Molecular Genetics and Metabolism xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

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

Frederic Tort [a,b], María Teresa García-Silva [c], Xènia Ferrer-Cortès [a], Aleix Navarro-Sastre [a,b], Judith Garcia-Villoria [a,b], Maria Josep Coll [a,b], Enrique Vidal [d], Jorge Jiménez-Almazán [d], Joaquín Dopazo [d,e,f], Paz Briones [a,b,g], Orly Elpeleg [h], Antonia Ribes [a,b,*]

[a] Secció d'Errors Congènits del Metabolisme, Servei de Bioquímica i Genètica Molecular, Hospital Clinic, IDIBAPS, 08028, Barcelona, Spain
[b] CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain
[c] Unidad de Enfermedades Mitocondriales- Enfermedades Metabólicas Hereditarias. Servicio de Pediatría. Hospital 12 de Octubre, Madrid, Spain
[d] BIER, CIBERER, Centro de Investigación Principe Felipe, Valencia, Spain
[e] Computational Medicine Institute, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain
[f] Functional Genomics Node, (INB) at CIPF, Valencia, Spain
[g] Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain
[h] Monique and Jacques Roboh Department of Genetic Research, Hadassah, Hebrew University Medical Center, Jerusalem, Israel

ARTICLE INFO

Article history:
Received 29 April 2013
Accepted 29 April 2013
Available online xxxx

Keywords:
SERAC1
3-Methylglutaconic
MEGDEL
Exome

ABSTRACT

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. Causative mutations in *TAZ*, *OPA3*, *DNAJC19*, *ATP12*, *ATP5E*, and *TMEM70* have been identified. After excluding the known genetic causes of 3-MGA-uria we used exome sequencing to investigate a patient with Leigh syndrome and 3-MGA-uria. We identified a homozygous variant in *SERAC1* (c.202C>T; p.Arg68*), that generates a premature stop codon at position 68 of SERAC1 protein. Western blot analysis in patient's fibroblasts showed a complete absence of SERAC1 that was consistent with the prediction of a truncated protein and supports the pathogenic role of the mutation. During the course of this project a parallel study identified mutations in *SERAC1* as the genetic cause of the disease in 15 patients with MEGDEL syndrome, which was compatible with the clinical and biochemical phenotypes of the patient described here. In addition, our patient developed microcephaly and optic atrophy, two features not previously reported in MEGDEL syndrome. We highlight the usefulness of exome sequencing to reveal the genetic bases of human rare diseases even if only one affected individual is available.

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 less 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 [1,10–12]. Three nuclear –*ATP12*, *ATP5E* and *TMEM70*–, and two mitochondrial –*ATP6* and *ATP8*– 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

* Corresponding author at: Secció d'Errors Congènits del Metabolisme -IBC, Servei de Bioquímica i Genètica Molecular, Hospital Clínic, Edifici Helios III, planta baixa, C/ Mejía Lequerica s/n, 08028 Barcelona, Spain. Fax: +34 93 227 56 68.
E-mail address: aribes@clinic.ub.es (A. Ribes).

1096-7192/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymgme.2013.04.021

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 neurosensorial 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 thalamus). 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 $^{14}CO_2$ production from [1-^{14}C]-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
Summary of clinical and biochemical features of the reported patients with *SERAC1* mutations [15] compared with the patient described here.

	Patient	Reported patients[a]
Clinical findings		
Age at first symptoms	4 d	1 d–6 y
Psychomotor delay	Yes	14/14
Neurosensorial deafness	Yes	13/14
Dystonia	Yes	13/14
Leigh-like brain imaging	Yes	13/13
Microcephaly	**Yes**	**0/15**
Optic atrophy	**Yes**	**0/15**
Alive	Yes	8/15
Present age	19 y	4 y–15 y
Biochemical data		
Range of 3-methylglutaconate in urine, mmol/mol creatinine	182–420 (C.V.<20)	16–196 (C.V.<20)
Range of 3-methylglurarate 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)
Filipin staining in fibroblasts	Abnormal	Abnormal (3/3)
Plasma cholesterol	Normal	Low (4/11)
Enzymatic activities		
3-Methylglutaconyl-CoA hydratase	Normal	NR (15/15)
PDH complex activity	Normal	NR (15/15)
Mitochondrial respiratory chain activities		
Muscle	Normal	Abnormal (9/11)
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.

Please cite this article as: F. Tort, et al., Exome sequencing identifies a new mutation in *SERAC1* in a patient with 3-methylglutaconic aciduria, Mol. Genet. Metab. (2013), http://dx.doi.org/10.1016/j.ymgme.2013.04.021