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SHORT COMMUNICATION

Novel mutations in five Japanese patients with 3-methylcrotonyl-CoA carboxylase deficiency

Mitsugu Uematsu · Osamu Sakamoto · Noriko Sugawara · Naonori Kumagai · Tetsuji Morimoto · Seiji Yamaguchi · Yuki Hasegawa · Hironori Kobayashi · Kenji Ihara · Makoto Yoshino · Yoriko Watanabe · Takahiro Inokuchi · Takato Yokoyama · Kohji Kiwaki · Kimitoshi Nakamura · Fumio Endo · Shigeru Tsuchiya · Toshihiro Ohura

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Abstract Isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency appears to be the most frequent organic aciduria detected in tandem mass spectrometry (MS/MS) screening programs in the United States, Australia, and Europe. A pilot study of newborn screening using MS/MS has recently been commenced in Japan. Our group detected two asymptomatic MCC deficiency patients by the pilot screening and collected data on another three MCC deficiency patients to study the molecular bases of the MCC deficiency in Japan. Molecular analyses revealed novel mutations in one of the causative genes, *MCCA* or *MCCB*, in all five of the patients: nonsense and frameshift mutations in *MCCA* (c.1750C > T/c.901_902delAA) in patient 1, nonsense and frameshift mutations in *MCCB* (c.1054_1055delGG/c.592C > T) in patient 2, frameshift and missense mutations in *MCCB* (c.1625_1626insGG/c.653_654CA > TT) in patient 3, a homozygous missense mutation in *MCCA* (c.1380T > G/ 1380T > G) in patient 4, and compound heterozygous missense mutations in *MCCB* (c.569A > G/ c.838G > T) in patient 5. No obvious clinical symptoms were observed in patients 1, 2, and 3. Patient 4 had severe neurological impairment and patient 5 developed Reye-like syndrome. The increasing use of MS/MS newborn screening in Japan will further clarify the clinical and genetic heterogeneity among patients with MCC deficiency in the Japanese population.

Keywords 3-Methylcrotonyl-CoA carboxylase · Newborn screening · Tandem mass spectrometry · Carnitine · Hyperammonemia

M. Uematsu · O. Sakamoto · N. Sugawara · N. Kumagai · T. Morimoto · S. Tsuchiya · T. Ohura (✉)
Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai, Japan
e-mail: tohura@mail.tains.tohoku.ac.jp

S. Yamaguchi · Y. Hasegawa · H. Kobayashi
Department of Pediatrics, Shimane University School of Medicine, 89-1 Enya-cho, Izumo, Shimane, Japan

K. Ihara
Department of Pediatrics,
Graduate School of Medical Science,
Kyushu University, 3-1-1 Maidashi,
Higashi-ku, Fukuoka, Japan

M. Yoshino · Y. Watanabe
Department of Pediatrics and Child Health, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Japan

T. Inokuchi
Research Institute of Medical Mass Spectrometry, Kurume University School of Medicine, 67 Asahimachi, Kurume, Japan

T. Yokoyama
Department of Pediatrics, St Mary's Hospital,
422 Tsubukuhonmachi, Kurume, Japan

K. Kiwaki · K. Nakamura · F. Endo
Department of Pediatrics, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto, Japan

Introduction

In 1977, medical institutions in Japan began to routinely perform newborn screening for the detection of disorders such as phenylketonuria, maple syrup urine disease, homocystinuria, and galactosemia. More recently, medical institutions in the United States, Australia, and Europe have adopted a new type of newborn screening capable of detecting many other metabolic diseases by tandem mass spectrometry (MS/MS) (Millington et al. 1990). Several medical centers in Japan are now demonstrating the MS/

MS system in pilot study programs for newborn screening using dried blood spots on Guthrie cards.

MS/MS newborn screening under the pilot study program in Japan has led to the detection of potentially mild and even asymptomatic disease in a growing number of cases. Isolated 3-methylcrotonyl-CoA carboxylase (MCC, EC 6.4.1.4) deficiency, an autosomal-recessive inheritance, appears to be the most frequent (approximately 1 in 50,000) organic aciduria (Koeberl et al. 2003; Schulze et al. 2003; Wilcken et al. 2003). The clinical course has been shown to vary considerably, ranging from entirely asymptomatic to death in infancy. MCC is a biotin-dependent enzyme in the L-leucine degradation pathway. The enzyme consists of two subunits, MCCα and MCCβ, encoded by *MCCA* and *MCCB*, respectively (Obata et al. 2001; Baumgartner et al. 2001; Gallardo et al. 2001; Holzinger et al. 2001). The *MCCA* gene is located on chromosome 3q26–q28 and consists of 19 exons, while the *MCCB* gene is located on chromosome 5q13 and consists of 17 exons. Enzyme deficiency can result from mutations in either the *MCCA* or *MCCB* gene (MIM 210200 and MIM 210210, respectively). Investigators have so far reported 28 *MCCA* and 41 *MCCB* mutation alleles, including missense, nonsense, frameshift, and splice site mutations (Stadler et al. 2006).

In this report we describe a molecular study of five Japanese patients with MCC deficiency, including two neonates detected by MS/MS newborn screening.

Materials and methods

Case report

Patient 1 was born to unrelated parents and MS/MS screening on day 5 revealed an elevated blood concentration of 3-hydroxyisovalerylcarnitine (12.1 μmol/l, cut-off point 1.0). He had no clinical problems and appeared entirely normal on physical examination. The blood ammonia concentration was significantly elevated to 111 μmol/l (normal 6–26) in a fasting condition and to 130 μmol/l 1 h after feeding. Serum leucine was also elevated (313 μmol/l, normal 50–198), and serum carnitine levels were decreased (total 13.3 μmol/l, normal 45–91; free 5.6 μmol/l, normal 36–74). A urinary organic acid analysis revealed markedly elevated concentrations of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid. From these results, we diagnosed his condition as an isolated MCC deficiency and prescribed treatment with a leucine-restricted diet and oral L-carnitine supplementation. Serum concentrations of leucine and ammonia were reduced to normal range after the therapy.

Patient 2 was born to non-consanguineous parents. MS/MS newborn screening revealed elevated levels of 3-hydroxyisovalerylcarnitine (6.98) and hypocarnitinemia (free carnitine 3.58). His condition was confirmed as MCC deficiency by urinary organic acid analysis, in the absence of any clinical symptoms. Serum ammonia was elevated slightly to 57 μmol/l. A family screening revealed an elevated concentration of 3-hydroxyisovalerylcarnitine in his asymptomatic brother (2 years old).

Patients 3 and 4 have both been reported previously (Ihara et al. 1997; Murayama et al. 1997). Patient 3 was born to unrelated parents and referred to Kyusyu University Hospital after a newborn screening by the Guthrie method detected an elevated level of leucine. His condition was diagnosed as MCC deficiency at the age of 5 months by gas chromatography/mass spectrometry (GC/MS) analysis of urine, though the patient remained free of symptoms (Ihara et al. 1997). Patient 4 was born to consanguineous parents. Her symptoms included marked growth retardation from birth, mental retardation, and epilepsy (Murayama et al. 1997). She was diagnosed as having MCC deficiency by urine GC/MS analysis at the age of 15 years.

Patient 5 was born to unrelated parents and grew up normally. At the age of 1 year she developed generalized convulsion and impairment of consciousness during gastroenteritis. Serum ammonia was markedly elevated to 240 μmol/l and carnitine levels were reduced (total 15.1; free 5.8). Her condition was diagnosed as Reye-like syndrome with serious aftereffects of motor and mental retardation. She was subsequently diagnosed as having MCC deficiency by urine GC/MS analysis.

Table 1 summarizes the clinical and genetic findings of these cases.

Genetic analysis of *MCCA* and *MCCB*

Total RNA was isolated from peripheral blood (patients 1, 2, and 5) or fibroblasts (patients 3 and 4). Complementary DNA (cDNA) was synthesized by 0.5 μg of total RNA. RT-PCR analysis was performed using the Thermoscript RT-PCR system (Invitrogen). The entire coding region of *MCCA* was amplified in six segments, and *MCCB* was amplified in five overlapping segments (the primer sequences are available upon request). The PCR products were directly sequenced using the Big Dye Primer Cycle Sequencing kit and ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). The detected mutations were confirmed by sequence analysis of the patient's genomic DNA. Written informed consent was obtained from the patient's parents. The Ethics

Table 1 Clinical findings and gene mutations in five patients with MCC deficiency

	Age at diagnosis	Clinical findings	Therapy	Gene mutations
Patient 1	NBS by MS/MS	No clinical symptoms	Restriction of leucine	MCCA
(1 year, male)	(32 days)	Elevation of plasma leucine	Carnitine supplementation	Ex 9: c.901_902delAA (p.Lys301AlafsX10)
		Mild hyperammonemia		
		Hypocarnitinemia		Ex16: c.1750C > T (Gln584X)
Patient 2	NBS by MS/MS	No clinical symptoms	Restriction of leucine	MCCB
(1 year, male)	(28 days)	Hypocarnitinemia	Carnitine supplementation	Ex 6: c.592C > T (Gln198X)
				Ex11: c.1054_1055delGG (p.Gly352ArgfsX26)
Patient 3	5 Months	Nystagmus (neonatal period)	Carnitine supplementation	MCCB
(9 years, male)	by urine GC/MS	Elevation of plasma leucine		Ex 7: c.653_654CA > TT (Ala218Val)
		Mild hyperammonia		Ex17: c.1625_1626insGG (p.Leu543ValfsX11)
		Hypocarnitinemia		
Patient 4	15 years	Failure to thrive from birth	Restriction of leucine	MCCA
(25 years, female)	by urine GC/MS	Mental retardation	Carnitine and glycine supplementation	Ex13: c.[1380T > G] + [1380T > G] (Ile460Met)
		Cerebral palsy, epilepsy		
Patient 5	1 year	Reye-like syndrome	Restriction of leucine	MCCB
(2 years, female)	by GC/MS		Carnitine and biotin supplementation	Ex6: c.569A > G (His190Arg) Ex9: c.838G > T (Asp280Tyr)

Committee of the Tohoku University School of Medicine approved this study.

Results

The sequencing analysis of patients revealed nine novel mutations in the coding exons of *MCCA* and *MCCB* (Table 1). According to direct sequencing analyses of the parents of patient 1, the father was heterozygous for the c.901_902delAA mutation, and the mother was heterozygous for the c.1750C > T mutation. Direct sequencing analyses of the parents and brother of patient 2 revealed that the brother had the same compound heterozygous mutations in *MCCB*, the father was heterozygous for the c.592C > T mutation, and the mother was heterozygous for the c.1054_1055delGG mutation. None of the four missense mutations (c.653_654CA > TT, c.1380T > G, c.569A > G and c.838G > T) was found in the DNA samples from 50 healthy volunteers by PCR-restriction fragment length polymorphism analysis (data not shown).

Discussion

As far as we know, this is the first report of mutations in MCC deficiency patients in Japan. Patients 1 and 2, the two patients detected by MS/MS newborn screening, were compound heterozygotes of a nonsense mutation and a frameshift mutation leading to a new stop codon. These mutations presumably led to significant reductions in MCC activity by destabilizing the mRNA. The previous report on patient 3 indicated that he had extremely low MCC activity (Ihara et al. 1997). Clinical findings of these cases were different from those we expected, however. Patients 1, 2 and 3 were almost entirely free of symptoms. Moreover, the affected elder brother of patient 2 was revealed to have been free of symptoms. On the other hand, patient 4, a homozygote for Ile460Met, and patient 5, a compound heterozygote for His190Arg and Asp280Tyr, were both severe phenotypes from the neonatal to infantile period and retained serious aftereffects.

Stadler et al. recently reported that there was no correlation between phenotypes and genotypes in MCC deficiency (Stadler et al. 2006). From these findings and our results, we conjecture that factors other than the genotype at the MCC loci, such as modifying genes and environmental factors, may have a major influence on the MCC deficiency phenotype. By expansion of MS/MS newborn screening, more and more asymptomatic subjects with MCC deficiency have been detected. Thus, earlier reports on patients with MCC deficiency might be biased toward symptomatic patients.

Although there is no established evidence about essential treatment for patients with MCC deficiency, long-term treatment with restriction of dietary leucine and L-carnitine supplementation has been recommended (Sweetman and Williams 2001). Most previous studies had reported a negative effect of biotin supplement therapy for MCC deficiency (Sweetman and Williams 2001), but recently it

was reported that the patient with heterozygote mutation in *MCCA* gene showed good clinical and biochemical response to biotin (Friebel et al. 2006). Patient 5 in this manuscript was treated with biotin, but she did not respond to it. Biotin supplementation has not been tried in the other four patients. It may be worthwhile to try biotin supplementation for all patients with MCC deficiency since there were no serious side effects of biotin supplementation reported.

Patients with MCC deficiency are usually symptom-free when in good health, but may succumb to ketoacidosis and hypoglycemia during acute stress such as infection (Ficicioglu and Payan 2006). The patients should therefore be treated early on with mild protein restriction and carnitine supplementation, with parental counseling to prevent catabolic episodes.

We expect newborn screening with MS/MS to help prevent the development of severe phenotypes by facilitating early diagnosis and treatment. Further increases in the number of MCC deficiency cases detected through the MS/MS screening program in Japan will help to clarify the prognostic indicators of MCC deficiency and beneficial therapy of this disease, especially in asymptomatic cases.

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