ESHG

BRIEF COMMUNICATION

Hypoketotic hypoglycemia without neuromuscular complications in patients with *SLC25A32* deficiency

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Mitochondrial flavin adenine dinucleotide (FAD) transporter deficiencies are new entities recently reported to cause a neuromyopathic phenotype. We report three patients from two unrelated families who presented primarily with hypoketotic hypoglycemia. They all had acylcarnitine profiles suggestive of multiple acyl-CoA dehydrogenase deficiency (MADD) with negative next-generation sequencing of electron-transfer flavoprotein genes (*ETFA*, *ETFB*, and *ETFDH*). Whole exome sequencing revealed a homozygous c.272 G > T (p.Gly91Val) variant in exon 2 of the *SLC25A32* gene. The three patients shared the same variant, and they all demonstrated similar clinical and biochemical improvement with riboflavin supplementation. To date, these are the first patients to be reported with hypoketotic hypoglycemia without the neuromuscular phenotype previously reported in patients with *SLC25A32* deficiency.

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INTRODUCTION

Riboflavin (Vitamin B2) is precursor of two essential cofactors namely flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which are utilized in numerous enzymatic reactions and in a wide range of metabolic pathways including the mitochondrial electron transport chain, mitochondrial and peroxisomal fatty acid β-oxidation, and branched-chain amino acids catabolism [1].

Primary defects of flavocoenzyme metabolism include genetically inherited disorders involving enzymes in the synthetic pathway of essential cofactors FMN and FAD and disorders of riboflavin or flavocoenzyme transport. Secondary flavocoenzyme defects refer to the functional disruption of cofactor-dependent reactions. Both primary and secondary flavocoenzyme deficiencies may present with abnormal biochemical features including characteristic elevations of plasma acylcarnitines and urinary organic acids abnormalities. Riboflavin therapy will be beneficial in both; hence early recognition of these disorders is clinically important [2, 3].

Mitochondrial FAD transporter deficiencies are entities that were first described in 2016 primarily in association with a variable myopathic presentation. Two patients have been reported so far. The first was a 14-year-old girl with riboflavin-responsive recurrent exercise intolerance and biochemical features of multiple acyl-CoA dehydrogenase deficiency (MAAD) [4]. The second patient, 51 years old at the time of reporting, presented at 3 years of age with muscle weakness following an episode of influenza, and subsequently had progressive exercise intolerance in childhood, together with neurological symptoms including early-onset ataxia, myoclonus, dysarthria, and dysphagia [5].

Both patients had a dramatic improvement in the clinical and biochemical abnormalities following oral riboflavin supplementation, including improved exercise tolerance.

We report three patients from two unrelated Omani families who presented primarily with hypoketotic hypoglycemia. They all had an acylcarnitine profile suggestive of MADD with negative next-generation sequencing of *ETFA, ETFB*, and *ETFDH* genes. A homozygous c.272 G > T (p.Gly91Val) variant was detected in exon 2 of the *SLC25A32* gene. The variant is shared by the three patients reported.

MATERIALS

Patient 1

This is a 25-year-old man who developed an episode of vomiting and hypoglycemia at the age of 6 months. Since then, he had recurrent admissions with episodes of vomiting and lethargy triggered by infections or febrile illness. He had no symptoms in between those episodes, and they became less frequent as he grew up. He was less tolerant to fasting, but never had any exercise intolerance, myalgia, or evidence of rhabdomyolysis (change in urine color). Family history was remarkable for consanguinity and a younger sister who died at the age of seven months while asleep during an intercurrent illness. She had a documented episode of hypoglycemia prior to this incidence.

Blood tests during an episode of hypoglycemia showed normal blood gas, normal ammonia with mildly elevated lactic acid at 5 mmol/L (0.5–1.6 mmol/l). His creatine kinase (CK) was normal as well. Initial acylcarnitine profile showed significantly increased short-chain, medium-chain acylcarnitines, and to a lesser extent long-chain acylcarnitines and glutarylcarnitines. Urine organic acids showed moderately increased urinary excretion of 2-hydroxyglutaric, ketoglutaric, ethylmalonic acids, and isovalerylglycine. Those results were thought to be suggestive of a diagnosis of MADD and hence he was started on riboflavin and

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carnitine supplementation. His free and total carnitines were significantly reduced at 3 umol/L (reference range; 30–50 umol/l) and 4 ummol/L (reference range; 43–65 umol/L), respectively. Currently, he takes riboflavin at a dose of 50 mg twice daily, carnitine at 1 g three times daily and Co-enzyme Q10 at a dose of 60 mg two times daily. There were no admissions with hypoglycemia episodes since then, he is now able to fast for more than 14 h, and his biochemical abnormalities including lactate have completely normalized. Echocardiography was normal. He presently runs an active lifestyle with regular strenuous exercises with no physical or neurological limitations.

Last physical examination at the age of 25 years showed a well-appearing young man, with a weight of 76.4 height of 162 cm. There was no hepatomegaly and the rest of the examination including neurological examination was unremarkable.

Next-generation sequencing with deletion/duplication analysis was carried out for electron-transfer flavoprotein genes (*ETFA* and *ETFB*) and the electron-transfer flavoprotein dehydrogenase-related gene (*ETFDH*) and the result showed no clinically significant DNA variants. Whole exome sequencing and mitochondrial DNA analysis, was done at Breda Genetics Laboratory in Italy and a homozygous c.272 G > T (p.Gly91Val) variant was detected in exon 2 of the *SLC25A32* gene (NM_030780).

Patient 2

This is a 4 year and 3-month old female child who was born to non-consanguineous Omani parents with unremarkable family history otherwise. She was born full-term via vaginal delivery with a good Apgar score. She had normal growth and development. At the age of one year, she presented with acute gastroenteritis and was found incidentally to have hepatomegaly and mildly elevated aminotransferases. Diagnostic work-up was negative for infectious mononucleosis and hepatitis. Her abdominal ultrasound showed a mildly enlarged liver with normal echogenicity. She also had photosensitivity since early infancy, but her eye exam was normal.

Subsequently, she had recurrent hospital admissions with hypoketotic hypoglycemia triggered by febrile illness associated with vomiting. The lowest blood sugar reading was 0.7 mmol and she only had trace ketonuria then. She also had associated recurrent lactic acidosis/acidemia with the lactate level being around 2.7–6.8 mmol/l (0.5–1.6 mmol/l). There was no evidence of hyperlipidemia or hyperuricemia and she always had normal CK. A next-generation sequencing panel for hypoglycemia that included Fructose 1,6 bisphophatase deficiency was outsourced to Fulgent Diagnostics laboratory with negative results for any causative variants.

Plasma acylcarnitine showed increased butyrylcarnitine (C4) and urine organic acids showed elevated methylsuccinic acid, methylfumaric acid, and ethylmalonic acid excretion. Ethylmalonic aciduria was ruled out with negative sequencing and deletion/duplication testing of *ETHE1*. Whole-exome sequencing outsourced to Medical Neurogenetics, Atlanta, USA showed a homozygous c.272 G > T (p.Gly91Val) variant in *SLC25A23*.

This patient initially was empirically managed with intravenous dextrose during acute febrile illness and hospital admissions and was kept on supplementations of uncooked corn starch (UCCS) at 1.5 g/kg/day at home with avoidance of fasting. After she was diagnosed with *SLC25A32*-related disorder, UCCS was stopped, and she was started on carnitine at 90 mg/Kg/day and riboflavin at 100 mg two times daily. She had no recurrence of hypoglycemia since then.

Patient 3

This is the brother of patient 2. Following the diagnosis of *SLC25A32*-related disorder in his sister, he was pre-symptomatically screened with acylcarnitine profiling in dried blood spots at the age of 19 months. He was also found to have an acylcarnitine profile suggestive of MADD (Table 1). Urine organic acids in a sample collected while normoglycemic was essentially unremarkable.

The parents did not come to collect prescribed riboflavin and they elected to wait for his sister's scheduled routine clinic appointment. He presented with symptomatic hypoglycemia in the context of a febrile illness before starting him on the supplementation. He was managed during that episode with intravenous dextrose 10% 0.9%NaCl solution at 1.5 × maintenance, and was kept on oral riboflavin at 100 mg per day and carnitine at 100 mg/Kg/day. Testing for the known familial variant confirmed homozygosity for c.272 G > T (p.Gly91Val) variant in *SLC25A32*, previously identified in his sister.

DISCUSSION

Mitochondrial FAD transporter deficiencies are a rare cause of MADD previously reported to cause exercise intolerance and muscle weakness with progressive neurological symptoms which improved after riboflavin supplementation [4, 5]. The clinical delineation in the two previously reported patients reported showed no associated hypoglycemia, which is instead the major presenting complication in the patients reported here. The limited number of cases reported so far argue for the limitation of knowing the full phenotypic spectrum associated with disease-causing variants in implicated genes like *SLC25A32* discussed here.

The three patients we report were all found to have a novel homozygous c.272 G > T (p.Gly91Val) in *SLC25A32*. This variant has not been reported yet in public databases like in dbSNP, gnomAD, 1000 Genomes, NHLI Exome Sequencing Project (ESP), or ClinVar. This variant is also absent from in-house 1562 population-specific exomes. The nucleotide position is conserved across 35 mammalian species. *In silico* analysis indicates that the reported variant is likely damaging (MutationTaster: disease-causing; FATHMM-MKL: damaging; Provean: damaging; DANN: 0.998, range 0–1, being 1 the most damaging). The three described patients are from two different Omani families and two distant geographical regions arguing for the possibility of a founder effect given the rarity of this variant in the Omani population otherwise. The diagnosis of FAD transporter defect is also supported with the acylcarnitine profiles and urine organic acids patterns suggestive of MADD in the absence of an alternative genetic diagnosis despite WES. The notable clinical response to riboflavin supplementation is also in supportive of this diagnosis.

FAD-linked dehydrogenases are essential in the mitochondrial fatty acid beta-oxidation pathway [6]. Stress or fasting-induced hypoglycemia would thus be an anticipated complication in patients with FAD transport defects given the expected impact of FAD availability to dependent dehydrogenases involved in fatty acid beta-oxidation [7]. The hypoketotic response to hypoglycemia seen in the patients we report agrees with this assumption. Hypoketotic hypoglycemia has not been reported in the previously published patients with *SLC25A32*-related defects. It is not clear if this complication was overridden by the predominant neuromuscular phenotype, or it was not reported on the assumption of it being a mild secondary or non-specific complication. It would be of interest to know whether patients with *SLC25A32*-related defects who presented at an older age with neuromuscular symptoms may have had fasting or stress-induced hypoglycemia at younger age enabling this diagnosis earlier. On the other hand, it is uncertain whether the patients we report here at a younger age with hypoketotic hypoglycemia are distant to develop neuromuscular complications related to this diagnosis when they grow older. One questions whether riboflavin supplementation alone would be sufficient to prevent this complication or additional supplementation with folate or formate [8] may be required for prevention of the neuromuscular phenotype associated with defective mitochondrial folate transport [9, 10].

Table 1. Clinical summary of the patients with FAD transporter deficiency reported to date.

Patient	Age When reported (years)	Sex	Ethnicity	Consan-guinity	Phenotype	Acylcarnitine Profile Abnormalities before Riboflavin Supplementation (DBS)	Urine Organic acid abnormalities in umol/mmol creatinine	Response to Riboflavin	Causative Variant/s in *SLC25A32*	Zygosity
1	25	Male	Omani	Yes	hypo-ketotic hypoglycemia	High C6, C8, and C10	Ethylmalonic acid :22 (<20) 2- OH glutaric acid:274 (<20)	clinical and biochemical improvement	c.272 G > T (p.Gly91Val)	homozygous
2	4	Female	Omani	No	hypo-ketotic Hypoglycemia & mild hepatomegaly	High C4, C5, C6, C8, and C10	Ethylmalonic acid :1011(<33) 2- OH glutaric acid :335(<65)	clinical and biochemical improvement	c.272 G > T (p.Gly91Val)	homozygous
3	1.5	Male	Omani	No	Hypo-ketotic Hypoglycemia	High C4, C6, C8, and C10	Ethylmalonic acid :44(<47) 2- OH glutaric acid:95 (<80)	clinical and biochemical improvement	c.272 G > T (p.Gly91Val)	homozygous
4[4]	14	Female	French	No data	recurrent exercise intolerance	features of multiple acyl–coenzyme A dehydrogenase deficiency (not specified)		clinical and biochemical improvement	Two heterozygous mutations, c.425 G > A (p.Trp142*) and c.440 G > A (p.Arg147His)	compound heterozygous
5[5]	51	Male	Dutch	Yes	early-onset ataxia, myoclonia, dysarthria, muscle weakness, and exercise intolerance	High C4, C5, C6, C8.C10		clinical improvement	c.264_31delinsCTCACAAATGCTCA	homozygous

DBS; Dried Blood Spots. Superscript numbers indicate the references where these patients are reported as respectively referenced. Patients 1, 2, and 3 are the patients reported in this manuscript.

Table 1 summarizes the patients with defects in *SLC25A32* presently known, including the patients reported here. There is still not enough data to support the phenotype-genotype correlation in FAD transporter defects because of the limited number of cases reported to date. The variant detected in patient 5 completely abolishes translation and it is therefore highly likely that the protein is either completely absent. Patient 4 was compound heterozygous for nonsense and a missense variant which may explain the milder phenotype compared to patient 5. Patients 1, 2, and 3 are all homozygous for the same missense variant. It is uncertain whether the neuromuscular complications related to defects in *SLC25A32* are distant to occur in the absence of at least one truncating variant.

The limited genotype–phenotype correlations, genetic heterogeneity, and impact of physiological status at the time of samples collection for biochemical testing are recognized challenges while investigating patients with hypoglycemia with absent immediate biochemical or endocrine clues. Whole-exome sequencing may arguably be a cost-effective strategy to identify the underlying genetic defects for these disorders [3].

Finally, we conclude that screening for *SLC25A32* defects should be included in the differential diagnosis of patients with hypoketotic hypoglycemia; particularly when the acylcarnitine profile or urine organic acids profiles are suggestive of MADD. More patients would need to be evaluated clinically before the full clinical spectrum of this disorder could be ascertained. Whether riboflavin alone is adequate for the prevention of long-term complications is yet to be answered.

DATA AVAILABILITY

Data available on request from the authors

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AUTHOR CONTRIBUTIONS

Bushra AL Shamsi: drafting the manuscript, Khalid Al-Thihli and Fathiya Al-Murshedi: supervising writing the manuscript and medical care of patients, Asila Al-Habsi: the medical care of patients.

ETHICAL APPROVAL

Given the retrospective nature of this study, and since no identifying data are included in this manuscript ethical approval was not required for the purpose of this study. Clinical consent forms were obtained.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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