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ORIGINAL ARTICLE

A *SLC39A8* variant causes manganese deficiency, and glycosylation and mitochondrial disorders

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Summary *SLC39A8* variants have recently been reported to cause a type II congenital disorder of glycosylation (CDG) in patients with intellectual disability and cerebellar atrophy. Here we report a novel *SLC39A8* variant in siblings with features of Leigh-like mitochondrial disease. Two sisters born to consanguineous Lebanese parents had profound developmental delay, dystonia, seizures and failure to thrive. Brain MRI of both siblings identified bilateral basal ganglia hyperintensities on T2-weighted imaging and cerebral atrophy. CSF lactate was elevated in patient 1 and normal in patient 2. Respiratory chain enzymology was only performed on patient 1 and revealed complex IV and II + III activity was low in liver, with elevated complex I activity. Complex IV activity was borderline low in patient 1 muscle and pyruvate dehydrogenase activity was reduced. Whole genome sequencing identified a homozygous Chr4(GRCh37):g.103236869C>G; c.338G>C; p.(Cys113Ser) variant in *SLC39A8*, located in one of eight regions identified by homozygosity mapping. *SLC39A8* encodes a manganese and zinc transporter which localises to the cell and mitochondrial membranes. Patient 2 blood and urine manganese levels were undetectably low. Transferrin electrophoresis of patient 2 serum revealed a type II CDG defect. Oral supplementation with galactose and uridine led to improvement of the transferrin isoform pattern within 14 days of treatment initiation. Oral manganese

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has only recently been added to the treatment. These results suggest SLC39A8 deficiency can cause both a type II CDG and Leigh-like syndrome, possibly via reduced activity of the manganese-dependent enzymes β-galactosyltransferase and mitochondrial manganese superoxide dismutase.

Introduction

Mitochondrial respiratory chain disorders are a heterogeneous group of disorders that can be difficult to diagnose. Leigh syndrome (OMIM #256000) is the most common childhood presentation, and is characterised by progressive neurodegeneration with brainstem and/or basal ganglia dysfunction, intellectual and motor developmental delay, elevated serum or cerebrospinal fluid (CSF) lactate, and mitochondrial respiratory chain and/or pyruvate dehydrogenase complex deficiency (Lake et al 2016). Patients with atypical features are classified as having Leigh-like syndrome. Mutations in over 75 genes have now been reported as causes of Leigh syndrome (Lake et al 2016). These genes may be in the mitochondrial or nuclear genomes or encode proteins with a mitochondrial function, although a few cases have been reported in genes which were previously unknown to have a role in mitochondrial function. Some of the Leigh syndrome disease genes have no known role in the activity of mitochondrial respiratory chain complexes or the PDH complex but appear to indirectly affect activity (Lake et al 2016). Despite the large number of genes already associated with Leigh syndrome, many cases remain without a genetic diagnosis (Lake et al 2016) indicating that variants in as yet unidentified genes are likely to be responsible.

In this study, we used whole genome sequencing (WGS) to identify the genetic cause of Leigh-like syndrome in two siblings with profound intellectual disability, T2-weighted bilateral symmetrical basal ganglia hyperintensities, cerebral atrophy, and deficiency of respiratory chain complexes IV and II + III in patient 1 liver and borderline low complex IV in muscle. We identified a novel variant in *SLC39A8* that encodes a zinc and manganese transporter. Previously reported cases of SLC39A8 deficiency resulted in a type IIn congenital disorder of glycosylation (OMIM #616721), with no reported mitochondrial involvement. In these patients, manganese deficiency results in reduced functioning of a manganese-dependent β-galactosyltransferase (EC 2.4.1.38). We propose that the mitochondrial dysfunction in our patients may result from reduced activity of the mitochondrial free radical scavenger manganese superoxide dismutase (MnSOD; EC 1.15.1.1) and that *SLC39A8* variants may cause a Leigh-like syndrome.

Materials and methods

Clinical information

Two sisters born to consanguineous (double first cousin) healthy Lebanese parents displayed similar presentations with profound developmental delay, dystonia, seizures, feeding difficulties and failure to thrive.

Patient 1: The female proband was born at 41 weeks gestation following an uncomplicated pregnancy with a birth weight of 3.61 kg and Apgar scores of 8 and 9 at 1 and 5 min respectively. Parental concerns were initially raised at 4 months of age when she had not reached age appropriate milestones and displayed floppiness. Medical consultation was sought at 13 months of age due to additional features of back arching and involuntary stiffening of limbs, and feeding difficulties of approximately 4 months' duration. Clinically, she was globally delayed, with a weight <3rd percentile, and had dysmorphic features including a broad forehead, hirsutism, anteverted nostrils, thin lips and a smooth philtrum. No overt skeletal abnormalities or ophthamological abnormalities were noted. Limb hypertonia with intermittent opisthotonic posturing was present. Brain MRI showed cerebral atrophy and bilateral basal ganglia hyperintensities on T2-weighted imaging. At 13 months, laboratory investigations included normal plasma amino acids, plasma acylcarnitines, very long chain fatty acids, white cell lysosomal enzymes, karyotyping, urine organic acids, amino acids, glycosaminoglycans and blood lactate. CSF lactate was 4.2 mmol/L (reference range <1.9). Brainstem auditory evoked potentials were normal. An electroencephalogram (EEG) was normal. Electromyography (EMG) showed evidence of denervation on the right tibialis anterior with fibrillation potentials at rest. Nerve conduction velocities (NCV) were absent for all sensory nerves studied including the right median, ulnar and sural nerves. These findings were consistent with an axonal neuropathy. Muscle histology identified atrophic fibres in all fasciculi with some subsarcolemmal lipid accumulation. No ragged-red or cytochrome oxidase negative fibres were present. Selected mitochondrial DNA (mtDNA) point mutation analysis (m.3243A>G, m.8344A>G, m.8993 T>G, m.8993 T>C) in skeletal muscle was normal. The fibroblast acylcarnitine profile showed a moderate elevation in butyrylcarnitine, which has previously been observed in patients with mitochondrial respiratory chain defects (Sim et al 2002).

Oral coenzyme Q10 was commenced at 10 mg QID due to reduced CII + CIII activity in liver (Table 1; coenzyme Q10 testing was not available), with additional multivitamin supplementation. She was also on oral cisapride and cimetidine for gastro-oesophageal reflux, and clobazam for seizures. At 19 months, she had shown minor developmental gains, having started babbling 2 to 3 months prior. She remained severely delayed in all other aspects and clinically displayed marked

Table 1 Respiratory chain enzyme activities for patient 1 expressed relative to protein and relative to citrate synthase (CS)

	Enzyme activity			CS ratio		
Assay	P1 fibroblasts	P1 muscle	P1 liver	P1 fibroblasts	P1 muscle	P1 liver
Complex I (nmol/min/mg)	34 (34–141)	33 (19–90)	**45** (10–21)	227 (180–520)	284 (100–470)	**1023** (270–570)
Complex II (nmol/min/mg)	46 (22–100)	21 (16–56)	98 (60–125)	307 (153–390)	181 (110–330)	2227 (1400–3200)
Complex II + III (nmol/min/mg)	41 (42–122)	25 (19–66)	**6.3** (15–30)	273 (135–510)	216 (115–460)	**143** (440–860)
Complex III (/min/mg)	**4.0** (4.9–28)	14 (14–67)	–	27 (25–112)	121 (100–300)	–
Complex IV (/min/mg)	2.7 (1.1–11.6)	1.8 (1.0–10.9)	**0.5** (1.1–1.6)	18 (12–46)	16 (12–64)	**11** (25–48)
Citrate synthase (nmol/min/mg)	150 (87–322)	116 (76–250)	44 (32–42)	–	–	–

Values outside the normal range are shown in bold. Reference ranges are shown in brackets

hypertonicity with significant back arching, scissoring and ankle clonus. Oral dichloroacetate was trialled at 75 mg/kg/day with oral thiamine at 1 mg/kg/day. Brainstem auditory evoked potentials performed at 21 months were broadly within normal limits, though it was reported that it was difficult to identify wave I on the right. A repeat study conducted at 24 months showed a mild increase in I-V interpeak latency bilaterally, with definite prolongation on the right, suggesting a disturbance of brainstem conduction. She died at 26 months of age during an intercurrent infection.

Patient 2: Six years after the birth of a healthy male, a second affected female sibling (patient 2) was born after an uneventful pregnancy. Immediately after delivery, mild tachypnoea necessitated brief supplemental oxygen. She fed well postnatally and was discharged home at 2 days of age. Early developmental milestones were reportedly normal, however by 3 months of age she had poor head control, increased peripheral tone, dystonia and episodes of eye rolling. She weighed 5.64 kg (10th–25th percentile), length was 57.1 cm (<3rd percentile) and head circumference was 42.0 cm (50th–75th percentile). She had a prominent forehead and blue sclerae. An EEG at that time was normal, however she was commenced on oral pyridoxine, carbidopa-levodopa, clobazam, and omeprazole for gastro-oesophageal reflux. Global psychomotor retardation was evident by 6 to 7 months of age. At 7 months, blood gas analysis performed during an admission showed a lactate of 8.7 mmol/L (ref range 0.7–2.0) and a repeat level done within several hours was 4.3 mmol/L. Whole blood lactate analysed approximately 30 mins after the latter sample was normal (1.2 mmol/L). Creatine kinase, ammonia, CSF glucose, lactate and neurotransmitters were all normal. Urine organic acid analysis showed a slight increase in 3-methylglutaconate. Lactate was not increased in the urine. Liver function was normal apart from a mildly increased alanine transaminase (ALT) 113 U/L (ref range 10–50). An MRI of the brain (Fig. 1) demonstrated bilateral symmetrical T2 hyperintensity in the basal ganglia, especially the globus pallidus and putamen with hypointensity on T1, consistent with mitochondrial dysfunction. Mild cerebral atrophy was present without cerebellar or brainstem atrophy. In infancy, seizures were initially controlled with clobazam, but lamotrigine needed to be added at 18 months of age. Over the years she remained profoundly globally delayed, subsequently developing scoliosis and obstructive sleep apnoea secondary to profuse oropharyngeal secretions, small jaw, mid-face hypoplasia and adenoid hypertrophy. Tonsillectomy and adenoidectomy was carried out to alleviate her stridor, but only offered temporary relief. A sleep study demonstrated a predominant central sleep apnoea with a significant degree of obstruction. Bi-level non-invasive ventilator (BIPAP) support was trialled but not tolerated by the patient. She had poor weight gain due to feeding difficulties. Gastrostomy and fundoplication were performed at 3 years of age. Recurrent hospital admissions occurred due to respiratory compromise from pooling of oral secretions and aspirations. An EEG was repeated at 5 years of age and showed a diffuse encephalopathy, however reports of upward eye deviation were not associated with epileptic activity. Dystonic posturing increased over the years, improving with the addition of chloral hydrate. At 8 years of age she was 107.8 cm tall (<3rd percentile). X-ray of her lower extremities performed due to suspected hip joint pain demonstrated diffuse osteopenia and bilateral coxa valga. Increasing stridor necessitated a mandibular tug procedure which was performed at 8 years of age. Persistent multilevel airway obstruction with significant velopharyngeal collapse, glossoptosis, lingual tonsillar hypertrophy, laryngomalacia and restrictive lung disease necessitated major surgery with palatoplasty, tongue base reduction and supraglottoplasty at 11 years. Postoperatively, she proved difficult to extubate, however subsequent dramatic improvements were noted with her breathing and sleep pattern. When last reviewed at 12 years of age, she remained profoundly delayed, having no discernible speech and occasionally communicating through gestures. Clinically, she had intermittent divergent strabismus, mid-face hypoplasia, short stature, thoracolumbar kyphoscoliosis and weak neck muscles requiring head support to minimise obstructed breathing. She displayed dystonic posturing with intermittent stiffening of limbs and was confined to a wheelchair, unable to sit without support. Bone mineral densitometry revealed decreased bone mineral content for bone area and lean

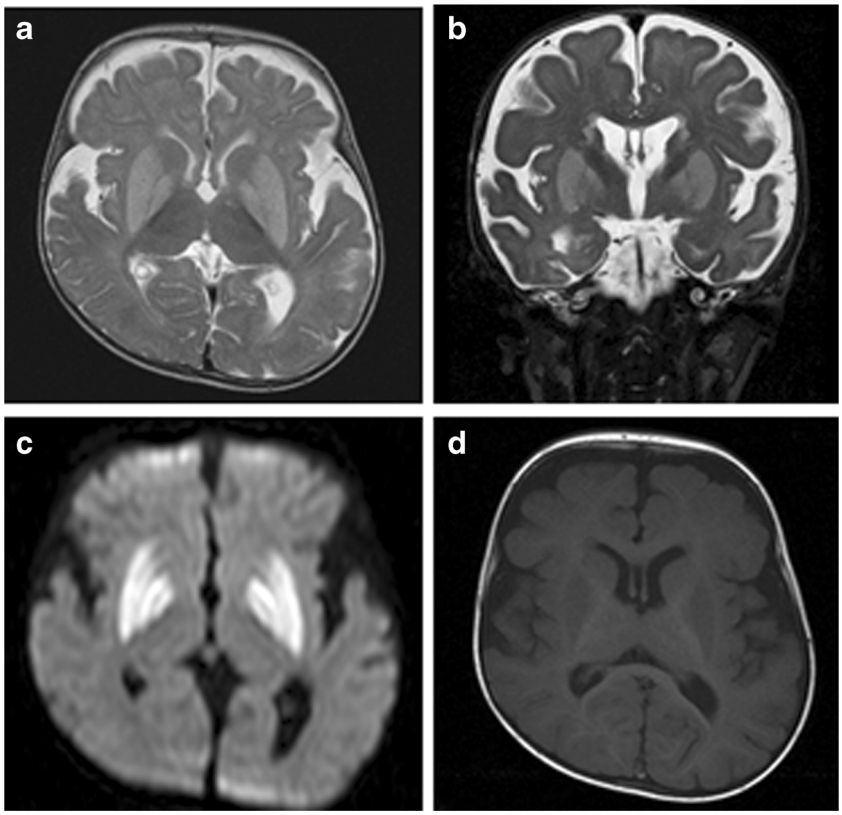


Fig. 1 Brain MRI of patient 2 at ~7 months old. **a** high signal intensity on T2 axial view of bilateral globus pallidus and putamen; **b** high signal intensity on T2 coronal view of bilateral globus pallidus, putamen and head of caudate; **c** diffusion restriction on axial view in the putamen and globus pallidus; **d** low signal intensity on T1 axial view of bilateral globus pallidus and putamen. Note also the brain atrophy. Cerebellar size and structure was normal (images not shown)

tissue mass. Laboratory investigations including thyroid function testing, coagulation profile, factor VII, factor XII, antithrombin III and protein S were all normal. Protein C was only marginally decreased at 64% (ref range 65–127%).

Oral therapy with galactose (Link Pharmaceuticals) was initiated at 12 years 8 months of age at a dose of 0.5 g/kg/day for the first 3 days, and subsequently increased to 1 g/kg/day. Simultaneous to the commencement of galactose, oral uridine (Link Pharmaceuticals) was supplemented at 120 mg/kg/day. Both were continued for 2 weeks before repeat transferrin electrophoresis was performed (Fig 2b). Oral manganese (Musashi ZMA capsules) was added next at 1.5 mg daily. Her dietary intake of manganese was estimated to be approximately 5 mg daily and the RDI for her age is 1.6 mg daily.

Respiratory chain enzyme activities

Respiratory chain enzyme activities were determined in patient 1 as previously described (Frazier and Thorburn 2012).

Genotyping and linkage analysis

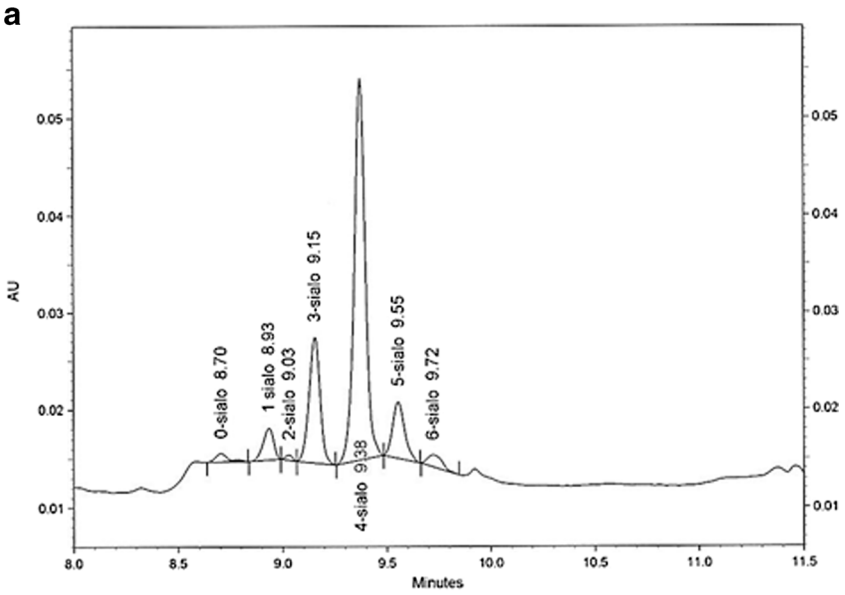
The affected siblings, their parents and unaffected brother were genotyped. Genome-wide SNP analysis was performed by the Australian Genome Research Facility (Melbourne, Australia) using Illumina Human 610-Quad SNP chips. Data files for linkage analysis were generated using LINKDATAGEN (Bahlo and Bromhead 2009) as previously described and parametric analysis performed using Merlin (Abecassis and Wiggington 2005). A completely penetrant autosomal recessive model was used with a rare disease allele frequency =0.0001. A list of candidate mitochondrial genes was generated from MitoCarta (Pagliarini et al 2008).

Whole genome sequencing

Whole genome sequencing was performed at the Kinghorn Centre for Clinical Genomics (Garvan Institute, Sydney) on genomic DNA extracted from blood of patient 2 and her parents. WGS sequencing libraries were prepared using Illumina TruSeq Nano HT v2.5 sample preparation kits and sequenced one lane per sample, on Illumina HiSeq X ten sequencers, via 2 × 150 bp reads, with >110Gb data per lane, >75% bases with at least Q30 base quality, and >30× mean coverage. At this coverage, 95% of the nuclear genome was covered to >15× depth. Reads were aligned to the b37d5 reference genome using BWA MEM v0.7.10, sorted using novosort v1.03.01, then realigned around known indels, and base quality scores recalibrated using GATK v3.3. Variants were identified using

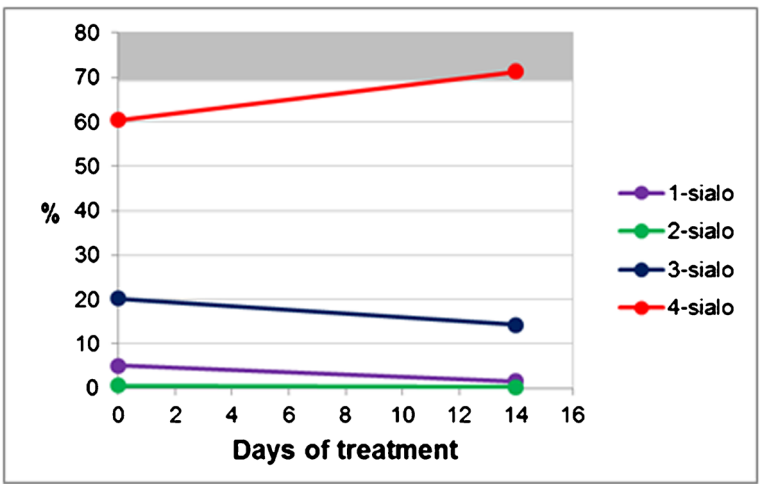
Fig. 2 Capillary electrophoresis of patient 2 serum transferrin isoforms. **a** tetrasialo-transferrrin levels are below the reference range; **b** tetrasialo-transferrin levels normalise post oral galactose and uridine supplementation for 14 days

a



Transferrin isoform	Migration time (min)	Corrected area (%)	Reference range (%)
0-sialo	8.70	1.67	
1-sialo	8.93	5.11	0.00-0.01
2-sialo	9.03	0.56	0.63-1.86
3-sialo	9.15	20.25	1.64-8.55
4-sialo	9.38	60.44	69.53-78.98
5-sialo	9.55	9.56	
6-sialo	9.72	2.40	

b



GATK HaplotypeCaller v3.3 and GenotypeGVCFs, and variant filters established using VQSR. Variants were annotated using VEP v79, converted into a database using Gemini v0.11.0 (Paila et al 2013). Variants were filtered using Seave, an in house variant filtration platform. Sanger sequencing was used to confirm variants in all family members.

Subsequent biochemical investigations

Further biochemical investigations were performed to confirm the pathogenicity of the identified variant. Serum transferrin isoforms from patient 2 were analysed by capillary electrophoresis using the Analis CEofix CDT kit (Legros et al 2002). The method employs dynamic coating of the capillary with an initiator (Tris-phosphate buffer pH 2.0), followed by separation at 28 kV in a Tris-borate buffer pH 8.5. The transferrin isoforms were detected at 200 nm. Manganese, zinc and cadmium levels were tested in whole blood and urine, and RBC glutathione levels determined.

Results

We investigated the cause of profound developmental delay, dystonia, seizures, failure to thrive and feeding difficulties in two children from a consanguineous Lebanese family. These clinical features together with the brain MRI findings of T2-weighted bilateral basal ganglia hyperintensities (Fig. 1) were consistent with a mitochondrial respiratory chain disorder.

Respiratory chain enzyme activity results confirmed the presence of a respiratory chain enzyme deficiency in patient 1. Respiratory chain enzymes in skeletal muscle homogenate were suggestive of an isolated complex IV defect with borderline low activity of complex IV (48% of control mean, relative to citrate synthase (CS); Table 1). Pyruvate dehydrogenase (PDH) activity in skeletal muscle was below the normal range (40% of same-day control relative to CS; data not shown). Respiratory chain enzymes in a liver homogenate had low activity of complexes IV and II + III but elevated activity of complex I (32, 24 and 244% of control mean respectively, relative to CS) (Table 1). In conjunction with the muscle enzymology, these results supported a primary respiratory chain defect, particularly affecting complex IV. Respiratory chain enzymes in fibroblasts were less affected, with complexes III and IV activities at 43 and 67% of control means respectively relative to CS (Table 1). Patient 2 did not undergo biopsies for measurement of respiratory chain enzyme activities, however FGF21 levels were elevated in serum from patient 2 (data not shown), consistent with a mitochondrial disorder (Montero et al 2016).

Genome wide homozygosity mapping of the family was undertaken when screening for common mtDNA mutations was negative. Eight regions of homozygosity common to the two affected siblings were identified (Table 2). Cross-referencing to the MitoCarta database (Pagliarini et al 2008) produced a list of 15 candidate genes (Table 2). Sanger sequencing of all exons of these genes using genomic DNA extracted from blood failed to identify any variants.

Whole genome sequencing was undertaken on patient 2 and her parents, to provide a genetic diagnosis for the family. No candidates were identified within genes in the MitoCarta2.0 database (Calvo et al 2015) or in the mitochondrial genome. Three candidate homozygous variants were identified in genes that were within the regions of homozygosity, *SLC39A8*, *ENPEP* and *SNTB1* (Table 2). *ENPEP* encoding glutamyl aminopeptidase, and *SNTB1* encoding syntrophin, beta 1, a dystrophin associated protein, were considered less likely candidates based on literature searches; however, we cannot exclude the possibility that these variants or an unidentified variant may contribute to the patient phenotype. The *SLC39A8* variant was considered to be the most likely candidate based on recent reports of SLC39A8 deficiency in patients with cerebellar atrophy and intellectual disability (Boycott et al 2015; Park et al 2015). *SLC39A8* encodes a manganese, zinc and cadmium transporter that has been found at both the cellular and mitochondrial membrane (Besecker

Table 2 Regions of homozygosity and WGS candidate genes

	Homozygosity region (hg19 based co-ordinates)				WGS
Chromosome	Start position (bp)	End position (bp)	Length (Mb)	MitoCarta genes	Candidate genes
chr4	84,888,283	113,278,307	28.4	*PPM1K*, *PIGY*, *PDHA2*, *PPA2*, *HADH*, *RPL34*	*SLC39A8*, *ENPEP*
chr4	175,484,896	177,341,328	1.9	–	–
chr7	154,934,251	157,550,088	2.6	–	–
chr8	121,267,486	141,507,153	20.2	*MRPL13*, *TMEM65*, *NDUFB9*,	*SNTB1*
chr11	0	2,980,972	3.0	*SIRT3*, *SLC25A22*, *MRPL23*	*DRD4*
chr17	4,242,139	6,844,664	2.6	*SLC25A11*, *CIQBP*	–
chr17	71,319,967	71,968,857	0.6	–	–
chr20	0	2,319,913	2.3	*SNPH*	–

et al 2008; He et al 2006). We identified a homozygous Chr4(GRCh37):g.103236869C>G; c.338G>C; p.(Cys113Ser) variant in *SLC39A8* that is not present in ExAC (Lek et al 2015). Cys113 is conserved among vertebrate species (Suppl. Fig. 1) and the p.(Cys113Ser) substitution is predicted to be deleterious (SIFT score = 0.03) or probably damaging (PolyPhen2 score = 0.988). Sanger sequencing confirmed the *SLC39A8* c.338 G>C variant was homozygous in both affected children and heterozygous in both parents and the unaffected sibling.

Following the identification of the *SLC39A8* variant, a number of biochemical investigations were performed on patient 2 to confirm pathogenicity of the variant. Serum transferrin isoforms were analysed by capillary electrophoresis and revealed an abnormal transferrin glycosylation pattern (Fig. 2a) consistent with a CDG type II defect as reported in other patients with SLC39A8 deficiency (Boycott et al 2015; Park et al 2015). The proportions of tetrasialo-transferrin 60.44% (ref range 65.53–78.98%) and disialo-transferrin 0.56% were both decreased, whilst trisialo-transferrin 20.25% (ref range 1.64–8.55%), monosialo-transferrin 5.11% (ref range 0.00–0.01%) and asialo-transferrin 1.67% (ref range 0.00–0.01%) were increased (Fig. 2a). Whole blood and urine manganese were undetectable at <0.10 μmol/ L (ref interval 0.11–0.30), and <0.10 nmol/mmol creat (ref ≤3.0) respectively. Blood cadmium was 2 nmol/L (ref interval ≤30), zinc was 12 μmol/ L (ref 10–18) and RBC glutathione 5.6 μmol/g (ref 4.2–9.8).

Treatment with galactose and uridine for 2 weeks demonstrated modest improvements in the glycosylation pattern, with normalisation of tetrasialo-transferrin 71.27% (ref range 65.53–78.98%) and reduction of hypogalactosylated transferrin isoforms; trisialo-transferrin 14.29% (ref range 1.64–8.55%), monosialo-transferrin 1.48% (ref range 0.00–0.01%) and asialo-transferrin to undetectable levels (Fig 2b). Blood manganese levels remained undetectable at <0.10 μmol/ L (ref interval 0.11–0.30) prior to manganese supplementation and will be repeated with transferrin electrophoresis after planned dose increments in galactose to 2 g/kg/day and uridine to 150 mg/kg/day.

Discussion

Here we report the identification of a novel *SLC39A8* variant as a cause of an apparent mitochondrial disorder in two siblings with profound developmental delay, dystonia and seizures. Our patients have many features in common with previously reported cases of SLC39A8 deficiency including developmental delay, brain atrophy, hypotonia and in some cases seizures (Boycott et al 2015; Park et al 2015). Patient 2 also had some skeletal abnormalities and strabismus, as reported in several previous cases. Unlike previously reported cases, our patients displayed dystonia and patient 1 had evidence of a mitochondrial respiratory chain deficiency, presenting as a Leigh-like syndrome. In addition, both affected individuals in our family had radiological features consistent with Leigh disease. Based on the Nijmegen criteria they would be defined as having a probable mitochondrial respiratory chain disorder (Morava et al 2006).

Interestingly, the patients reported by Boycott and colleagues (Boycott et al 2015) had cerebellar atrophy, which is typical of CDG disorders (the affected individuals in our study had normal cerebellar size and structures), and one also had a lactate peak on MRS, which is not typical of CDG disorders. It would be very interesting to determine if the patients in the first study also had functional abnormalities of the mitochondrial respiratory chain.

SLC39A8 is a member of the solute carrier 39 metal transporter family and imports manganese, zinc and cadmium, with a higher affinity for manganese (He et al 2006). It is primarily located at the cell membrane but has also been detected at the mitochondrial membrane (Besecker et al 2008). Inhibition of SLC39A8 expression in human airway epithelial cells using siRNA resulted in mitochondrial dysfunction (Besecker et al 2008). Patient 2 had undetectable levels of manganese in blood and urine supporting pathogenicity of the identified *SLC39A8* variant. The undetectable levels of manganese in patient 2 blood and urine and normal levels of zinc in blood, are consistent with the cases reported by Park et al 2015, and that the primary physiological role of SLC39A8 is manganese transport (He et al 2006). However, five of eight cases due to a p.Gly38Arg SLC39A8 variant reported by Boycott et al 2015 had modestly reduced zinc levels in blood in addition to severe manganese deficiency in blood, while urine manganese levels were elevated in two cases, possibly indicating different effects of this *SLC39A8* variant or influences of other genetic and/or environmental factors.

SLC39A8 deficiency causes a type II CDG with patient 2 showing serum transferrin patterns consistent with those described for other patients with *SLC39A8* variants (Park et al 2015). The glycosylation disorder is believed to arise due to reduced functioning of the β-galatosyltransferase enzyme, which requires manganese as a cofactor (Park et al 2015; Ramakrishnan et al 2006). β-galatosyltransferase transfers UDP-galactose to *N*-acetylglucosamine of a glycan, hence manganese deficiency inhibits this process resulting in reduced glycosylation, as evidenced by the reduced levels of the most common tetrasialo-form of transferrin on electrophoresis of patient 2.

Increasing the intracellular UDP-galactose pool by galactose and uridine supplementation completely restored galactosylation in a severely affected patient (Park et al 2015). Correction of transferrin glycosylation prior to attempting manganese supplementation may be reasonable because transferrin is the major manganese binding protein in the vascular circulation (Herrera

et al 2014; Park et al 2015), and manganese uptake across the blood–brain-barrier is known to be transferrin dependent (Tuschl et al 2013; Park et al 2015). Patient 2 showed partial correction of her glycosylation pattern after only 2 weeks of combined galactose and uridine therapy. We are, however, unable to associate this biochemical improvement with any overt clinical correlation, as she has only been on treatment for 4 weeks thus far. Additionally, our patient is more advanced in her disease progression in contrast to the infant reported by Park and colleagues (Park et al 2015). Longer term assessments, particularly with regard to the clinical course, will be necessary to evaluate whether the promising biochemical effects observed from dietary galactose and uridine supplementation on glycosylation translate to clinical stability or even improvement. It remains uncertain whether manganese supplementation will have any clinical impact on these patients as this will be dependent on the residual ability of the mutant SLC39A8 to transport manganese and/or whether alternative transporters are able to compensate (Boycott et al 2015). Manganese is also a cofactor for MnSOD, a reactive oxygen species scavenger in mitochondria (Holley et al 2011). In one MnSOD knockout mouse model, mice showed neural degeneration in the basal ganglia and brainstem that was characterised by extensive mitochondrial damage (Lebovitz et al 1996). Manganese deficiency in yeast leads to reduced activity of MnSOD and elevated levels of superoxide (Irazusta et al 2006). Reactive oxygen species cause damage to enzymes containing Fe-S clusters including complex I, II and III of the respiratory chain, and also damage mtDNA which encodes some subunits of complex I, III, IV and V (Holley et al 2011). Reduced activity of MnSOD due to manganese deficiency may explain the complexes II + III and IV deficiencies in patient 1 however it is unclear why complex I activity was elevated in liver. Unusual patterns of combined mitochondrial respiratory chain deficiency have also been reported in patients with *BOLA3*, *NFU1*, *ISCU* and *LYRM4* variants which encode proteins involved in Fe-S cluster biogenesis (Cameron et al 2011; Haack et al 2013; Lim et al 2013). Some of these patients also showed reduced complex IV activity despite its lack of Fe-S clusters. In addition, some of these patients had a PDH complex enzyme deficiency as seen in our patient 1. This was found to result from reduced levels of lipoic acid, a coenzyme of the PDH complex, which is synthesised by the Fe-S cluster containing enzyme lipoate synthase (Cameron et al 2011; Haack et al 2013). We measured MnSOD activity in patient 1 fibroblast mitochondrial extracts but did not see any significant difference compared to controls (data not shown). However, no respiratory chain deficiency was detected in patient 1 fibroblasts and this result does not preclude effects in other tissues. It is not known whether the previously reported cases of SLC39A8 deficiency also had a mitochondrial disorder as to our knowledge respiratory chain enzyme activities were not determined (Boycott et al 2015; Park et al 2015).

Our patients represent the second case report of a glycosylation disorder associated with a mitochondrial disorder. Recently in a case where a *C10orf2* (*Twinkle*) variant was identified, the patient was also found to have elevated alpha-fetoprotein and a type I CDG pattern (Bouchereau et al 2015). The authors speculated that sialyltransferases and glycosyltransferases, which are found on the outer mitochondrial membrane, may be affected by membrane disruption caused by mitochondrial DNA depletion.

In conclusion, we present a novel case of SLC39A8 deficiency causing a type II CDG in association with a mitochondrial disorder. This case highlights the utility of WGS in providing a genetic diagnosis for complex rare diseases, and is particularly imperative when potential therapeutic avenues are available. We recommend that SLC39A8 deficiency be considered as a possible cause of Leigh-like syndrome in other cases of mitochondrial disorders lacking a genetic diagnosis.

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Compliance with ethical standards

Conflict of interest LR, MC, VG, TR, DT, KP, MB, CS and SB declare they have no conflict of interest. JC is a communicating editor of the Journal of Inherited Metabolic Disease.

Ethics All procedures followed in this study 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), and this project was approved by the Sydney Children's Hospitals Network Human Research Ethics Committee (reference number 10/CHW/113). Informed consent was obtained for all participants included in the study.

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