Expanding the Clinical Spectrum of Mitochondrial Citrate Carrier (SLC25A1) Deficiency: Facial Dysmorphism in Siblings with Epileptic Encephalopathy and Combined D,L-2-Hydroxyglutaric Aciduria

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Abstract Recessive mutations in *SLC25A1* encoding mitochondrial citrate carrier cause a rare inherited metabolic disorder, combined D,L-2-hydroxyglutaric aciduria (D,L-2-HGA), characterized by epileptic encephalopathy, respiratory insufficiency, developmental arrest and early death. Here, we describe two siblings compound heterozygotes for null/missense *SLC25A1* mutations, c.18_24dup (p.Ala9Profs*82), and c.134C>T (p.Pro45Leu). These children presented with classic clinical features of D,L-2-HGA, but also showed marked facial dysmorphism. Additionally, there was prominent lactic acidosis in one of the siblings. Our observations suggest that facial dysmorphism is a previously unrecognized but an important diagnostic

feature of *SLC25A1* deficiency and expand the clinical phenotype linked to *SLC25A1* mutations.

Introduction

Combined D-2- and L-2-hydroxyglutaric aciduria (D,L-2-HGA; OMIM:615182) is a rare inborn error of metabolism characterized by elevated levels of both D-2- and L-2-hydroxyglutaric acids (HG) in body fluids which typically manifests as severe neonatal epileptic encephalopathy, respiratory insufficiency, global hypotonia, lack of developmental progress, and early death. Deficiency of the mitochondrial citrate carrier as a result of recessive mutations in the *SLC25A1* gene was recently recognized as the underlying cause of D,L-2-HGA (Nota et al. 2013). *SLC25A1* belongs to the inner mitochondrial membrane transporters of SLC25 family and promotes the efflux of citrate/isocitrate from mitochondria to cytoplasm (Gutiérrez-Aguilar and Baines 2013; Palmieri 2013, 2014). It plays key roles in mitochondrial function, lipid biosynthesis, and inhibition of glycolysis (Catalina-Rodriguez et al. 2012). The origin of elevated D and L enantiomers of 2-HG due to *SLC25A1* deficiency is still unclear, but has been attributed to accumulation of citrate and other tricarboxylic acid (TCA) cycle intermediates in the mitochondria including 2-ketoglutarate (2-KG) which in turn is converted to D and L, 2-HG (Kranendijk et al. 2012). We report two siblings with classic clinical features of D,L-2-HGA, and additionally facial dysmorphism expanding the phenotype of *SLC25A1* deficiency. Also, there was marked lactic

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acidosis in one of the siblings. These observations suggest the importance of testing for SLC25A1 mutations and/or 2-HG enantiomers in patients with facial dysmorphism, encephalopathy, and lactic acidosis.

Case Reports

The patients were siblings born from healthy nonconsanguineous Hispanic parents. Patient 1 was born at full term via cesarean section. Global hypotonia and facial dysmorphism consisting of hypertelorism, broad depressed nasal bridge, micrognathia, and retrognathia were noted at birth. The patient developed encephalopathy and seizures at approximately 24 h of life. The patient had to be intubated for respiratory failure and altered mental status. Subsequently, the patient remained ventilator dependent. Magnetic resonance imaging of the brain showed diffuse atrophy and dilation of both ventricles. The patient died at 2 months of age.

Patient 2 was born at term by spontaneous vaginal delivery and also exhibited hypertelorism, broad depressed nasal bridge, micrognathia, and retrognathia, accompanied by bilateral ptosis and global hypotonia. The patient had respiratory failure and seizures with encephalopathy on day 1 of life. Intubation of this patient for respiratory failure was difficult due to narrow anteriorly placed vocal cords. This patient too remained ventilator dependent. An ultrasound of the head revealed bilateral ventriculomegaly and increase in extra axial space in the right frontal region. Echocardiography showed ventricular and atrial septal defects, patent ductus arteriosus, and bicommissural aortic valve. The patient died at 3 weeks of age.

Molecular and Metabolic Studies

A chromosomal microarray (performed using the Affymetrix Cytoscan HD array; this array consisted of nearly 2.7

million genetic markers incorporating 743,304 single nucleotide polymorphism probes as well as 1,953,246 nonpolymorphic copy number variation probes) for both patients was normal. Blood lactate of patient 1 on day 8 of life was 3.6 mmol/L (reference range 0.6–2.5). Blood lactate of patient 2 was initially 9.8 mmol/L (day 1 of life) and increased to 25 mmol/L on day 5. Plasma amino acids and acylcarnitine profile assays for both patients were normal. Urine organic acid assay for patient 1 showed elevated 2-HG, lactate, and fumarate. Further analysis of the urine for the differentiation of D and L, 2-HG enantiomers by liquid chromatography tandem mass spectrometry (LC-MS/MS) (Struys et al. 2004) showed elevations of both metabolites (Table 1). Initial qualitative urine organic acid analysis for patient 2 did not show an elevation of 2-HG, while further analysis by LC-MS/MS showed mild to modest elevations in four of five urine samples. Urinary citrate was low for patient 2 (Table 1).

Both patients were compound heterozygous for the mutations c.18_24dup (p.Ala9Profs*82) and c.134C>T (p.Pro45Leu) in *SLC25A1*. The mutational status of patient 1 was previously reported in the study by Nota et al., which first identified *SLC25A1* mutations in D,L-2-HGA (Nota et al. 2013). The mother was heterozygous for c.134C>T mutation, while the father was heterozygous for c.18_24dup.

Discussion

Only few cases of mitochondrial citrate carrier deficiency have been reported in the literature thus far (Chaouch et al. 2014; Edvardson et al. 2013; Nota et al. 2013). The hallmark biochemical alteration caused by *SLC25A1* mutations is D,L-2-hydroxyglutaric aciduria, accompanied by higher urinary concentrations of 2-KG and variable elevations of other TCA cycle intermediates (succinate,

Table 1 Urinary concentrations of D and L, 2-hydroxyglutaric acids (2-HG) and citrate

Patient	Day of life	D-2-HG (mmol/mol creatinine)	L-2-HG (mmol/mol creatinine)	Citrate (mmol/mol creatinine)
Control		2.8–17	1.3–19	72–1,449
Patient 1	20	449 ^a	110 ^a	ND
Patient 2	1	29 ^a	40 ^a	ND
	3	11	17	ND
	9	61 ^a	35 ^a	ND
	12	57 ^a	30 ^a	19
	17	84 ^a	38 ^a	33

^a Indicates elevations compared with the reference range. Please note normal concentrations in the sample obtained on day of life 3 for patient 2
ND not determined

fumarate, and malate) (Kranendijk et al. 2012). These patients typically present with severe neonatal epileptic encephalopathy, respiratory insufficiency, hypotonia, and developmental arrest. However, a relatively milder presentation as congenital myasthenia has also been reported (Chaouch et al. 2014).

Our described siblings carried compound heterozygous mutations in *SLC25A1* that generate early truncated (p.Ala9Profs*82) and missense (p.Pro45Leu) alleles. It was shown previously that the patient homozygous for p.Ala9Profs*82 had no detectable SLC25A1 on immunoblot analysis of fibroblasts (Nota et al. 2013). Pro45 residue is highly conserved throughout evolution in all SLC25A1 members, and the 134 C>T transition has an unfavorable score (Pierri et al. 2014). From a structural point of view, it belongs to the region involved in conformational changes of the citrate carrier occurring during the translocation mechanism. Therefore, this substitution is likely to affect the transport rate of citrate (Palmieri and Pierri 2010).

The clinical presentation of these patients is typical for D,L-2-HGA. The hallmark biochemical alteration of increased D and L, 2-HG enantiomers in urine was also present in these children, although not persistently in one of the siblings. The facial dysmorphism present in both siblings is a hitherto unrecognized feature of this disease. One of the siblings had severe lactic acidosis and cardiac malformations. Our findings suggest that in newborns with facial dysmorphism, multiple malformations, epileptic encephalopathy, and lactic acidosis, SLC25A1 deficiency should be suspected. The initial qualitative urine organic assay in the younger sibling was normal during the acute presentation implying that excretion of these metabolites may be intermittent or may potentially be overlooked when using the traditional urinary organic acid screening method. A dedicated analytical procedure that is able to quantify both enantiomers of 2-HG and *SLC25A1* sequencing is required to confirm the diagnosis.

The severity of the clinical findings in these siblings is likely accounted for by the key roles played by SLC25A1 in carbohydrate and lipid metabolism and in promoting mitochondrial function (Catalina-Rodriguez et al. 2012). Citrate is produced predominantly in mitochondria and is oxidized via the TCA cycle and oxidative phosphorylation (OXPHOS). The entry of malate (another TCA cycle intermediate) in exchange for citrate is coupled with the transport of one proton to maintain electroneutrality across the mitochondrial membrane (Palmieri 2013). Thus, the mitochondrial citrate transporter maintains the integrity of the TCA cycle as well as mitochondrial inner membrane potential, the stability of which is linked to proton flux and electron transport chain activity. In the cytoplasm, citrate functions as an allosteric inhibitor of the glycolytic enzyme, phosphofructokinase-1 (Newsholme

Fig. 1 Cytoplasmic and mitochondrial effects due to SLC25A1-dependent citrate export. See also text for explanation. In the cytoplasm, citrate promotes fatty acid (FA) synthesis. Malate oxidation in the mitochondria generates NADH, which donates its electrons to the electron transport chain thereby promoting oxidative phosphorylation (OXPHOS). Malate/citrate shuttle is also crucial in stabilizing mitochondrial membrane potential (MMP). Cytoplasmic citrate acts as an allosteric inhibitor of the glycolytic enzyme phosphofructokinase-1. Pyruvate can either be converted to lactate in anaerobic glycolysis or enter the mitochondria where through the action of pyruvate dehydrogenase (PDH) is converted to acetyl coenzyme A (not shown) which then binds with oxaloacetate to form citrate. In red are the major input and output pathways linked to SLC25A1 activity

et al 1977). Therefore, it is likely that when SLC25A1 is severely compromised, glycolysis proceeds unchecked and mitochondrial respiration is impaired leading to lactic acidosis (Fig. 1). The high lactate, global hypotonia, and encephalopathy observed in patients with SLC25A1 deficiency are similar to clinical findings in patients with mitochondrial encephalomyopathies caused by respiratory chain defects.

SLC25A1 also plays a crucial role in lipogenesis (Gnoni et al. 2009; Iacobazzi and Infantino 2014; Watson and Lowenstein 1970). Studies on skin fibroblasts from patients with SLC25A1 deficiency showed low citrate in culture medium (Nota et al. 2013). Similar observation in cell lines suggests that the cytoplasmic pool of citrate is maintained by the mitochondrial citrate carrier (Catalina-Rodriguez et al. 2012). Cytosolic citrate is cleaved by citrate lyase into acetyl-CoA – the main carbon source for fatty acid and cholesterol biosynthesis – and oxaloacetate (Gnoni et al. 2009; Iacobazzi and Infantino 2014; Watson and Lowenstein 1970). Cytoplasmic citrate is a positive allosteric modulator of acetyl-CoA carboxylase, a key enzyme in fatty acid synthesis (Halestrap and Denton 1974; Iacobazzi and Infantino 2014).

The etiology of facial dysmorphism and other developmental malformations is unclear. There is inadequate

phenotypic description of previous patients with D,L-2-HGA in the literature. One patient with D,L-2-HGA reported to have dysmorphism was initially diagnosed as D-2-hydroxyglutaric aciduria (D-2-HGA) (Kranendijk et al. 2012). Inhibition of the mitochondrial citrate carrier in zebrafish showed reduced jaw and small brain (Catalina-Rodriguez et al. 2012) implicating SLC25A1 deficiency in the dysmorphism and developmental defects seen in these patients. It may be secondary to abnormal epigenetic control of nuclear gene expression. Levels of histone acetylation are dependent upon availability of acetyl-CoA which is produced from cleavage of citrate exported from the mitochondria (Wellen et al. 2009). Histone acetylation plays essential roles in cell cycle progression, gene expression, and silencing. Inhibition of the drosophila ortholog of *SLC25A1* resulted in extensive chromosomal breakage, cell cycle arrest, and global reduction in histone acetylation (Morciano et al. 2009). Facial dysmorphism and brain malformations are also recognized features of D-2-HGA (Kranendijk et al. 2012). Accumulation of D-2-HG in both these conditions may be a causative factor as it acts as a competitive inhibitor of 2-KG (a TCA cycle intermediate)-dependent enzymes including histone demethylase and prolyl hydroxylase leading to global alteration in histone and DNA methylation patterns (Xu et al. 2011). Epigenetic dysregulation leading to disruption of embryogenesis is the most plausible mechanism for malformations in D,L-2-HGA. However, it may also be the result of impaired lipogenesis, particularly cholesterol synthesis. Agenesis of corpus callosum and optic nerve hypoplasia, previously reported in a patient with SLC25A1 deficiency (Edvardson et al. 2013), and the dysmorphism observed in our patients are reminiscent of patients with Smith-Lemli-Opitz syndrome and other defects of cholesterol biosynthesis (Herman and Kratz 2012). Interestingly, heterozygous deletion of the entire *SLC25A1* gene occurs in 22q.11.2 microdeletion syndrome which shares many of the facial features of these patients. However, the facial features of the parents of these patients were unremarkable. Loss of single copy of *SLC25A1* by itself is unlikely to be of any major consequences. However, its modifying role cannot be denied. This aspect needs to be further explored.

In summary, our observations suggest that facial dysmorphism and lactic acidosis are important diagnostic markers of SLC25A1 deficiency along with neonatal epileptic encephalopathy, global hypotonia, and D,L-2-HGA. An early diagnosis may be helpful from a therapeutic perspective as citrate therapy was found to reduce the frequency and severity of seizures in a patient with mitochondrial citrate carrier deficiency (Mühlhausen et al. 2014).

Compliance with Ethics Guidelines

This clinical report is a retrospective clinical observation that does not require ethics committee approval at this institution.

There are no prior publications of this manuscript.

The work is not and will not be submitted to any other journal while under consideration by "JIMD."

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Dr. Maria Laura Avantaggiati was involved in laboratory interpretation, drafting of cartoon, and revising the manuscript critically for important intellectual content.

Dr. Marie McDonald supervised the case report and was involved in patient care, laboratory interpretation, and revising the manuscript critically for important intellectual content.

References

- Catalina-Rodriguez O, Kolukula VK, Tomita Y et al (2012) The mitochondrial citrate transporter, CIC, is essential for mitochondrial homeostasis. *Oncotarget* 3:1220–1235
- Chouch A, Porcelli V, Cox D et al (2014) Mutations in the mitochondrial citrate carrier SLC25A1 are associated with impaired neuromuscular transmission. *J Neuromuscul Dis* 1:75–90

- Edvardson S, Porcelli V, Jalas C et al (2013) Agenesis of corpus callosum and optic nerve hypoplasia due to mutations in SLC25A1 encoding the mitochondrial citrate transporter. *J Med Genet* 50:240–245
- Gnoni GV, Priore P, Geelen MJ, Siculella L (2009) The mitochondrial citrate carrier: metabolic role and regulation of its activity and expression. *IUBMB Life* 61:987–994
- Gutiérrez-Aguilar M, Baines CP (2013) Physiological and pathological roles of mitochondrial SLC25 carriers. *Biochem J* 454:371–386
- Halestrap AP, Denton RM (1974) Hormonal regulation of adipose-tissue acetyl-Coenzyme A carboxylase by changes in the polymeric state of the enzyme. The role of long-chain fatty acyl-Coenzyme A thioesters and citrate. *Biochem J* 142:365–377
- Herman GE, Kratz L (2012) Disorders of sterol synthesis: beyond Smith-Lemli-Opitz syndrome. *Am J Med Genet C Semin Med Genet* 160C:301–321
- Iacobazzi V, Infantino V (2014) Citrate-new functions for an old metabolite. *Biol Chem* 395:387–399
- Kranendijk M, Struys EA, Salomons GS, Van der Knaap MS, Jakobs C (2012) Progress in understanding 2-hydroxyglutaric acidurias. *J Inherit Metab Dis* 35:571–587
- Morciano P, Carrisi C, Capobianco L et al (2009) A conserved role for the mitochondrial citrate transporter Sea/SLC25A1 in the maintenance of chromosome integrity. *Hum Mol Genet* 18:4180–4188
- Mühlhausen C, Salomons GS, Lukacs Z et al (2014) Combined D2-/L2-hydroxyglutaric aciduria (SLC25A1 deficiency): clinical course and effects of citrate treatment. *J Inherit Metab Dis* 37:775–781
- Newsholme EA, Sugden PH, Williams T (1977) Effect of citrate on the activities of 6-phosphofructokinase from nervous and muscle tissues from different animals and its relationships to the regulation of glycolysis. *Biochem J* 166:123–129
- Nota B, Struys EA, Pop A et al (2013) Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. *Am J Hum Genet* 92:627–631
- Palmieri F (2013) The mitochondrial transporter family SLC25: identification, properties and physiopathology. *Mol Aspects Med* 34:465–484
- Palmieri F (2014) Mitochondrial transporters of the SLC25 family and associated diseases: a review. *J Inherit Metab Dis* 37:565–575
- Palmieri F, Pierri CL (2010) Structure and function of mitochondrial carriers – role of the transmembrane helix P and G residues in the gating and transport mechanism. *FEBS Lett* 584:1931–1939
- Pierri CL, Palmieri F, De Grassi A (2014) Single-nucleotide evolution quantifies the importance of each site along the structure of mitochondrial carriers. *Cell Mol Life Sci* 71:349–364
- Struys EA, Jansen EE, Verhoeven NM, Jakobs C (2004) Measurement of urinary D- and L-2-hydroxyglutarate enantiomers by stable-isotope-dilution liquid chromatography-tandem mass spectrometry after derivatization with diacetyl-L-tartaric anhydride. *Clin Chem* 50:1391–1395
- Watson JA, Lowenstein JM (1970) Citrate and the conversion of carbohydrate into fat. Fatty acid synthesis by a combination of cytoplasm and mitochondria. *J Biol Chem* 245:5993–6002
- Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* 324:1076–1080
- Xu W, Yang H, Liu Y et al (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α -ketoglutarate-dependent dioxygenases. *Cancer Cell* 19:17–30