Japanese Male Siblings with 2-Methyl-3-Hydroxybutyryl-CoA Dehydrogenase Deficiency (HSD10 Disease) Without Neurological Regression

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Abstract 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (HSD10 disease) is a rare X-linked disorder caused by a mutation in the *HSD17B10* gene. Fewer than 30 patients with this disorder have been reported worldwide. The classical infantile form of HSD10 disease is characterized by a progressive neurodegenerative course with retinopathy and cardiomyopathy, although HSD10 disease has broad clinical heterogeneity. However, several male patients have not shown neurological regression. Here, we describe two Japanese siblings with HSD10 disease without neurological regression. A 4-year-old boy presented with unconsciousness due to severe hypoglycemia. Laboratory testing on admission showed mild metabolic acidosis and mild hyperammonemia. Urinary organic acid analysis in the acute phase showed elevated excretion of 2-methyl-3-hydroxybutyric acid, tiglylglycine, and ketones. However, 2-methylacetoacetate was not elevated. HSD10 disease was suspected based on urinary organic

acid data. The patient had a novel hemizygous c.470C>T (p.A157V) mutation in the *HSD17B10* gene. His mother was a heterozygous carrier of this mutation. The patient's older brother also had the c.470C>T (p.A157V) mutation. Neurological development was normal at the time of evaluation. The pilot newborn screening results using tandem mass spectrometry of the proband were reevaluated retrospectively and showed a high C5:1 carnitine level of 0.070 nmol/mL (upper cutoff limit, 0.05 nmol/mL) and a normal C5-OH carnitine level of 0.290 nmol/mL (upper cutoff limit, 1.0 nmol/mL). His affected brother and another patient with the atypical form of HSD10 disease having p.A154T also showed elevated C5:1 but not C5-OH in serum acylcarnitine analysis. Thus, these data suggested that some patients with this disorder may be identified using newborn screening.

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Introduction

2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (HSD10 disease) is a rare X-linked disorder caused by a mutation in the *HSD17B10* gene (Zschocke et al. 2000; Ofman et al. 2003). Fewer than 30 patients have been reported worldwide. Classical infantile form of HSD10 disease is characterized by a progressive neurodegenerative course with retinopathy and cardiomyopathy, leading to death at the age of 2–4 years or later (Zschocke 2012); however, this disorder has wide clinical heterogeneity. Only three patients in two families have been reported to have the atypical presentation of the disease, without neurological regression (Rauschenberger et al. 2010; Fukao et al.

2014). Moreover, one patient without neurological regression during childhood was reported to develop Parkinsonism in adulthood, as an abstract form (Lorea et al. 2015).

Here, we describe the fourth and fifth case of HSD10 disease without neurological regression in two Japanese siblings. The proband developed ketotic hypoglycemia three times at 4 years of age.

Case Presentation

A 4-year-old Japanese boy, who had been well and exhibited normal development until admission, presented with unconsciousness. One day before admission, he had a high fever and appetite loss. His parents were unrelated, and he had a 10-year-old brother and an 8-year-old sister. Both of his siblings exhibited normal growth and development.

On physical examination on admission, the patient had a height of 90.5 cm (−1.1 SD), body weight of 12.7 kg (−1.7 SD), heart rate of 114 bpm, and body temperature of 36.8°C. The Glasgow Coma Scale was E4V1M1. Laboratory testing on admission showed mild metabolic acidosis (blood gas pH, 7.337; pCO₂, 33.8 mmHg; HCO₃[−], 17.7 mM), mild hyperammonemia (156 μM), and severe hypoglycemia (blood glucose less than 1.1 mM). Other data were as follows: white blood cell count, 26,900/μL; hemoglobin, 12.5 g/dL; blood urea nitrogen, 0.57 mM; aspartate aminotransferase, 49 IU/L; lactate dehydrogenase, 353 IU/L; lactate, 2.7 mM; total ketone bodies, 5.4 mM; and insulin, 2.2 μIU/mL. Three minutes after bolus infusion of 18 mL of 20 % glucose, blood glucose was increased to 6.61 mM, and the patient became conscious. After continuous glucose infusion, his symptoms improved, and he began to take oral food. On day 2 of hospitalization, the patient exhibited duodenal ulcers, with complaints of abdominal pain and melena. He was successfully medicated and was discharged from the hospital on day 22 of hospitalization. Urinary organic acid analysis in the acute phase showed elevated excretion of 2-methyl-3-hydroxybutyrate to 830 μg/mg Cr (mean ± SD, 5.1 ± 5.3) and tiglylglycine to 252.6 μg/mg Cr (1.1 ± 0.6). However, 2-methylacetoacetate was not elevated (0.1 μg/mg Cr; 1.0 ± 1.6). Urinary organic acid analysis 1 month later under nonsymptomatic conditions also showed increased excretion of 2-methyl-3-hydroxybutyrate (91.8 μg/mg Cr) and tiglylglycine (207.7 μg/mg Cr). HSD10 disease was suspected based on urinary organic acid data and harmonized elevation of blood ketone bodies with hypoglycemia.

Mutation analysis was then carried out at the genomic level. The results showed that the patient had a novel hemizygous c.470C>T (p.A157V) mutation in the *HSD17B10* gene. His mother was a heterozygous carrier of this mutation, and his older brother also had the c.470C>T (p.A157V) mutation.

This mutation was not present in a population-based variation database consisting of 1,208 Japanese individuals (Human Genetic Variation Database, www.genome.med.kyoto-u.ac.jp/SnpDB/index.html).

2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD) activity was assayed using fibroblasts, as previously described (Zschocke et al. 2000). 2M3HBDH activity was low (0.25 ± 0.05 nmol/min/mg protein; control, 1.33 ± 0.08 nmol/min/mg protein). Immunoblot analysis showed that fibroblasts from patients with p. A157V or p.A154T mutations had similar levels of HSD17B10 protein as control fibroblasts (Fig. 1).

Pilot newborn screening using tandem mass spectrometry at 5 days of age showed C5:1 levels of 0.070 nmol/mL and C5-OH of 0.290 nmol/mL. Screening criteria for beta-ketothiolase deficiency at newborn screening were 0.05 nmol/mL for C5:1 and 1.0 nmol/mL for C5-OH. Reexamination showed C5:1 of 0.090 nmol/mL and C5-OH of 0.590 nmol/mL. Therefore, screening criteria for beta-ketothiolase deficiency was not carried out and was judged as normal at that time.

The patient developed two additional ketotic hypoglycemic episodes at 4 years of age. His neurological development was normal, with an IQ of 90 (Wechsler Intelligence Scale for Children). No abnormal findings were identified in echocardiographic or ophthalmological examinations.

Urinary and blood analyses were also performed for his older brother at 11 years of age. Urinary organic acid analysis showed elevated 2-methyl-3-hydroxybutyrate to 25.0 μg/mg Cr and tiglylglycine to 44.1 μg/mg Cr. Serum acylcarnitine profile analysis showed an elevated C5:1 of 0.108 nmol/mL (mean ± SD, 0.012 ± 0.005) and a normal C5-OH of 0.06 nmol/mL (0.06 ± 0.03). His IQ was 74, classifying him as borderline intelligence; however, he was enrolled in normal classes in school and had never experienced any apparent neurological problems.

Discussion

Typical HSD10 disease is suspected when patients with neurological degeneration or psychomotor retardation show similar urinary organic acid or blood acylcarnitine profiles with beta-ketothiolase deficiency. Our patient developed severe ketotic hypoglycemia, and HSD10 disease was suspected based on metabolic evaluation of this episode. His neurological development was normal, and he had still not exhibited any neurological symptoms at the latest follow-up (5 years of age). Moreover, his older brother was shown to have the same mutation and an IQ indicating borderline intelligence at 11 years of age, without the occurrence of neurological problems. The older brother had never experienced hypoglycemic episodes.

Fig. 1 Immunoblot analysis: Fibroblasts were cultured in Eagle's minimum essential medium containing 10% fetal calf serum. Immunoblot analysis for 2M3HBD was carried out using anti-rat 2M3HBD antibodies and antihuman glyceraldehyde 3-phosphate

dehydrogenase (GAPDH) antibodies (sc-25778; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a reference. *Each lane represents 30 µg of fibroblast extracts*

Based on urinary organic acid profiles and normal mitochondrial acetoacetyl-CoA thiolase activity in the patient's fibroblasts, we assumed that the function of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase was blocked in this patient. In accordance to this, we identified a novel hemizygous mutation c.470C>T (p.A157V) in the *HSD17B10* gene, which encodes the HSD10 protein, a multifunctional mitochondrial 17β-hydroxysteroid dehydrogenase and one of the three components of mitochondrial RNaseP. The latter is essential for mitochondrial tRNA processing (Holzmann et al. 2008). Several studies have shown that defects in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase activity are not associated with clinical severity of HSD10 disease, whereas defects in RNaseP function are associated with clinical severity (Rauschenberger et al. 2010; Yang et al. 2009).

Patients with HSD10 disease exhibit broad clinical heterogeneity. Zschocke subdivided HSD10 patients into four groups: the neonatal form, infantile form, juvenile form, and atypical presentation, as shown in Table 1 (Zschocke 2012). The classical presentation, which is called the infantile form, is characterized by a progressive neurological regression and cardiomyopathy, leading to death. However, several patients have been described who did not show neurological regression, a presentation classified as the atypical presentation (Table 2). A patient with the c.495A>C (p. Q165H) mutation was shown to have normal development up to his current age of 5 years, although he previously exhibited growth retardation and feeding diffi-

culties during infancy. Moreover, his male cousin, who had the same mutation, achieved normal neurodevelopment until his current age of 8 years (Rauschenberger et al. 2010). We recently reported another patient with the c.460G>A (p.A154T) mutation who showed no neurological regression until his current age of 6.5 years (Fukao et al. 2014). Thus, in this report, we described an additional two patients with c.470C>T (p.A157V) mutations, leading to the atypical presentation of HSD10 disease. However, another patient with the p.A158V mutation was reported to have developed Parkinsonism and was diagnosed with HSD10 disease at the age of 27 years (Lorea et al. 2015). Because the main characteristic of these patients was the lack of neurological regression during childhood, we propose that these patients should be classified as having a nonregressive form of HSD10 disease. Some patients may have developmental delays or neurological problems without neurological regression. Therefore, careful follow-up is needed for young patients without neurological regression because we cannot exclude the possibility of neurological problems and other symptoms later in life.

The mutations identified in the four patients with atypical HSD10 (Table 2) are located in close proximity in the tertiary structure of the HSD17B10 protein subunit (PDB ID: 2O23). Moreover, the catalytic triad of this subunit is formed by Ser155, Tyr168, and Lys172. All of these four mutations are very close to one of the catalytic residues. Thus, catalytic activity is expected to be affected by these mutations. Polyphen2 software suggests that

Table 1 Clinical presentation of HSD10 disease and mutations in the *HSD17B10* gene

Classification	Number of previous cases	Typical mutations	Clinical presentation	Reference
Neonatal form	3	p.N247S, p.R226Q, p.D86D	Immediate postnatal onset of severe metabolic lactic acidosis, little psychomotor development, seizures, progressive cardiomyopathy, death in the first month of life	Perez-Cerda et al. (2005), García-Villoria et al. (2009), and Rauschenberger et al. (2010)
Infantile form	More than 13	p.R130C, p.P210S, p.L122V	Normal development in the first 6–18 months of life, followed by a progressive neurodegenerative course with retinopathy and cardiomyopathy, leading to death at the age of 2–4 years or older	Zschocke et al. (2000), Ensenauer et al. (2002), Sutton et al. (2003), Sass et al. (2004), Perez-Cerda et al. (2005), Cazorla et al. (2007), Lenski et al. (2006), García-Villoria et al. (2009), Rauschenberger et al. (2010), and Zschocke (2012)
Juvenile form	1	p.E249Q	Normal development in the first 5 years and slow deterioration of speech and motor skills linked to measles infection	Olpin et al. (2002)
Nonregressive form	Six cases from four families, including the siblings in the current report	p.R165H, p.A154T, p.A157V, p.A158V	Normal development in childhood, with typical urinary organic acid profiles and potential transient metabolic decompensation	Rauschenberger et al. (2010), Fukao et al. (2014), and Lorea et al. 2015; Present case

Table 2 Mutations and clinical presentation of nonregressive form of HSD10 disease

Mutation	First presentation	Neurological problem	Family members with the same mutation	Reference
p.Q165H	Pre- and postnatal failure to thrive	None at 8 years of age	Brother and male cousin	Rauschenberger et al. (2010)
p.A154T	Five-day period of appetite loss and vomiting with severe ketoacidosis due to gastroenteritis at 6 years of age	None at 7 years of age		Fukao et al. (2014)
p.A158V	Parkinsonism at 27 years of age	None before diagnosis		Lorea et al. (2015)
p.A157V	Unconsciousness due to severe hypoglycemia at 4 years of age	None at 5 years of age	Older brother	Present case

p.A157V may be classified as probably damaging. Additionally, immunoblot analysis showed that fibroblasts from patients with p. A157V or p.A154T mutations had similar levels of HSD17B10 protein as control fibroblasts (Fig. 1) (Fukao et al. 2014). These mutations may affect catalytic activity but not stability.

Our proband and the patient with the p.A154T mutation developed ketotic hypoglycemic episodes several times, leading to further urinary organic acid analyses and thereby facilitating the final diagnosis (Fukao et al. 2014). However, it is not clear whether patients with HSD10 disease may tend to develop ketotic hypoglycemia owing to the lack of sufficient cases; further studies are needed to determine the associations between ketotic hypoglycemia and HSD10 disease.

Notably, C5:1 was particularly elevated, whereas C5-OH was within the upper cutoff limit in newborn screening in

our probands. Such high C5:1 levels were not observed in more than 10,000 newborns in Gifu, Japan (unpublished observation). However, the patient's older brother and our other patient with the p.A154T mutation also showed elevated C5:1 but not C5-OH in serum acylcarnitine analysis. Thus, these data suggest that this disorder may be identifiable during newborn screening.

Conclusion

Here, we present two male siblings with HSD10 disease without neurological regression (the nonregressive form) having a novel hemizygous c.470C>T (p.A157V) mutation in the *HSD17B10* gene. The younger sibling had several ketotic hypoglycemic events at 4 years of age, whereas the older brother showed no symptoms. The nonregressive

form of HSD10 disease may not be as rare as was previously thought, and careful follow-up is needed for young patients with atypical HSD10 disease.

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Take-Home Message

Patients of HSD10 disease without neurological regression during childhood may not be as rare as was previously thought.

Conflict of Interest

Shohei Akagawa, Toshiyuki Fukao, Yuko Akagawa, Hideo Sasai, Urara Kohdera, Minoru Kino, Yosuke Shigematsu, Yuka Aoyama, and Kazunari Kaneko declare that they have no conflicts of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the family for inclusion in this report. The molecular study was approved by the Ethical Committee of the Graduate School of Medicine, Gifu University, Gifu, Japan, and carried out with written approval.

Compliance with Ethics guidelines

This article does not contain any studies with animal subjects performed by the any of the authors.

Author Contributions

SA is the attending doctor of the siblings. YA, UK, and MK followed and treated the patients with SA. YS chemically diagnosed these patients by GC-MS. TF, HS, and YA performed molecular analysis and contributed to molecular diagnosis. TF, YS, and KK helped SA to diagnose and treat

the patient as advising doctors. SA and TF contributed to the conception and design of this report. YS and KK contributed to drafting of the article. SA, TF, YS, YA, and KK contributed to critical editing of the article. All authors approved the final version of the article.

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