D．考察

βケトチオラーゼ（BKT）欠損症とHSD10欠損症は、イソロイシンの代謝過程では近接した部位の代謝障害であるが、臨床像は大きく異なる（表2）。BKT欠損症の欠損酵素T2（アセトアセチル-CoAチオラーゼ）は、イソロイシン代謝過程のメチルアセトアセチル-CoAチオラーゼとβ酸化系のアセチル-CoAチオラーゼとして両方に触媒作用がある。アセトアセチル-CoAが代謝されないためにケトーシス発作を起こしやすい。従ってBKT欠損症の主症状は「ケトアシドーシス発作」である。

一方HSD10欠損症では、イソロイシンの中間代謝過程の障害のみならずコレステロール代謝、RNasePの障害が起こるために、進行性の精神運動発達異常、失調、アテトーゼ、高乳酸血症などBKT欠損症の臨床像とは大きく異なる。また遺伝形式もβケトチオラーゼ欠損症では常染色体劣性遺伝形式で、HSD10欠損症ではX連鎖性劣性遺伝であり男児のみに起こる。

表2. βケトチオラーゼ欠損症とHSD10欠損症の鑑別点

	βケトチオラーゼ欠損症	HSD10欠損症
遺伝	AR 11q22.3-23.1	XR Xp11.2
臨床症状	ケトアシドーシス発作	発達遅滞 神経変性 網膜変性
一般生化学	ケトン陽性	,高乳酸血症
アシルカルニチン分析	C5:1 C5-OH	C5:1 C5-OH
尿中有機酸分析	TG 2M3HBA 3MAA	TG 2M3HBA —
酵素・遺伝子	T2	2M3HBD

略字：AR、常染色体性劣性遺伝；XR、X連鎖性劣性遺伝；TG＝チグリルグリシン；2M3HBA＝2-メチル-3-ヒドロキシ酪酸；2MAA＝2-メチルアセト酢酸；T2＝アセトアセチル-CoAチオラーゼ；2M3HBD＝2-メチル-3-ヒドロキシブチリル-CoA脱水素酵素。

両者は代謝産物のプロフィールが類似しているが、代謝マップからみて尿中有機酸分析で2MAAの検出の有無が重要な鑑別点である。BKT欠損症では2MAAが検出されるが、HSD10欠損症では検出されない。しかし我々の行ったβケトチオラーゼ欠損症（インド、ベトナムおよび日本人症例）患者の尿ではいずれも検出されなかった。本研究でこの原因について検討した。

BKT欠損症3症例のいずれも2MAAが検出されなかった理由として、インド、ベトナムからの検体搬送方法の要因が考えられる。すなわち図4に示すように、乾燥尿ろ紙で搬送すると一部のαケト酸やβケト酸は非常に不安定となることがわかっている。おそらく2MAAもβケト酸の一種であり室温で尿ろ紙の形で搬送された検体では2MAAが分解したものと考えられる。

また日本人患者ではアジア諸国の患者に比べて酵素欠損の程度が軽く異常代謝産物の量が著しく少ない傾向がある。表1に示したアシルカルニチン所見でもアジアの症例ではC5-OH、C5:1の上昇がみられたのに対し症例3（日本人症例）ではいずれも正常範囲であった。2MAAはTGや2M3HBAに比べて量が少なくまた不安定な傾向があるため、この症例3（日本人症例）では2MAAが検出感度以下になったものと思われる。

BKT欠損症の日本人初例は1980年代に発見されたが、この症例では診断マーカーである2M3HBA、TG、および2MAAが明瞭に検出されていた。しかしその当時に保存された尿を分析したところ図2Bに示すように、2MAAは検出されなかった。一方同じ患者で6か月前（2013年12月）に採取された尿を分析したところ、図2、図3に示すように2MAAが検出された。図3に示すようにマススペクトルも2MAAのoxime-TMS誘導体と推定されるものに一致した。

以上の事実から、BKT欠損症患者で2MAAが検出されなかった理由として、①インド、ベトナムからの検体が尿ろ紙として室温で送られてきたこと、②日本人症例が軽症型のため2MAAの量が少なすぎて検出感度以下になったこと、および③一定期間以上経過した古い検体では2MAAが分解することが考えられた。有機酸分析所見からβケトチオラーゼ欠損症とHSD10欠損症を鑑別する際には、尿検体が室温で1週間以上経過してないこと、および古い検体で

ないことを確認して評価する必要がある。少なくとも検体採取から 6 か月以上たっていないことを確認する必要がある。

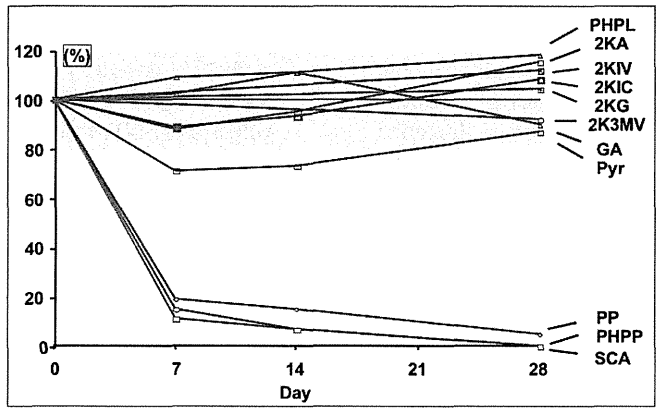


図 4. 尿ろ紙中の代謝産物の安定性

Fu XW et al, J Chromat B 758: 87-94, 2001 らより引用。尿ろ紙を室温に放置した。縦軸は%変化、横軸は放置した日数。略字：PHPL＝p-ヒドロキシフェニル乳酸；2KA＝αケトアジピン酸；2KIV＝αケトイソバレリン酸；2KIC=αケトイソカプロン酸；2KG=αケトグルタル酸；2K3MV=αケト-3-メチルバレリン酸；GA=グルタル酸；Pyr＝ピルビン酸；PP＝フェニルピルビン酸；PHPP＝p-ヒドロキシフェニルピルビン酸；
SCA＝サクシニルアセトン。

E. 結論

βケトチオラーゼ欠損症と HSD10 欠損症は臨床症状が大きく異なるが、代謝障害部位が近接しているためアシルカルニチンや有機酸所見には類似点がある。アシルカルニチン分析では両者ともに C5-OH と C5:12 の上昇がみられ、尿中有機酸分析では両者ともに 2M3HBA と TG の上昇がみられる。が、2-メチルアセト酢酸の有無で鑑別される。しかし 2MAA の量が著しく少なかったり、尿検体が古かったり、尿ろ紙検体の場合 2MAA が分解して検出できなくなる可能性を念頭に置いて、異常代謝産物のプロフィールから両者の鑑別を進める必要がある。

F. 健康危険情報

特になし。

G. 研究発表

1. 論文発表

1) Yasuno T, Osafune K, Sakurai H, Asaka I, Tanaka A, Yamaguchi S, Yamada K, Hitomi H, Arai S, Kurose Y, Higaki Y, Sudo M, Ando S, Nakashima H, Saito T, Kaneoka H: Functional analysis of iPSC-derived myocytes from a patient with carnitine palmitoyltransferase II deficiency. Biochemical and Biophysical Research Communications 448(2): 175-181, 2014 (May)
2) Shioya A, Takuma H, Yamaguchi S, Ishii A, Hiroki M, Fukuda T, Sugiee H, Shigematsu Y, Tamaoka A: Amelioration of acylcarnitine profile using bezafibrate and riboflavin in a case of adult-onset glutaric acidemia type 2 with novel mutations of the electron transfer flavoprotein dehydrogenase (ETFDH) gene. Journal of The Neurological Sciences 346(1-2): 350-352, 2014 (November)
3) Sakai C, Yamaguchi S, Sasaki M, Miyamoto Y, Matsushima Y, Goto YI: ECHS1 mutations cause combined respiratory chain deficiency resulting in Leigh syndrome. Human Mutation, inpress
4) Kobayashi T, Minami S, Mitani A, Tanizaki Y, Booka M, Okutani T, Yamaguchi S, Ino K: Acute fatty liver of pregnancy associated with fetal mitochondrial trifunctional protein deficiency. J Obstet Gynaecol Res, inpress (November)
5) 山口清次: タンデムマスを導入した新生児マススクリーニングの社会的意義と課題. 公衆衛生情報 44(3): 5-8, 2014 (6 月)
6) Naiki M, Ochi N, Kato YS, Purevsuren J, Yamada K, Kimura R, Fukushi D, Hara S, Yamada Y, Kumagai T, Yamaguchi S, Wakamatsu N: Mutations in HADHB, which encodes the β-subunit of mitochondrial trifunctional protein, cause infantile onset hypoparathyroidism and peripheral polyneuropathy. American Journal of Medical Genetics A 164(5): 1180-1187, 2014 (May)
7) Mine J, Taketani T, Yoshida K, Yokochi F, Kobayashi J, Maruyama K, Nanishi E, Ono M, Yokoyama A, Arai H, Tamaura S, Suzuki Y, Otsubo S, Hayashi T, Kimura M, Kishi K, Yamaguchi S: Clinical and genetic investigation of 17 Japanese patients with

hyperekplexia. Developmental Medicine & Child Neurology: Online, 2014 (OCT)
8) Tomatsu S, Shimada T, Mason RW, Kelly J, LaMarr WA, Yasuda E, Shibata Y, Futatsumori H, Montano AM, Yamaguchi S, Suzuki Y, Orii T: Assay for glycosaminoglycans by tandem mass spectrometry and its applications. Journal of Analytical Bioanalytical Techniques Special Issue 2: Online, 2014 (Febrauary)
9) Vatanavicharn N, Yamada K, Aoyama Y, Fukao T, Densupsoontorn N, Jirapinyoe P, Sathienkijkanchai A, Yamaguchi S, Wasant P: Carnitine-acylcarnitine translocase deficiency: two neonatal cases with common splicing mutation and in vitro bezafibrate response. Brain and Development, inpress
10) 坊岡美奈, 比嘉明日美, 津野嘉伸, 熊谷健, 奥谷貴弘, 吉川徳茂, 城道久, 太田菜美, 八木重孝, 南佐和子, 井篦一彦, 山田健治, 山口清次: 胎児心不全で発症したミトコンドリア三頭酵素欠損症の1例. 日本周産期・新生児医学学会雑誌 50(3): 1015-1021, 2014 (9月)
11) 山口清次: ミトコンドリア脂肪酸β酸化異常症. 編: 別冊日本臨床　新領域別症候群シリーズ No.29 神経症候群（第2版）Ⅲ -その他の神経疾患を含めて- －Ⅶ 先天代謝異常－, 日本臨床社, 大阪, p627-631, 2014 (6月, 883)
12) 山口清次: 有機酸代謝異常. 編: 別冊日本臨床 新領域別症候群シリーズ No.29 神経症候群（第2版）Ⅲ -その他の神経疾患を含めて- －Ⅶ 先天代謝異常－, 日本臨床社, 大阪, p622-626, 2014 (6月, 883)
13) 山口清次: 有機酸・脂肪酸代謝異常症. 編: 別冊日本臨床　新領域別症候群シリーズ No.31 神経症候群（第2版）Ⅵ -その他の神経疾患を含めて- －ⅩⅣてんかん症候群 全般てんかんおよび症候群 症候性 特異症候群 先天代謝異常－, 日本臨床社, 大阪, p205-211, 2014 (12月)

2. 学会発表

1) Yamaguchi S: Pediatric emergency and inbron metabolic disease. Seminar: Updates on Inborn Errors of Metabolism セミナー. Kubang Kerian Kelantan, Malaysia, April 2014
2) Yamaguchi S: Current topics in mass screening and collaboration studies with Asian countries. Seminar: Updates on Inborn Errors of Metabolism セミナー. Kubang Kerian Kelantan, Malaysia, April 2014
3) Vatanavicharn N, Taketani T, Nabangchang C, Yamaguchi S: Isolated sulfite oxidase deficiency: A rare metabolic disorder with neuroimaging mimicking perinatal asphyxia. 第56回日本先天代謝異常学会. 仙台, 11 2014

H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得　なし
2. 実用新案登録　なし
3. その他

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表(分担報告書)

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Fukao T, Akiba K, Goto M, Kuwayama N, Morita M, Hori T, Aoyama Y, Venkatesan R, Wierenga R, Moriyama Y, Hashimoto T, Usuda N, Murayama K, Ohtake A, Hasegawa Y, ShigematsuY, Hasegawa Y	The first case in Asia of 2-methyl-3-hydroxybutyryl-Co A dehydrogenase deficiency (HSD10 disease) with atypical presentation.	J Hum Genet	59	609-614	2014
Fukao T, Mitchell G, Saas JO, Hori T, Orii K, Aoyama Y	. Ketone body metabolism and its defects.	J Inherited Metab Dis	37	541-551	2014
Hori T, Yamaguchi S, Shintaku H, Horikawa R, Shigematsu Y, Hakayanagi M, **Fukao T**	Inborn errors of ketone body utilization.	Pediatr Int	I57	41-48	2015

Ⅳ. 研究成果の刊行物・別刷

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

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 (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 μg 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).

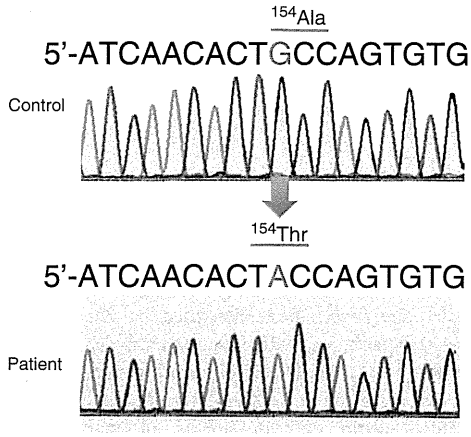


Figure 1 HSD17B10 mutation. Genomic direct sequencing of exon 5. A hemizygous c.460G>A (p.A154T) substitution was identified. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

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.

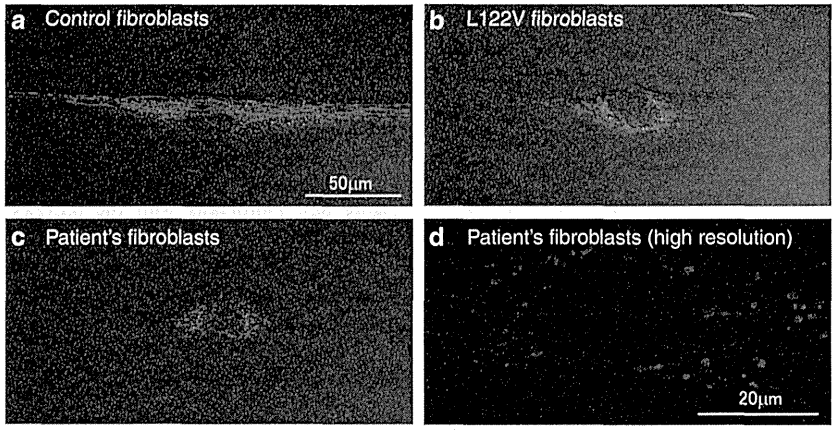


Figure 2 Mitochondrial morphology. (**a**–**c**) Merged images from differential interference contrast (DIC) and MitoTracker Red. (**a**) Control fibroblast. (**b**) Fibroblast with the p.L122V mutation. (**c**) Fibroblast with the p.A154T mutation. (**d**) Fluorescent image of MitoTracker Red from the 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.

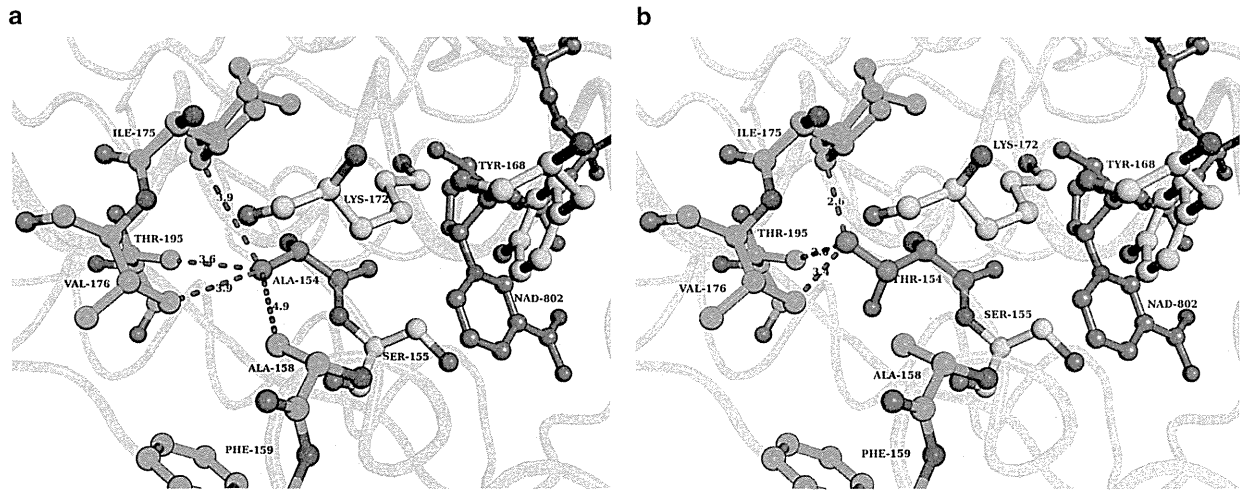


Figure 3 Structural analysis. (**a**) Environment of residue Ala154 as seen in PDB ID 2023. Oxygen atoms are shown in red, nitrogen in blue and carbon is color coded as follows: Ala154 in magenta, the catalytic triad comprising residues Ser155, Tyr168 and Lys172 in yellow, and NAD in blue. Ala158, Phe159, 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.

1 Zschocke, J., Ruiter, J. P., Brand, J., Lindner, M., Hoffmann, G. F., Wanders, R. J. *et al.* Progressive infantile neurodegeneration caused by 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency: a novel inborn error of branched-chain fatty acid and isoleucine metabolism. *Pediatr. Res.* **48**, 852–855 (2000).
2 Ofman, R., Ruiter, J. P., Feenstra, M., Duran, M., Poll-The, B. T., Zschocke, J. *et al.* 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency is caused by mutations in the HADH2 gene. *Am. J. Hum. Genet.* **72**, 1300–1307 (2003).
3 Yang, S. Y., He, X. Y. & Miller, D. HSD17B10: a gene involved in cognitive function through metabolism of isoleucine and neuroactive steroids. *Mol. Genet. Metab.* **92**, 36–42 (2007).
4 Yang, S. Y., He, X. Y. & Schulz, H. Multiple functions of type 10 17beta-hydroxysteroid dehydrogenase. *Trends Endocrinol. Metab.* **16**, 167–175 (2005).
5 Zschocke, J. HSD10 disease: clinical consequences of mutations in the HSD17B10 gene. *J. Inherited Metab. Dis* **35**, 81–89 (2012).
6 Holzmann, J., Frank, P., Loffler, E., Bennett, K. L., Gerner, C. & Rossmanith, W. RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. *Cell* **135**, 462–474 (2008).
7 Fukao, T., Scriver, C. R. & Kondo, N. The clinical phenotype and outcome of mitochondrial acetoacetyl-CoA thiolase deficiency (beta-ketothiolase or T2 deficiency) in 26 enzymatically proved and mutation-defined patients. *Mol. Genet. Metab.* **72**, 109–114 (2001).
8 Mitchell, G. A. & Fukao, T. in *The Metabolic & Molecular Basis of Inherited Disease* Vol. 2, Ch. 102 (eds Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle D.) 2327–2356 (McGraw-Hill, New York, 2001).
9 Cazorla, M. R., Verdu, A., Perez-Cerda, C. & Ribes, A. Neuroimage findings in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency. *Pediatr. Neurol.* **36**, 264–267 (2007).
10 Ensenauer, R., Niederhoff, H., Ruiter, J. P., Wanders, R. J., Schwab, K. O., Brandis, M. *et al.* Clinical variability in 3-hydroxy-2-methylbutyryl-CoA dehydrogenase deficiency. *Ann. Neurol.* **51**, 656–659 (2002).
11 Garcia-Villoria, J., Gort, L., Madrigal, I., Fons, C., Fernandez, C., Navarro-Sastre, A. *et al.* X-inactivation of HSD17B10 revealed by cDNA analysis in two female patients with 17beta-hydroxysteroid dehydrogenase 10 deficiency. *Eur. J. Hum. Genet* **18**, 1353–1355 (2010).
12 Garcia-Villoria, J., Navarro-Sastre, A., Fons, C., Perez-Cerda, C., Baldellou, A., Fuentes-Castello, M. A. *et al.* Study of patients and carriers with 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency: difficulties in the diagnosis. *Clin. Biochem.* **42**, 27–33 (2009).
13 Lenski, C., Kooy, R. F., Reyniers, E., Loessner, D., Wanders, R. J., Winnepenninckx, B. *et al.* The reduced expression of the HADH2 protein causes X-linked mental retardation, choreoathetosis, and abnormal behavior. *Am. J. Hum. Genet.* **80**, 372–377 (2007).
14 Olpin, S. E., Pollitt, R. J., McMenamin, J., Manning, N. J., Besley, G., Ruiter, J. P. *et al.* 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency in a 23-year-old man. *J. Inherited Metab. Dis* **25**, 477–482 (2002).
15 Perez-Cerda, C., Garcia-Villoria, J., Ofman, R., Sala, P. R., Merinero, B., Ramos, J. *et al.* 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency: an X-linked inborn error of isoleucine metabolism that may mimic a mitochondrial disease. *Pediatr. Res.* **58**, 488–491 (2005).
16 Poll-The, B. T., Wanders, R. J., Ruiter, J. P., Ofman, R., Majoie, C. B., Barth, P. G. *et al.* Spastic diplegia and periventricular white matter abnormalities in 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, a defect of isoleucine metabolism: differential diagnosis with hypoxic-ischemic brain diseases. *Mol. Genet. Metab.* **81**, 295–299 (2004).
17 Rauschenberger, K., Scholer, K., Sass, J. O., Sauer, S., Djuric, Z., Rumig, C. *et al.* A non-enzymatic function of 17beta-hydroxysteroid dehydrogenase type 10 is required for mitochondrial integrity and cell survival. *EMBO Mol. Med* **2**, 51–62 (2010).
18 Sass, J. O., Forstner, R. & Sperl, W. 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency: impaired catabolism of isoleucine presenting as neurodegenerative disease. *Brain Dev* **26**, 12–14 (2004).
19 Seaver, L. H., He, X. Y., Abe, K., Cowan, T., Enns, G. M., Sweetman, L. *et al.* A novel mutation in the HSD17B10 gene of a 10-year-old boy with refractory epilepsy, choreoathetosis and learning disability. *PLoS ONE* **6**, e27348 (2011).
20 Sutton, V. R., O'Brien, W. E., Clark, G. D., Kim, J. & Wanders, R. J. 3-Hydroxy-2-methylbutyryl-CoA dehydrogenase deficiency. *J. Inherited Metab. Dis* **26**, 69–71 (2003).
21 Yang, S. Y., He, X. Y., Olpin, S. E., Sutton, V. R., McMenamin, J., Philipp, M. *et al.* Mental retardation linked to mutations in the HSD17B10 gene interfering with neurosteroid and isoleucine metabolism. *Proc. Natl Acad. Sci. USA* **106**, 14820–14824 (2009).
22 Fukao, T., Song, X. Q., Mitchell, G. A., Yamaguchi, S., Sukegawa, K., Orii, T. *et al.* Enzymes of ketone body utilization in human tissues: protein and messenger RNA levels of succinyl-coenzyme A (CoA):3-ketoacid CoA transferase and mitochondrial and cytosolic acetoacetyl-CoA thiolases. *Pediatr. Res.* **42**, 498–502 (1997).
23 Fukao, T., Nakamura, H., Song, X. Q., Nakamura, K., Orii, K. E., Kohno, Y. *et al.* Characterization of N93S, I312T, and A333P missense mutations in two Japanese families with mitochondrial acetoacetyl-CoA thiolase deficiency. *Hum. Mutat.* **12**, 245–254 (1998).

24 Kirby, D. M., Crawford, M., Cleary, M. A., Dahl, H. H., Dennett, X. & Thorburn, D. R. Respiratory chain complex I deficiency: an underdiagnosed energy generation disorder. *Neurology* **52**, 1255–1264 (1999).
25 Schagger, H. & von Jagow, G. Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Anal. Biochem.* **199**, 223–231 (1991).
26 Kirby, D. M., Salemi, R., Sugiana, C., Ohtake, A., Parry, L., Bell, K. M. *et al.* NDUFS6 mutations are a novel cause of lethal neonatal mitochondrial complex I deficiency. *J. Clin. Invest.* **114**, 837–845 (2004).
27 Bernier, F. P., Boneh, A., Dennett, X., Chow, C. W., Cleary, M. A. & Thorburn, D. R. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* **59**, 1406–1411 (2002).
28 Benach, J., Filling, C., Oppermann, U. C., Roversi, P., Bricogne, G., Berndt, K. D. *et al.* Structure of bacterial 3beta/17beta-hydroxysteroid dehydrogenase at 1.2 A resolution: a model for multiple steroid recognition. *Biochemistry.* **41**, 14659–14668 (2002).
29 Persson, B., Kallberg, Y., Bray, J. E., Bruford, E., Dellaporta, S. L., Favia, A. D. *et al.* The SDR (short-chain dehydrogenase/reductase and related enzymes) nomenclature initiative. *Chemico-Biological Interactions* **178**, 94–98 (2009).
30 Yang, S. Y., He, X. Y. & Miller, D. Hydroxysteroid (17beta) dehydrogenase X in human health and disease. *Mol. Cell Endocrinol.* **343**, 1–6 (2011).
31 Deutschmann, A. J., Amberger, A., Zavadil, C., Steinbeisser, H., Mayr, J. A., Feichtinger, R. G. *et al.* Mutation or knock-down of 17beta-hydroxysteroid dehydrogenase type 10 cause loss of MRPP1 and impaired processing of mitochondrial heavy strand transcripts. *Hum. Mol. Genet.* **23**, 3618–3628 (2014).

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

Supplemental Table S1. Genomic PCR primers

exon 1	sense	5'-g.470ATCCCCATCCCGTGGAGTGG
	antisense	5'-g.683AGTGCTGACTTTCACCTCTTGA
exon 2	sense	5'-g.810GGAGAAGCAGCACACCTAGT
	antisense	5'-1279TCCCACAGTGCTTGAAGGCT
exon 3,4, 5	sense	5'-g.2309CCTCTCCCTTCTCACAAATCT
	antisense	5'-g.3139TGCTGCTGCTTAGGTGGTGGAT
exon 6	sense	5'-g.3130AGCAGCAGCAGCCTTTTATCT
	antisense	5'-g.3565ATTAGGCACAGAGGGCGACT

Nucleotides are numbered according to NG_008153 RefSeqGene.

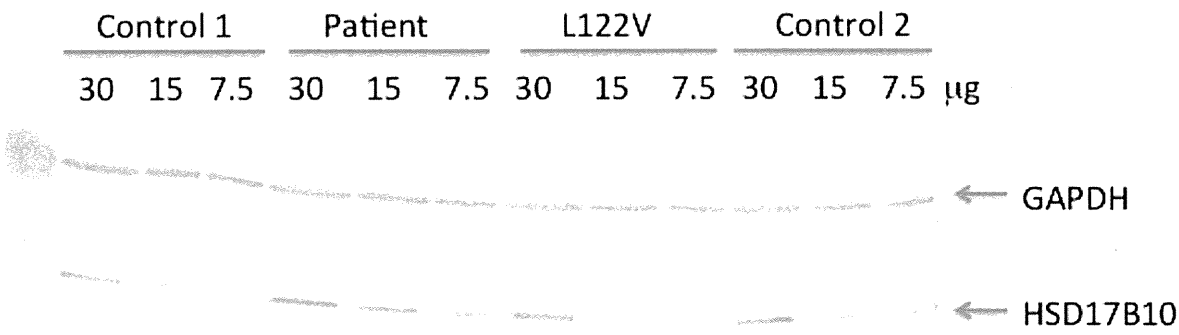
Supplemental Table S2. Enzyme assay using blood mononuclear cells.

	acetoacetyl-CoA thiolase			SCOT
	-K+	+K+	+K/-K	
After 1st episode				
Control	6,4	16.1	2.5	12.1
Patient	10.8	21.2	2.0	13.7
After 2nd episode				
Control	6.9	12.7	1.8	14.3
Patient	5.8	13.1	2.3	17.0

Enzyme activities were expressed as nmol/min/mg protein.

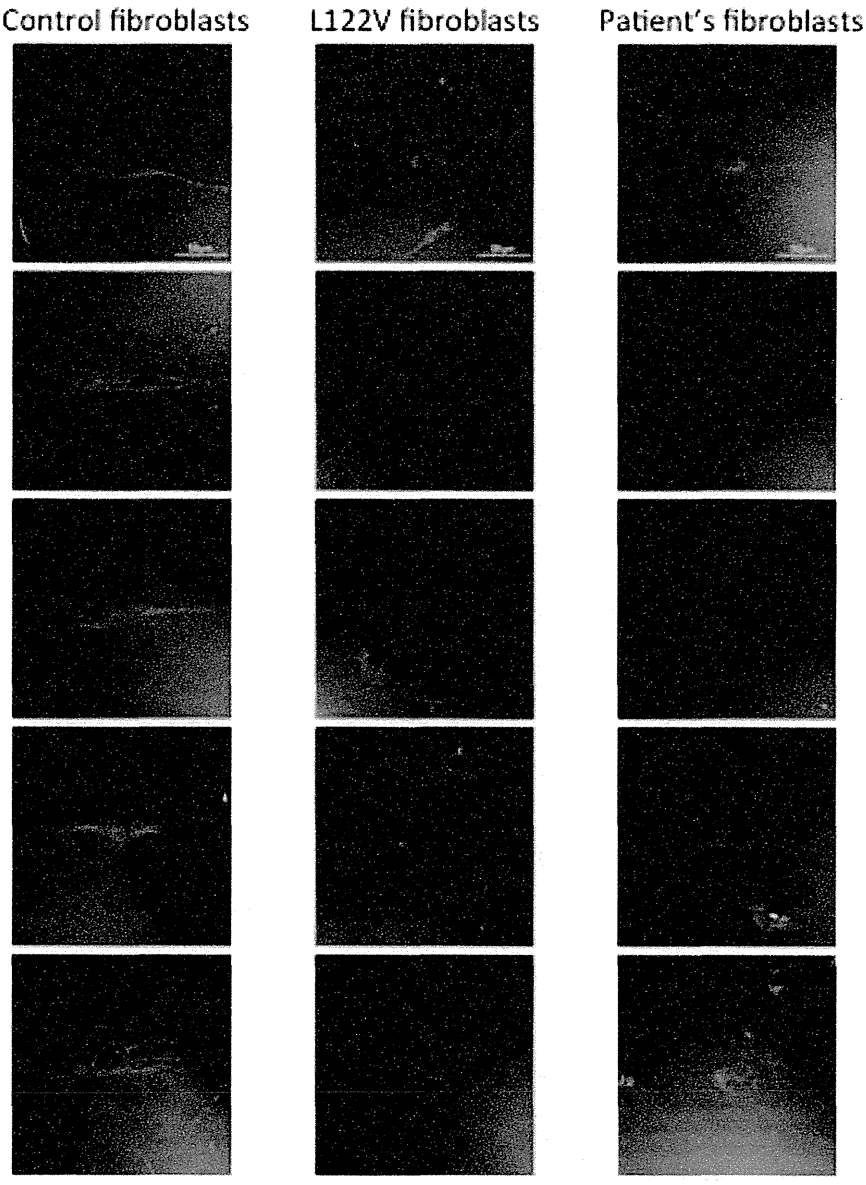
Supplemental Figure 1

Immunoblot analysis. Serially diluted samples 30, 15, and 7.5 micrograms of fibroblast protein extracts were applied. The lane L122V indicates fibroblasts with HSD10 disease in which the mutation was p. L122V. A mixture of anti-rat HSD17B10 antibody and anti-human GAPDH antibody was used as the first antibody.



Supplemental Figure 2

Fig. 2 only shows representative photos for mitochondrial staining. This supplemental figure shows that similar findings were observed in other fibroblasts.

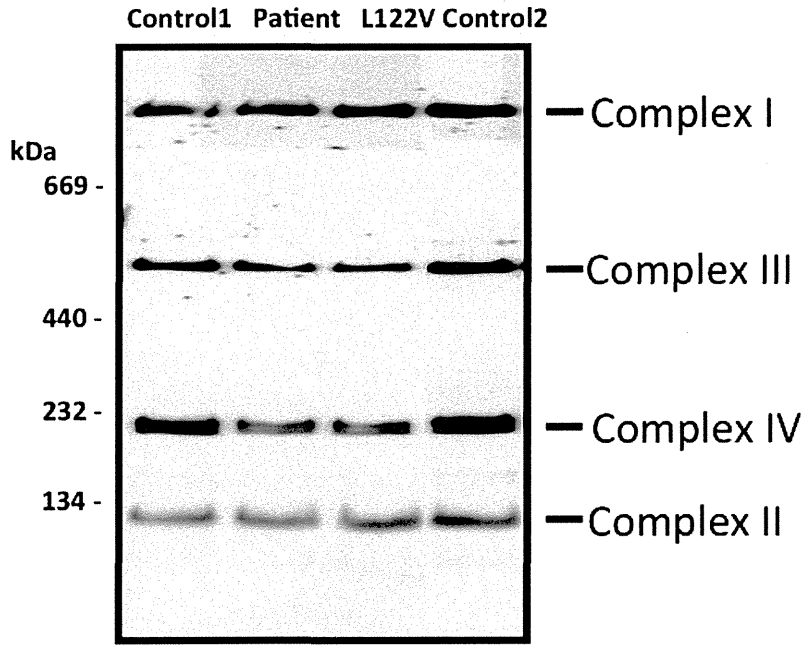


Supplemental Table S3. Respiratory chain enzyme activities

	COI	COII	COII+III	COIII	COIV	CS
Control fibroblasts						
Crude activity (%)	176.1	134.4	207.2	185.6	123.2	186.4
CS ratio (%)	91.7	69.5	105.2	93.7	64.9	
COII ratio (%)	130.3		149.3	137.5	93.3	
L122V fibroblasts						
Crude activity (%)	117.9	152.1	161.2	143.7	55.5	111.2
CS ratio (%)	103.0	131.9	137.2	121.7	49.0	
COII ratio (%)	77.0		102.6	94.0	37.1	
Patient's fibroblasts						
Crude activity (%)	118.0	117.4	101.6	166.0	51.5	98.0
CS ratio (%)	117.0	115.5	98.2	159.5	51.6	
COII ratio (%)	99.9		83.8	140.8	44.6	

Supplemental Figure 3

Blue native polyacrylamide gel electrophoresis analysis of skin fibroblasts. Twenty μg proteins of isolated mitochondria from fibroblasts were solubilized in dodecyl maltoside and subjected to blue native polyacrylamide gel electrophoresis and Western blotting. The amount of fully assembled complex IV was shown to be slightly decreased both in this patient and another one with L122V.



J Inherit Metab Dis
DOI 10.1007/s10545-014-9704-9

ICIEM SYMPOSIUM 2013

Ketone body metabolism and its defects

Toshiyuki Fukao · Grant Mitchell · Jörn Oliver Sass · Tomohiro Hori · Kenji Orii · Yuka Aoyama

Received: 1 January 2014 / Revised: 1 March 2014 / Accepted: 10 March 2014

Abstract Acetoacetate (AcAc) and 3-hydroxybutyrate (3HB), the two main ketone bodies of humans, are important vectors of energy transport from the liver to extrahepatic tissues, especially during fasting, when glucose supply is low. Blood total ketone body (TKB) levels should be evaluated in the context of clinical history, such as fasting time and ketogenic stresses. Blood TKB should also be evaluated in parallel with blood glucose and free fatty acids (FFA). The FFA/TKB ratio is especially useful for evaluation of ketone body metabolism. Defects in ketogenesis include mitochondrial HMG-CoA synthase (mHS) deficiency and HMG-CoA lyase (HL) deficiency. mHS deficiency should be considered in non-ketotic hypoglycemia if a fatty acid beta-oxidation defect is suspected, but cannot be confirmed. Patients with HL deficiency can develop hypoglycemic crises and neurological symptoms even in adolescents and adults. Succinyl-CoA-3-oxoacid CoA transferase (SCOT) deficiency and beta-ketothiolase (T2) deficiency are two defects in ketolysis. Permanent ketosis is pathognomonic for SCOT deficiency. However, patients with "mild" SCOT mutations may have nonketotic periods. T2-deficient patients with "mild" mutations may have normal blood acylcarnitine profiles even in ketoacidotic crises. T2 deficient patients cannot be detected in a reliable manner by newborn screening using acylcarnitines. We review recent data on clinical presentation, metabolite profiles and the course of these diseases in adults, including in pregnancy.

Communicated by: Matthias Baumgartner

Presented at the 12th International Congress of Inborn Errors of Metabolism, Barcelona, Spain, September 3-6, 2013.

T. Fukao (✉) · T. Hori · K. Orii
Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan
e-mail: toshi-gif@umin.net

T. Fukao · Y. Aoyama
Medical Information Sciences Division, United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Gifu, Japan

G. Mitchell
Division of Medical Genetics, Department of Pediatrics, CHU Sainte-Justine and Université de Montréal, Montreal, Canada

J. O. Sass
Division of Clinical Chemistry & Biochemistry, University of Zurich Children's Hospital, Zurich, Switzerland

Published online: 08 April 2014

Ketone body metabolism

Acetoacetate (AcAc) and 3-hydroxybutyrate (3HB) are the two main ketone bodies. They are 4-carbon carboxylic acids, hence, accumulation results in ketoacidosis. Under normal physiological conditions, ketone bodies are the only energy vectors from the liver to brain when glucose supply is low (Mitchell and Fukao 2001; Sass 2012). It should be noted that brain can use ketone bodies as fuels. In special conditions, other substrates are used. An example is the abnormal hyperlactacidemia that accompanies hypoglycemia in patients with glycogen storage disease type 1. In this case, lactate may be an important source of energy for the brain. Ketogenic diets, which have low carbohydrate and high fat content, have been used to treat GLUT1 deficiency (Klepper et al 2002; Klepper and Voit 2002; Morris 2005) and pyruvate dehydrogenase deficiency (Falk et al 1976; Morris 2005). Intractable epilepsy is the best-known indication of the ketogenic diet (Morris 2005; Neal et al 2008). Oral 3HB supplementation has also been used experimentally to treat conditions such as hyperinsulinemic hypoglycemia and multiple acyl-CoA dehydrogenase deficiency (Plecko et al 2002; Van Hove et al 2003).

Figure 1 provides an overview of ketone body metabolism. Free fatty acids (FFA) are supplied from adipose tissues. In the

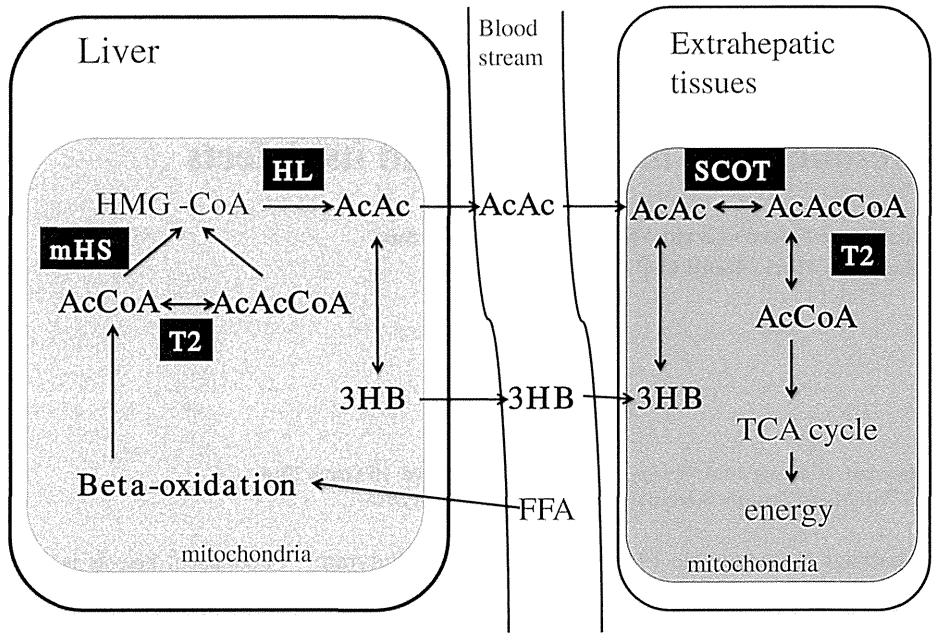


Fig. 1 Summary of ketone body metabolism. *left* Ketogenesis in liver. The HMG-CoA pathway of ketone body formation is much more active in liver than elsewhere. *center* The ketone bodies, 3HB and AcAc, diffuse from liver mitochondria to the circulation and then to extrahepatic tissues including brain. *right* In extrahepatic tissues, SCOT and T2 mediate the production of acetyl-CoA for use in energy production or synthesis. Abbreviations are the same as those in the text except for Ac-CoA (acetyl-CoA), AcAc-CoA (acetoacetyl-CoA), TCA (tricarboxylic acid cycle)

hepatocytes, fatty acid beta-oxidation produces plenty of acetyl-CoA and acetoacetyl-CoA. They are condensed to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)by mitochondrial HMG-CoA synthase (mHS). AcAc is produced from HMG-CoA by HMG-CoA lyase (HL). AcAc is in part reduced to form 3HB. Both AcAc and 3HB diffuse to the bloodstream. In extrahepatic tissues, 3HB is changed back into AcAc, which then is activated to acetoacetyl-CoA by succinyl-CoA:3-oxoacid CoA transferase (SCOT). Next, mitochondrial acetoacetyl-CoA thiolase (T2) transfers an acetyl group to free CoA, producing two molecules of acetyl-CoA. These steps are essential for energy production from ketones in extrahepatic tissues. Brain has no other fatty acid-derived source of energy and ketone bodies are an essential aspect of brain metabolism during fasting (Mitchell and Fukao 2001).

In this article we review ketone body metabolism and the four reported inborn errors of ketone body synthesis and utilization, concentrating on new findings of clinical importance.

Control of ketone body synthesis

Ketogenesis is controlled by hormones. Glucagon and catecholamines induce FFA mobilization from adipose tissue and fatty acid oxidation and ketogenesis. Insulin suppresses these steps (Fukao et al 2004a). Ketogenic stresses including fasting, febrile illnesses, vomiting and diarrhea, induce both FFA oxidation and ketone body synthesis. Gastroenteritis is one of the most common causes of ketosis in children.

Evaluation of ketone body metabolism

Circulating ketone body levels are an important parameter of energy metabolism. They must be interpreted in relation to the clinical state and to the levels of other energy metabolites at the time when the ketone body level was obtained. Clinical history must include the duration of fasting, previous nutritional status and the presence of any acute stress. The most important other energy metabolites are blood glucose and FFA level. Use of the following considerations will allow most patients to be rapidly assigned to a general diagnostic category, from which further investigation can lead to a definitive diagnosis.

In this review, we discuss plasma total ketone body (TKB) levels. In some centers, 3HB and AcAc are measured separately. Their sum provides the TKB level. Some centers measure only 3HB, which is more chemically stable than AcAc and which is not volatile. AcAc accounts for a variable fraction of TKB, depending upon the redox state of the mitochondrial matrix (Mitchell and Fukao 2001). Therefore, TKB level cannot be accurately estimated from the 3HB level alone.

Figure 2 shows blood TKB levels as a function of fasting time for control children (Bonnefont et al 1990). Young children (defined as less than 7 years of age in the study shown) develop ketosis faster than older children. A TKB level of 2 to 5 mM is seen in control young children after a 24 h fast. At least two reasons may explain the effect of aging to progressively delay the increase of ketone body levels during fasting. First, energy demands as a function of body weight decrease more than two-fold between infancy and adulthood (Eckert 1988; http://www.health.gov/dietaryguidelines/2010.asp) and

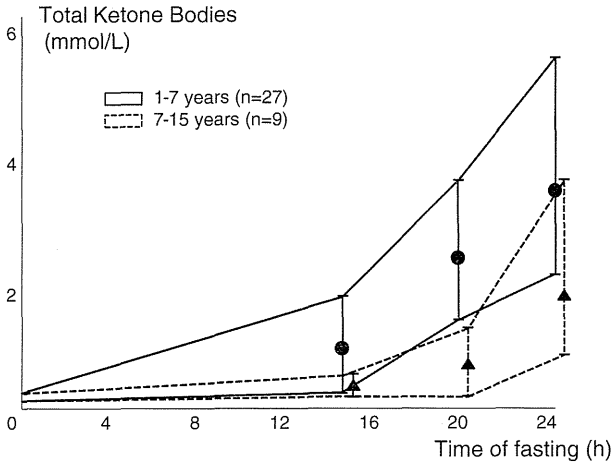


Fig. 2 Plasma total ketone body (TKB) levels as a function of fasting time and age, in groups of children aged 1–7 years and 7–15 years. Results are expressed as 10–90 percentiles with mean values. Redrawn from the data of Bonnefont et al 1990

second, the increase of muscle mass during childhood and adolescence provides a reservoir of protein that can serve for gluconeogenesis.

Blood TKB should be interpreted in relation to blood glucose, insulin and plasma FFA levels. Unfortunately, FFA analysis is not widely performed, despite its diagnostic value, and FFA data are not available in all case reports of defects in fatty acid oxidation and ketone body metabolism. The ratio of FFA/TKB is especially useful for the evaluation of ketone body metabolism. Defects in ketogenesis and fatty acid oxidation are suggested by a ratio above 2.5 and defects in ketolysis, by a ratio of less than 0.3 (Bonnefont et al 1990). Examples of clinical evaluation of ketone body metabolism in acutely ill children in Fig. 3.

Inborn errors of ketogenesis

Two inherited disorders directly affect ketogenesis, deficiency of mitochondrial HMG-CoA synthase (mHS, *HMGCS2* gene) and deficiency of HMG-CoA lyase (HL, *HMGCL* gene) (Table 1).

mHS deficiency

mHS deficiency was first described in 1997 (Thompson et al 1997). We are aware of 12 case reports that contain sufficient detail to be summarized data in Table 2 (Thompson et al 1997; Morris et al 1998; Aledo et al 2001, 2006; Bouchard et al 2001; Zschocke et al 2002; Wolf et al 2003; Pitt et al 2009; Carpenter et al 2010; Hogg et al 2012; Loughrey et al 2013; Ramos et al 2013; Sass et al 2013). This disorder has been characterized clinically by hypoglycemic crises. Most patients presented with symptomatic hypoglycemia, often during a gastroenteritis, and showed an absence of clinical symptoms between acute episodes. Hepatomegaly was noted at hypoglycemic crises in most patients. Severe metabolic acidosis was noted in several patients (Wolf et al 2003; Carpenter et al 2010; Sass et al 2013). The predominant laboratory finding is non(hypo)ketotic hypoglycemia with high FFA levels. Table 3 shows high FFA and low ketone body levels at hypoglycemic crises or monitored fasting tests. This is similar to long-chain fatty acid beta-oxidation defects, but in contrast to these conditions, blood CK level is not usually elevated in mHS deficiency. Fasting tests are usually unnecessary for diagnosis but may be useful for assessing fasting intolerance. So far, there are no established specific markers in urinary organic acids and blood acylcarnitine profiles, although the presence of urinary 4-hydroxy-6-methylpyrone (Pitt et al 2009; Carpenter et al 2010; Hogg et al 2012) and of elevated acetylcarnitine (Aledo et al 2006) has been suggested as a possible marker in decompensated patients. Ketonuria does not preclude the diagnosis of mHS deficiency (Hogg et al 2012; Sass et al 2013). If a patient has non-ketotic hypoglycemia and acidosis, but no other metabolic abnormality suggestive of a fatty acid oxidation defect, mHS deficiency should be considered. Usually, patients have experienced only one hypoglycemic crisis (Table 2), suggesting that early diagnosis may permit effective prevention of crises. Notably, two of these 12 patients died, each before 2 years of age, and permanent brain damage can result from the hypoglycemic crises of mHS deficiency (Sass et al 2013; Loughrey et al 2013) .

HL deficiency

More than 100 patients have been reported since the first description in 1976 (Faull et al 1976a, b); nine of these were from Japan (Muroi et al 2000a, b). Two pathways, ketogenesis from fatty acid oxidation and leucine catabolism, are affected. In most patients the first hypoglycemic crisis occurs before 1 year of age. One third may have neonatal onset. In acute episodes, laboratory tests show non(hypo)-ketotic hypoglycemia with high FFA and severe metabolic acidosis with liver dysfunction and hyperammonemia. Urinary organic acid analysis is often diagnostic because leucine metabolites, 3-hydroxy-3-methylglutarate, 3-methylglutaconate, 3-methylglutarate, 3-hydroxyisovalerate, and 3-methylcrotonylglycine are present.

In the Japanese series (Muroi et al 2000a, b), five of nine patients had neonatal onset. Two patients experienced hypoglycemia even after 10 years of age. Developmental delay was noted in three patients and epilepsy was recorded in three patients.

Patients with HL deficiency may develop hypoglycemia and other complications even in their teens and adulthood and HL deficiency may be diagnosed only as adults. We are aware of three such reports. The first describes a 36-year-old woman

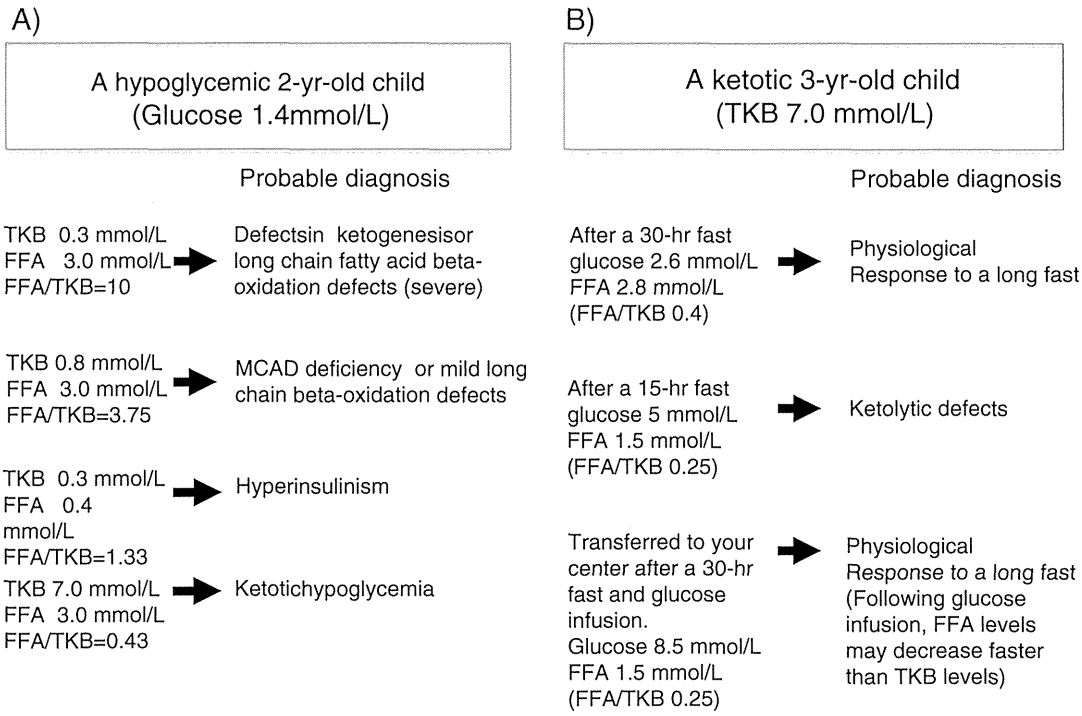


Fig. 3 Examples of the clinical evaluation of ketone body metabolism in acutely ill children. **a** A 2-year-old child has hypoglycemia (glucose 1.4 mmol/L). Possible diagnoses are shown if he has TKB and FFA levels as indicated. **b** A 3-year-old child has hyperketonemia (TKB 7.0 mmol/L). Possible diagnoses are shown if he has TKB and FFA levels as indicated. These examples illustrate the importance of combining clinical history and defining metabolite patterns, as described in the text

with seizures, recurrent metabolic disturbances, and severe leukoencephalopathy (Bischof et al 2004). The other reports describe a 23-year-old man with dilated cardiomyopathy (Leung et al 2009), and a previously asymptomatic 29 year-old man who presented with hypoglycemic coma (Reimao et al 2009).

Inborn errors of ketolysis

Two inherited disorders of ketolysis are known, succinyl-CoA:3-oxoacid CoA transfease (SCOT, *OXCT1* gene) deficiency and mitochondrial acetoacetyl-CoA thiolase (T2, *ACAT1* gene) deficiency (Table 1). T2 deficiency is known as beta-ketothiolase deficiency and also as an inborn error of isoleucine catabolism (Daum et al 1971, 1973). The step catalyzed by T2 in ketolysis can also be catalyzed to some extent by another mitochondrial enzyme, medium-chain 3-ketoacyl-CoA thiolase (Middleton 1973). If SCOT is completely lacking, ketolysis is completely blocked, but if functional T2 is completely absent, some ketolysis is still possible. This may explain in part why permanent ketosis is often observed in SCOT deficiency but not in T2 deficiency.

SCOT deficiency

SCOT deficiency was first described in 1972 (Tildon and Cornblath 1972) and follows an autosomal recessive mode of inheritance. More than 30 patients have been reported or are known to the authors (Cornblath et al 1971; Tildon and Cornblath 1972; Perez-Cerda et al 1992; Sakazaki et al 1995; Kassovska-Bratinova et al 1996; Pretorius et al 1996; Niezen-Koning et al 1997; Rolland et al 1998; Snyderman et al 1998; Song et al 1998; Fukao et al 2000, 2004b, 2006, 2007a, 2010b, 2011; Baric et al 2001; Berry et al 2001; Longo et al 2004; Yamada et al 2007; Merron and Akhtar 2009; Shafqat et al 2013). This disorder is clinically characterized by intermittent ketoacidotic episodes and asymptomatic intervals between episodes. There are no characteristic urinary organic acids except for large amounts of 3HB and AcAc. If present, permanent ketosis, i.e., the existence of ketosis at all times, even during asymptomatic periods when the patient is well-nourished and not fasting, is pathognomonic for SCOT deficiency but is not present in all SCOT-deficient patients. SCOT enzyme activity should be assayed in all suspected patients. About one half of patients develop their first ketoacidotic crisis in the neonatal period.

Table 4 summarizes five Japanese patients. GS02 and his younger sister (GS02s) are typical SCOT-deficient patients