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Brief Communication

Milder clinical course of Type IV 3-methylglutaconic aciduria due to a novel mutation in *TMEM70*

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ABSTRACT

Mitochondrial disorders are a large and genetically heterogeneous group of disorders posing a significant diagnostic challenge. Only approximately 10–20% of patients have identifiable alterations in their mitochondrial DNA (mtDNA). The remaining ~80–90% of affected patients likely harbor mutations in nuclear genes, most of which are still poorly characterized, and therefore not amenable to efficient screening using currently available molecular methods.

Here we present a patient, who has been followed since birth after presenting with neonatal hyperammonemia, lactic acidosis, Reye-like syndrome episodes, and ventricular tachyarrhythmia. Initial biochemical work-up revealed hyperalaninemia, normal plasma glutamine, mild orotic aciduria and significant amounts of urinary 3-methylglutaconic (3-MGC) and 3-methylglutaric (3-MGA) acids. Muscle biopsy demonstrated the presence of ragged-red fibers and non-specific structural abnormalities of mitochondria. The activities of respiratory chain enzymes (complexes I–IV) showed no deficiency. Mutational analysis of the entire mitochondrial genome did not reveal deleterious point mutations or large deletions. Long-term follow-up was significant for a later-onset hypertrophic cardiomyopathy, muscle weakness, and exercise intolerance. Although she had frequent episodes of Reye-like episodes in infancy and early childhood, mostly triggered by illnesses, these symptoms improved significantly with the onset of puberty.

In the light of recent reports linking cases of type IV 3-methylglutaconic aciduria (3-MGCA) and hypertrophic cardiomyopathy to mutations in *TMEM70*, we proceeded with sequencing analysis of this gene. We identified one previously reported splice site mutation, c.317-2A>G and a novel mutation c.494G>A (p.G165D) in an evolutionarily conserved region predicted to be deleterious. This variant was not identified in 100 chromosomes of healthy control subjects and 200 chromosomes of patients with cardiomyopathies. Western blotting using a polyclonal antibody against ATP5J, subunit F6 of ATP synthase, on patient's skin fibroblasts showed undetectable amount of the ATP5J protein. In comparison to the previously reported cases, we note that our patient had normal growth parameters and cognitive development, absence of structural heart and urinary tract defects, no dysmorphic features, improvement of symptoms with age, and persistence of hypertrophic cardiomyopathy.

1. Introduction

Only approximately 10–20% of pediatric patients with definite mitochondrial disorders have identifiable mtDNA abnormalities by using a panel of common mtDNA point mutations and single deletions [1,2]. The vast majority among the remaining 80–90% of patients likely harbor mutations in their nuclear genes. Poor characterization of nuclear genes implicated in mitochondrial dysfunction and insufficiently understood genotype–phenotype correlations result in delayed diagnosis, hampering the counseling of the affected families.

One of the recently described nuclear genes implicated in mitochondrial disorders is *TMEM70* encoding an ancillary factor of mammalian F_OF_1-ATP synthase [3,4]. Eukaryotic F_OF_1-ATP synthase assembly is a complex process regulated on multiple levels: mRNA translation, processing and stability, or involvement of chaperones needed for the correct assembly of the complex. TMEM70 is a 30-kDa mitochondrial transmembrane protein thought to play an important role in the ATP synthase biogenesis, likely at the stage of the F_1 unit assembly [4]. *TMEM70* is ubiquitously present in the genomes of higher eukaryotic species and plants, but is absent in yeast.

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Mutations in *TMEM70* (MIM *612418) have been linked to isolated ATP synthase deficiency [3]. Deficiency of ATP synthase due to defects in nuclear genes usually leads to the loss of synthetic and hydrolytic activities of ATP synthase. Clinically, these individuals present with hypertrophic cardiomyopathy, structural cardiovascular anomalies (aortic and pulmonic stenosis), hypospadias in males, failure to thrive, and normal to mildly delayed cognitive development. Shared dysmorphic features have been described (e.g. tall forehead, curved eyebrows, flattening of midface, long philtrum, low-set ears and thin lips) [5–8]. Biochemically, patients exhibit 3-MGCA, lactic acidosis, and mild intermittent hyperammonemia [7]. Most affected individuals reported thus far belong to the Roma ethnic group [3].

Due to the limited number of reported patients, the full mutational spectrum and natural history of this condition are still poorly understood. Here we describe the case of a girl aged 14 years, who presented in the newborn period with a Reye-like syndrome episode, 3-MGCA, ventricular tachycardia, and later-onset hypertrophic cardiomyopathy. She was subsequently found to have mutations in *TMEM70*.

2. Case report

Clinical and biochemical findings in this patient have been previously described elsewhere [9]. The patient presented on day of life 1 with symptoms resembling Reye-like syndrome: lethargy, hepatomegaly, hypoglycemia, seizures, and metabolic acidosis. The biochemical workup revealed hyperammonemia of 357 μmol/L (reference range, 22–48 μmol/L), lactic acidosis of 10 mmol/L (0.2–2 mmol/L), mildly elevated alanine aminotransferase of 69 U/L (10–25 U/L) and aspartate aminotransferase of 162 U/L (15–50 U/L), and ventricular tachyarrhythmia. Initial biochemical work-up revealed, elevated alanine of 631 μmol/L (141–343 μmol/L) with normal plasma glutamine, slightly elevated plasma propionylcarnitine of 1.4 μmol/L (0–0.4 μmol/L). Urine organic acids assay revealed mild orotic aciduria of 81 nmol/mg creatinine (<60 nmol orotic acid/mg creatinine) and significant amounts of urinary 3-methylglutaconic (3-MGC), 121 mmol/mol of creatinine (0–8 mmol/mol of creatinine) and 3-methylglutaric (3-MGA) acids, 33 mmol/mol of creatinine (0–2 mmol/mol of creatinine). The hyperammonemia was controlled with protein-restricted diet (2 g/kg/day) and intravenous sodium benzoate and sodium phenylacetate. At age 4 days the patient developed ventricular arrhythmia. At 10 days of age an echocardiogram revealed moderate hypertrophy and decreased shortening fraction of the left ventricle. Assays using skin fibroblasts to measure the activity of carnitine-acylcarnitine translocase, pyruvate dehydrogenase complex, pyruvate carboxylase, and mitochondrial respiratory chain enzymes (complexes II–IV) revealed no abnormalities. Fatty acid oxidation studies by using an *in vitro* probe in cultured fibroblasts were normal. Sequencing of *TAZ* (G4.5) revealed no deleterious mutations. Gomori staining of the skeletal muscle demonstrated ragged red fibers. Electron microscopy showed abnormal concentric arrangements of mitochondrial cristae with rare dense bodies, excess glycogen, and fat deposition. Mitochondrial respiratory chain enzymes (complexes I–IV) on skeletal muscle were normal.

Since her initial presentation, she had multiple admissions for Reye-like symptoms as a child always precipitated by intercurrent infections. These episodes subsequently subsided. Her last episode occurred at 8 years of age. The heart arrhythmia resolved, but she developed fatigue, exercise intolerance, and hypertrophic cardiomyopathy. At age 7 years, her brain MRS showed a small upright doublet at 1.3 parts/million in the left mesial occipital region, frontal regions anterior to corpus callosum, head of the right caudate nucleus consistent with a small elevation of lactate. Choline, creatine, and NAA peaks were normal. There was no hearing loss or visual abnormalities.

She continued to be followed at the Genetics Clinic for the suspected mitochondrial cytopathy and by the Pediatric Cardiology Service for her hypertrophic cardiomyopathy. Her medications include atenolol, carnitine, coenzyme Q10, thiamine, ascorbate, and riboflavin. Her most recent visit to our clinic was at age 14 years. At that time, her physical exam and review of systems revealed no dysmorphic features, ophthalmological manifestations, hearing loss, muscle tenderness, rhabdomyolysis, epilepsy, movement disorders, or ataxia. She had normal growth parameters and was doing well academically. She participated in track, but due to increasing exercise intolerance, she had to quit and switch to less strenuous physical regimen. She described a “second wind” phenomenon after a short rest.

3. Materials and methods

Spectrophotometric analysis of the respiratory chain complexes on fibroblasts and skeletal muscle was performed according to previously described protocols [10–13]. Mitochondrial genome was screened by the temporal temperature gradient electrophoresis followed by sequencing of abnormal amplicons [14]. Each coding exon and 50 bp of its flanking intronic regions of the *TMEM70* gene was PCR amplified using FastStart DNA polymerase (Roche, IN) and sequence-specific oligonucleotides primers were linked to the M13 universal primers at the 5'–ends. PCR products were purified using ExcelaPure 96-well UF PCR purification plates (Edge BioSystems, Gaithersburg, MD). Sequencing reactions were performed using the BigDye Terminator Cycle Sequencing kit (version 3.1) and analyzed on an ABI3730XL automated DNA sequencer with the Sequencing Analysis Software version 5.1.1 (Applied Biosystems, CA). The sequencing results were compared to the GenBank *TMEM70* sequence (NT_008183.19) by using the Mutation Surveyor version 3.24 (SoftGenetics, PA). The potential effects of mutations on the protein structure were estimated by comparing the local environments of mutants among the homologues of the *TMEM70* protein family according to the previously described methods [15–17]. Postranslational secondary protein analysis was performed using web-base tools assembled on page http://www.expasy.ch/tools/#proteome. Western blotting was performed using skin fibroblasts. Following centrifugation, cell pellets were sonicated using XL-2000 Misonix Sonicator (Qsonica, Newtown, CT) for 2 minutes. The total protein concentration was measured using Bradford method. Seventy mg of protein per sample were loaded to a 6–20% gradient acrylamide gel and ran for 100 minutes at 120 V. The proteins were then transferred to a nitrocellulose membrane at 300 mA for 1 h. Western blot for ATP5J, an F6 subunit of ATP synthase, was performed using polyclonal ATP5J (C-18) antibody (Santa Cruz Biotechnology, CA). GAPDH was included as a loading control. Two samples from apparently healthy controls were included as positive control for complex V/ATP5J.

4. Results

The patient was found to be heterozygous for one previously reported splice site mutation (c.317-2A>G) and a novel, previously unclassified missense variant c.494G>A (p.G165D) with inconsistent prediction regarding their pathogenicity by PolyPhen (deleterious) and SIFT (benign). Glycine at amino acid residue 165 was found to be conserved in fish, chicken and mammal species. This variant was not identified in 100 chromosomes of healthy control subjects and 200 chromosomes of patients with cardiomyopathies. Her parents were carriers for the identified DNA alterations, and appeared to be in good health. Both parents were of Northern European descent, and had no knowledge of Roma ancestry among their distant relatives. Posttranslational protein analysis of TMEM70 revealed no potential role of residue p.G165 in secondary protein modifications. The activities of respiratory chain enzyme complexes I–IV in skeletal muscle and II–IV