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Case Report

Flavin adenine dinucleotide synthase deficiency due to *FLAD1* mutation presenting as multiple acyl-CoA dehydrogenation deficiency-like disease: A case report

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Abstract

Multiple acyl-CoA dehydrogenase deficiency (MADD), also known as glutaric acidemia type II, is classically caused by a congenital defect in electron transfer flavoprotein (ETF) or ETF dehydrogenase (ETFDH). Flavin adenine dinucleotide synthase (FADS) deficiency caused by mutations in *FLAD1* was recently reported as a novel riboflavin metabolism disorder resembling MADD. Here, we describe a Japanese boy with FADS deficiency due to a novel mutation (p.R249*) in *FLAD1*. In the asymptomatic male infant born at full term, newborn screening showed positive results with elevated C5 and C14:1 acylcarnitine levels and an increased C14:1/C2 ratio. Biochemical studies were unremarkable except for lactic acidosis (pH 7.197, lactate 61 mg/dL). A diagnosis of MADD was suspected because of mild abnormalities of the acylcarnitine profile and apparent abnormalities of urinary organic acids, although mutations in the *ETFA*, *ETFB*, *ETFDH*, and riboflavin transporter genes (*SLC52A1*, *SLC52A2*, and *SLC52A3*) were not detected. Administration of riboflavin and L-carnitine was initiated at one month of age based on the diagnosis of "biochemical MADD" despite a lack of symptoms. Nevertheless, the acylcarnitine profile was not normalized. Symptoms resembling bulbar palsy, such as vocal cord paralysis and dyspnea with stridor, were present from 3 months of age. At 4 months of age, he became bedridden because of hypoxic-ischemic encephalopathy due to fulminant respiratory failure with aspiration pneumonia. At 2 years and 5 months of age, a homozygous c.745C > T (p.R249*) mutation in the *FLAD1* gene was identified, confirming the diagnosis of FADS deficiency. His severe clinical course may be caused by this nonsense mutation associated with poor responsiveness to riboflavin. Persistent lactic acidosis and neuropathy, such as bulbar palsy, may be important for diagnosing FADS deficiency. Although the biochemical findings in FADS deficiency are similar to those in MADD, their clinical symptoms and severity may not be identical.
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Keywords: Flavin adenine dinucleotide synthase deficiency; Multiple acyl-CoA dehydrogenase deficiency; Glutaric acidemia type II; FLAD1; Riboflavin; Newborn screening; Infantile onset; Lactic acidosis; Mitochondrial disease; Bulbar palsy

1. Background

Multiple acyl-CoA dehydrogenase deficiency (MADD, OMIM 231680), also known as glutaric acidemia type II, is an autosomal recessive disease caused

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by a congenital defect in electron transfer flavoprotein (ETF) or ETF dehydrogenase (ETFDH, EC 1.5.5.1) [1]. The defect in ETF (ETFα and ETFβ) or ETFDH disturbs short- to long-chain acyl-CoA dehydrogenases and other mitochondrial dehydrogenases such as glutaryl-CoA, isovaleryl-CoA, and sarcosine dehydrogenases. MADD has been clinically classified into 2 types: 1) a neonatal-onset type that leads to the development of severe respiratory failure, cardiomyopathy, hypotonia, metabolic acidosis, and profound hypoglycemia during the neonatal period or early infancy and is often fatal, and 2) a late-onset type that leads to the development of intermittent episodic attacks of lethargy, hypoglycemia, and muscle cramping/weakness after the infantile period [2]. Treatment with riboflavin (vitamin B_2) is effective in some cases, particularly in the late-onset type of MADD, and improves both symptoms and biochemical abnormalities including acylcarnitine profiles in the responders [3].

Flavin adenine dinucleotide (FAD), a metabolite of riboflavin, serves as a cofactor in reactions involving FAD-dependent mitochondrial dehydrogenases such as short-, medium-, and long-chain acyl-CoA dehydrogenases. Therefore, riboflavin-insufficient conditions and riboflavin metabolism disorders can impair these dehydrogenation processes [4]. Indeed, a riboflavin transporter defect, which is induced by mutations in genes such as *SLC52A1, SLC52A2*, and *SLC52A3*, reportedly caused biochemical abnormalities mimicking MADD [5]. Moreover, a novel disorder resembling MADD due to mutations in *FLAD1*, which encodes FAD synthase (FADS), has also been described [6]. To our knowledge, only three articles involving eleven patients with FADS deficiency due to *FLAD1* mutations have been reported thus far [6–8]. Therefore, the classification of clinical severity and differences in clinical features between classical MADD and FADS deficiency remain poorly understood. However, mitochondrial respiratory chain deficiency is one of the characteristics of patients with FADS deficiency, while symptoms such as lactic acidosis, hyperammonemia, and hypoglycemia, which are often observed in classical neonatal-onset MADD, have never been reported in FADS deficiency [7]. Here, we describe a Japanese boy with FADS deficiency associated with a novel mutation in *FLAD1* who was biochemically suspected to have MADD by expanded newborn screening (ENBS).

2. Case report

The patient was born to a non-consanguineous Japanese couple at 39 weeks of gestation via normal vaginal delivery without asphyxia. His birth weight was 2862 g. He had been asymptomatic with an uneventful course until the 4th day of life when ENBS revealed positive results suggestive of both very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency and isovaleric acidemia based on the following findings: mildly elevated levels of C14:1 (0.55 μM; cut off < 0.35 μM) and C5 (1.30 μM, cut off < 1.0 μM) and an increased C14:1/C2 ratio (0.037, cut off < 0.013).

At 32 days of age, he had no symptoms such as failure to thrive, and routine blood studies showed unremarkable results except for lactic acidosis (Table 1). We had never examined the lactate level in cerebrospinal fluid. The acylcarnitine (AC) profiles based on dried blood spots as well as serum revealed mild elevations in several short- to long-chain ACs (Table 2), and a urinary organic acid analysis performed using gas chromatography-mass spectrometry revealed mild elevations in lactate, ethylmalonate, 2-hydroxyglutarate, and isovalerylglycine, suggesting MADD biochemically. However, ETFA, ETFB, and ETFDH proteins were found to be normal by immunoblotting, and direct sequencing revealed no mutations in *ETFA, ETFB*, and *ETFDH* in the patient. No mutation was detected in the riboflavin transporter genes (*SLC52A1, SLC52A2,* and *SLC52A3*) by a multigene panel using next-generation sequencing. To evaluate the β-oxidation capacity, a fatty acid oxidation (FAO) flux

Table 1
Routine blood examination performed on day 32.

			(Reference*)	Unit
CBC				
	WBC	99	(35–85)	x 10^2/μL
	RBC	383	(430–570)	x 10^4/μL
	Hb	11.7	(13.5–17.0)	g/dL
	Plt	44.8	(15–35)	x 10^4/μL
Biochemical data				
	TP	6.4	(6.7–8.3)	g/dL
	Alb	4.5	(3.5–5.0)	g/dL
	T-Bil	5.9	(0.3–1.2)	mg/dL
	AST	45	(13–33)	IU/L
	ALT	27	(8–42)	IU/L
	LDH	323	(119–229)	IU/L
	ALP	965	(115–359)	IU/L
	BUN	6.1	(8–22)	mg/dL
	Cre	0.22	(0.6–1.1)	mg/dL
	Na	139	(138–146)	mEq/L
	K	5.3	(3.6–4.9)	mEq/L
	Cl	105	(99–109)	mEq/L
	Ca	10.2	(8.7–10.3)	mg/dL
	BS	86	(69–104)	mg/dL
	NH_3	94	(<75)	μg/dL
Blood gas (venous)				
	pH	7.197		
	pCO_2	54		mmHg
	HCO_3^-	20.2		mmol/L
	BE	−7.7		mmol/L
	Lac	61		mg/dl

* The reference values are based on healthy adults at the Shikoku Medical Center for Children and Adults. Obvious abnormal findings are underlined.

Table 2
Acylcarnitine profiles in DBS and serum obtained on day 32.

	DBS	(Reference)	Serum	(Reference)
C0	42.26	(20–70)	70.49	(25–100)
C2	14.06	(5–45)	11.46	(4–60)
C4	0.66	(<1.4)	1.13	(<1.00)
C5	1.25	(<0.7)	1.27	(<0.7)
C5-DC	0.09	(<0.25)	0.24	(<0.25)
C6	0.22	(<0.15)	0.55	(<0.15)
C8	0.38	(<0.3)	0.85	(<0.3)
C10	0.49	(<0.25)	1.19	(<0.3)
C12	0.21	(<0.3)	0.43	(<0.2)
C14	0.28	(<0.4)	0.23	(<0.2)
C16	0.81	(0.4–3.0)	0.23	(<0.3)
C18	0.29	(<2.0)	0.0	(<0.3)

unit: μmol/L.

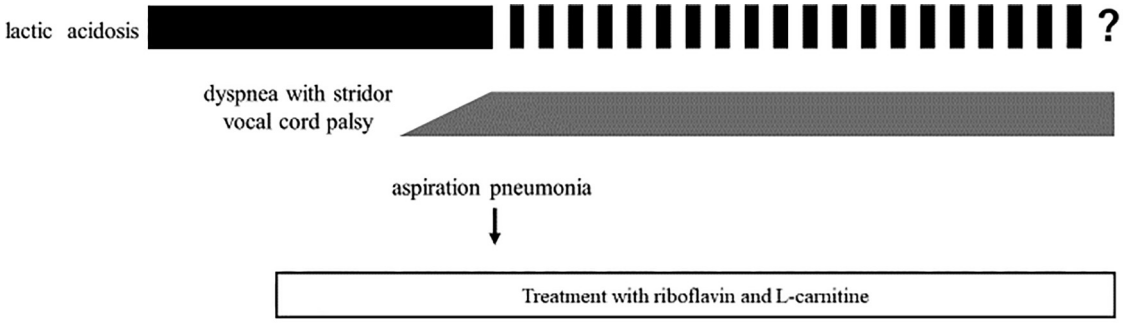
analysis and an *in vitro* probe acylcarnitine (IVP) assay were performed using fibroblasts. Although a mild decrease in the FAO flux was observed, the IVP assay showed a normal pattern. Although we could not make a definite diagnosis at that time, administration of riboflavin and L-carnitine was initiated from 1 month of age based on the probable diagnosis of MADD. However, this treatment did not normalize the AC profile (Fig. 1). At 3 months of age, dyspnea with stridor gradually developed, and paralysis of the vocal cords was diagnosed. At 4 months old, he suffered from hypoxic-ischemic encephalopathy due to fulminant respiratory failure associated with aspiration pneumonia and vocal cord paralysis. He has been bedridden with a tracheotomy since this episode.

The *FLAD1* gene sequence study was conducted at 2 years and 5 months of age, and a homozygous novel c.745C > T (p.R249*) mutation was identified, thus establishing the diagnosis of FADS deficiency.

3. Discussion

In this article, we reported a Japanese case of infantile-onset FADS deficiency caused by a novel *FLAD1* mutation. The patient was initially diagnosed with MADD biochemically based on ENBS and urinary organic acid analysis. Treatment with riboflavin and L-carnitine was initiated but failed to improve his symptoms and biochemical abnormalities. At 4 months of age, he became bedridden after an episode of hypoxic-ischemic encephalopathy due to vocal cord paralysis and aspiration pneumonia.

Our case is the first case of FADS deficiency identified by ENBS and treated with riboflavin before symptom onset. Nevertheless, the patient's neurological outcome was not favorable even after the initiation of riboflavin treatment. Riboflavin-dependent FADS deficiency has been previously reported [6,8]. Responsiveness to riboflavin may be dependent on the genotype as patients with the earlier-onset type with severe mutations did not respond to riboflavin [6]. Although the novel p.R249* mutation in *FLAD1* has not been functionally evaluated, this genotype is considered a severe mutation because it is a nonsense mutation, which



	25 d	40 d	48 d	3 m	4 m	6 m	9 m	1 y 2 m	1 y 7 m	2 y	2 y 4 m	2 y 7 m	(cut off*)
C0	31.8	26.8	39.0	47.8	38.4	33.0	26.4	22.0	24.9	24.4	22.0	30.80	(10-60)
C2	12.5	17.6	22.8	28.4	31.2	25.7	23.8	17.8	19.5	17.3	18.1	26.90	(<45)
C5	1.1	1.1	2.1	1.1	0.9	1.3	1.0	1.2	1.2	1.0	1.2	1.8	(<1.0)
C8	0.49	0.35	0.45	0.68	0.53	0.35	0.36	0.38	0.35	0.37	0.32	0.37	(<0.3)
C10	0.88	0.61	0.87	1.36	1.26	0.95	0.82	0.96	0.93	0.81	0.67	0.77	(<0.4)
C12	0.27	0.20	0.28	0.61	0.73	0.54	0.55	0.54	0.55	0.58	0.58	0.600	(<0.4)
C14:1	0.22	0.19	0.23	0.44	0.52	0.39	0.40	0.44	0.37	0.45	0.42	0.43	(<0.27)
C16	0.7	0.8	1.1	1.8	1.6	1.5	1.4	0.9	1.0	0.9	0.8	1.09	(<2.2)

Fig. 1. Clinical course of symptoms, treatments, and acylcarnitine profiles in DBS from birth to 2 years and 7 months of age.
* Cut off values were used at another institution for newborn screening. The unit for acylcarnitin was μmol/L. Abnormal values are underlined. d, days; m, months; y, years.

may explain why our patient experienced early infantile onset, did not respond to riboflavin, and exhibited severe and fulminant symptoms.

Our case suggests that FADS deficiency can be identified by ENBS based on positive results suggestive of fatty acid oxidation disorders and/or organic acidemias, such as VLCAD deficiency and/or isovaleric acidemia even though MADD is not included in the ENBS target panel. Furthermore, early diagnosis of FADS deficiency by ENBS may not improve the prognosis of patients, particularly those with early onset who are poor responders to riboflavin.

Both persistent lactic acidosis and biochemical findings resembling MADD are important clues for diagnosing FADS deficiency, while episodic lactic acidosis is often observed in the acute phase of classical MADD. Lactic acidosis was continuously observed in our patient for at least several months after birth. Curiously, although lactic acidosis has not been reported in patients with FADS deficiency, lactic acidosis was considered a reasonable complication of FADS deficiency because FADS deficiency is associated with a respiratory chain deficiency [6]. Moreover, because FAD is a coenzyme for succinate dehydrogenase, FADS deficiency may also disrupt the Krebs cycle, impairing mitochondrial function. In our patient, although respiratory chain activity was not analyzed, a respiratory chain deficiency was likely concomitant. We were unable to determine whether lactic acidosis was truly permanent because blood gas results had not been inspected after a certain period. However, we believe that persistent lactic acidosis distinguished FADS deficiency from classical MADD.

Moreover, neurological disturbances such as bulbar palsy, including vocal cord paralysis, dyspnea with stridor, and dysphagia, may be unique symptoms of FADS deficiency. Neuropathy is not generally observed in classical MADD [9]; in particular, bulbar palsy has not been reported as a complicating illness. Meanwhile, pontobulbar palsy was reported as a complication of Brown-Vialetto-Van Laere and Fazio Londe syndrome, which is characterized as a riboflavin transporter deficiency. Therefore, we speculated that bulbar palsy may be one of the characteristics of riboflavin metabolism disorders. In this regard, we could not accurately estimate whether FADS deficiency directly involved the central nervous system because we did not examine brain imaging before our patient was bedridden. However, stridor, shallow breathing, and aspiration pneumonia were observed within a few months after birth in Turkish cases diagnosed postmortem as well as in our case [7].

Finally, AC profiles failed to predict the severity of the patient's condition. The levels of various short-, medium-, and long-chain ACs were mildly elevated in our case, differing from the typical AC profile of classical early infantile-onset MADD. Furthermore, the normal results of the IVP assay also suggested that the AC profile did not reflect the clinical condition. We previously described that the IVP assay findings of late-onset myopathic MADD were similar to those of healthy controls [2]. Specifically, not only the blood AC profile but also the results of both the IVP assay and FAO flux analysis indicated that this patient had a milder form of MADD. Nevertheless, the patient's condition was clinically severe and the early-onset type. Therefore, we speculate that the main pathological condition of FADS deficiency may be defined as a mitochondrial disease rather than fatty acid oxidation disorder. In fact, the patient's condition deteriorated from 3 months of age even though his AC profiles did not worsen at that time (Fig. 1). In conclusion, AC profiles may not reflect the deteriorating pathological condition, unlike observations in other fatty acid oxidation disorders.

4. Conclusions

FADS deficiency is a novel disorder that should be considered in the differential diagnosis of MADD, particularly in patients without a defect in ETF or ETFDH. Although FADS deficiency demonstrates biochemical findings similar to those observed in MADD, its clinical symptoms and severity may be different, especially symptoms indicative of mitochondrial malfunction, such as lactic acidosis and neuropathy, which may be important for distinguishing FADS deficiency from other fatty acid oxidation disorders.

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