246188) to chr6:159.8 Mb (rs947311). In this, genomics region included SERAC1, which is located at chr6:158.3–158.9 Mb. However, this homozygous region was not initially considered to be associated with the disease since it was interrupted by several heterozygous SNPs. We may now speculate that those SNPs were probably acquired after the mutation in SERAC1.

During the course of this project a parallel study also identified mutations in SERAC1 as the genetic cause of patients with MEDEL syndrome [15], which had originally been reported in a cohort of patients with 3-MGA-uria and oxidative phosphorylation defects presenting with a particular association of clinical symptoms including Leigh-like brain imaging, hearing loss and dystonia [14]. The clinical and biochemical features of our patient compared with those previously reported are summarized in Table 1. Clinically, all the patients had very similar presentations during the neonatal period or later in life during infancy. Psychomotor delay neurosensorial deafness, dystonia and Leigh-like brain imaging were the characteristic findings in almost all of them. In addition, our patient presents microcephaly (detected at 20 months of age) and developed optic atrophy at 16 years of age. The most consistent biochemical finding in our patient is the persistently high excretion of 3-methylglutaconate and 3-methylglutarate, with normal plasma lactate, while lactate was increased in 14 among 15 reported patients. This finding may be related to the normal respiratory chain activities in our patient (Table 1).

The identification of SERAC1 as the genetic cause of MEDEL syndrome led to the description of 14 different mutations in 15 patients, but the c.202C>T variant we identified was not present in any of the 15 patients reported in that study [15]. The newly identified mutation is a nonsense substitution that generates a premature stop codon at position 68 of SERAC1 protein (c.202C>T, p.Arg68*). To investigate the effect of this mutation we performed western blot analysis in protein lysates from patient fibroblasts using anti-SERAC1 antibody (HPA025716, Sigma) raised against an immunogenic peptide comprising residues 321–394. Results showed a complete absence of SERAC1 (Fig. 1C) that was consistent with the prediction of a truncated protein. Therefore the newly identified SERAC1 mutation causes lack of protein and consequently it is, with high probability, disease causing.

SERAC1 encodes for a protein with a serine-lipase domain. The function of this protein was completely unknown until the mentioned disease-causing mutations were identified [15]. Studies in patients' fibroblasts showed that SERAC1 is localized at the contact sites between mitochondria and endoplasmic reticulum and plays a role in the phosphatidylglycerol remodeling pathways. Biochemically, patients carrying SERAC1 mutations showed an altered composition of phosphatidylglycerol 34:1 and 36:1 species and low levels of bis (monoaoylglycerol) phosphate, with subsequent accumulation of free cholesterol, pointing to a role for this protein in both mitochondrial function and cholesterol trafficking [15]. However, in contrast to patients with Barth syndrome, total cardiolipin levels were normal and only imbalances in some species were found. Cardiolipin alteration in Barth syndrome leads to defects in the assembly and stability of the mitochondrial respiratory chain complexes [22], but cardiolipin composition in patients with SERAC1 defects is only slightly altered. Therefore, the heterogeneous pattern of respiratory chain defects found in SERAC1 patients may be related to these slight alterations in cardiolipin composition and may explain why some patients, including the case reported here, showed non-detectable defects in the respiratory chain (Table 1).
Wortmann and co-workers analyzed the accumulation of free cholesterol in three patients using filipin staining [15]. These authors observed that SERAC1 patients clearly had more staining than control individuals. The patient we report here also showed increased filipin staining (Fig. 1D), though it was less prominent than that of a Niemann Pick type C patient (used as a positive control), and also showed a different cell distribution pattern (Fig. 1D). Our observations suggest that filipin staining may not be useful to screen for SERAC1 deficiency in patients with 3-MGA-uria. On the contrary, the analysis of the composition of phosphatidylglycerol and cardiolipin species seems to be an emerging additional and promising approach to stratify patients with 3-MGA-uria [15].

4. Conclusions

Regarding the clinical aspects we would like to remark that although the symptoms of our patient are consistent with MEGDEL syndrome, our report expands the clinical spectrum associated to SERAC1 deficiency [15]. To this effect, in addition to the main clinical symptoms of dystonia, deafness and Leigh-like brain imaging, the patient described here developed microcephaly and optic atrophy, two features not previously reported in MEGDEL syndrome.

According to our experience we highlight the usefulness of exome sequencing to reveal the genetic bases of human rare diseases even if only one affected individual is available. In such a situation an accurate biochemical characterization of the patient together with the exclusion of other possible genetic causes and the availability of the healthy parents are important factors to be considered when using an exome sequencing approach. The identification of the altered gene could in turn provide new mechanistic insights into the biological processes involved in the disease [23]. In the case of 3-MGA-uria such identifications have allowed scientists to gain insight into the complex mitochondrial metabolic pathways.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2013.04.021.

References
