Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2014.05.008.

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H. Niizeki*
Department of Dermatology, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

A. Shiohama[a,b]
[a]Department of Dermatology, Keio University School of Medicine, Tokyo, Japan, [b]Laboratory of Gene Medicine, Keio University School of Medicine, Tokyo, Japan

T. Sasaki
Center for Integrated Medical Research, Keio University School of Medicine, Tokyo, Japan

A. Seki
Department of Orthopedics, National Center for Child Health and Development, Tokyo, Japan

K. Kabashima, A. Otsuka
Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

K. Kosaki
Department of Orthopedic Surgery, University of Tokyo, Tokyo, Japan

A. Ogo
Department of Metabolism and Endocrinology, Clinical Research Institute, National Hospital Organization Kyushu Medical Center, Fukuoka, Japan

T. Yamada
Department of Dermatology, Himeji Red Cross Hospital, Hyogo, Japan

M. Miyasaka
Department of Radiology, National Center for Child Health and Development, Tokyo, Japan

K. Matsuoka
Department of Pathology, National Center for Child Health and Development, Tokyo, Japan

A. Hirakiyama, T. Okuyama
Department of Laboratory Medicine, National Center for Child Health and Development, Tokyo, Japan

M. Matsuda
Department of Dermatology, National Center for Child Health and Development, Tokyo, Japan

K. Nakabayashi
Department of Reproductive Biology, National Research Institute for Child Health and Development, Tokyo, Japan

K. Tanese
Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

A. Ishiko
Department of Dermatology, School of Medicine, Toho University, Ota-ku, Tokyo, Japan

M. Amagai
Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

J. Kudoh
Laboratory of Gene Medicine, Keio University School of Medicine, Tokyo, Japan

* Corresponding author. Tel.: +81 3 3416 0181; fax: +81 3 5494 7909.
E-mail address: niizeki-h@ncchd.go.jp (H. Niizeki).

Received 9 December 2013
Received in revised form 12 May 2014
Accepted 13 May 2014

http://dx.doi.org/10.1016/j.jdermsci.2014.05.008

Letter to the Editor

The natural trait of the curvature of human hair is correlated with bending of the hair follicle and hair bulb by a structural disparity in the root sheath

The shape of human hair is one of the most distinguishable characteristics among various populations and commonly classified according to geographic regions and ethnic differences. Two hypotheses have been proposed as key determinants of human hair shape based on morphological features. One proposes that hair shape is determined solely by the shape of the hair follicle (HF) [1], straight hair being generated from straight cylindrical HFs whereas highly curled hair is produced by curved HFs. The second hypothesis proposes that hair shape is programmed by the hair bulb (HB) based on results from organ culture, suggesting that an asymmetric differentiation and proliferation of each follicular layer beginning in the HB and mechanical stress on the concave side of the HB are closely related to the formation of hair shape [2].

Journal of Human Genetics (2014), 1–6

www.nature.com/jhg

ORIGINAL ARTICLE

The first case in Asia of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (HSD10 disease) with atypical presentation

Toshiyuki Fukao[1,2], Kazuhisa Akiba[3], Masahiro Goto[4], Nobuki Kuwayama[1], Mikiko Morita[1], Tomohiro Hori[1], Yuka Aoyama[2], Rajaram Venkatesan[5], Rik Wierenga[5], Yohsuke Moriyama[6], Takashi Hashimoto[6], Nobuteru Usuda[6], Kei Murayama[7], Akira Ohtake[4,8], Yuki Hasegawa[9], Yosuke Shigematsu[10] and Yukihiro Hasegawa[4]

2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD) deficiency (HSD10 disease) is a rare inborn error of metabolism, and <30 cases have been reported worldwide. This disorder is typically characterized by progressive neurodegenerative disease from 6 to 18 months of age. Here, we report the first patient with this disorder in Asia, with atypical clinical presentation. A 6-year-old boy, who had been well, presented with severe ketoacidosis following a 5-day history of gastroenteritis. Urinary organic acid analysis showed elevated excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine. He was tentatively diagnosed with β-ketothiolase (T2) deficiency. However, repeated enzyme assays using lymphocytes showed normal T2 activity and no T2 mutation was found. Instead, a hemizygous c.460G>A (p.A154T) mutation was identified in the *HSD17B10* gene. This mutation was not found in 258 alleles from Japanese subjects (controls). A normal level of the HSD17B10 protein was found by immunoblot analysis but no 2M3HBD enzyme activity was detected in enzyme assays using the patient's fibroblasts. These data confirmed that this patient was affected with HSD10 disease. He has had no neurological regression until now. His fibroblasts showed punctate and fragmented mitochondrial organization by MitoTracker staining and had relatively low respiratory chain complex IV activity to those of other complexes.
Journal of Human Genetics advance oline publication, 18 September 2014; doi:10.1038/jhg.2014.79

INTRODUCTION

HSD10 disease, originally described as 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD) deficiency,[1] is a rare X-linked recessive disorder caused by a mutation in the *HSD17B10* gene.[2–5] This gene encodes a multifunctional protein that has 17β-hydroxysteroid dehydrogenase activity as well as 2M3HBD activity,[3–5] and which is also an essential component of mitochondrial RNase P, being required for tRNA processing in mitochondria.[6]

This disorder was first identified in a patient with progressive infantile neurodegeneration whose urinary organic acid profile was suspected to be due to β-ketothiolase (mitochondrial acetoacetyl-CoA thiolase; T2) deficiency in isoleucine catabolism.[1] However, the clinical presentation of that patient was different from that of typical T2 deficiency, which is characterized by intermittent ketoacidosis and no clinical symptoms between crises, and typically normal development.[7,8] Fewer than 30 patients have been reported to date.[1,2,5,9–21] Typically, HSD10 disease is characterized by a progressive neurodegenerative course from 6 to 18 months of age, in conjunction with retinopathy and cardiomyopathy, leading to death at the age of 2–4 years or later.[5] However, clinical heterogeneity is noted in this disorder.[5] An atypical milder presentation was reported in three families.[13,14,17]

Here, we describe a 6-year-old Japanese boy with the HSD10 disease, who had no neurodegeneration and developed severe ketoacidosis at the age of 6 years. This is believed to be the first report of HSD10 disease in Asia.

MATERIALS AND METHODS

Case presentation

We report the case of a boy who had been well and achieved normal development until 6 years of age when he presented with severe ketoacidosis following

[1]Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, Japan; [2]Medical Information Sciences Division, United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Gifu, Japan; [3]Department of General Pediatrics, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan; [4]Department of Endocrinology and Metabolism, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan; [5]Faculty of Biochemistry and Molecular Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland; [6]Department of Anatomy and Cell Biology, Fujita Health University School of Medicine, Toyoake, Japan; [7]Department of Metabolism, Chiba Children's Hospital, Chiba, Japan; [8]Department of Pediatrics, Saitama Medical University, Moroyama, Japan; [9]Department of Pediatrics, Shimane University School of Medicine, Izumo, Japan and [10]Department of Health Science, Faculty of Medical Sciences, University of Fukui, Eiheiji-cho, Japan
Correspondence: Professor T Fukao, Department of Pediatrics, Graduate School of Medicine, Medical Information Sciences Division, United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Gifu 501-1194, Japan.
E-mail: toshi-gif@umin.net
Received 21 January 2014; revised 24 April 2014; accepted 20 August 2014

a 5-day period of appetite loss and vomiting due to gastroenteritis. Physical examination at admission showed a height of 108 cm, body weight of 18.3 kg (2 kg loss), heart rate of 128 per min and respiratory rate of 32 per min. Unconsciousness was not noted. Laboratory testing showed blood gas pH 7.01, pCO_2 9.2 mm Hg, HCO_3^- 2.8 mEq l^{-1}, blood glucose 5.9 mmol l^{-1}, white blood cell count 16 180 μl^{-1}, hemoglobin 14.3 g dl^{-1}, blood urea nitrogen 14.5 mg dl^{-1}, aspartate aminotransferase 29 IU l^{-1}, alanine aminotransferase 17 IU l^{-1}, lactate dehydrogenase 238 IU l^{-1}, ammonia 65 $\mu g\ dl^{-1}$ and lactate 2.4 mmol l^{-1}.

After bolus infusion of 20 ml kg^{-1} 5% glucose and electrolytes, blood total ketone body level was 14 mmol l^{-1} and free fatty acid was 0.97 mmol l^{-1}. He responded to intravenous fluid infusion (including 5% glucose), and blood gas showed pH 7.48 and HCO_3^- 23.7 mmol l^{-1} on day 2 of hospitalization. He became well and started oral food intake on that day. He was discharged from the hospital on day 7 of hospitalization. Semiquantitative urinary organic acid analysis in the acute phase showed elevated excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine, as well as ketones. He was tentatively diagnosed with T2 deficiency. One month later, he developed an episode of abdominal pain and lethargy in which hypoglycemia (1.4 mmol l^{-1}) and mild metabolic acidosis (blood pH 7.29, pCO_2 36.4 mm Hg, HCO_3^- 17.5 mmol l^{-1} and lactate 5.5 mmol l^{-1}) were noted. He responded quickly to intravenous infusion of electrolytes and glucose. Urinary organic acid analysis at the acute phase of this episode showed elevated concentrations of 2-methyl-3-hydroxybutyrate but not of tiglylglycine and 2-methylacetoacetate (Table 1). Blood acylcarnitine analysis using tandem mass spectrometry showed elevated C5:1 carnitine but not C5-OH carnitine (Table 1). After this episode, he did not experience another metabolic event until now (6.5 years of age).

His mother claimed that his gross motor development was slow and he could walk alone after the age of 1 year and 6 months. He also had some clumsiness with fine motor skills. His growth was normal. His height and weight were 111.5 cm (−1.2 s.d.) and 22.2 kg (0 s.d.), respectively. His neurological development was slightly below normal with a verbal IQ of 112, performance IQ of 64 and a full scale IQ of 88 (Wechsler Intelligence Scale for Children). Cerebral magnetic resonance imaging and magnetic resonance spectroscopy yielded normal findings at the age of 6.5 years. No abnormal findings were identified in echocardiography and ophthalmological examinations at the age of 7 years.

Enzyme assay and immunoblot analysis

Peripheral blood mononuclear cells were isolated from heparinized blood by gradient centrifugation in Ficoll–Paque medium (GE Healthcare, Uppsala, Sweden). The fibroblasts were cultured in Eagle's minimum essential medium containing 10% fetal calf serum. Acetoacetyl-CoA thiolase and succinyl-CoA:3-ketoacid CoA transferase were assayed in lymphocytes and fibroblasts, as described previously.[22] 2M3HBD activity in fibroblasts was measured as described previously.[1] Immunoblot analysis for 2M3HBD was done using anti-rat 2M3HBD antibody, which was originally made by us (TH) and anti-human glyceraldehyde 3-phosphate dehydrogenase antibody (sc-25778; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a reference. We used fibroblasts from an HSD10-deficient patient,[16] as a positive disease control.

Mutation analysis

This study was approved by the Ethical Committee of the Graduate School of Medicine, Gifu University, Gifu, Japan. Genomic DNA was purified from the fibroblasts with Sepa Gene kits (Sanko Junyaku, Tokyo, Japan). Mutation screening was performed at the genomic level by PCR and direct sequencing, using primer sets for fragments including each exon and its intron boundaries. Primers and PCR conditions for *ACAT1* gene were as previously described.[23] For *HSD17B10*, we amplified each genomic region with the primer pairs shown in Supplementary Table S1.

Screening of A154T mutation in the Japanese population

The presence of A145T mutations was screened using TaqMan triplet genotyping in 92 Japanese men and 83 women, according to the manufacturer's protocol (Life Technologies, Carlsbad, CA, USA).

Mitochondrial morphology

Fibroblasts from HSD10 patients and control fibroblasts were cultured in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% fetal calf serum at 37 °C and 5% CO_2. The mitochondria in living fibroblasts were stained with 100 nM MitoTracker Red CMRXRos (Life Technologies) for 30 min at 37 °C. Fluorescent images were captured and analyzed with an LSM710 laser scanning confocal microscope equipped with an incubation system (Carl Zeiss, Oberkochen, Germany).

Respiratory chain enzyme analysis

An *in vitro* respiratory chain enzyme activity assay[24] and blue native polyacrylamide gel electrophoresis[25,26] were used to quantify the activity and amount of respiratory chain enzyme complexes. The diagnostic criteria of Bernier *et al.*[26,27] were used to judge the activity.

Structural analysis of the A154 mutation

The crystal structure of human HSD17B10 complexed with NAD^+ (PDB ID: 2O23, deposited in the RCSB protein databank; www.rcsb.org)[28] was used for

Table 1 Urinary organic acid and serum acylcarnitine analyzes

		This patient		*T2D (severe)*	*T2D (mild)*
	Mean (s.d.)	*Hypoglycemic*	*Asymptomatic*	*Asymptomatic*	*Symptomatic*
Urinary organic acids					
Lactic acid	37.9±28.1	7755.8	7.3	5.1	195.0
3-OH butyric acid	27.8±21.5	17116.1	3.0	5.4	6295.0
Acetoacetic acid	0.2±0.4	72.5	0.7	1.0[a]	16.7[a]
2-Me-3-OH butyric acid	4.4±4.0	296.2	132.6	130.4	121.6
2-Methylacetoacetic acids	0±0	0.0	0.7	69.4[a]	2.8[a]
Tiglylglycine	2.2±4.3	0.1	298.9	212.4	3.7
Serum acylcarnitines					
C0	31.3±8.4	13.4		67.4	79.2
C2	6.2±2.1	16.2		7.7[a]	2.1[a]
C5:1	0.012±0.005	0.63		0.72	0.079
C5OH	0.06±0.03	0.11		0.34	0.06

T2D (severe) was GK01, and T2D (mild) was GK77.
Amounts of urinary organic acids are expressed as mmol per mol Cr.
Amounts of serum acylcarnitine are expressed as nnom ml^{-1}.
[a]Values may be low because of degradation due to long storage at −30 °C.

structural analysis. The program COOT was used to analyze the structure and PyMOL Molecular Graphics System, version 1.4.1 (Schrödinger, LLC; www.pymol.org/citing), was used to make the figures.

RESULTS

Exclusion of the diagnosis of T2 deficiency

We first made a tentative diagnosis of T2 deficiency, based on the severe ketoacidotic event with elevated 2-methyl-3-hydroxybutyrate and tiglylglycine in urinary organic acid analysis. However, repeated enzyme assays showed normal T2 activity (Supplementary Table S2). Furthermore, no T2 mutation was identified by genomic PCR followed by direct sequencing.

Mutation analysis of HSD17B10 gene

Urinary organic acid analysis showed blockade at the T2 or 2M3HBD level in the isoleucine catabolic pathway. Therefore, we investigated the possibility of an *HSD17B10* gene mutation, although the clinical course of this patient was different from that of typical HSD10 patients. A hemizygous c.460G>A (p.A154T) mutation was identified in *HSD17B10* gene (Figure 1). His mother was a heterozygous carrier of this mutation. His maternal uncle did not have this mutation. Samples from maternal grandparents were not available for the study. TaqMan analysis showed that this mutation was not found in 258 alleles from Japanese subjects (controls).

Enzyme assay and immunoblot analysis for 2M3HBD

We used a fibroblast cell line from a Dutch patient whose mutation was c.364C>G (p.L122V) as a positive disease control. He was classified with the infantile form of HSD10 disease because he had shown motor delay and spastic diplegia since infancy.[16] The patient was able to walk but had psychomotor retardation with spasticity and minimal language development (Bwee Tien Poll-The, personal communication), and hence his clinical manifestations were milder than for the typical infantile form of the disease.

2M3HBD activity was absent from the patient's fibroblasts, as well as HSD10-deficient fibroblasts with p.L122V mutation,[16] designated as L122V fibroblasts (Table 2). However, the control samples showed 2M3HBD activity, which was in accordance with reported control values for the assay.[1] Immunoblot analysis showed that fibroblasts from our patient and the previous HSD10-deficient patient had an almost similar amount of HSD17B10 protein to the controls (Supplementary Figure S1).

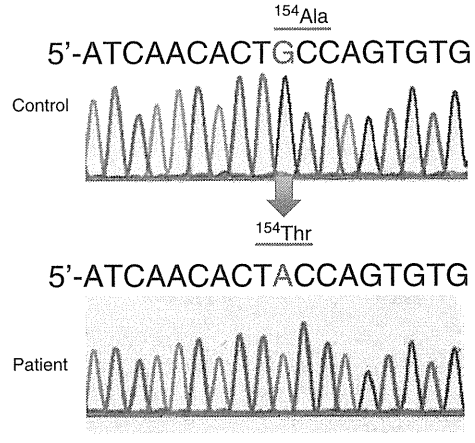

Figure 1 HSD17B10 mutation. Genomic direct sequencing of exon 5. A hemizygous c.460G>A (p.A154T) substitution was identified. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Mitochondrial staining

MitoTracker staining revealed a filamentous network-like structure of the mitochondria in control fibroblasts (Figure 2 and Supplementary Figure S2). Fibroblasts with the p.L122V and p.A154T mutations showed punctate and fragmented mitochondrial organization. This finding is the same as that previously reported in fibroblasts with R130C and D86G mutations.[17] Furthermore, mitochondria in A154T mutated cells had highly variable diameters, ranging from thin tubes to swollen bulbs.

Respiratory chain enzyme assay

Respiratory chain enzyme assay of the patient's fibroblasts showed normal activity of complexes I, II and III (98–159% relative to citrate synthase) (Supplementary Table S3). Complex IV activity was also within the normal range but significantly lower than that of other complexes (51.6% relative to citrate synthase and 44.6% relative to complex II). In blue native polyacrylamide gel electrophoresis, the band corresponding to assembled complex IV was slightly decreased too (Supplementary Figure S3). These tendencies were also detected in fibroblasts with L122V mutation.

Mutation site in the tertiary structure of human HSD17B10

HSD17B10 is a tetramer consisting of four identical subunits, each having the fold of short-chain dehydrogenase/reductase superfamily. Inspection of the human HSD17B10 structure (PDB ID: 2O23) revealed that residue Ala154 is close to the active site (Figure 3a). Ala154 is completely buried and the Cβ atom of Ala154 faces a hydrophobic (apolar) pocket created by residues such as Ile175, Val176 and Cγ of Thr195. The residue next to Ala154, Ser155, is one of the catalytic residues, and part of the catalytic triad formed by Ser155, Tyr168 and Lys172. The mutation of Ala154 to Thr154, that is, from a small, hydrophobic side chain to a larger, polar side chain results in steric clashes with residues Ile175, Val176 and Thr195 in the current conformation (Figure 3b). To avoid these steric clashes, main and side chain conformational changes are expected in the region around Ile175 and Ala/Thr154. The changes around Ile175 may also affect the catalytically competent conformation of the active site residue Lys172. In addition, the changes around Ala/Thr154 are expected to cause structural changes of the catalytic residue Ser155, which has to interact with the substrate for the reaction to occur. Therefore, all these rearrangements resulting in the non-optimal conformations of Ser155 and Lys172 may severely affect the catalytic capability of this enzyme. The substrate binding may not be affected as much because the catalytic triad is only at the beginning of the much larger substrate binding pocket[28] extending outward. Therefore, catalysis of both the steroid substrates such as allopregnanolone[21]

Table 2 2M3HBD assay using fibroblasts

	2M3HBD	*AcAcCoA thiolase*
Control fibroblasts 1	0.75±0.40	15.6
Control fibroblasts 2	0.90±0.58	28.1
L122V fibroblasts	0.19±0.08	28.0
Patient's fibroblasts	0.04±0.11	34.0

Acetoacetyl-CoA (AcAcCoA) thiolase activity was measured in the presence of potassium ion at 37 °C.

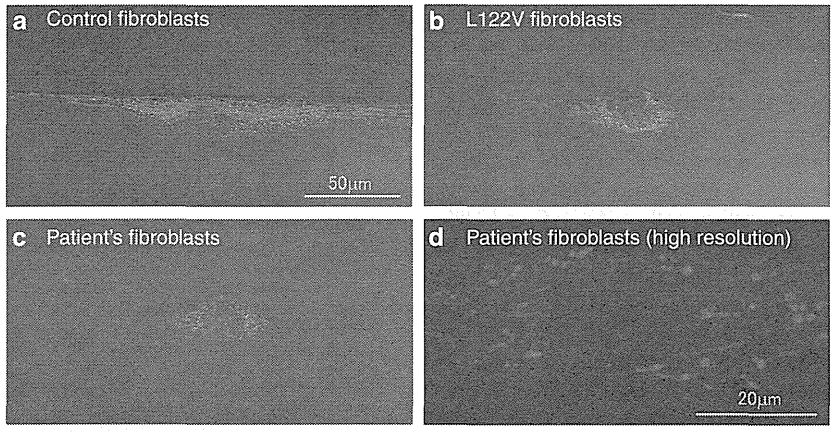

Figure 2 Mitochondrial morphology. (**a**–**c**) Merged images from differential interference contrast (DIC) and MitoTracker Red. (**a**) Control fibroblast. (**b**) Fibroblast with the p.L122V mutation. (**c**) Fibroblast with the p.A154T mutation. (**d**) Fluorescent image of MitoTracker Red from the p.A154T mutated cell. Bars: **a**–**c**, 50 μm; **d**, 20 μm. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

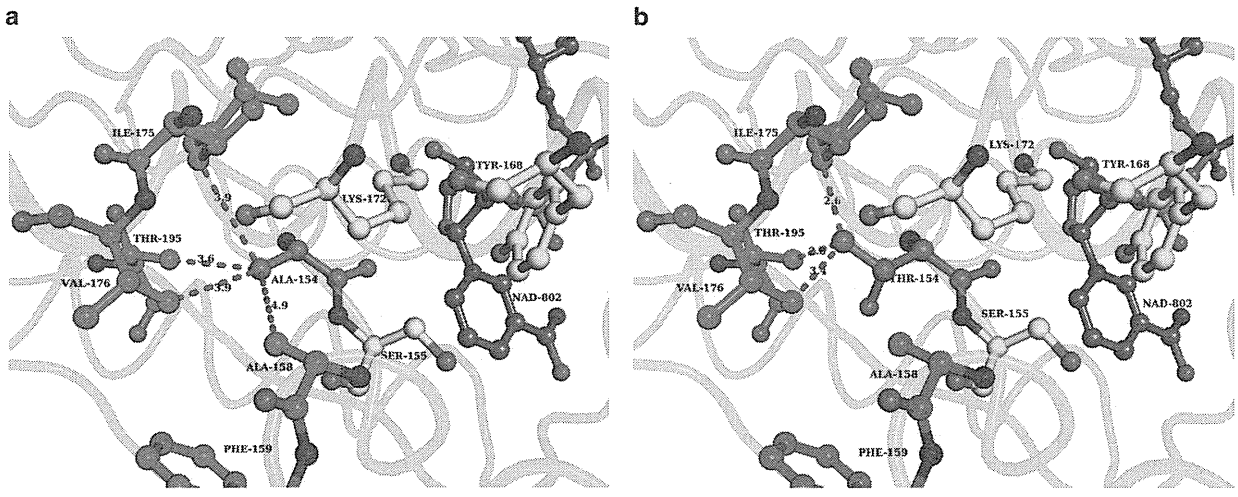

Figure 3 Structural analysis. (**a**) Environment of residue Ala154 as seen in PDB ID 2023. Oxygen atoms are shown in red, nitrogen in blue and carbon is color coded as follows: Ala154 in magenta, the catalytic triad comprising residues Ser155, Tyr168 and Lys172 in yellow, and NAD in blue. Ala158, Phe159, Ile175, Val176 and Thr195 (Cγ) are some of the residues pointing toward the side chain of Ala154, creating a hydrophobic pocket. These are highlighted in green. Ile175 has a double conformation. The relevant distances are shown with red dashes. (**b**) Possible steric clashes in HSD10 disease due to mutation of Ala154 into Thr154. Thr154 is shown in magenta. Ala154 was mutated to Thr154 using PDB-entry 2023 by the program COOT. The expected steric clashes of the Thr154 side chain with Ile175, Val176 and Thr195 are highlighted by red dashes.

and fatty acyl-CoA substrates such as 2-methyl-3-hydoxybutyryl-CoA are predicted to be equally affected.

DISCUSSION

This is believed to be the first report of HSD10 disease in Asia. Since the discovery of the first patient in 2000,[1] fewer than 30 patients have been described.[1,2,5,9–21] Typically, this disorder is suspected when patients with neurological degeneration or psychomotor retardation show similar urinary organic acid or blood acylcarnitine profiles with T2 deficiency. However, our patient experienced a severe ketoacidotic episode with blood pH 7.01 and blood total ketone level of 14 mM after a 5-day history of gastroenteritis. This clinical picture is similar to T2 deficiency, although the onset of the first severe ketoacidotic episode at the age of 6 years is late compared with that in typical T2-deficient patients who develop such crises around the age of 6 months to 2 years.[7,8] The first patient described by Zschocke *et al.*[1] had metabolic decompensation with ketonuria on day 2 of life. Disturbance in isoleucine catabolism may be attributed to such reversible metabolic decompensation in HSD10 disease, and appears to be independent from pathophysiology of neurodegeneration in HSD10 disease.

In the patients with HSD10 disease described thus far, broad clinical heterogeneity has been found.[5,30] The classical presentation that is observed in most patients, which was called the infantile form by

Zschocke,[5] is characterized by a period of more or less normal development in the first 6–18 months of life. This is followed by a progressive neurodegenerative disease course in conjunction with progressive cardiomyopathy, leading to death at the age of 2–4 years or older. Patients with a common mutation c.388C>T (p.R130C) present with the infantile form. Some patients with other mutations have more severe neonatal forms. Atypical presentation was reported in three families. (1) Only one patient with c.745G>C (p.E249Q) mutation developed normally in the first 5 years of life and then showed neurological deterioration.[14] This was classified as the juvenile form by Zschocke.[5] (2) The proband of a family with c.495A>C (p.Q165H) mutation showed growth retardation, feeding difficulty and microcephaly but his neurological status remained normal at up to age 5 years. Moreover, his male cousin with the same mutation achieved normal neurodevelopment until his current age of 8 years, with a height and weight in the 25th percentile.[17] (3) Four boys in a large family showed X-linked intellectual disability, choreoathetosis and abnormal behavior with a normal urinary organic acid profile, and they had an apparent synonymous mutation that affected splicing efficiency in the HSD17B10 gene.[13] Our patient with a novel c.460G>A (p.A154T) mutation showed no neurological degeneration, at least until age 6.5 years, and normal growth. Hence, our patient had a milder phenotype than in patients with juvenile HSD10 disease.

There is evidence that the neurological degeneration observed in HSD10 disease is not caused by a deficiency in the isoleucine metabolism-related 2M3HBD activities of HSD17B10.[17,21] Instead, defects in neuroactive steroid metabolism[21] and/or the non-enzymatic function of the protein required for mitochondrial integrity and cell survival[17] may be responsible for the neurological manifestations. The HSD17B10 protein is one of three component proteins of mitochondrial RNase P, which is essential for mitochondrial translation.[6] Reduced function as a component of RNase P may contribute to clinical severity. The p.R130C mutation common for infantile form reduced not only its mutant HSD10 level but also that of another RNase P component, MRPP-1, suggesting that HSD10 is important for the maintenance of the MRPP1–HSD10 subcomplex of RNase P.[31] Analysis of the consequences of the A154T mutation on the tertiary structure suggests that A154T mutation affects enzyme activity of both 2-methyl-3-hydroxybutyryl-CoA and neurosteroids. The enzymological characterization of the expressed HSD17B10 A154T variant is required to confirm this observation. Mitochondrial morphological changes using MitoTracker staining have been reported,[17] and we also observed punctate and fragmented mitochondrial organization in our patient. Mitochondrial respiratory chain complex IV activity was decreased in both fibroblasts with A154T and those with L122V, although the decreased level did not fulfill the minor diagnostic criteria of Bernier *et al.*[27] Mitochondrial respiratory chain enzyme assay was reported to be normal in fibroblasts with V65A mutation. Further investigation in other fibroblasts with HSD10 disease is necessary to confirm that reduced complex IV activity is one of the characteristics in HSD10 disease.

We have described a patient with mild phenotype HSD10 disease with a novel A154T mutation, who is believed to be the first patient with HSD10 disease in Asia. Accumulation of more data on phenotype–genotype correlation of HSD10 disease is important to understand the molecular basis of the disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We sincerely thank Dr Jos PN Ruiter, Professor Ronald JA Wanders and Professor Bwee Tien Poll-The for providing the fibroblast cell line from an HSD10-deficient patient as a positive control and giving a protocol for the 2M3HBD enzyme assay.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

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SHORT COMMUNICATION

A girl with West syndrome and autistic features harboring a *de novo TBL1XR1* mutation

Hirotomo Saitsu[1], Jun Tohyama[2], Tom Walsh[3], Mitsuhiro Kato[4], Yu Kobayashi[2], Ming Lee[3], Yoshinori Tsurusaki[1], Noriko Miyake[1], Yu-ichi Goto[5], Ichizo Nishino[6], Akira Ohtake[7], Mary-Claire King[3] and Naomichi Matsumoto[1]

Recently, *de novo* mutations in *TBL1XR1* were found in two patients with autism spectrum disorders. Here, we report on a Japanese girl presenting with West syndrome, Rett syndrome-like and autistic features. Her initial development was normal until she developed a series of spasms at 5 months of age. Electroencephalogram at 7 months showed a pattern of hypsarrhythmia, which led to a diagnosis of West syndrome. Stereotypic hand movements appeared at 8 months of age, and autistic features such as deficits in communication, hyperactivity and excitability were observed later, at 4 years and 9 months. Whole exome sequencing of the patient and her parents revealed a *de novo TBL1XR1* mutation [c.209 G>A (p.Gly70Asp)] occurring at an evolutionarily conserved amino acid in an F-box-like domain. Our report expands the clinical spectrum of *TBL1XR1* mutations to West syndrome with Rett-like features, together with autistic features.
Journal of Human Genetics advance online publication, 7 August 2014; doi:10.1038/jhg.2014.71

TBL1XR1 at 3q26.32 encodes transducin β-like 1 X-linked receptor 1, a co-repressor of nuclear hormone transcription factors that is required for β-catenin–Tcf-mediated Wnt signaling.[1–3] Recently, two *de novo TBL1XR1* mutations were found in 2 of 2446 patients with autism spectrum disorders, suggesting an association between *TBL1XR1* mutations and autism.[4,5] Here, we present a third case with a *de novo TBL1XR1* mutation, showing West syndrome, Rett-like and autistic features.

CASE REPORT

This report concerns a 5-year-old girl who is the offspring of unrelated healthy Japanese parents. She was born at 39 weeks of gestation without asphyxia after an uneventful pregnancy. Her birth weight, birth length and head circumference were 3088 g (+0.3 standard deviation (s.d.)), 51.1 cm (+1.0 s.d.) and 34.0 cm (+0.5 s.d.), respectively. She showed social smiling and head control at 3 and 4 months of age, respectively. Then at 5 months she developed a series of spasms occurring 5–6 times a day, when her head control became unstable. Electroencephalography at 7 months of age showed hypsarrhythmia patterns (Figure 1a), which led to a diagnosis of West syndrome. Brain magnetic resonance imaging showed no structural brain anomalies (Figures 1a and c). Administration of adrenocorticotropic hormone therapy only temporarily reduced the frequency of spasms.

On examination at 7 months of age, the patient's weight, height and head circumference were 8720 g (+1.2 s.d.), 69.5 cm (+0.9 s.d.) and 42 cm (−0.6 s.d.), respectively. Mild dysmorphic features were observed, including a long palpebral fissure, thick eyebrows and downturned corners of the mouth. The patient showed no eye fixation and pursuit, as well as no social smile. Her muscle tone was mildly hypotonic. Despite being given extensive treatments including adrenocorticotropic hormone therapy in combination with valproic acid, nitrazepam, vitamin B6, topiramate, clonazepam and clobazam, she continued to exhibit frequent subtle tonic seizures with eyelid opening. At 8 months of age, stereotypical hand movements appeared that resembled hand-washing.

Laboratory examination revealed elevated serum levels of several components: lactic acid (39.9 mg dl^{-1}, compared with a normal range of 5.0–20.0 mg dl^{-1}), pyruvate (2.79 mg dl^{-1}, normal range 0.3–0.9 mg dl^{-1}) and alanine (1447 nmol ml^{-1}, normal range 180–470 nmol ml^{-1}). However, following vitamin B1 treatment, both pyruvate and alanine serum levels returned to normal. The levels of lactic acid and pyruvate in cerebrospinal fluid appeared normal (14.6 and 0.65 mg dl^{-1}, respectively). Respiratory-chain enzymes, using muscle homogenates and fibroblasts, and mitochondrial DNA sequence analysis were all normal, and pathological examination of muscle specimens revealed no specific findings. Repeated examination of both serum lactic acid and pyruvate levels also showed no abnormalities.

[1]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan; [2]Department of Pediatrics, Nishi-Niigata Chuo National Hospital, Niigata, Japan; [3]Department of Genome Sciences and Department of Medicine, University of Washington, Seattle, WA, USA; [4]Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan; [5]Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Japan; [6]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Japan and [7]Department of Pediatrics, Faculty of Medicine, Saitama Medical University, Saitama, Japan
Correspondence: Dr H Saitsu or Professor N Matsumoto, Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
E-mail: hsaitsu@yokohama-cu.ac.jp or naomat@yokohama-cu.ac.jp
Received 12 June 2014; revised 7 July 2014; accepted 17 July 2014

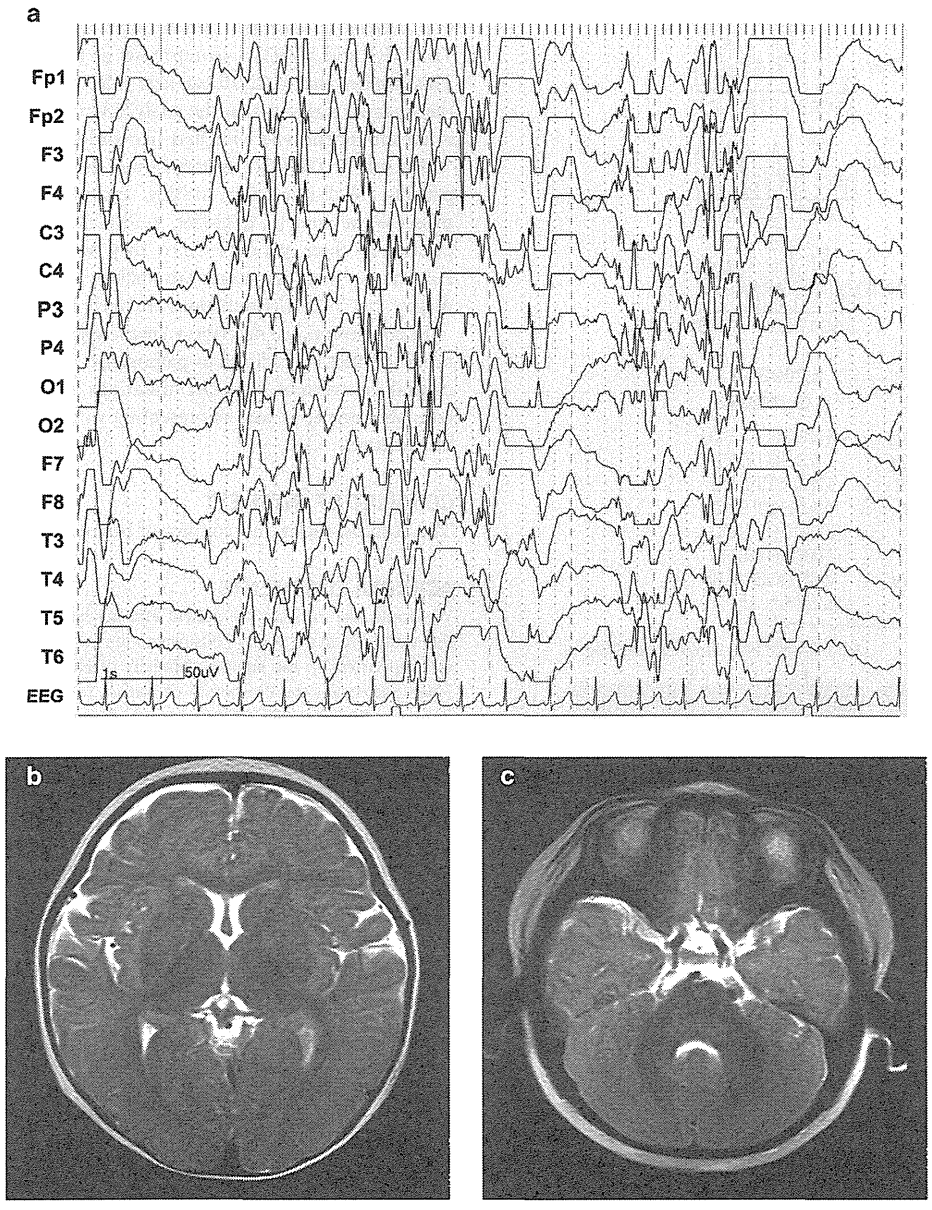

Figure 1 Electroencephalogram (EEG) and brain magnetic resonance imaging in the patient at 7 months of age. (**a**) Interictal EEG showed high-amplitude multifocal spikes with irregular slow waves consistent with a finding of hypsarrhythmia. T2-weighted axial images through the basal ganglia (**b**) and the cerebellum (**c**) showed no abnormalities.

After contracting a fever at the age of 1 year and 10 months, her seizures were controlled using a combination therapy of topiramate, valproic acid, clobazam and vitamin B1. However, the patient still could not speak any meaningful words at 4 years and 9 months of age but could walk with support; she had a developmental quotient of 13. At this age she still showed stereotypic hand movements as well as autistic features such as deficits in communication, hyperactivity and excitability.

RESULTS AND DISCUSSION

G-banded karyotyping was normal (46,XX). No pathological copy number aberrations were detected by the 2.7M Array (Affymetrix, Santa Clara, CA, USA). Whole exome sequencing of the patient and her parents was performed. Genomic DNA of blood leukocytes was captured using the SeqCap EZ Exome Library v2.0 (Roche NimbleGen, Madison, WI, USA), and sequenced with on HiSeq2000 (Illumina, San Diego, CA, USA). Variants were detected as previously described.[6] Variants with a Phred-like consensus quality score of >100 were considered as candidate variants. We found a total of four *de novo* candidate variants, in which two mutations were further validated as *de novo* by Sanger sequencing: *SELPLG* NM_001206609.1: c.794C>T (p.Thr265Met) and *TBL1XR1* NM_024665.4:c.209G>A (p.Gly70Asp). The other two variants were transmitted from her mother, demonstrating that the two variants were falsely uncalled in the mother. Neither of the two *de novo* mutations was found in the 6500 National Heart, Lung, and Blood Institute exomes nor in our

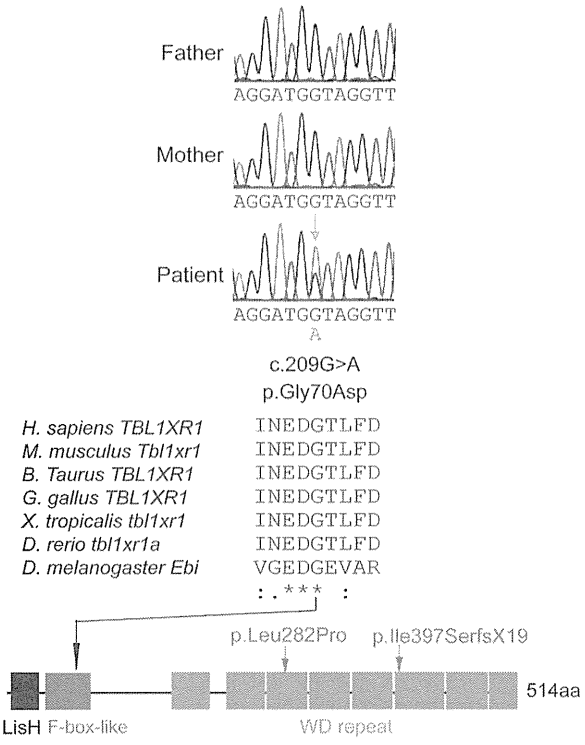

Figure 2 *De novo TBL1XR1* mutation. The c.209G>A (p.Gly70Asp) mutation occurred *de novo* at an evolutionarily conserved amino acid in an F-box-like domain. Multiple amino-acid sequences of TBL1XR1 proteins were aligned with tools available on the CLUSTALW web site. Two previously reported mutations (p.Leu282Pro and p.Ile397SefsX19) are highlighted in red. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

575 in-house control exomes. Both mutations were predicted as damaging by SIFT and PolyPhen2. However, MutationTaster classified only p.Gly70Asp in *TBL1XR1* as damaging whereas p.Thr265Met in *SELPLG* was predicted as a polymorphism. We found no recessive mutations in known early-onset epileptic encephalopathy genes including *SLC25A22*, *PNPO*, *PNKP* and *PLCB1*.[7] All experimental protocols used were approved by the Institutional Review Board of Yokohama City University School of Medicine.

SELPLG encodes P-selectin glycoprotein ligand 1, for which a knock-out mouse study showed that *Selplg* is required for leukocyte adhesion and rolling.[8,9] No neurological abnormalities were reported, suggesting that the *SELPLG* mutation is less likely to be involved in the phenotype of this patient.

TBL1XR1, also denoted as *TBLR1*, is required for β-catenin–Tcf-mediated Wnt signaling.[1,2] Mutations in *TCF4*, an essential mediator of Wnt signaling, have been shown to cause Pitt–Hopkins Syndrome, which presents with severe intellectual disability, seizures and stereotypic movements.[10,11] This suggests that the β-catenin–Tcf-mediated Wnt pathway of signaling is essential for normal brain function. Moreover, two *de novo TBL1XR1* mutations (p.Leu282Pro and p.Ile397SerfsX19) were found in 2 of 2446 patients with autism spectrum disorders.[4,5] In our case, the p.Gly70Asp mutation occurred in an evolutionarily conserved amino acid within an F-box-like domain (Figure 2). Indeed, the F-box-like domain of TBLR1 (TBL1XR1) is essential for a high affinity interaction between TBL1XR1 and SMRT, a co-repressor of nuclear hormone receptors.[3] This implies that p.Gly70Asp may affect this interaction. Therefore, the evidence suggests that the p.Gly70Asp mutation may cause a West syndrome phenotype with Rett-like and autistic features.

The role of *TBL1XR1* mutations was also investigated in 280 epileptic patients. High-resolution melting analysis revealed that three rare missense variants (p.Ala116Ser, p.Gly405Glu and p.Asn407Ser) were present in three patients. Since they were predicted as benign by PolyPhen-2, these mutations are unlikely to be pathogenic. These data suggest that *TBL1XR1* mutations are rarely involved in epileptic patients.

In conclusion, we describe a Japanese girl with a *de novo TBL1XR1* mutation that is predicted as pathogenic. Our report suggests that the clinical spectrum of *TBL1XR1* mutations includes autistic features as a core phenotype, as well as presenting with West syndrome and Rett-like features.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank the patient and her family for their participation in this study. We thank Kiyomi Nishiyama for her technical assistance. This study was supported by: the Japanese Ministry of Health, Labour, and Welfare; the Japan Society for the Promotion of Science (a Grant-in-Aid for Scientific Research (B) (25293085, 25293235) and a Grant-in-Aid for Scientific Research (A) (13313587)); the Takeda Science Foundation; the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems; the Strategic Research Program for Brain Sciences (11105137); and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (12024421).

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Journal of Human Genetics (2014) 59, 609–614

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ORIGINAL ARTICLE

The first case in Asia of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency (HSD10 disease) with atypical presentation

Toshiyuki Fukao[1,2], Kazuhisa Akiba[3], Masahiro Goto[4], Nobuki Kuwayama[1], Mikiko Morita[1], Tomohiro Hori[1], Yuka Aoyama[2], Rajaram Venkatesan[5], Rik Wierenga[5], Yohsuke Moriyama[6], Takashi Hashimoto[6], Nobuteru Usuda[6], Kei Murayama[7], Akira Ohtake[4,8], Yuki Hasegawa[9], Yosuke Shigematsu[10] and Yukihiro Hasegawa[4]

2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD) deficiency (HSD10 disease) is a rare inborn error of metabolism, and <30 cases have been reported worldwide. This disorder is typically characterized by progressive neurodegenerative disease from 6 to 18 months of age. Here, we report the first patient with this disorder in Asia, with atypical clinical presentation. A 6-year-old boy, who had been well, presented with severe ketoacidosis following a 5-day history of gastroenteritis. Urinary organic acid analysis showed elevated excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine. He was tentatively diagnosed with β-ketothiolase (T2) deficiency. However, repeated enzyme assays using lymphocytes showed normal T2 activity and no T2 mutation was found. Instead, a hemizygous c.460G>A (p.A154T) mutation was identified in the *HSD17B10* gene. This mutation was not found in 258 alleles from Japanese subjects (controls). A normal level of the HSD17B10 protein was found by immunoblot analysis but no 2M3HBD enzyme activity was detected in enzyme assays using the patient's fibroblasts. These data confirmed that this patient was affected with HSD10 disease. He has had no neurological regression until now. His fibroblasts showed punctate and fragmented mitochondrial organization by MitoTracker staining and had relatively low respiratory chain complex IV activity to those of other complexes.
Journal of Human Genetics (2014) **59**, 609–614; doi:10.1038/jhg.2014.79; published online 18 September 2014

INTRODUCTION

HSD10 disease, originally described as 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD) deficiency,[1] is a rare X-linked recessive disorder caused by a mutation in the *HSD17B10* gene.[2–5] This gene encodes a multifunctional protein that has 17β-hydroxysteroid dehydrogenase activity as well as 2M3HBD activity,[3–5] and which is also an essential component of mitochondrial RNase P, being required for tRNA processing in mitochondria.[6]

This disorder was first identified in a patient with progressive infantile neurodegeneration whose urinary organic acid profile was suspected to be due to β-ketothiolase (mitochondrial acetoacetyl-CoA thiolase; T2) deficiency in isoleucine catabolism.[1] However, the clinical presentation of that patient was different from that of typical T2 deficiency, which is characterized by intermittent ketoacidosis and no clinical symptoms between crises, and typically normal development.[7,8] Fewer than 30 patients have been reported to date.[1,2,5,9–21] Typically, HSD10 disease is characterized by a progressive neurodegenerative course from 6 to 18 months of age, in conjunction with retinopathy and cardiomyopathy, leading to death at the age of 2–4 years or later.[5] However, clinical heterogeneity is noted in this disorder.[5] An atypical milder presentation was reported in three families.[13,14,17]

Here, we describe a 6-year-old Japanese boy with the HSD10 disease, who had no neurodegeneration and developed severe ketoacidosis at the age of 6 years. This is believed to be the first report of HSD10 disease in Asia.

MATERIALS AND METHODS

Case presentation

We report the case of a boy who had been well and achieved normal development until 6 years of age when he presented with severe ketoacidosis following

[1]Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, Japan; [2]Medical Information Sciences Division, United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Gifu, Japan; [3]Department of General Pediatrics, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan; [4]Department of Endocrinology and Metabolism, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan; [5]Faculty of Biochemistry and Molecular Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland; [6]Department of Anatomy and Cell Biology, Fujita Health University School of Medicine, Toyoake, Japan; [7]Department of Metabolism, Chiba Children's Hospital, Chiba, Japan; [8]Department of Pediatrics, Saitama Medical University, Moroyama, Japan; [9]Department of Pediatrics, Shimane University School of Medicine, Izumo, Japan and [10]Department of Health Science, Faculty of Medical Sciences, University of Fukui, Eiheiji-cho, Japan
Correspondence: Professor T Fukao, Department of Pediatrics, Graduate School of Medicine, Medical Information Sciences Division, United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Gifu 501-1194, Japan.
E-mail: toshi-gif@umin.net
Received 21 January 2014; revised 24 April 2014; accepted 20 August 2014; published online 18 September 2014

a 5-day period of appetite loss and vomiting due to gastroenteritis. Physical examination at admission showed a height of 108 cm, body weight of 18.3 kg (2 kg loss), heart rate of 128 per min and respiratory rate of 32 per min. Unconsciousness was not noted. Laboratory testing showed blood gas pH 7.01, pCO_2 9.2 mm Hg, HCO_3^- 2.8 mEq l^{-1}, blood glucose 5.9 mmol l^{-1}, white blood cell count 16 180 μl^{-1}, hemoglobin 14.3 g dl^{-1}, blood urea nitrogen 14.5 mg dl^{-1}, aspartate aminotransferase 29 IU l^{-1}, alanine aminotransferase 17 IU l^{-1}, lactate dehydrogenase 238 IU l^{-1}, ammonia 65 $\mu g\ dl^{-1}$ and lactate 2.4 mmol l^{-1}.

After bolus infusion of 20 ml kg^{-1} 5% glucose and electrolytes, blood total ketone body level was 14 mmol l^{-1} and free fatty acid was 0.97 mmol l^{-1}. He responded to intravenous fluid infusion (including 5% glucose), and blood gas showed pH 7.48 and HCO_3^- 23.7 mmol l^{-1} on day 2 of hospitalization. He became well and started oral food intake on that day. He was discharged from the hospital on day 7 of hospitalization. Semiquantitative urinary organic acid analysis in the acute phase showed elevated excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine, as well as ketones. He was tentatively diagnosed with T2 deficiency. One month later, he developed an episode of abdominal pain and lethargy in which hypoglycemia (1.4 mmol l^{-1}) and mild metabolic acidosis (blood pH 7.29, pCO_2 36.4 mm Hg, HCO_3^- 17.5 mmol l^{-1} and lactate 5.5 mmol l^{-1}) were noted. He responded quickly to intravenous infusion of electrolytes and glucose. Urinary organic acid analysis at the acute phase of this episode showed elevated concentrations of 2-methyl-3-hydroxybutyrate but not of tiglylglycine and 2-methylacetoacetate (Table 1). Blood acylcarnitine analysis using tandem mass spectrometry showed elevated C5:1 carnitine but not C5-OH carnitine (Table 1). After this episode, he did not experience another metabolic event until now (6.5 years of age).

His mother claimed that his gross motor development was slow and he could walk alone after the age of 1 year and 6 months. He also had some clumsiness with fine motor skills. His growth was normal. His height and weight were 111.5 cm (−1.2 s.d.) and 22.2 kg (0 s.d.), respectively. His neurological development was slightly below normal with a verbal IQ of 112, performance IQ of 64 and a full scale IQ of 88 (Wechsler Intelligence Scale for Children). Cerebral magnetic resonance imaging and magnetic resonance spectroscopy yielded normal findings at the age of 6.5 years. No abnormal findings were identified in echocardiography and ophthalmological examinations at the age of 7 years.

Enzyme assay and immunoblot analysis

Peripheral blood mononuclear cells were isolated from heparinized blood by gradient centrifugation in Ficoll–Paque medium (GE Healthcare, Uppsala, Sweden). The fibroblasts were cultured in Eagle's minimum essential medium containing 10% fetal calf serum. Acetoacetyl-CoA thiolase and succinyl-CoA:3-ketoacid CoA transferase were assayed in lymphocytes and fibroblasts, as described previously.[22] 2M3HBD activity in fibroblasts was measured as described previously.[1] Immunoblot analysis for 2M3HBD was done using anti-rat 2M3HBD antibody, which was originally made by us (TH) and anti-human glyceraldehyde 3-phosphate dehydrogenase antibody (sc-25778; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a reference. We used fibroblasts from an HSD10-deficient patient,[16] as a positive disease control.

Mutation analysis

This study was approved by the Ethical Committee of the Graduate School of Medicine, Gifu University, Gifu, Japan. Genomic DNA was purified from the fibroblasts with Sepa Gene kits (Sanko Junyaku, Tokyo, Japan). Mutation screening was performed at the genomic level by PCR and direct sequencing, using primer sets for fragments including each exon and its intron boundaries. Primers and PCR conditions for *ACAT1* gene were as previously described.[23] For *HSD17B10*, we amplified each genomic region with the primer pairs shown in Supplementary Table S1.

Screening of A154T mutation in the Japanese population

The presence of A145T mutations was screened using TaqMan triplet genotyping in 92 Japanese men and 83 women, according to the manufacturer's protocol (Life Technologies, Carlsbad, CA, USA).

Mitochondrial morphology

Fibroblasts from HSD10 patients and control fibroblasts were cultured in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% fetal calf serum at 37 °C and 5% CO_2. The mitochondria in living fibroblasts were stained with 100 nM MitoTracker Red CMRXRos (Life Technologies) for 30 min at 37 °C. Fluorescent images were captured and analyzed with an LSM710 laser scanning confocal microscope equipped with an incubation system (Carl Zeiss, Oberkochen, Germany).

Respiratory chain enzyme analysis

An *in vitro* respiratory chain enzyme activity assay[24] and blue native polyacrylamide gel electrophoresis[25,26] were used to quantify the activity and amount of respiratory chain enzyme complexes. The diagnostic criteria of Bernier *et al.*[26,27] were used to judge the activity.

Structural analysis of the A154 mutation

The crystal structure of human HSD17B10 complexed with NAD^+ (PDB ID: 2O23, deposited in the RCSB protein databank; www.rcsb.org)[28] was used for

Table 1 Urinary organic acid and serum acylcarnitine analyzes

		This patient		*T2D (severe)*	*T2D (mild)*
	Mean (s.d.)	*Hypoglycemic*	*Asymptomatic*	*Asymptomatic*	*Symptomatic*
Urinary organic acids					
Lactic acid	37.9±28.1	7755.8	7.3	5.1	195.0
3-OH butyric acid	27.8±21.5	17116.1	3.0	5.4	6295.0
Acetoacetic acid	0.2±0.4	72.5	0.7	1.0[a]	16.7[a]
2-Me-3-OH butyric acid	4.4±4.0	296.2	132.6	130.4	121.6
2-Methylacetoacetic acids	0±0	0.0	0.7	69.4[a]	2.8[a]
Tiglylglycine	2.2±4.3	0.1	298.9	212.4	3.7
Serum acylcarnitines					
C0	31.3±8.4	13.4		67.4	79.2
C2	6.2±2.1	16.2		7.7[a]	2.1[a]
C5:1	0.012±0.005	0.63		0.72	0.079
C5OH	0.06±0.03	0.11		0.34	0.06

T2D (severe) was GK01, and T2D (mild) was GK77.
Amounts of urinary organic acids are expressed as mmol per mol Cr.
Amounts of serum acylcarnitine are expressed as nnom ml^{-1}.
[a]Values may be low because of degradation due to long storage at −30 °C.

structural analysis. The program COOT was used to analyze the structure and PyMOL Molecular Graphics System, version 1.4.1 (Schrödinger, LLC; www.pymol.org/citing), was used to make the figures.

RESULTS

Exclusion of the diagnosis of T2 deficiency

We first made a tentative diagnosis of T2 deficiency, based on the severe ketoacidotic event with elevated 2-methyl-3-hydroxybutyrate and tiglylglycine in urinary organic acid analysis. However, repeated enzyme assays showed normal T2 activity (Supplementary Table S2). Furthermore, no T2 mutation was identified by genomic PCR followed by direct sequencing.

Mutation analysis of HSD17B10 gene

Urinary organic acid analysis showed blockade at the T2 or 2M3HBD level in the isoleucine catabolic pathway. Therefore, we investigated the possibility of an *HSD17B10* gene mutation, although the clinical course of this patient was different from that of typical HSD10 patients. A hemizygous c.460G>A (p.A154T) mutation was identified in *HSD17B10* gene (Figure 1). His mother was a heterozygous carrier of this mutation. His maternal uncle did not have this mutation. Samples from maternal grandparents were not available for the study. TaqMan analysis showed that this mutation was not found in 258 alleles from Japanese subjects (controls).

Enzyme assay and immunoblot analysis for 2M3HBD

We used a fibroblast cell line from a Dutch patient whose mutation was c.364C>G (p.L122V) as a positive disease control. He was classified with the infantile form of HSD10 disease because he had shown motor delay and spastic diplegia since infancy.[16] The patient was able to walk but had psychomotor retardation with spasticity and minimal language development (Bwee Tien Poll-The, personal communication), and hence his clinical manifestations were milder than for the typical infantile form of the disease.

2M3HBD activity was absent from the patient's fibroblasts, as well as HSD10-deficient fibroblasts with p.L122V mutation,[16] designated as L122V fibroblasts (Table 2). However, the control samples showed 2M3HBD activity, which was in accordance with reported control values for the assay.[1] Immunoblot analysis showed that fibroblasts from our patient and the previous HSD10-deficient patient had an almost similar amount of HSD17B10 protein to the controls (Supplementary Figure S1).

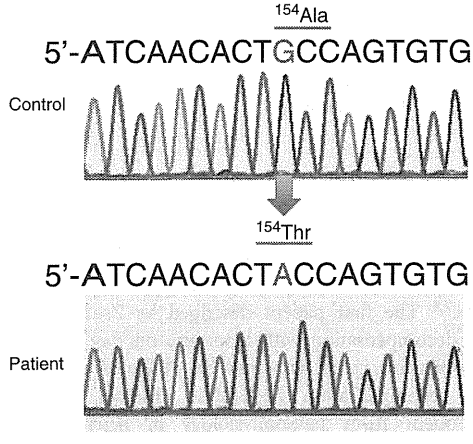

Figure 1 HSD17B10 mutation. Genomic direct sequencing of exon 5. A hemizygous c.460G>A (p.A154T) substitution was identified. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Mitochondrial staining

MitoTracker staining revealed a filamentous network-like structure of the mitochondria in control fibroblasts (Figure 2 and Supplementary Figure S2). Fibroblasts with the p.L122V and p.A154T mutations showed punctate and fragmented mitochondrial organization. This finding is the same as that previously reported in fibroblasts with R130C and D86G mutations.[17] Furthermore, mitochondria in A154T mutated cells had highly variable diameters, ranging from thin tubes to swollen bulbs.

Respiratory chain enzyme assay

Respiratory chain enzyme assay of the patient's fibroblasts showed normal activity of complexes I, II and III (98–159% relative to citrate synthase) (Supplementary Table S3). Complex IV activity was also within the normal range but significantly lower than that of other complexes (51.6% relative to citrate synthase and 44.6% relative to complex II). In blue native polyacrylamide gel electrophoresis, the band corresponding to assembled complex IV was slightly decreased too (Supplementary Figure S3). These tendencies were also detected in fibroblasts with L122V mutation.

Mutation site in the tertiary structure of human HSD17B10

HSD17B10 is a tetramer consisting of four identical subunits, each having the fold of short-chain dehydrogenase/reductase superfamily. Inspection of the human HSD17B10 structure (PDB ID: 2O23) revealed that residue Ala154 is close to the active site (Figure 3a). Ala154 is completely buried and the Cβ atom of Ala154 faces a hydrophobic (apolar) pocket created by residues such as Ile175, Val176 and Cγ of Thr195. The residue next to Ala154, Ser155, is one of the catalytic residues, and part of the catalytic triad formed by Ser155, Tyr168 and Lys172. The mutation of Ala154 to Thr154, that is, from a small, hydrophobic side chain to a larger, polar side chain results in steric clashes with residues Ile175, Val176 and Thr195 in the current conformation (Figure 3b). To avoid these steric clashes, main and side chain conformational changes are expected in the region around Ile175 and Ala/Thr154. The changes around Ile175 may also affect the catalytically competent conformation of the active site residue Lys172. In addition, the changes around Ala/Thr154 are expected to cause structural changes of the catalytic residue Ser155, which has to interact with the substrate for the reaction to occur. Therefore, all these rearrangements resulting in the non-optimal conformations of Ser155 and Lys172 may severely affect the catalytic capability of this enzyme. The substrate binding may not be affected as much because the catalytic triad is only at the beginning of the much larger substrate binding pocket[28] extending outward. Therefore, catalysis of both the steroid substrates such as allopregnanolone[21]

Table 2 2M3HBD assay using fibroblasts

	2M3HBD	*AcAcCoA thiolase*
Control fibroblasts 1	0.75±0.40	15.6
Control fibroblasts 2	0.90±0.58	28.1
L122V fibroblasts	0.19±0.08	28.0
Patient's fibroblasts	0.04±0.11	34.0

Acetoacetyl-CoA (AcAcCoA) thiolase activity was measured in the presence of potassium ion at 37 °C.

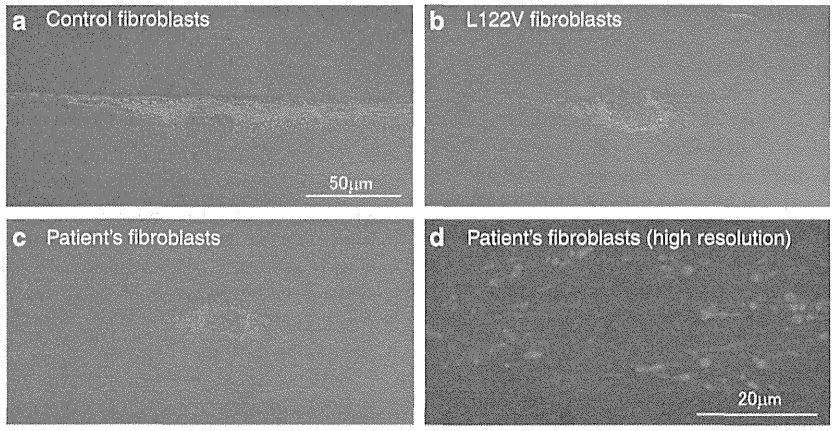

Figure 2 Mitochondrial morphology. (**a**–**c**) Merged images from differential interference contrast (DIC) and MitoTracker Red. (**a**) Control fibroblast. (**b**) Fibroblast with the p.L122V mutation. (**c**) Fibroblast with the p.A154T mutation. (**d**) Fluorescent image of MitoTracker Red from the p.A154T mutated cell. Bars: **a**–**c**, 50 μm; **d**, 20 μm. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

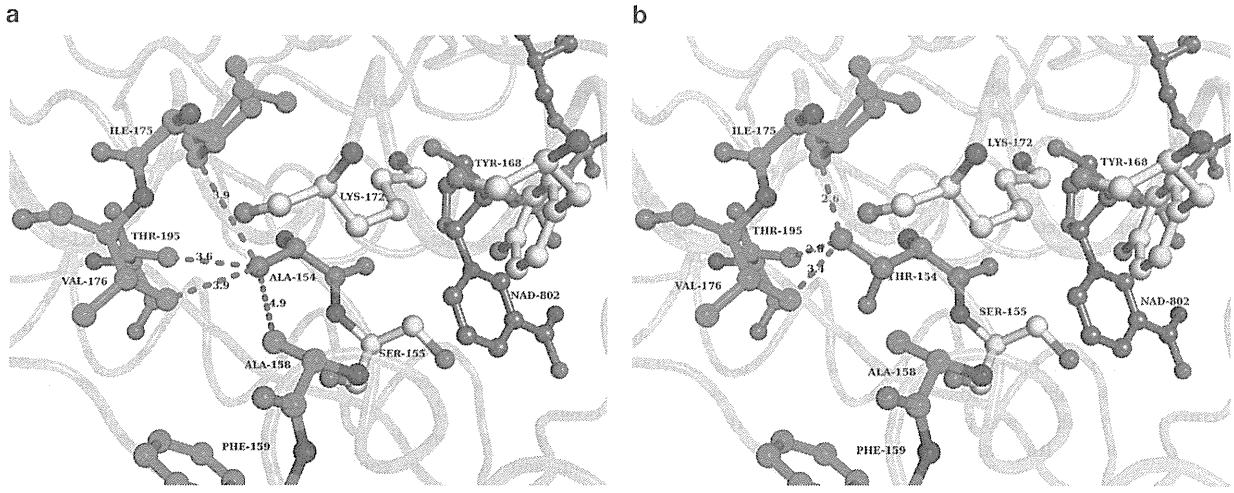

Figure 3 Structural analysis. (**a**) Environment of residue Ala154 as seen in PDB ID 2023. Oxygen atoms are shown in red, nitrogen in blue and carbon is color coded as follows: Ala154 in magenta, the catalytic triad comprising residues Ser155, Tyr168 and Lys172 in yellow, and NAD in blue. Ala158, Phe159, Ile175, Val176 and Thr195 (Cγ) are some of the residues pointing toward the side chain of Ala154, creating a hydrophobic pocket. These are highlighted in green. Ile175 has a double conformation. The relevant distances are shown with red dashes. (**b**) Possible steric clashes in HSD10 disease due to mutation of Ala154 into Thr154. Thr154 is shown in magenta. Ala154 was mutated to Thr154 using PDB-entry 2023 by the program COOT. The expected steric clashes of the Thr154 side chain with Ile175, Val176 and Thr195 are highlighted by red dashes.

and fatty acyl-CoA substrates such as 2-methyl-3-hydoxybutyryl-CoA are predicted to be equally affected.

DISCUSSION

This is believed to be the first report of HSD10 disease in Asia. Since the discovery of the first patient in 2000,[1] fewer than 30 patients have been described.[1,2,5,9–21] Typically, this disorder is suspected when patients with neurological degeneration or psychomotor retardation show similar urinary organic acid or blood acylcarnitine profiles with T2 deficiency. However, our patient experienced a severe ketoacidotic episode with blood pH 7.01 and blood total ketone level of 14 mM after a 5-day history of gastroenteritis. This clinical picture is similar to T2 deficiency, although the onset of the first severe ketoacidotic episode at the age of 6 years is late compared with that in typical T2-deficient patients who develop such crises around the age of 6 months to 2 years.[7,8] The first patient described by Zschocke *et al.*[1] had metabolic decompensation with ketonuria on day 2 of life. Disturbance in isoleucine catabolism may be attributed to such reversible metabolic decompensation in HSD10 disease, and appears to be independent from pathophysiology of neurodegeneration in HSD10 disease.

In the patients with HSD10 disease described thus far, broad clinical heterogeneity has been found.[5,30] The classical presentation that is observed in most patients, which was called the infantile form by

Zschocke,[5] is characterized by a period of more or less normal development in the first 6–18 months of life. This is followed by a progressive neurodegenerative disease course in conjunction with progressive cardiomyopathy, leading to death at the age of 2–4 years or older. Patients with a common mutation c.388C>T (p.R130C) present with the infantile form. Some patients with other mutations have more severe neonatal forms. Atypical presentation was reported in three families. (1) Only one patient with c.745G>C (p.E249Q) mutation developed normally in the first 5 years of life and then showed neurological deterioration.[14] This was classified as the juvenile form by Zschocke.[5] (2) The proband of a family with c.495A>C (p.Q165H) mutation showed growth retardation, feeding difficulty and microcephaly but his neurological status remained normal at up to age 5 years. Moreover, his male cousin with the same mutation achieved normal neurodevelopment until his current age of 8 years, with a height and weight in the 25th percentile.[17] (3) Four boys in a large family showed X-linked intellectual disability, choreoathetosis and abnormal behavior with a normal urinary organic acid profile, and they had an apparent synonymous mutation that affected splicing efficiency in the HSD17B10 gene.[13] Our patient with a novel c.460G>A (p.A154T) mutation showed no neurological degeneration, at least until age 6.5 years, and normal growth. Hence, our patient had a milder phenotype than in patients with juvenile HSD10 disease.

There is evidence that the neurological degeneration observed in HSD10 disease is not caused by a deficiency in the isoleucine metabolism-related 2M3HBD activities of HSD17B10.[17,21] Instead, defects in neuroactive steroid metabolism[21] and/or the non-enzymatic function of the protein required for mitochondrial integrity and cell survival[17] may be responsible for the neurological manifestations. The HSD17B10 protein is one of three component proteins of mitochondrial RNase P, which is essential for mitochondrial translation.[6] Reduced function as a component of RNase P may contribute to clinical severity. The p.R130C mutation common for infantile form reduced not only its mutant HSD10 level but also that of another RNase P component, MRPP-1, suggesting that HSD10 is important for the maintenance of the MRPP1–HSD10 subcomplex of RNase P.[31] Analysis of the consequences of the A154T mutation on the tertiary structure suggests that A154T mutation affects enzyme activity of both 2-methyl-3-hydroxybutyryl-CoA and neurosteroids. The enzymological characterization of the expressed HSD17B10 A154T variant is required to confirm this observation. Mitochondrial morphological changes using MitoTracker staining have been reported,[17] and we also observed punctate and fragmented mitochondrial organization in our patient. Mitochondrial respiratory chain complex IV activity was decreased in both fibroblasts with A154T and those with L122V, although the decreased level did not fulfill the minor diagnostic criteria of Bernier *et al.*[27] Mitochondrial respiratory chain enzyme assay was reported to be normal in fibroblasts with V65A mutation. Further investigation in other fibroblasts with HSD10 disease is necessary to confirm that reduced complex IV activity is one of the characteristics in HSD10 disease.

We have described a patient with mild phenotype HSD10 disease with a novel A154T mutation, who is believed to be the first patient with HSD10 disease in Asia. Accumulation of more data on phenotype–genotype correlation of HSD10 disease is important to understand the molecular basis of the disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We sincerely thank Dr Jos PN Ruiter, Professor Ronald JA Wanders and Professor Bwee Tien Poll-The for providing the fibroblast cell line from an HSD10-deficient patient as a positive control and giving a protocol for the 2M3HBD enzyme assay.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

Journal of Human Genetics (2014) 59, 292–295

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SHORT COMMUNICATION

De novo WDR45 mutation in a patient showing clinically Rett syndrome with childhood iron deposition in brain

Chihiro Ohba[1,2,5], Shin Nabatame[3,5], Yoshitaka Iijima[4], Kiyomi Nishiyama[1], Yoshinori Tsurusaki[1], Mitsuko Nakashima[1], Noriko Miyake[1], Fumiaki Tanaka[2], Keiichi Ozono[3], Hirotomo Saitsu[1] and Naomichi Matsumoto[1]

Rett syndrome (RTT) is a neurodevelopmental disorder mostly caused by *MECP2* mutations. We identified a *de novo WDR45* mutation, which caused a subtype of neurodegeneration with brain iron accumulation, in a patient showing clinically typical RTT. The mutation (c.830 + 1G > A) led to aberrant splicing in lymphoblastoid cells. Sequential brain magnetic resonance imaging demonstrated that iron deposition in the globus pallidus and the substantia nigra was observed as early as at 11 years of age. Because the patient showed four of the main RTT diagnostic criteria, *WDR45* should be investigated in patients with RTT without *MECP2* mutations.
Journal of Human Genetics (2014) **59,** 292–295; doi:10.1038/jhg.2014.18; published online 13 March 2014

Keywords: β-propeller protein-associated neurodegeneration (BPAN); neurodegeneration with brain iron accumulation; Rett syndrome; static encephalopathy of childhood with neurodegeneration in adulthood; *WDR45*

INTRODUCTION

Rett syndrome (RTT) is a neurodevelopmental disorder characterized by regression, loss of acquired purposeful hand skill and language, gait abnormalities and stereotypic hand movements.[1] In typical RTT, *MECP2* mutations can be found in 95–97% of cases, and *CDKL5* and *FOXG1* mutations have been found in atypical RTT.[1] Recently, mutations in *WDR45*, which plays an important role in autophagy, have been identified in a novel subtype of neurodegeneration with brain iron accumulation, called β-propeller protein-associated neurodegeneration (BPAN), which is formerly designated as static encephalopathy of childhood with neurodegeneration in adulthood.[2–4] BPAN shows an unprogressive course during childhood, sudden-onset severe dystonia-parkinsonism and progressive dementia in adulthood. Characteristic brain magnetic resonance imaging findings include iron deposition in the globus pallidus and substantia nigra, and hyperintensity of the substantia nigra with a central band of hypointensity in T1-weighted images.[3] Interestingly, 7 of 23 patients with a *WDR45* mutation showed Rett-like features, suggesting a possible involvement of *WDR45* mutations in RTT.[5] Here we report a patient with typical RTT possessing a *de novo WDR45* mutation.

MATERIALS AND METHODS

A 14-year-old Japanese girl was born to non-consanguineous parents as a first child after an uneventful pregnancy. There was no familial history of neurological diseases. Although her initial development was normal as she controlled her head at 4 months of age, developmental milestones were gradually delayed and she learned to walk at 1 year and 7 months. During infancy, she played with toys and started to talk at ~12 months of age. Her walking developed repetitive atonic episodes and an electroencephalogram showed focal irregular polyspikes and waves, and hence she was diagnosed with epilepsy. She was administered antiepileptic agents and carbamazepine was found to be effective. She gradually lost hand function and verbal communications by 4 years of age, and developed stereotypic hand movements such as continuous rubbing and licking, and dystonia. Although she now walks alone with an ataxic gait, her hand skills have regressed and she cannot talk. She shows hyperventilation, abnormally deep breathing, bruxism during waking periods, hypotonia, peripheral vasomotor disturbance, kyphosis, small cold hands and feet, sudden inappropriate laughing, diminished response to pain and intense eye communication. Sleep disturbance and microcephaly were unobserved. Brain magnetic resonance imaging at 3 and 4 years of age showed no remarkable findings (Figures 1a–d). However, T2-weighted images (WI) revealed mild hypointensity in the globus pallidus and the substantia nigra at 11 years of age (Figures 1e and f). At 14 years, this T2 shortening progressed (Figures 1g and h), and this hypointensity was obvious in T2*WI (Figures 2c

[1]Department of Human Genetics, Graduate School of Medicine, Yokohama City University, Yokohama, Japan; [2]Department of Clinical Neurology and Stroke Medicine, Yokohama City University, Yokohama, Japan; [3]Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan and [4]Division of Pediatrics, Osaka Developmental Rehabilitation Center, Osaka, Japan
Correspondence: Professor N Matsumoto and Dr H Saitsu, Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
E-mail: naomat@yokohama-cu.ac.jp or hsaitsu@yokohama-cu.ac.jp
[5]These authors contributed equally as first authors.
Received 13 January 2014; revised 10 February 2014; accepted 18 February 2014; published online 13 March 2014

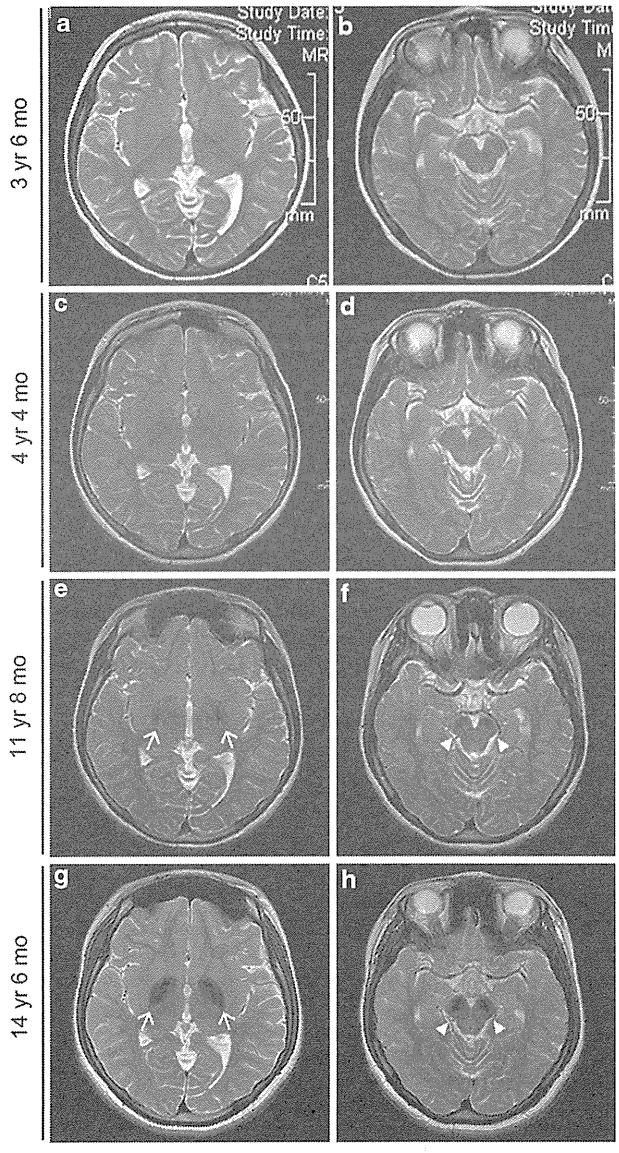

Figure 1 T2-weighted axial images of the patient with a *WDR45* mutation. (**a–h**) T2-weighted axial images of the patient. ((**a**, **b**) at 3 years and 6 months; (**c**, **d**) at 4 years and 4 months; (**e**, **f**) at 11 years and 8 months; (**g**, **h**) at 14 years and 6 months) Brain magnetic resonance imaging (MRI) at 3 and 4 years revealed no remarkable findings in the brain structure, volume and signal intensity (**a–d**). Mild T2 hypointensity in the globus pallidus (**e**, arrow) and the substantia nigra (**f**, arrowhead) was noticed at 11 years of age. At 14 years, T2WI showed strong hypointensity in the globus pallidus (**g**, arrow) and the substantia nigra (**h**, arrowhead). mo, months; yr, years.

and d). T1WI showed hyperintensity of the substantia nigra with a weak central band of T1WI hypointensity (Figure 2b). Experimental protocols for genetic analysis were approved by the Institutional Review Board of Yokohama City University School of Medicine. Clinical information and peripheral blood samples were acquired from family members after obtaining written informed consent.

Sanger sequencing

MECP2 mutation was sequenced by Sanger method. Parental samples were also sequenced with respect to identified variants.

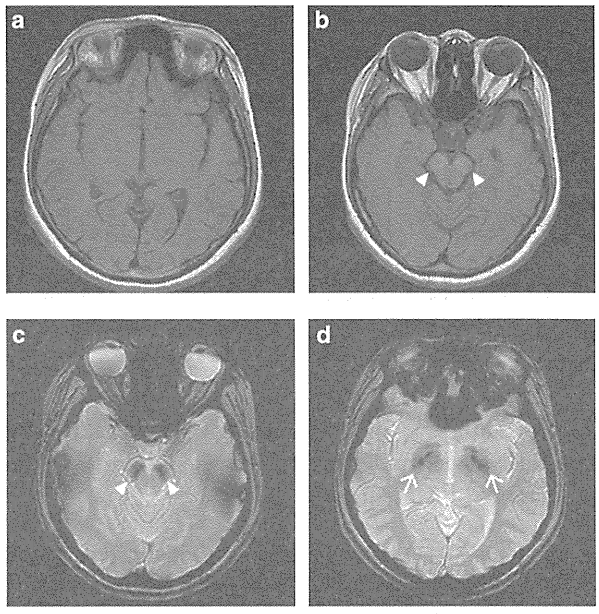

Figure 2 Brain magnetic resonance imaging (MRI) of the patient at 14 years of age. T1-weighted (**a**, **b**), and T2*-weighted (**c**, **d**) axial images of the patient at 14 years and 6 months of age. T1-weighted images show normal findings in the globus pallidus (**a**), but hyperintensity of the substantia nigra with a weak central band of T1WI hypointensity (**b**, arrowhead). T2*-weighted images show strong hypointensity in the substantia nigra (**c**, arrowhead) and the globus pallidus (**d**, arrow).

Whole exome sequencing

Genomic DNA was isolated from peripheral blood leukocytes using QuickGene 610L (Wako, Osaka, Japan), captured using the SureSelect Human All Exon v4 Kit (51 Mb; Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina HiSeq2000 (Illumina, San Diego, CA, USA) with 101 bp paired-end reads. Four samples were run in one lane of the flow cell. Exome data processing, variant calling and variant annotation were performed as previously described.[4] The *WDR45* mutation was confirmed by Sanger sequencing.

Reverse transcriptase-PCR

Lymphoblastoid cell lines were established from the patient. Reverse transcriptase-PCR using total RNA extracted from lymphoblastoid cell lines was performed as previously described.[6] Briefly, total RNA was extracted using the RNeasy Plus Mini kit (Qiagen, Tokyo, Japan), and 4 μg subjected to reverse transcription. For PCR, 2 μl of complementary DNA was used with primer ex8-9-F spanning exons 8 and 9 (5′-GTGGACCTGGCGAGCACAAAG-3′) and primer ex11-12-R spanning exons 11 and 12 (5′-AACTCTGTCATT GCCATCTGCGTAG-3′). The PCR reaction consisted of five cycles of 98 °C for 10 s, 72 °C for 30 s, five cycles of 98 °C for 10 s, 70 °C for 30 s, five cycles of 98 °C for 10 s, 68 °C for 30 s and 30 cycles of 98 °C for 10 s, 66 °C for 30 s. PCR products were electrophoresed on a 10% polyacrylamide gel and sequenced. PCR products were purified using the QIAEXII Gel extraction kit (Qiagen).

RESULTS AND DISCUSSION

We detected c.602C>T (p.Sla201Val) mutation of *MECP2* in the patient by Sanger sequencing. However, we consider this mutation was not pathogenic because the mutation was inherited from her healthy mother. The mutation was also found in 3 males and 8 females of our 574 in-house control exomes (281 males and 293 females). By whole exome sequencing, we

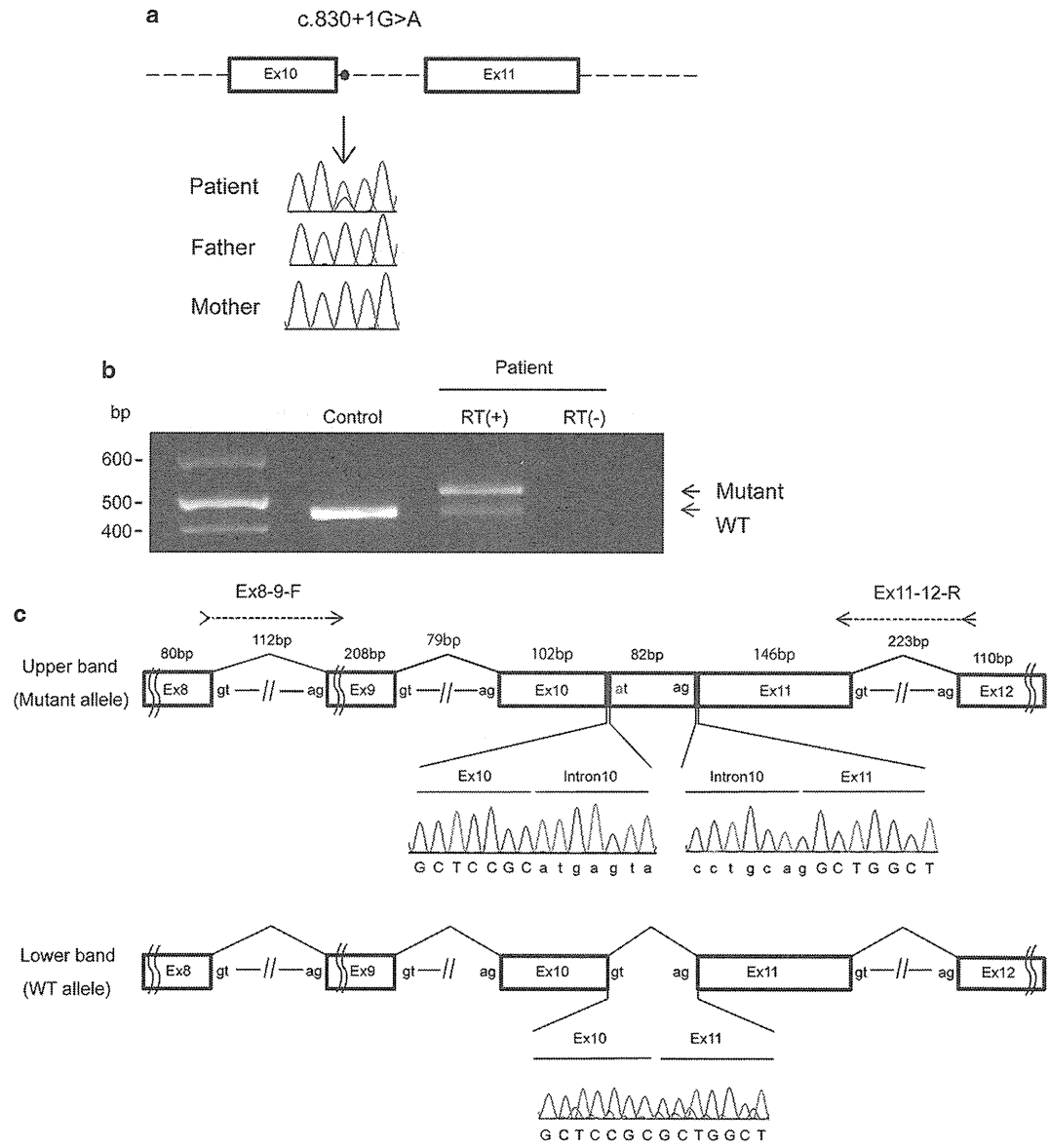

Figure 3 Mutation leading to aberrant splicing in the patient's lymphoblastoid cell lines. (**a**) Location of the *WDR45* mutation. A splice donor site mutation (c.830 + 1G > A) in the patient, which was absent from her parents, was confirmed by Sanger sequencing. (**b**) Reverse transcriptase (RT)-PCR analysis using lymphoblastoid cell lines derived from the patient and a control. A single band (472 bp), corresponding to the wild-type allele, was amplified using a control complementary DNA (cDNA) template. A longer aberrant band was detected from the patient's cDNA. (**c**) Schematic representation of the wild-type and mutant transcripts determined by sequencing PCR products, and primers used for the analysis. The upper band (551 bp) has a 82-bp insertion of the entire intron 10 sequence, leading to a frameshift. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

identified a splice donor site mutation (c.830 + 1G > A) in the *WDR45* gene, which is absent from the proband's parents, indicating that the mutation occurred *de novo* (Figure 3a). The mutation was absent in the 6500 exomes sequenced by the National Heart, Lung, and Blood Institute exome project and our 574 in-house control exomes. Reverse transcriptase-PCR and sequencing revealed aberrant splicing in which 82 bp intronic sequences were retained by the use of a cryptic splice donor site within intron 10, generating a premature stop codon (p.Leu278*) (Figures 3b and c).

Although the brain magnetic resonance imaging at 11 years of age showed iron deposition in the globus pallidus and the substantia nigra, clinical features showed no aggravation at this time. Thus, there is no correlation between iron deposition and clinical phenotype. The fact that iron deposition preceded neurological decline is important information for elucidating BPAN pathogenesis that is caused by autophagy impairment.

The patient showed regression and stabilization, and fulfilled four of the main revised RTT diagnostic criteria,[1] indicating that she clinically showed typical RTT. In autopsied RTT brains, tyrosine hydroxylase activity is reduced in the substantia nigra, and may cause hypofunction of the nigrostriatal dopamine neurons involved in modulating posture and locomotion.[7] Dystonia and parkinsonism are also seen in older RTT patients as well as BPAN.[8]

Therefore, substantia nigra dysfunction might be involved in both RTT and BPAN, facilitating our understanding of the pathomechanism of RTT caused by *MECP2* mutations. We recommend that *WDR45* should be checked in RTT patients without *MECP2* mutation.

ACKNOWLEDGEMENTS

We thank the patient's family for their participation in this study. We also thank Nobuko Watanabe for her technical assistance. This work was supported by the Ministry of Health, Labour, and Welfare of Japan; the Japan Society for the Promotion of Science (a Grant-in-Aid for Scientific Research (B) from (25293085, 25293235), a Grant-in-Aid for Scientific Research (A) (13313587)); the Takeda Science Foundation; the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems; the Strategic Research Program for Brain Sciences (11105137); and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (12024421).

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