Clinical and molecular investigation of 37 Japanese patients with multiple acyl-CoA dehydrogenase deficiency: p.Y507D in ETFDH, a common Japanese variant, causes a mortal phenotype

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1. Introduction

Multiple acyl-CoA dehydrogenase deficiency (MADD) is an inherited metabolic disease caused by a defect in electron transfer flavoprotein alpha (ETFα), ETF beta (ETFβ), or ETF dehydrogenase (ETFΔH) [1,2]. Defects in ETF or ETFΔH cause deficiencies in multiple acyl-CoA dehydrogenases, such as short-, medium-, and long-chain acyl-CoA dehydrogenases, as well as isovaleryl-CoA dehydrogenase, glutaryl-CoA dehydrogenase, and sarcosine-CoA dehydrogenase [3]. Although the exact prevalence of MADD is not known, it is reported to be 1:250,000 births [4] (1:480,000 births in Japan [5]), while it is more common in China (1:22,000 births) [6].

MADD has been roughly classified into neonatal and later-onset forms. The neonatal-onset form is further subdivided into two types: a neonatal-onset form with congenital anomalies, such as polyhydramnios, kidney and dysmorphic facial features (type 1), and a neonatal-onset form without any anomalies (type 2) [7]. Patients with type 1 or 2 develop severe respiratory failure, cardiomyopathy, hypotonia, metabolic acidosis, and profound hypoglycemia soon after birth, and almost all die in the neonatal period or early infancy regardless of treatment. In the later-onset form (type 3), myopathic symptoms, such as hypotonia, is reported to present as mimicking MADD. MADD is roughly classified into neonatal (type 1 or 2) and later-onset (type 3) forms. To identify clinicogenetic characteristics in Japan, we investigated 37 Japanese patients with MADD diagnosed from 1997 to 2020. The causes of MADD were ETFΔH deficiency in 26 patients, ETFα deficiency in four, ETFβ deficiency in six, and riboflavin metabolism disorder in one. All 15 patients with the neonatal-onset type died by 2 years of age, while five of 22 patients with the later-onset form died by 3 years of age. Furthermore, 8 of 15 patients with the later-onset form of ETFΔH deficiency treated with riboflavin were riboflavin non-responders. p.Y507D in ETFΔH was identified as the most common variant (9 of 48 alleles, 18.8%). Of two patients with a homozygous p.Y507D variant, one experienced disease onset and died in the neonatal period, while the other experienced disease onset at two months of age and died at two years old, suggesting that the p.Y507D variant results in fatal outcomes. Our study concluded that more than half of Japanese patients with MADD died by three years old, and more than half of patients with the later-onset form had poor responsiveness to riboflavin, partly due to the unique Japanese p.Y507D variant in ETFΔH.
myalgia, and exercise intolerance, are the most common, followed by intermittent episodic attacks of lethargy, hypoglycemia, and hyperammonemia; occasionally, acute encephalopathy or even sudden death can occur from infancy to adulthood [8].

Furthermore, riboflavin-insufficient conditions and riboflavin metabolism disorders have been recently reported to mimic MADD [9]. Flavin adenine dinucleotide (FAD), a metabolite derived from riboflavin, is a cofactor for electron transfer via the ETF and ETFDH complex resulting from the oxidation of fatty acids and amino acids to the electron transport chain in the inner mitochondrial membrane [10]. Riboflavin metabolism disorders include defects in riboflavin transporters 1, 2, and 3; FAD synthase; and FAD transporters encoded by SLC25A1, SLC25A2, and SLC25A3; FLAD1; and SLC25A32, respectively [11].

Newborn screening (NBS) for MADD is widespread in the United States and several European countries [12–14]. However, NBS for this disease has only been performed in a few regions of Japan because a Japanese pilot study of expanded NBS published in a Japanese domestic journal (S. Yamaguchi, et al., The Journal of Japanese Society for Neonatal Screening, 2013) revealed poor outcomes and diagnostic accuracy for MADD.

The treatment approach includes a protein- and fat-restricted diet, avoidance of long fasting and exercise, and carnitine supplementation [15]. Most patients respond well to high-dose riboflavin therapy [16]. Coenzyme Q10 (CoQ) supplementation can also be therapeutic for riboflavin-responsive MADD [17]. The efficacy of D-3-hydroxybutyrate has also been reported [18]. Additionally, we reported that bezafibrate improved the symptoms and biochemical abnormalities of patients with MADD [19,20].

Recently, the clinical, biochemical, and genetic characteristics of MADD, such as common variants in China, were reported, showing that the majority of type 3 disease cases are caused by defects in ETFDH [6,16,21]. However, the clinical features and genetic characteristics of Japanese patients are different from those of previous reports. Although the genetic aspects of 15 Japanese MADD patients were reported in 2008 [22], the clinical characteristics have not been well investigated, and the number of patients was insufficient. Herein, we aimed to elucidate the clinigenetic characteristics of Japanese patients with MADD by investigating a larger number of cases.

2. Materials and methods

Thirty-seven Japanese patients with MADD identified by Shimane University during the period from 1997 to 2020 were included in this study. Of the 37 patients, 18 were reported in our previous studies [19,22–25]. All patients with ETFA, ETFB, or ETFDH deficiency were diagnosed by genetic analysis and Western blotting except for one patient, whose Western blotting could not be performed due to the lack of fibroblasts. If ETFA, ETFB, or ETFDH protein was absent in Western blotting, the patients were enrolled even if the variant was found to be heterozygous by genetic analysis. Riboflavin metabolism disorders were confirmed only by genetic analysis. Exclusion criteria was as follows: (1) patients whose parents were not Japanese, (2) patients who had typical acylcarnitine profile for MADD but could not be diagnosed by genetic analysis, and (3) patients who were diagnosed by institutions other than our laboratory.

Genetic tests for ETFA, ETFB, ETFDH, FLAD1, and SLC25A32 were performed as previously reported [24,25]. Genomic DNA was extracted from fibroblasts or mononuclear cells using the QIAamp DNA Micro Kit (Qiagen GmbH, Hilden, Germany) and was directly sequenced. Additionally, SLC25A1, SLC25A2, and SLC25A32 were analyzed by multigene panel genetic testing using next-generation sequencing at Gifu University (by Prof. Fukao and Dr. Sasai). Western blotting of ETFA, ETFB, and ETFDH was performed as previously reported [24,27]. Rabbit polyclonal antibodies against ETFA and ETFB were obtained from the late Dr. T. Hashimoto (Professor Emeritus of Shinshu University, Matsumoto, Japan), and an antibody against ETFDH was purchased from Japan Bio Services Co., Ltd. (Saitama, Japan).

This study was retrospectively performed through direct communication and information from clinical reports kindly provided by attending physicians. The information included sex, age at onset and diagnosis, clinical course, neurological development, life outcomes, and responses to riboflavin and bezafibrate. The response to drugs was defined based on the subjective opinions of the attending physicians as follows: “excellent” indicated no symptoms and no abnormal biochemical findings, including AST (aspartate aminotransaminase), ALT (alanine aminotransferase), LDH (lactate dehydrogenase), CK (creatine kinase), and acylcarnitine profile; “good” was defined as almost no symptoms or biochemical abnormalities with occasional metabolic attacks, such as muscle pain due to infections and hard exercise; “partial” was defined as a small decrease in the frequency of metabolic attacks, an improvement in the degree of muscle pain and weakness, or a slight improvement in biochemical abnormalities; and “no” was defined as a lack of improvement in both clinical symptoms and biochemical abnormalities. For some patients, we followed up further and confirmed the information not written in the inspection request paper by personal communication when attending physicians could be contacted. The relevance of the clinical information, clinical phenotypes, and genotypes was investigated without a statistical procedure.

This study was approved by the Ethics Committee of Shimane University Faculty of Medicine (#202000715–2), and the participants had the opportunity to decline participation in this study.

3. Results

Clinical and genetic findings regarding the 37 participants are summarized in Tables 1 and 2. Detailed information on all patients is shown in Table 3. In this study, 21 males and 15 females participated, and the sex of one patient was unknown. The median age at onset was 3 months (0 days to 40 years old). Regarding the clinical forms, the numbers of patients with type 1, type 2, and type 3 were 9, 6, and 22, respectively (Table 1). The causes of MADD were ETFDH deficiency in 26 patients, ETFA deficiency in 4 patients, ETFB deficiency in 6 patients, and FAD synthase deficiency in one patient (Table 2). No patients with RVFT1, RVFT2, RVFT3, or FAD transporter deficiency were identified.

Table 1

| Type | Clinical severity of participants (n = 37) |
|------|----------------------------------------|
| Type 1 (neonatal onset with anomalies) | 9 |
| Type 2 (neonatal onset without anomalies) | 6 |
| Type 3 (later onset) | 22 |

3.1. Clinical features and outcomes of ETFDH deficiency

Of 26 patients with ETFDH deficiency, 4 patients presented with neonatal onset with anomalies (type 1), 5 presented with neonatal onset without anomalies (type 2), and the remaining 17 presented with later onset (type 3) (Table 3). All patients with type 1 or 2 died in the neonatal period or early infancy regardless of treatment, but one patient with type 1 (#4) and three with type 2 (#7–9) survived beyond the neonatal period. Among them, all but one patient (#4, #7, and #8) underwent early diagnosis and intervention soon after birth and partially responded to bezafibrate but could not be discharged due to continuous intensive care. The other patient (#9) unexpectedly died at two months of age despite early diagnosis and intervention. Although he was enrolled as a type 2 form, his true phenotype might have been type 3 because his symptoms during the neonatal period, such as transient hypoglycemia and hyperammonemia, immediately improved with a dextrose infusion.

Among 17 patients with type 3 ETFDH deficiency, all but two survived for a long period. In this group, 11 of the 15 patients with known neurological outcomes had normal development. Of the two patients who died, one (#12) was detected by NBS and exhibited hypoglycemia
Two patients with type 3 (#35, #36) were siblings and were previously reported [22]. The older patient (#35) became bedridden due to a metabolic attack at 4 months after birth, and died at two years of age. The oldest patient (#24) began to use a wheelchair in his 50s due to progressive muscle weakness and myalgia, as previously described [24]. One patient (#20) first became pregnant at 31 years of age and successfully delivered two healthy children. Patients #10 and #22 were clinically (not genetically) diagnosed with osteogenesis imperfecta and Sotos syndrome, respectively, but no other patients had other congenital diseases. Two sisters (#25 and #26) were diagnosed by NBS and remained asymptomatic.

3.2. Clinical findings and outcomes of ETFA deficiency

All four patients with ETFA deficiency were previously reported by Yotsumoto et al. [22]. Two patients with type 1 (#27, #28) and one with type 3 (#29) died during the neonatal period and infancy, respectively. The other patient with type 3 (#30), who was judged as a riboflavin responder, survived with persisting muscle weakness and biochemical abnormalities but was able to work without problems.

3.3. Clinical findings and outcomes of ETFB deficiency

Of six ETFB-deficient patients, four patients (#31 and #34–36) were previously reported [22]. Three patients had type 1, one had type 2, and two had type 3, and all six patients died in infancy. The two patients newly participating in this study (#32 and #33) suffered from severe metabolic acidosis and some congenital anomalies soon after birth and were posthumously diagnosed as having type 1 of MADD. Although no pathological variants were identified in patient #32, he was finally diagnosed with ETFB deficiency because the bands of ETFA and ETFB were absent in Western blotting, and exon 1 of ETFB could not be amplified by PCR (polymerase chain reaction) using its cDNA and conventional primers. Two patients with type 3 (#35, #36) were siblings and were previously reported by Yamaguchi et al. [23]. The older brother (#35) became bedridden due to a metabolic attack at 4 months of age and died at 3 years. Although his younger brother (#36) was preeventually diagnosed and treated with riboflavin and l-carnitine soon after birth, he developed metabolic decompensation following diarrhea at 10 months of age and gradually deteriorated, becoming bedridden. He had a poor outcome similar to his older brother.

3.4. Riboflavin metabolic disorders

We identified one patient with FAD synthase deficiency (#37) as having riboflavin metabolic disorder, and this patient was previously reported [25]. Although he was diagnosed by NBS and treated early with riboflavin and l-carnitine, he experienced persistent lactic acidosis and exhibited symptoms of bulbar palsy, such as vocal cord paralysis and dysphagia, at approximately 3 months old. Subsequently, he became bedridden due to hypoxic-ischemic encephalopathy following aspiration pneumonia at 4 months of age.

3.5. Responses to riboflavin and bezafibrate

Of the 23 participants treated with riboflavin, none had an excellent response, 6 had a good response, 2 had a partial response, and 15 patients had no response (Tables 2 and 3). The eight good or partial riboflavin responders all had type 3 of MADD. Among them, five patients with type 3 ETFDH deficiency (#14, 16, 18, 20 and 22) and one with type 3 ETFA deficiency (#30) were good responders. Patient #30 had ETFA deficiency and was judged a good responder by his attending physician because his lethargy improved immediately after supplementation with riboflavin. However, the single effect of riboflavin effect was not objectively evaluated because other treatments, such as supplementation with carnitine and dietary restriction, commenced simultaneously with riboflavin. Two patients with type 3 ETFDH deficiency (#13 and #17) were judged as partial riboflavin responders because their muscle weakness or biochemical findings were improved but insufficient after treatment with riboflavin. Meanwhile, riboflavin was ineffective for all four patients with the neonatal-onset form (type 1 or type 2) and 8 of 15 patients with type 3 ETFDH deficiency. Except for one patient (#30), all patients with ETFA, ETFB, or FLAD1 deficiency were riboflavin non-responders. The patients with poor riboflavin responsiveness did not have specific genotypes except for p.Y507D, but all patients with onset before 6 months of age were non-responders.

Bezafibrate was administered as an off-label use to eight patients with ETFDH deficiency (one with type 1, two with type 2, and five with type 3) after the efficacy of riboflavin was judged to be lacking or insufficient. Nevertheless, all patients administered bezafibrate continued riboflavin treatment because attending physicians were concerned that patients’ conditions would worsen after stopping treatment with riboflavin. Regarding the responsiveness of bezafibrate, there were no excellent responders, two good responders, three partial responders, and three non-responders. Patient #19 was one of the two good responders, had the most effective response and was previously reported by Yamaguchi et al. [19]. He was diagnosed by NBS and managed early with riboflavin and l-carnitine. At one year of age, several episodes of hypotonia following infection and lethargy, such as hypoglycemia before breakfast, occurred. He was admitted to the intensive care unit (ICU) due to respiratory failure following aspiration pneumonia at two years of age and subsequently could not walk alone or speak well. His DQ (developmental quotient) was less than 70 at the time. However, his gait and speech disturbance recovered to pre-ICU hospitalization levels a few weeks after the initiation of treatment with bezafibrate, and his biochemical data, such as blood AST, ALT, CK, and some acylcarnitine (C4, C6, and C8) levels, returned to normal. He is now 13 years old and is a top performer in mathematics in his junior high school, although he has issues with long-distance running and a few other study subjects. The other good responder (#23) had the later-onset form (type 3) and experienced fatigue induced by minor exercise at 29 years of age, but his exercise intolerance was improved by bezafibrate. Although all three partial responders died, bezafibrate partially improved clinical features, such as hepatomegaly and hypotonia, for one patient with type 1 (#4) and two patients with type 2 (#7 and #8). The three patients (#12, 20, and 24) who were non-responders all had type 3 ETFDH deficiency.

3.6. Variants

Genetic variations were identified in 24, 4, and 5 unrelated patients with ETFDH, ETFA, and ETFB deficiencies, respectively. The most
| No. | Sex | Age at onset | Type | Age at diagnosis | Life outcome | Clinical features | Neurological development | Mutation | Response to treatment |
|-----|-----|--------------|------|-----------------|-------------|------------------|------------------------|----------|---------------------|
| 1   | M   | 0d           | Type 1 | Postmortem      | Dead (2d)   | Heart failure    | –                      | c.577G > A (p.E193K) | Riboflavin | Good                |
|     |     |              |       |                 |             | Poly cystic kidney Small for gestational age |                      | exon 1-6 deletion    | Bezafibrate | No                  |
| 2   | F   | 0d           | Type 1 | Postmortem      | Dead (2d)   | Heart failure    | –                      | c.972-2A>G | Partial improvement of clinical features |
|     |     |              |       |                 |             | Poly cystic kidney Pulmonary hypertension Respiratory failure Renal anomaly |                      | large deletion? |                       |
| 3   | N/A | 1d           | Type 1 | Postmortem      | Dead (1d)   | Heart failure    | –                      | c.946G > A (p.E316K) | –                     | –                   |
|     |     |              |       |                 |             | Lactic acidemia |                      | unknown            | –                     | –                   |
| 4   | M   | 1d           | Type 1 | 18d             | Dead (1y)   | Heart failure    | Delay                 | c.2 T > C (p. M1T)  | No                   | Partial improvement of hepatomegaly |
|     |     |              |       |                 |             | Lactic acidemia Potter’s face |                      | c.809G > T (p.W297L) |                       |                     |
| 5   | M   | 0d           | Type 2 | Postmortem      | Dead (4d)   | Heart failure    | –                      | c.1519 T > G (p.Y507D) | No                   | Partial improvement of hepatomegaly |
|     |     |              |       |                 |             | Severe Developmental delay |                        | Abnormal Splicing? |                       |
| 6   | F   | 0d           | Type 2 | Postmortem (>24h)| N/A | Heart failure    | –                      | c.1519 T > G (p.Y507D) | N/A                  | –                   |
|     |     |              |       |                 |             | Severe Developmental delay |                        | c.1519 T > G (p.Y507D) |                       |                     |
| 7‡  | F   | 0d           | Type 2 | 5d              | Dead (2y)   | Hypoglycemia    | Delay                 | c.1787A > G (p.D596G) | No                   | –                   |
| 8‡  | M   | 1d           | Type 2 | 5d              | Dead (1y)   | Hypoglycemia    | Delay                 | c.1787A > G (p.D596G) | No                   | –                   |
| 9   | M   | 1d           | Type 2 | 1m              | Dead (2 m)  | Hyper ammonomania Sudden death |               | c.1084G > A (p.G362R) | No                   | –                   |
|     |     |              |       |                 |             | Generalized |                        | c.1601C > T (p.P534L) |                       |                     |
| 10  | M   | 2 m          | Type 3 | Sibling screening | Alive (20y) | Hypo pigment | Normal               | –                      | –                     | –                   |
| 11  | M   | 2 m          | Type 3 | 2 m             | Dead (2y)   | Liver dysfunction Myopathy |               | Normal               | –                     | –                   |
| 12  | F   | 4 m          | Type 3 | NBS             | Dead (3y)   | Hypoglycemia    | Delay                 | Normal               | –                     | –                   |
| 13  | M   | 5 m          | Type 3 | 7 m             | Alive (23y) | Lethargy Muscle weakness |               | Normal               | –                     | Partial improvement of clinical features |
| 14  | M   | 6 m          | Type 3 | N/A             | Alive (27y) | Muscle weakness | Normal               | c.1208C > T (p.A403V) | Good                  | –                   |
| 15  | F   | 7 m          | Type 3 | NBS             | Alive (2y)  | Normal           | N/A                  | c.1487 T > A (p.L496H) | No                   | –                   |
| 16  | F   | 7 m          | Type 3 | 7 m             | Alive (13y) | Erythropoietin | Normal               | c.251C > T (p.A84V)  | Good                  | –                   |
| 17  | M   | 10 m         | Type 3 | 11 m            | Alive (4y)  | Lethargy Muscle weakness |               | c.1601C > T (p.P534L) | Partial improvement of biochemical data | – |
| 18  | F   | 1 y          | Type 3 | N/A             | Alive (19y) | Hypoglycemia    | Normal               | c.1096C > T (p.L366F) | Good                  | –                   |
| 19  | M   | 1 y          | Type 3 | NBS             | Alive (12y) | Muscle weakness | Mild delay           | c.1217G > A (p.5406N) | No                    | Good                |
| 20  | F   | 1y10 m       | Type 3 | 11 y            | Alive (33y) | Hypoglycemia    | Normal               | c.1675C > T (p.R559*) | Good                  | No                  |
| 21  | F   | 13 y         | Type 3 | 13 y            | Alive (26y) | Muscle weakness | Normal               | c.1774 T > C (p.C929R) | N/A                   | N/A                 |
| 22  | M   | 20 y         | Type 3 | 24 y            | Alive (28y) | Liver dysfunction | Delay               | c.1519 T > G (p.Y507D) | Unknown               | Good                |
| 23  | M   | 29 y         | Type 3 | 31 y            | Normal      | Normal           | No                   | Good                  | –                     |

(continued on next page)
### Table 3 (continued)

| Patient No. | Sex | Age at onset | Age at diagnosis | Life outcome | Clinical features | Neurological development | Mutation Allele 1 (amino acid change) | Mutation Allele 2 (amino acid change) | Response to Riboflavin | Bezoilate |
|-------------|-----|--------------|-----------------|--------------|-------------------|--------------------------|--------------------------------------|--------------------------------------|----------------------|----------|
| 24<sup>ii</sup> | M  | 40s y        | 58y             | Alive (41y)  | Rhabdomyolysis    | Normal                   | c.890G > T (p.W297L)                  | c.950C > G (p.P317R)                  | No                   | No       |
| 25<sup>i</sup> | F  | –            | NBS             | Alive (3y)   | Liver dysfunction  | Normal                   | c.389A > T (p.D130V)                  | c.389A > T (p.D130V)                  | No                   | –        |
| 26<sup>i</sup> | F  | –            | NBS             | Alive (1y)   | None (pre-symptomatic status) | Normal                   | c.389A > T (p.D130V)                  | c.389A > T (p.D130V)                  | No                   | –        |

**ETFA deficiency**

| Type | No. | Sex | Age at onset | Age at diagnosis | Life outcome | Clinical features | Neurological development | Mutation | Response |
|------|-----|-----|--------------|-----------------|--------------|-------------------|--------------------------|----------|----------|
| 1    | 27<sup>i</sup> | M  | 0d           | Postmortem      | Dead (3d)    | Respiratory failure | –                        | c.7C > T (p.R3*)                   | N/A                   | –        |
|      | 28<sup>i</sup> | F  | 0d           | 14d             | Dead (21d)   | Heart failure      | –                        | c.IV36-1G > G                   | N/A                   | –        |
| 3    | 29<sup>i</sup> | M  | 8 m          | N/A             | Dead (1y)    | Polycystic kidney  | N/A                      | c.283 T > G (p.L95V)              | –                     | –        |
|      | 30<sup>i</sup> | M  | 1y           | 2y              | Alive (28y)  | Lethargy          | Normal                   | c.478delG (truncated)             | Good                  | –        |

**ETFB deficiency**

| Type | No. | Sex | Age at onset | Age at diagnosis | Life outcome | Clinical features | Neurological development | Mutation | Response |
|------|-----|-----|--------------|-----------------|--------------|-------------------|--------------------------|----------|----------|
| 1    | 31<sup>i</sup> | F  | 0d           | Postmortem      | Dead (3d)    | Heart failure      | –                        | c.77delG (p.T27Pfs*34)             | –                     | –        |
|      | 32<sup>i</sup> | M  | 0d           | Postmortem      | Dead (5d)    | Polycystic kidney  | –                        | c.490C > T (p.R164W)              | –                     | –        |
|      | 33<sup>i</sup> | F  | 0d           | Postmortem      | Dead (<10d)  | Brain anomaly      | –                        | c.138-140delGA (p.F47del)          | –                     | –        |
| 2    | 34<sup>i</sup> | F  | 5d           | Postmortem      | Dead (5d)    | Sudden death       | –                        | c.491G > A (p.R164Q)              | –                     | –        |
| 3    | 35<sup>iii</sup><sup>ii</sup> | M  | 4 m          | 10 m            | Dead (3y)    | Liver dysfunction  | Delay                    | c.IV5S + 1G (C.P.G148-M200del)    | No                    | –        |
|      | 36<sup>iii</sup><sup>ii</sup> | M  | 10 m         | Sibling screening | Dead (3y)    | Liver dysfunction  | Delay                    | c.IV5S + 1G (C.P.G148-M200del)    | No                    | –        |

**FLAD1 deficiency**

| Type | No. | Sex | Age at onset | Age at diagnosis | Life outcome | Clinical features | Neurological development | Mutation | Response |
|------|-----|-----|--------------|-----------------|--------------|-------------------|--------------------------|----------|----------|
| 3    | 37<sup>i</sup> | M  | 3 m          | Detected by NBS | Alive (3y)   | Lactic acidosis   | Delay                    | c.745C > T (p.R249*)              | –                     | –        |

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“i, ii, and iii” indicate the number of siblings of the patients. “-” indicates not performed or not evaluated.

N/A, data not available; y, year(s); m, month(s); d, day(s); DQ, developmental quotient.

Common deletion“ and “abnormal splicing” are suspected when samples cannot be well amplified by PCR using cDNA.

* Yotsumoto Y, et al. Mol Genet Metab. 2008 19.
* Yamaguchi S, et al. Mol Genet Metab. 2012 19.
* Yamada K, et al. Brain Dev. 2015 24.
* Yotsumoto Y, et al. Mol Genet Metab. 2008 21.
* Yamaguchi S, et al. Pediatric Research. 1991 23.
* Yamada K, et al. Brain Dev. 2019 25.

Common variant was p.V507D in ETFDH, which was present in 9 of 48 identified alleles (18.8%). The second most common variants were p.W297L and p.P534L in ETFDH (each 3/48, 6.3%). Furthermore, two variants in ETFDH, p.L366F and p.R559*, were identified in two patients each. No common variant was observed in patients with ETF and ETF deficiency.

Six novel variants, p.193 K, p.E316K, p.Y333*, p.A360P, and p.L496H in ETFDH and p.F47del in ETFB, were identified in this study. According to ACMG (American College of Medical Genetics and Genomics) guidelines, p.Y333* in ETFDH and p.F47del in ETFB were classified as “Very Strong (PV51)”, while the other 4 variants in ETFDH were classified as “Moderate (PM2 or PM3)”. These variants were not detected in 100 alleles from unaffected Japanese individuals and were all determined to be “disease causing” by Mutation Taster (https://www.mutationtaster.org/), while p.E316K and p.A360P were determined to be “benign” and “possibly damaging”, respectively, by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and the other variants were designated “probably damaging”. All the affected amino
acids are highly conserved in most higher eukaryotes.

4. Discussion

The clinical and molecular aspects of 37 Japanese patients with MADD were determined in this study. No cohort study of this scale on MADD has ever been reported except in China, where MADD is a common lipid storage disease. In Japan, the proportion of patients with neonatal onset (type 1 and type 2) was not high compared with published data; however, the prognosis of the later-onset form (type 3) was relatively poor, and riboflavin-responsive cases were fewer than those of other countries. A Japanese-specific common variant causing MADD, p.Y507D in ETFDH was identified in 9 (18.8%) of the 48 alleles of 24 unrelated patients with ETFDH deficiency.

To our knowledge, p.Y507D in ETFDH has been reported in only Japan [24,28], but clinical findings about this variant are lacking. In our study, there were two patients with homozygous p.Y507D; one developed MADD during the neonatal period (type 2), while the other developed type 3 and died at two years of age. Furthermore, all six patients with compound heterozygous p.Y507D variant presented with relatively severe symptoms, including heart failure, sudden death, and developmental delay from the neonatal period to early infancy, and 5 of them were riboflavin non-responders and died by three years of age; even though they were diagnosed by NBS. Because MADD is an autosomal recessive disease, a compound heterozygous combination of a mild genotype and a severe genotype results in a mild phenotype. Therefore, the true clinical phenotype caused by p.Y507D may be underestimated in the compound heterozygous patients; nevertheless, our results suggest that p.Y507D is strongly associated with mortal phenotypes. Although many patients with ETFDH deficiency present with type 3 riboflavin-responsive MADD and the prognosis is favorable [6,16,21], the unique variant of p.Y507D could be the cause of the poor prognosis of Japanese patients with type 3 ETFDH deficiency.

Genotypes of Japanese MADD other than p.Y507D also differed from those in other countries. Only one Korean MADD patient has been reported, and the genotype and prevalence characteristics are unknown [5,29]. In China, almost all patients with MADD are ETFDH deficient, and the genotypes differ by region. For example, the most common variant of ETFDH was c.250G > A (p.A84T) for the Han Chinese population, particularly in southern China, while c.770A > G (p.Y257C) and c.1227A > C (p.L409F) were more common in northern China [21,30]. These Chinese-specific common variants were not identified in Japanese patients in this study. Thus, the genotype of east Asian patients with MADD varies markedly between regions in spite of the same Asian race. Additionally, eight Portuguese patients with MADD (seven with ETFDH deficiency and one with ETFB deficiency) were recently reported to have no common variants [31]. Three of seven Portuguese patients with ETFDH deficiency had neonatal-onset presentation (two type 1 and one type 2), and all of them died by three years of age; three of four patients with type 3 ETFDH deficiency had good life outcomes with normal development, but the other case with type 3 died at two years of age. Although the majority of Portuguese were Caucasian or mixed race, the variety of clinical phenotypes and genotypes in MADD is caused by differences in races and regions.

Regarding defective enzymes, ETFA and ETFB deficiencies were relatively common in Japanese patients. Although the worldwide proportions of the genotypes associated with MADD are not exactly known [15,32], patients with ETFA, ETFB, and ETFDH deficiencies were determined to be 11%, 16% and 70%, respectively, in our study. According to the report by Grunert S.C., the proportions of late-onset MADD were 5%, 2% and 93% for deficiencies of ETFA, ETFB, and ETFDH, respectively [16]; those of later-onset MADD in our study were 9% each for ETFA and ETFB deficiencies and 77% for ETFDH deficiency. Because ETFA and ETFB deficiencies tend to cause the neonatal-onset form and poor riboflavin responsiveness compared with ETFDH deficiency [22,33,34], a relatively large number of Japanese patients with ETFA or ETFB deficiency might have a poor prognosis.

Interestingly, only a small number of Japanese MADD patients exhibited riboflavin responsiveness compared to patients in other regions. In fact, more than half of the patients with type 3 responded poorly to riboflavin in our study. However, it has been claimed that the majority of cases of ETFDH deficiency generally manifest as a late-onset form and that riboflavin responsiveness is determined by the genotype of ETFDH [34]. Moreover, Grunert S.C. reported that “almost all patients with late-onset MADD (98%) are clearly responsive to riboflavin” [16], and the clinical and biochemical findings of almost all Chinese patients with the common variants c.250G > A, c.770A > G, and c.1227A > C of ETFDH were dramatically improved by supplementation with riboflavin [6,21]. We first considered that this discrepancy was caused by the influence of disease severity. Riboflavin-non-responsive patients are likely to present with life-threatening symptoms and laboratory abnormalities, such as respiratory distress, unconsciousness, hypoglycemia, metabolic acidosis, and hyperammonemia [25]. Conversely, patients with severe clinical features may tend to have poor riboflavin responsiveness. As mentioned above, Japanese patients with type 3 had relatively severe presentations, probably resulting in poor riboflavin responsiveness. In particular, among eight patients with the p.Y507D variant, causing a severe phenotype in at least one allele, all but one were nonresponsive. However, the poor riboflavin response cannot be explained only by disease severity and p.Y507D because riboflavin was not effective even for half of the patients with mild symptoms. In comparing our genotype data with published data by Olsen et al. [34], there were no known variants that could be expected to respond to riboflavin except for p.456 L. Strangely, the patient with the homozygous p.456 L variant (#24) in our study did not respond to riboflavin. Therefore, we newly hypothesized that a complication of CoQ deficiency might be involved. It was recently reported that a patient with MADD improved after taking riboflavin and CoQ in combination [36]. Because CoQ is not traditionally administered in Japan, no patients were treated with CoQ in our study. Although we could not investigate the serum concentration of CoQ, a considerable number of Japanese patients might have CoQ deficiency. Nevertheless, monotherapy with riboflavin is generally effective for most patients with type 3 ETFDH deficiency. The reason why riboflavin is not effective in many Japanese patients remains unknown, and poor responsiveness often worsens the life and neurological outcomes in Japanese patients.

Bezafibrate was apparently effective for some cases in this study, but we could not conclude the true efficacy of bezafibrate because the evaluation of responsiveness to bezafibrate was based on the subjective opinions of the attending physicians. Additionally, the single effect of bezafibrate could not be evaluated due to the combined use of riboflavin in all cases. Because bezafibrate was administrated as an off-label use (not a clinical trial), the objective findings and criteria were lacking to evaluate its effect. It has been reported that bezafibrate is not clinically effective for patients with very long-chain acyl-CoA dehydrogenase deficiency and carnitine palmitoyltransferase-2 deficiency [37], and bezafibrate would be ineffective for MADD as well. However, further case reports and studies will be necessary to investigate the true efficacy of bezafibrate because only a few studies on the clinical effect of bezafibrate in MADD have been reported [19,20,38].

Our results suggest that the significance of NBS for MADD is still limited in Japan because approximately 40% of patients had the neonatal-onset form and did not survive infancy. Additionally, even in type 3, many patients died before 3 years of age. Ado did not respond to riboflavin. Therefore, it may often be difficult to save lives even with early diagnosis and intervention. Nevertheless, our results also revealed favorable life and neurological outcomes for patients with type 3 who did survive for over 3 years. The number of patients with infantile onset, who were considered to substantially benefit from NBS, was relatively large in Japan, while that of patients with the adult-onset form, who were adversely considered to receive little benefit from NBS, was relatively small. Further cost–benefit studies are also essential to determine
the effect of NBS on MADD in Japan.

This study has a few limitations. First, the classification of response to riboflavin and bezafibrate was based on the subjective evaluation of the attending physicians. In particular, the effect of bezafibrate might be overestimated due to interpretation bias of the attending physicians because bezafibrate was expected to be a new promising drug at the time. Next, our study involved the majority of Japanese patients with MADD but not all. In fact, patients who were recently diagnosed (after 2015) tended to drop out, resulting in a small number of patients who were diagnosed after onset and were typical cases, which might have led to overestimation due to interpretation bias of the attending physicians. In particular, the effect of bezafibrate might have been underestimated.

Finally, our results were unique. The prognosis of patients with the late-onset form (type 3) of MADD is generally favorable in reports outside of Japan. As with all inborn errors of metabolism, the clinical phenotypes and characteristics differ among regions because the genotypes are substantially different in each region. More investigations on MADD are needed in each region and country.

5. Conclusion

Japanese patients with MADD with the exclusive variant p.Y507D in ETFDH had a relatively poor riboflavin response and poor prognosis. Because the prevalence, genotypes, phenotypes, and outcomes of MADD range widely across regions or countries, additional worldwide studies should be performed.

Sources of funding

This report was partially supported by AMED (grant numbers JP16ek0109050, JP19ek0109276, and JP20ek0109482) and JSPS KAKENHI (grant numbers 19K08300 and 19K08347). The authors confirm that the sponsors had no role in the study, and the content of the article was not influenced by the sponsors.

Declaration of Competing Interest

The authors have no conflicts of interest to declare regarding the publication of this manuscript.

Data availability

Data will be made available on request.

Acknowledgments

We are grateful to M. Furui, K. Konada, H. Kajitani, and T. Esumi for their technical assistance and all the attending physicians of the participants for providing the needed clinical information.

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