Pathologic Variants of the Mitochondrial Phosphate Carrier *SLC25A3*: Two New Patients and Expansion of the Cardiomyopathy/Skeletal Myopathy Phenotype With and Without Lactic Acidosis

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Abstract Variants in the *SLC25A3* gene, which codes for the mitochondrial phosphate transporter (PiC), lead to a failure of inorganic phosphate (Pi) transport across the mitochondrial membrane, which is required in the final step of oxidative phosphorylation. The literature described two affected sibships with variants in *SLC25A3*; all cases had skeletal myopathy and cardiomyopathy (OMIM 610773). We report here two new patients who had neonatal cardiomyopathy; one of whom did not have skeletal myopathy nor elevated lactate. Patient 1 had a homozygous splice site variant, c.158-9A>G, which has been previously reported in a Turkish family. Patient 2 was found to be a compound heterozygote for two novel variants, c.599T>G (p.Leu200Trp) and c. 886_898delGGTAGCAGTGCTTinsCAGATAC (p.Gly296_Ser300delinsGlnIlePro). Protein structure analysis indicated that both variants are likely to be pathogenic. Sequencing of *SLC25A3* should be considered in patients

with isolated cardiomyopathy, even those without generalized skeletal myopathy or lactic acidosis.

Introduction

SLC25A3 encodes the mitochondrial phosphate transporter (PiC), which transports inorganic phosphate (Pi) across the mitochondrial membranes, thereby allowing oxidative phosphorylation by the addition of Pi to ADP to yield ATP. The driving force for this reaction is the proton gradient across the inner mitochondrial membrane generated by complexes I, III, and IV of the electron transport chain. The terminal steps of ATP production require three enzymes. First, the *SLC25A3*-encoded PiC imports Pi into the mitochondrial matrix. Next, the F_1F_0 -ATP synthase catalyzes the addition of the Pi to ADP to form ATP. Finally, the adenine nucleotide translocase transports the new ATP molecule out of the matrix in exchange for an ADP molecule.

There are multiple publications describing pathogenic variants in several nuclear-encoded F_1F_0 -ATP synthase components, including *TMEM70* and *ATP5E*; these lead to a wide variety of phenotypes, including cardiomyopathy, neuropathy, ataxia, and lactic acidosis (Cizkova et al. 2008; Mayr et al. 2010; Tort et al. 2011). Variants in *MT-ATP6*, a mitochondrial-encoded component of the F_1F_0 -ATP synthase, can also cause a variety of phenotypes in the Leigh syndrome spectrum (OMIM 256000) (Pitceathly et al. 2012). Recently, reports have emerged describing the first examples of pathogenic variants in the mitochondrial PiC (Mayr et al. 2007; Mayr et al. 2011).

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SLC25A3, located on chromosome 12q23, encodes a protein 361 amino acids in length. There are two isoforms of the protein differing by alternative splicing of exons 3A and 3B. PiC-A includes exon 3A and is expressed in heart and skeletal muscle. PiC-B contains exon 3B and is expressed in a wide variety of other tissues, including the liver, kidney, and thyroid (Dolce et al. 1996). Exons 3A and 3B are more than 70% identical and encode 42 and 41 amino acids, respectively. In the two previously reported Turkish families, one had a homozygous c.158-9A>G transition creating a novel splice acceptor site in intron 2 (family 1), and the other has a homozygous c.215G>A (p.G72E) transition within exon 3A (family 2) (Mayr et al. 2007; Mayr et al. 2011). Of the five affected children in these families, three died within the first year of life from hypertrophic cardiomyopathy and lactic acidosis (Patients 1-1, 2-1, and 2-2), and two brothers were alive at nine and seventeen years with stable hypertrophic cardiomyopathy, proximal muscle weakness, and exercise intolerance (Patients 1-2 and 1-3) (Table 1). Of note, these patients demonstrated phenotypes that were consistent with decreased PiC-A in the tissues where the isoform is known to be expressed. In addition, in these cases, the retained PiC-B isoform was unable to compensate for the loss of exon 3A despite being expressed in those tissues, which may suggest alternate functions for the two isoforms.

Materials and Methods

Patients

Patient 1

This patient was a 12-month-old male born after a 41-week gestation to a 31-year-old Guatemalan mother and father. Pregnancy, including prenatal ultrasounds, was uncomplicated. Family history was negative for neonatal death, recurrent miscarriage, significant cardiac disease, or genetic abnormalities. There were two healthy siblings. There was no known parental consanguinity, but the parents were from the same small Guatemalan town. The baby was born by routine repeat Cesarean section at a community hospital. His Apgar scores were 7 at one minute of life and 8 at five minutes of life; his birth weight was 3,990 g (75th%). He was initially transferred to the well-baby nursery but within two hours of birth was found to have decreased tone, a weak cry, and hypoxia. He then quickly decompensated with profound hypotonia, respiratory failure requiring intubation, severe lactic acidosis, a coagulopathy, and poor cardiac contractility. His initial pH was 6.8 with a base deficit of −30, which was largely unresponsive to treatment with intravenous saline and sodium bicarbonate. Urine

ketones were negative, lactate was 19.2 mmol/L (reference range 0.5–2.2 mmol/L), ammonia was 24 mg/L (reference range 15–45 mg/L), and plasma betahydroxybutyrate was 0.1 mcg/mL (reference range <0.5 mcg/mL). An echocardiogram showed a structurally normal heart with a large patent ductus arteriosus and a right-to-left shunt coupled with pressures greater on the right side than on the left.

His respiratory status declined further, requiring treatment with nitrous oxide for 2 days. While intubated, he appeared strikingly and atypically alert out of proportion to his hypotonia, respiratory failure, and lactic acidosis. A repeat echocardiogram at 48 h of life showed right ventricular dilation and hypertrophy with a transitional patent ductus arteriosus. A head ultrasound showed increased echogenicity in the globus pallidus and thalami bilaterally, with normal video electroencephalogram. Brain magnetic resonance imaging (MRI) was grossly normal with MRI spectroscopy showing significantly elevated lactate levels. Normal results were obtained from newborn screening, an SNP microarray, a newborn hearing screen, and an ophthalmology exam. He was started on coenzyme Q, levocarnitine, riboflavin, thiamine, creatine, and biotin while his testing was pending.

He was extubated at 7 days of age but then had progressive severe lactic acidosis and was reintubated. Sequencing of *PHOX2B* was negative for the central hypoventilation syndrome (OMIM 209880), which was tested due to his prolonged ventilator dependence. Muscle biopsy at 2 weeks of age showed normal histology and normal mitochondrial DNA content; electron transport chain assays were not possible because of insufficient sample. His third attempt at extubation was successful and he was able to tolerate room air by ten weeks of age. Lactate levels throughout his admission ranged from 3 to 11 mmol/L (reference range 0.5–2.2 mmol/L). He did not have any significant renal, hepatic, or hematologic issues. On discharge, he was only prescribed carvedilol, sodium bicarbonate, and vitamin D supplementation. He was transferred to a long-term care facility where he has done well, although occasional readmissions were necessary for intubation when he suffered from viral infections.

Patient 2

The patient was a 10-month-old male born after a full-term pregnancy to a 32-year-old Haitian mother and a Dominican father. Pregnancy was unremarkable except for hypertrophic cardiomyopathy noted on the second trimester ultrasound examination and confirmed by fetal echocardiogram. Family history was negative for significant cardiac disease or genetic abnormalities, and there was no known parental consanguinity. He was born via scheduled Cesarean section due to the concerns of cardiac disease at a

Table 1 All reported cases of patients with variants in the mitochondrial phosphate carrier, *SLC25A3*, including the two new patients reported here (Patients 3-1 and 4-1.) Notice that patient 4-1, who carries two novel variants, does not demonstrate clinical myopathy nor lactic acidosis seen in all the previously reported patients

	Family 1				Family 2		Family 3		Family 4	
	Patient 1-1	Patient 1-2	Patient 1-3	Patient 2-1	Patient 2-3	Patient 3-1	Patient 4-1			
Report	Previous	Previous	Previous	Previous	Previous	New	New			
Ethnicity	Turkish	Turkish	Turkish	Turkish	Turkish	Guatemalan	Haitian/Dominican			
Age	Died at 6 months	9 years	17 years	Died at 4 months	Died at 9 months	12 months	10 months			
Gender	Female	Male	Male	Female	Female	Male	Male			
Variant	Homozygous c.158-9A>G affecting exon 3A	Homozygous c.158-9A>G affecting exon 3A	Homozygous c.158-9A>G affecting exon 3A	Homozygous c.215G->A within exon 3A	Homozygous c.215G->A within exon 3A	Homozygous c.158-9A>G affecting exon 3A	Compound heterozygous c.599T>G (exon 4) c. 886-898delins7 (exon 6)			
Clinical course	Hypertrophic cardiomyopathy and lactic acidosis, death at 6 months. Limited information	Prenatal hypertrophic cardiomyopathy, elevated lactate. At 9 years skeletal myopathy, compensated hypertrophic cardiomyopathy	Neonatal hypertrophic cardiomyopathy, elevated lactate. At 17 years skeletal myopathy, nonprogressive hypertrophic cardiomyopathy	Neonatal hypertrophic cardiomyopathy, severe skeletal myopathy, elevated lactate, progressive cardiomyopathy resulting in death at 4 months	Neonatal hypertrophic cardiomyopathy, severe skeletal myopathy, elevated lactate, progressive cardiomyopathy resulting in death at 9 months	Persistent lactic acidosis, neonatal cardiorespiratory failure, moderate skeletal myopathy, hypertrophic cardiomyopathy, stable at 12 months of age	Prenatal hypertrophic cardiomyopathy; cardiac transplant at age 7 months, no lactic acidosis, no clinical myopathy			

community hospital and then transferred to a high-acuity neonatal intensive care unit for the first month of life. His birth weight was 2,849 g (50%), and Apgar score was 8 at 1 min of life and 9 at 5 min of life.

He was initially asymptomatic and had normal tone, but echocardiogram at birth confirmed the presence of biventricular hypertrophy, left greater than right, with increased trabeculation and decreased left ventricular function, mild to moderate right ventricular dilation, and a moderate-sized atrial septal defect with left-to-right shunting. Cardiac catheterization and endomyocardial muscle biopsy at three weeks of life revealed nonspecific findings of cardiomyopathy with muscle disarray; there was no evidence of glycogen accumulation. Additional metabolic workup showed the following to be normal: plasma acylcarnitine profile, urine amino acids, plasma amino acids, plasma ammonia, serum cholesterol, plasma and urine carnitine, and serum creatinine kinase. Notable, screening lactate drawn while in cardiac failure was normal at 1.2 mmol/L (normal 0.5–1.6 mmol/L). Repeat lactate was also well within the normal range at 1.05 mM (normal range 0.80–2.0 mM), pyruvate level was slightly low at 0.02 mM (normal range 0.05–0.14 mM), and the lactate/pyruvate ratio was elevated at 53 (reference range 10–20). Newborn screening test and lysosomal screening for acid beta glucosidase, sphingomyelinase, alpha glucosidase, galactocerebrosidase, and alpha galactosidase were also normal. Imaging included normal head and renal ultrasounds. Medical treatment during the initial evaluation included courses of diuretics, beta blockers, and inotropes. He was eventually transferred at four weeks of life to a second tertiary care facility for cardiac transplant evaluation due to worsening heart failure and respiratory status. At 3 months of life, he underwent tracheostomy placement. MRI of the brain at 4 months of life showed prominence of the sulci, extra-axial spaces, and ventricles as well as thinning of the corpus callosum. Genetic and metabolic evaluation included a Noonan Spectrum panel for his cardiomyopathy, which did not reveal detectable point variants in the coding regions or intron/exon junctions of *PTPN11*, *SOS1*, *KRAS*, *NRAS*, *HRAS*, *RAF1*, *BRAF*, *SHOC2*, *MAP2K2*, *MAP2K1*, or *CBL* genes and no evidence of Pompe disease with normal acid alpha-glucosidase activity levels.

A muscle biopsy performed at 4 months of life showed no morphologic features of mitochondrial myopathy, but electron microscopy demonstrated minimal enlargement in size of mitochondria with slightly abnormal cristae. Electron transport chain enzyme function studies showed only increased citrate synthase levels suggestive of mitochondrial proliferation, thought to represent an adaptive response to mitochondrial dysfunction. Even when corrected for the increased citrate synthase levels, there were no deficiencies of the respiratory chain activities found.

At 7 months of life, the patient underwent an orthotopic cardiac transplant. The postoperative period was complicated by circulatory compromise and ventilator-dependent respiratory failure, significant gastroesophageal reflux, and seizures. He never experienced lactic acidosis during the course of his hospitalization. He was able to be discharged home at 11 months of age and was doing well with close follow-up.

Sequencing for Patient 1

Sequencing for patient 1 was performed by GeneDx (Gaithersburg, MD). Using genomic DNA from a blood specimen, all coding exons and the flanking splice junctions of a commercial panel of 101 genes involved in mitochondrial function and structure were PCR-amplified and sequenced simultaneously by next-generation sequencing. The DNA sequence was assembled, aligned against reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed. The entire mitochondrial genome from the blood sample was also amplified and sequenced using next-generation sequencing, with droplet-based multiplex PCR and library preparation with Illumina HiSeq2000. Exon level deletion/duplication analysis using Array CGH was performed concurrently. Mitochondrial DNA sequence was assembled and analyzed against the revised Cambridge Reference Sequence (rCRS) and the reported mutations and polymorphisms listed in the MITOMAP database (<http://www.mitomap.org>). The presence of the disease-associated sequence variant was confirmed by Sanger sequence analysis. A reference library of more than 6,000 samples from different ethnic groups and online databases for mtDNA variations was used to evaluate variants of unknown clinical significance.

Sequencing for Patient 2

The target sequences of a commercial panel of 162 genes involved in mitochondrial structure and function were enriched by using custom-designed NimbleGen SeqCap probe hybridization (Roche NimbleGen Inc., Madison, WI, USA). The captured target sequences included all coding exons and 20 bp of flanking intronic regions. The sample preparation followed the manufacturer's recommendation. Equal molar ratios of 10 indexed samples were pooled to be loaded to each lane of the flow cells for sequencing on a HiSeq2000 (Illumina Inc., San Diego, CA, USA) with 75 cycle single-end reads. The reads were aligned against human genome build hg19, and the variant calls were analyzed with the filter of dbSNP, HGMD, and NHLBI GO Exome Sequencing Project (ESP). The average sequence depth was 700× per base. All coding exons with <20× coverage were completed by PCR/Sanger sequencing to ensure 100% coverage. Sanger sequencing of the *SLC25A3* gene was also performed on the parental blood samples.

Fig. 1 Ribbon representation of human mitochondrial phosphate solute carrier (SLC25A3, NP_005879). **(a)** The overall structure of human mitochondrial phosphate solute carrier. Residues from 60 to 338 of the phosphate solute carrier are modeled using crystal structure of bovine mitochondrial ADP/ATP carrier as a template. The *top panel* is the side view, and the *lower panel* indicates the bottom view of the protein from the intermembrane space of mitochondria to the matrix. The *bottom view* illustrates the threefold symmetric organization of this protein, which is maintained through the human mitochondrial carrier superfamily. The helices and matrix loops were labeled according to the nomenclature of the original human mitochondrial ADP/ATP carrier publication. The *two dashed circles* indicate the locations of the two variants reported here. **(b)** The close-up atomic configuration of the p.L200W substitution. These hydrophobic

residues L133, I160, L180, and L200 are represented as a stick model and their carbon atoms are colored in *grey*. Substitution of W (carbon atoms in purple) for L at position 200 indicates a number of small but significant steric clashes (*red*). Moreover, the large hydrophobic side chain atoms of W are exposed to hydrophilic solvent. These two unfavorable factors contribute to intolerance of W substitution for L at this position. **(c)** The GSSAS sequence (from 296 to 300) is located at matrix loop M3 which connects H5 and H6. This segment connects transmembrane helices 5 and 6 to reinforce the closed conformation of the carrier for channel formation. As indicated by the bovine mitochondrial ADP/ATP carrier protein, there are many important residues in this loop M3 dictating nucleotide-binding phosphate stoichiometry and determining the threefold symmetric organization at the matrix side

Model Building for PiC

The initial search using full-length SLC25A3 protein sequence (NP_005879) identified the bovine mitochondrial ADP/ATP carrier as the top candidate from X-ray diffraction studies (PDB code: 1OKC at resolution of 2.20 Å). The sequence search also indicated human SLC25A3 protein shares a similar fold to other mitochondrial carrier protein from the CATH sequence domain search (www.cathdb.info). After excluding the disordered regions as predicted by the DISOPRED server (<http://bioinf.cs.ucl.ac.uk/disopred>), the sequence identity was improved to 19.1% and sequence similarity improved to

55.7% between human and bovine SLC25A3 proteins for residues 60–338. A model of human mitochondrial phosphate solute carrier was constructed based on the crystal structure of 1OKC with pGenTHREADER (<http://bioinf.cs.ucl.ac.uk>). The structure model was also subjected to energy minimization before performing structure analysis (Fig. 1a). The quality and accuracy of the stereochemical model were demonstrated with ProCheck (<http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/>) to have more than 90% favorable conformations of residues on Ramachandran plots. The key PX(D/E)XX(K/R) motif was also accurately modeled to overlap in the bovine mitochondrial ADP/ATP carrier.

Fig. 2 Next-generation sequencing result for the c.886_898delins7 (p.G296_S300delinsQIP) mutation. Thirteen nucleotides, GGTAGCAGTGCTT, at position c.886_898 were deleted and replaced by 7 new nucleotides, CAGATAC. This deletion/insertion change results in

the replacement of 5 amino acid residues, Gly-Ser-Ser-Ala-Ser with 3 amino acids, Gln-Ile-Pro, at position p.296_300 (p.G296_S300delGSSASinsQIP)

Results

Patient 1

Next-generation sequencing revealed a previously reported homozygous c.158-9A>G variant in *SLC25A3*. The c.158-9A>G is located in intron 2 which is next to exon 3A. This variant creates a novel splice site in intron 2 that leads to the inclusion of eight nucleotides on the 5' side of exon 3A, predicted to result in a frame shift and early termination in the first quarter of the protein (Mayr et al. 2011). Parental testing was not available for this patient.

Patient 2

A comprehensive next-generation sequencing analysis of 162 nuclear genes and the whole mitochondrial genome revealed two novel heterozygous variants in *SLC25A3*: c.599T>G (p.L200W) and c. 886_898delGGTAGCAGTGCTTinsCAGATAC (p.G296_S300delGSSASinsQIP) (Fig. 2). L200 is highly conserved, and the computer-based algorithms, SIFT and PolyPhen-2, predict the p.L200W substitution to be deleterious. This in frame amino acid change is categorized as an unclassified variant but is likely pathogenic. Subsequent sequence analyses of the parents indicate that the c.599T>G (p.L200W) was maternally inherited and c.886_898delinsCAGATAC (p.G296_S300delGSSASinsQIP) was paternally inherited; therefore, this patient's *SLC25A3* variants are in a transconfiguration.

Discussion

Here, we present two new unrelated patients with pathologic variants in *SLC25A3* predicted to cause dysfunction of the mitochondrial PiC. These patients are informative as only two families with variants in *SLC25A3* have been previously reported, and one of our patients has two novel variants and an expanded phenotype of cardiomyopathy without skeletal myopathy nor elevated lactate (Mayr et al. 2007; Mayr et al. 2011). In addition, these are the first patients reported who are not of Turkish descent, which indicates a larger widespread prevalence.

These patients had very distinct phenotypic presentations despite variants in the same gene. Patient 1 had a normal echocardiogram at birth but had profound respiratory failure with severe lactic acidosis. Patient 2 had prenatally diagnosed hypertrophic cardiomyopathy, which progressed to cardiac transplant, but never had any clinical signs of skeletal myopathy or lactic acidosis. There has not yet been any reported patients with dysfunction of the PiC without lactic acidosis, which is typically a finding that would lead clinicians to consider mitochondrial disorder. Our patient 2 had extensive testing, but mitochondrial disease was not initially considered as a differential diagnosis as he had isolated cardiomyopathy without lactic acidosis or skeletal myopathy.

We would also like to note the striking disconnect between the alert wakeful state of patient 1, which was felt to be the most unusual aspect of his presentation and is not

seen with more common diagnoses that cause neonatal lactic acidosis. We feel that this may be a distinguishing feature in the neonatal period between this disorder and other mitochondrial disorders that include more severe neurocognitive outcomes.

Patient 2, presented with a novel variant: c.886_898delinsCAGATAC (p.G296_S300delGSSASinsQIP), which resulted in a deletion of five amino acids GSSAS, at position p.296_300, and insertion of three amino acids, QIP. The five amino acid (GSSAS) sequences were located at matrix loop M3 which, according to the helix nomenclature of the ADP/ATP carrier, connects transmembrane helices 5 and 6. The relative threefold symmetric organization of this protein is maintained within the human mitochondrial carrier superfamily. It has been shown in the bovine ATP/ADP carrier structure that these connecting helices are positioned to be parallel to the membrane surface and their configuration reinforces the closed conformation of the carrier for channel formation on the matrix side (Fig. 1a, lower panel). There are many important residues in loop M3 that dictate nucleotide binding phosphate stoichiometry and interface with the threefold symmetric organization at the matrix side. These residues interact with the protein moiety as well as the hydrophobic membrane lipid bilayer. Thus, the replacement of flexible and small amino acids of GSSAS with a bulky glutamine and hydrophobic isoleucine is likely not tolerated (Fig. 1c).

The model of the human mitochondrial phosphate solute carrier suggests that L at position 200 is located at the small matrix helix which connects the transmembrane helices 3 and 4 according to the helix nomenclature of the ADP/ATP carrier. L200 interacts with adjacent hydrophobic residues L133, I160, and L180, which are in the close vicinity of the consensus sequence of all mitochondrial carrier proteins, PX(D/E)XX(K/R) motif. When L200 is changed to tryptophan, the bulky side chain is likely to introduce stereochemical clashes to these critical regions. Substitution of W (carbon atoms in purple) for L at position 200 indicates a number of small but significant steric clashes (red) (Fig. 1b). Moreover, the large hydrophobic side chain atoms of W are exposed to hydrophilic solvent. These two unfavorable factors contribute to intolerance of W substitution for L at this position. The motif, PMEAAK from amino acid position 182 to 197 in the phosphate carrier protein, also helps to maintain the protein structure as a channel for solute transport (PMID: 8132484). Replacement of L by a more bulky, aromatic W would be expected to interfere with the interaction among the segment containing W200, helix 3, and helix 4, thereby disrupting the structure of the channel and impeding solute transport (Figure 1b). There-

fore, we feel that this variant would be pathogenic and responsible for patient 2's presentation.

As next-generation sequencing becomes increasingly utilized for clinical diagnosis, it will allow for improved clinical management for sick patients presenting in infancy. Many life-altering choices for these patients were predicated on having an accurate diagnosis. For example, before the genetic diagnosis was ascertained, patient 1 had a do-not-resuscitate (DNR) order. Once the genetic diagnosis was discovered, we found examples in the literature of surviving children with only mild impairment; the family then removed the DNR order. For patient 2, the molecular diagnosis allowed him to be considered for cardiac transplant, as it was previously thought he may have a global mitochondrial defect that would make him ineligible.

Both of these patients were diagnosed through next-generation sequencing with commercial panels targeting a variety of mitochondrial diseases. Neither of these patients had enough distinguishing features of PiC dysfunction to make the diagnosis clinically, especially patient 2 who presented with isolated cardiomyopathy. It is interesting that this phenotype of just hypertrophic cardiomyopathy without lactic acidosis is found in the patient with the variants that affects exons 4 and 6, which would be expected to be included in all the splice forms. All of the previously reported patients and our patient 1 had mutations affecting only exon 3A found in PiC-A, which is expressed in just skeletal and cardiac muscle. Perhaps there is more global mitochondrial dysfunction leading to lactic acidosis from a disruption of the normal ratio of the PiC-A and PiC-B isoforms than from a decrease in both isoforms as would be expected by mutations in obligate exons. It has been shown that the bovine forms of PiC-A and PiC-B have different transport affinities, with PiC-A three times as high as PiC-B, but the maximal transport rate in PiC-A is three times less than PiC-B, suggesting that appropriate tissue-specific ratios of the isoforms may play a role in total-body phosphate allocation (Fiermonte et al. 1998). It is also possible that there is a higher level of retained activity with these new variants, which is leading to the milder skeletal muscle phenotype and lack of lactic acidosis.

In this report, we describe the clinical characteristics of two new patients with variants in *SLC25A3*, which codes for the mitochondrial phosphate carrier. We also report two novel mutations in exons four and six, consider the genotype-phenotype correlation of the variants, and describe the protein modeling of the novel mutation. In conclusion, we recommend that variants in the phosphate

transporter gene should be considered in a variety of conditions, including patients with isolated cardiomyopathy like our patient 2, with skeletal myopathy and lactic acidosis like our patient 1.

Synopsis

We present two new cases of apparent disruption of the mitochondrial phosphate transporter, encoded by *SLC25A3*, which has been reported to present with cardiomyopathy, skeletal myopathy, and lactic acidosis; we describe an expanded phenotype of isolated hypertrophic cardiomyopathy.

Compliance with Ethical Guidelines

Conflict of Interest

Elizabeth Bhoj, Mindy Li, Louisa Pyle, Rebecca Ahrens-Nicklas, Colleen Clarke, Lee-Jun Wong, Jing Wang, Victor Zhang, Neal Sondheimer, Can Ficicioglu, and Marc Yudkoff declare that they have no conflict of interest.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Contributions of Each Author

Bhoj EJ, Li M, Clarke C, Sondheimer N, Ficicioglu C, and Yudkoff M were involved in the clinical care and diagnosis of these patients.

Bhoj EJ, Li M, Ahrens-Nicklas R, and Pyle LC were involved in writing the manuscript.

Wang J, Zhang VW and Wong LJ were involved in the sequencing and protein modeling.

References

- Cizkova A, Stranecky V et al (2008) TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. *Nat Genet* 40(11):1288–1290
- Dolce V, Fiermonte G et al (1996) Tissue-specific expression of the two isoforms of the mitochondrial phosphate carrier in bovine tissues. *FEBS Lett* 399(1–2):95–98
- Fiermonte G, Dolce V, Palmieri F et al (1998) Expression in *Escherichia coli*, functional characterization, and tissue distribution of isoforms A and B of the phosphate carrier from bovine mitochondria. *J Biol Chem* 273:2782–2787
- Mayr JA, Merkel O et al (2007) Mitochondrial phosphate-carrier deficiency: a novel disorder of oxidative phosphorylation. *Am J Hum Genet* 80(3):478–484
- Mayr JA, Havlickova V et al (2010) Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit. *Hum Mol Genet* 19(17):3430–3439
- Mayr JA, Zimmermann FA et al (2011) Deficiency of the mitochondrial phosphate carrier presenting as myopathy and cardiomyopathy in a family with three affected children. *Neuromuscul Disord* 21(11):803–808
- Pitceathly RD, Murphy SM et al (2012) Genetic dysfunction of MT-ATP6 causes axonal Charcot-Marie-Tooth disease. *Neurology* 79(11):1145–1154
- Stappen R, Kramer R (1994) Kinetic mechanism of phosphate/phosphate and phosphate/OH⁻ antiports catalyzed by reconstituted phosphate carrier from beef heart mitochondria. *J Biol Chem* 269(15):11240–11246
- Tort F, Del Toro M et al (2011) Screening for nuclear genetic defects in the ATP synthase-associated genes TMEM70, ATP12 and ATP5E in patients with 3-methylglutaconic aciduria. *Clin Genet* 80(3):297–300