Case Report

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Detection of *FLAD1* mutations and lipid storage myopathy in a 5-year-old boy: a case report study

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Introduction and importance: Lipid storage myopathy due to flavin adenine dinucleotide synthetase 1 (*FLAD1*) deficiency is an autosomal recessive error of metabolism that causes variable mitochondrial dysfunction.

Case presentation: At the age of 3, the patient was found to have movement problems, such as difficulty rising from a chair (Gower's sign) and climbing stairs, which led to hospital admission and diagnosis. At the age of 4, carrier detection for spinal muscular atrophy was normal; however, at the age of 5, whole-exome sequencing revealed a pathogenic variant of Chr1: 154960762: A > T c.A554T:p.D185V in exon-2 of *FLAD1* gene was identified as homozygous.

Clinical discussion: In general, it is expected that the treatment of type 2 *FLAD1* gene mutation with riboflavin has a better prognosis, but these interventions may not be sufficient for the survival of the patient. Treatment with riboflavin has increased various functions, including skeletal-muscular, and cardiovascular function. As a result, like the patient in our study, the mutation in exon-2 is more severe and less responsive to riboflavin treatment.

Conclusion: Checking the *FLAD1* gene is recommended in all people with multiple acyl-CoA dehydrogenase deficiency.

Keywords: case report, *FLAD1*, mutation

Introduction

Disorders of lipid metabolism are a heterogeneous class of diseases with autosomal recessive inheritance. Delay in the diagnosis of these disorders is common due to its asymptomatic nature[1]. Multiple acyl-CoA dehydrogenase deficiency (MADD) is an autosomal recessive disease caused by a congenital defect in electron transfer flavoprotein (ETF)[2,3].

Flavin adenine dinucleotide (FAD), a metabolite of riboflavin, serves as a cofactor in reactions involving FAD-dependent mitochondrial dehydrogenases[4]. In recent years biallelic variants in *FLAD1* encoding FADS have been identified as causing a potentially treatable neuromuscular disease manifesting with lipid storage myopathy and metabolic abnormalities suggestive of MADD in association with multiple respiratory chain enzyme deficiencies[5]. Lipid storage myopathy due to *FLAD1* deficiency is an autosomal recessive inborn error of metabolism that manifests with variable mitochondrial dysfunction. The phenotype is extremely heterogeneous; some patients have a severe disorder with early onset, cardiac and respiratory failure resulting in early death, whereas others have a milder course with onset of muscle weakness in adulthood. Although there is no gold standard treatment, some patients show significant improvement with riboflavin treatment[6,7]. In this study, we report a *FLAD1* mutations in a 5-year-old boy, which led to lipid storage myopathy due to FAD synthetase deficiency. The work has been reported in line with the SCARE (Surgical CAre REport) criteria[8].

HIGHLIGHTS

- Checking the *FLAD1* gene is recommended in all people with multiple acyl-CoA dehydrogenase deficiency (MADD).
- Also, if no pathological changes are observed in A, B, and C, it is recommended to treat with riboflavin in all patients with changes in the *FLAD1* gene.

Case presentation

The patient was a 5-year-old boy living in Golestan province, Iran. The first child of the family, he was born by cesarean section, full term (39 weeks, birth weight: 3150 g, height (HT): 61 cm, Head circumference (HC): 42 cm), and complete vaccination. There was no history of hereditary, neurological, psychological, or congenital disease in the family members, and the parents were cousins.

The child, at the age of 3, was noticed to have movement problems by parents, such as difficulty in climbing stairs and placing hands on knees when standing up, which was when he

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Table 1

Routine blood examination performed.

Lab data	Count	Reference	Unit
Hematology			
WBC	7.90	4000–10 000	10^3/µl
RBC	4.64	4.5–6.3	10^6/µl
Hemoglobin	12.3	14–18	g/dl
Hematocrit	35.6	39–52	%
MCV	76.5	80–97	fl
MCH	26.5	26–32	pg
MCHC	34.6	32–36	g/dl
RDW-CV	11.7	11.5–16	%
Plateletes	358	140 000–400 000	10^3/µl
MPV	7.2	6.5–12	
PDW	15.5	9–17	–
PCT	2.56	–	–
Hormone studies			
T4	8.8	4.4–11.7	mg/dl
TSH	2.9	0.39–6.16	mIU/ml
F-thyroxine F-T4	1.6	0.8–2.2	ng/dl
Vitamin D3 (25-OH)	8.8	4.4–11.7	ng/ml
Blood biochemistry			
Alkaline phosphates	672	180–1200	U/l
Calcium	10.35	8.6–10.3	mg/dl
Phosphorous	5.5	3.5–5.5	mg/dl

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PCT, patient Care Technician; PDW, platelet distribution width; RBC, red blood cell; RDW-CV, red cell distribution width – coefficient of variation; TSH, thyroid-stimulating hormone; WBC, white blood cell.

was admitted for the process of diagnosis. Laboratory results of a child are recorded in Table 1.

This child was suspected for the diagnosis of Duchenne muscular dystrophy (DMD). DNA was extracted from peripheral blood leukocytes for Multiple Ligation-dependent Probe Amplification (MLPA) (P034 and P035 MRC Holland) screening for deletions and duplication in the 79 exons of the Dystrophin gene. The MLPA analysis did not reveal any deletion or duplication in the DMD gene. Pathology of muscle biopsy revealed that hematoxylin and eosin (H&E) stain reveals striated muscle tissue with bimodal fiber size; atrophic fibers are wound and arranged in groups, round hypertrophied are also seen, some necrosis/regeneration are seen with myophagocytosis, internalized nuclei are not increased, the endomysial connective tissue is normal, no inflammation, and adipose tissue replacement was noted. Gomori trichrome stain reveals no ragged red fiber, no rod, and no rimmed vacuole. Congo red stain reveals no congophilic inclusion. Oil Red O (ORO) stain reveals no lipid excess in muscle fibers. Periodic acid-Schiff (PAS) and PAS + Diastasis stains reveal no glycogen excess in muscle fibers, and pale fibers are seen. NAHD-TR (nicotinamide adenine dinucleotide dehydrogenase-tetrazolium reductase) reaction reveals some differentiation of muscle fibers, whorled fibers are noted, rare cytoplasmic cores are seen, small dark fibers are also seen, and no typical target fiber is seen. Succinate dehydrogenase (SDH) reaction reveals no prominent abnormal mitochondrial proliferation. Cytochrome *c* oxidase (COX) reaction reveals no COX-negative fiber. Also, severe atrophy of mainly type 2 and also some type 1 fibers is associated with a few necrosis-/regeneration and no fiber type grouping.

The patient, at the age of 4, the child was referred for carrier detection for spinal muscular atrophy (SMA); DNA was extracted for analysis by MLPA (P021 MRC Holland) for exons VII and VIII in *SMN1* and *SMN2* genes. The results of this analysis are shown in Table 2.

Finally, this child at the age of 5 was evaluated to determine the genetic cause of the neuromuscular disorder; advanced techniques such as next-generation sequencing on all genes related to this group of diseases were done. The results of the analysis are shown in Table 3.

According to Table 3, whole-exome sequencing (WES) revealed a pathogenic variant of Chr1: 154960762:A > T c. A554T:p.D185V in exon-2 gene *FLAD1* was identified as homozygous. The patient was given high-dose vitamins B1, B6, and Q10 and was followed up every 3 months to check the patient's condition and functional improvement screening.

Discussion

In recent years variations in *FLAD1* encoding FADS have been recognized as causing a potentially treatable neuromuscular disease manifesting with lipid storage myopathy and metabolic abnormalities suggestive of MADD[9].

In general, it is expected that the treatment of type 2 *FLAD1* gene mutation with riboflavin has a better prognosis, but these interventions may not be sufficient for the survival of the patient[10,11]. In the study of Olsen *et al*.[12], treatment with riboflavin has increased various functions, including skeletal-muscular and cardiovascular function. As a result, like the patient in our study, the mutation in exon-2 is more severe and less responsive to riboflavin treatment. However, our patient was partially responsive to riboflavin treatment based on plasma acylcarnitine profile and urine organic acid excretion. In other studies, treatment with riboflavin supplements in seven children improved clinical symptoms, including significant improvement in muscle symptoms and increased muscle strength, and in one adult patient, improved biochemical abnormalities[10–12].

The two siblings presented here contribute to the clinical profiling of the newly-discovered disease, FAD synthase deficiency. Despite the presence of biochemical hallmarks of classical early-onset MADD in at least one of the patients, these infants presented not with acute metabolic decompensations but with severe hypotonia and its complications after 2 months of age. Differential diagnoses included ETF and dehydrogenase ETF (ETFDH) deficiencies, riboflavin transporter deficiencies, spinal muscular atrophy, and primary mitochondrial respiratory chain deficiency. These patients support the observation by Olsen *et al*. that biallelic frameshift mutations in exon-2 of *FLAD1* gene may still allow partial clinical response to riboflavin supplementation, but this intervention may not be sufficient for survival[10].

Riboflavin-dependent FADS deficiency has been previously reported[12]. Responsiveness to riboflavin may be dependent on

Table 2

Results of analysis for carrier detection for SMA.

SMN1 copy number exon 7	*SMN1* copy number exon 8	*SMN2* copy number exon 7	*SMN2* copy number exon 8	Phenotype
Two copies	Two copies	Two copies	Two copies	Normal

SMA, spinal muscular atrophy.

Table 3

Results of whole-exome sequencing test to find the genetic cause of disorders.

Gene and transcript	Variant	Disease	exon	Zogosity
FLAD1	Chr1: 154960762: A > T c.A554T:p. D185V	Lipid storage myopathy due to flavin adenine dinucleotide synthetase deficiency (MIM:255100)	2	Homozygote AR

the genotype, as patients with the earlier-onset type with severe mutations did not respond to riboflavin. In general, all patients with FADS deficiency should receive high-dose riboflavin therapy, and if the diagnosis is suspected, riboflavin supplementation should be administered while waiting for mutation analysis and final diagnosis confirmation. If the disease is not confirmed, riboflavin should be stopped[12]. Prenatal or postnatal treatment of patients with FADS deficiency with high-dose riboflavin, which may prevent or improve disease symptoms, has been suggested, but further investigation and studies are needed[13].

Conclusion

Description of malformations and dysmorphic features in future cases similar to this patient may extend the clinical spectrum of FAD synthase deficiency due to *FLAD1* mutations. Checking the *FLAD1* gene is recommended in all people with MADD. Also, if no pathological changes are observed in A, B, and C, it is recommended to treat with riboflavin in all patients with changes in the *FLAD1* gene.

Ethical approval

Not applicable.

Consent

Written informed consent was obtained from the patient for the publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request. The whole research was done under the permission of the Ethics committee of Golestan University of Medical Sciences.

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Author contribution

S.A.H.: diagnosed and managed this patient and interpretation; L.S.H. and M.G.G.: revised the manuscript and finalized the draft.

Conflicts of interest disclosure

There are no conflicts of interest.

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