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Screening of MCAD deficiency in Japan: 16 years' experience of enzymatic and genetic evaluation

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ABSTRACT

Background: Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is a representative disorder of fatty acid oxidation and is one of the most prevalent inborn errors of metabolism among Caucasian populations. In Japan, however, it was as late as 2000 when the first patient was found, and enzymatic and genetic evaluation of MCAD deficiency began.
Methods: We measured octanoyl-CoA dehydrogenase activity in lymphocytes of symptomatic children and newborn screening (NBS)-positive subjects who showed elevated levels of C8-acylcarnitine in blood. The results were further confirmed by direct sequencing of the *ACADM* gene.
Results: The disease was diagnosed in 9 out of 18 symptomatic children. The affected patients showed residual activities from 0% to 3% of the normal average value, except for one patient with 10% activity. Concerning 50 NBS-positive subjects, 18 with enzymatic activities around 10% or lower and 14 with activities ranging from 13% to 30% were judged to be affected patients, and biallelic variants were detected in most of the cases tested. Newborns with higher enzymatic activities were estimated to be heterozygous carriers or healthy subjects, though biallelic variants were detected in 5 of them. Genetic analysis detected 22 kinds of variant alleles. The most prevalent was c.449_452delCTGA (p.T150Rfs), which was followed by c.50G>A (p.R17H), c.1085G>A (p.G362E), c.157C>T (p.R53C), and c.843A>T (p.R281S); these five variants accounted for approximately 60% of all the alleles examined.
Conclusion: Our study has revealed the unique genetic backgrounds of MCAD deficiency among Japanese, based on the largest series of non-Caucasian cases. A continuous spectrum of severity was also observed in our series of NBS-positive cases, suggesting that it is essential for every nation and ethnic group to accumulate its own information on gene variants, together with their enzymatic evaluation, in order to establish an efficient NBS system for MCAD deficiency.

1. Introduction

Medium-chain acyl-CoA dehydrogenase (MCAD; EC 1.3.8.7) deficiency is one of the most representative disorders of the fatty acid oxidation system (FAOD), which in infants and young children can lead to hypoglycemia, Reye-like encephalopathy and, in worst cases, cardiopulmonary arrest. Since the first case report on the condition in 1982 [1], followed by the cloning of the *ACADM* gene in 1989 [2], MCAD deficiency emerged as a cause of sudden infantile death with an unexpectedly high incidence among populations of European descent, which was due to the prevalent variant c.985A>G (p.K329E) [3–6]. These findings led to newborn screening (NBS) of this potentially fatal disease, and the introduction of tandem mass spectrometry (MS/MS) has enabled detection of affected patients by analyzing the profile of acylcarnitines in dried blood specimens (DBS).

In Japan, the c.985A>G allele was not found in a multilateral survey [7], nor in another study of patients with suspicious symptoms [8], and

Abbreviations: MCAD, medium-chain acyl-CoA dehydrogenase; FAOD, fatty acid oxidation disorder; MS/MS, tandem mass spectrometry; NBS, newborn screening; DBS, dried blood specimen.

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no affected patient had been found before MS/MS-based screening for FAOD was put into practice. This method was introduced into Japan in 1997, and the first patient was found in 2000 after repetitive hypoglycemic episodes [9]. Pilot studies of NBS also commenced in several areas, and a second patient was successfully detected in 2001 [9]. After these initial achievements, pilot studies gradually expanded across the country, and official nationwide NBS was finally realized in 2014.

Since the discovery of the first two patients, we have continued enzymatic and genetic evaluation of newborns and symptomatic children who showed suspicious blood acylcarnitine profiles. In this report, we present the characteristics of Japanese patients with MCAD deficiency.

2. Methods

2.1. Screening of MCAD deficiency

Blood samples were analyzed by MS/MS (LCMS-8030, Shimadzu, Kyoto, Japan; API 4000, AB Sciex, Framingham, MA, USA; ACQUITY TQD, Waters, Milford, MA, USA, *etc.*) following the protocol described in our previous report [10]. In order to set indices and cutoff values for NBS, we referred to an earlier study [11], which proposed a level of octanoylcarnitine (C8) ≧ 0.3 nmol/mL and C8/C10 ≧ 2.0 in DBS collected within 72 h after birth. As DBS are generally collected as late as the fourth or fifth day after birth in Japan, we considered that newborns with C8 ≧ 0.3 nmol/mL and C8/C10 ≧ 1.4 might be affected with MCAD deficiency. For selective screening, serum specimens were collected from patients who presented with suggestive clinical symptoms, and those with C8 ≧ 0.2 nmol/mL and C8/C10 ≧ 1.0 were suspected to have MCAD deficiency.

2.2. Measurement of MCAD activity

Activity of MCAD was measured according to our previously reported method [9]. In brief, production of 2-octenoyl-CoA from n-octanoyl-CoA (Sigma Chemical, St. Louis, MO) and ferrocenium hexafluorophosphate (Aldrich, St. Louis, MO) by crude lysate of peripheral lymphocytes was detected by a system of high-performance liquid chromatography (Shimadzu, Kyoto, Japan) and quantified according to ultraviolet absorbance at 260 nm. The activities were calculated as picomoles of 2-octenoyl-CoA formation/minute/10^6 lymphocytes and evaluated as a percentage of the average of normal control values.

2.3. Sequence analysis of the ACADM gene

Genomic DNA was extracted from peripheral white blood cells. All exons and flanking intron regions comprising the *ACADM* gene were PCR-amplified, and the products were sequenced directly using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM 310 genetic analyzer (Applied Biosystems).

3. Results

3.1. Confirmation of diagnosis

We confirmed the diagnosis of MCAD deficiency primarily based on results of enzymatic assay, which were further evaluated by genetic analysis when informed consent was obtained. The results are listed in Tables 1–4 in the order of residual enzymatic activities.

3.1.1. Symptomatic patients (Table 1)

We enzymatically evaluated 18 symptomatic patients. According to the results, MCAD deficiency was diagnosed in 9 patients, of whom residual activities ranged from 0% to 3% of the average of normal control values (S-01–08), except for a patient that showed activity of 10.3% (S-09). MCAD activity in another patient (S-10) was 47.0%; although it was as high as those of obligate heterozygous carriers tested in our study, two types of single-base substitutions were detected in this subject. The other patients were judged to be normal with regard to MCAD. Clinical onset of the affected patients ranged from 2 days after birth to 2 years and 3 months. Though no deaths were documented in our series of cases, neurological sequelae remained in two patients (S-02, 03).

3.1.2. Screening positive newborns (Table 2)

We enzymatically evaluated 50 C8-positive newborns. Eighteen newborns showed MCAD activities around 10% or lower, and were therefore judged to be associated with the actual risk of acute metabolic failure (N-01–18). Fourteen newborns who showed residual activities ranging from 13% to 30% were also judged to be affected patients (N-19–32), and biallelic variants were detected in most of the cases tested. Eight newborns with enzymatic activities between 37% and 60% appeared to be heterozygous carriers (N-33–40), though biallelic variants were detected in three of them (N-33, 36, 39). Ten newborns who retained enzymatic activities higher than 70% were judged to be normal

Table 1
Clinical, biochemical and genetic characteristics of symptomatic cases.

Case	MCAD activity (%)[a]	Newborn dried blood (retrospective data)		Serum (after clinical onset)		*ACADM* variants		Age of clinical onset	Symptoms
		C8 (μM) (≧0.3)	C8/C10 (≧1.4)	C8 (μM) (≧0.2)	C8/C10 (≧1.0)	Nucleotide	Amino acid		
S-01	BLQ[b]	Not available		8.89	13.5	c.[449_452del];[(449_452del)]	p.[T150Rfs];[(T150Rfs)]	1y1m	Hypoglycemia
S-02[c]	BLQ[b]	Not available		7.90	11.1	Deletion of exons 11, 12	Deletion of exons 11, 12	1y1m	Hypoglycemic encephalopathy
S-03	BLQ[b]	1.57	8.77	4.11	9.34	c.[1085G>A];[(1085G>A)]	p.[G362E];[(G362E)]	2d	Hypoglycemic encephalopathy
S-04[c]	BLQ[b]	Not available		1.71	15.5	c.[449_452del];[(449_452del)]	p.[T150Rfs];[(T150Rfs)]	1y4m	Hypoglycemia
S-05	BLQ[b]	Not available		Not available		Detected elsewhere	Detected elsewhere	1y5m	Hypoglycemia
S-06	1.9	3.95	6.07	3.67	5.28	c.[449_452del];[555T>G]	p.[T150Rfs];[I185M]	2y3m	Hypoglycemia
S-07	3.0	2.52	10.1	6.35	12.2	Not analyzed		1y3m	Hypoglycemia
S-08	3.1	Not available		18.2	13.4	Not analyzed		1y4m	Hypoglycemia
S-09	10.3	0.62	3.7	5.97	3.49	c.[157C>T];[449_452del]	p.[R53C];[T150Rfs]	8m	ALTE[d]
S-10	47.0	0.11	0.4	0.36	1.3	c.[50G>A](;)[1247T>C]	p.[R17H](;)[I416T]	1m	Vomiting
S-11–18	64.3–114.1					Not analyzed		Various	Various atypical symptoms

[a] The average value of MCAD activities in 60 normal control subjects was 50.93 ± 13.46 pmol/min/10^6 lymphocytes (mean ± SD).
[b] BLQ: below the limit of quantification.
[c] Variants of cases S-02 and S-04 were detected by Purevsuren J et al. [12,13].
[d] ALTE: an apparent life-threatening event.