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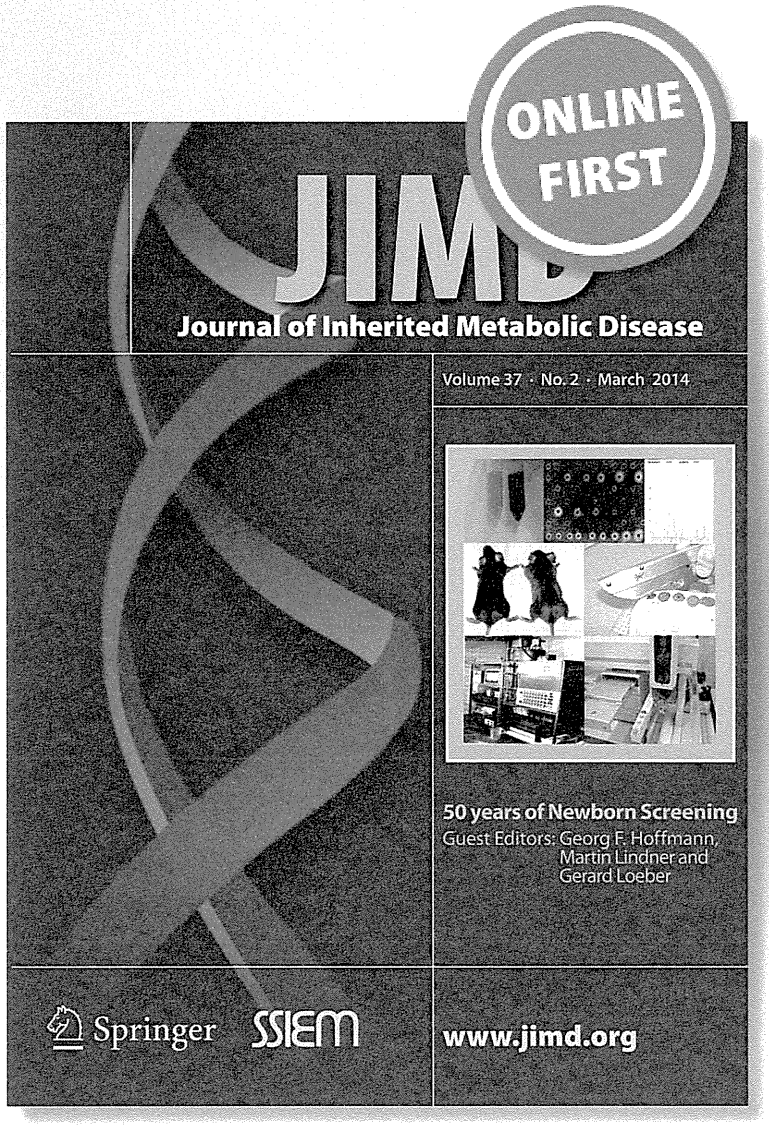

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Abstract Acetoacetate (AcAc) and 3-hydroxybutyrate (3HB), the two main ketone bodies of humans, are important vectors of energy transport from the liver to extrahepatic tissues, especially during fasting, when glucose supply is low. Blood total ketone 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.

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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

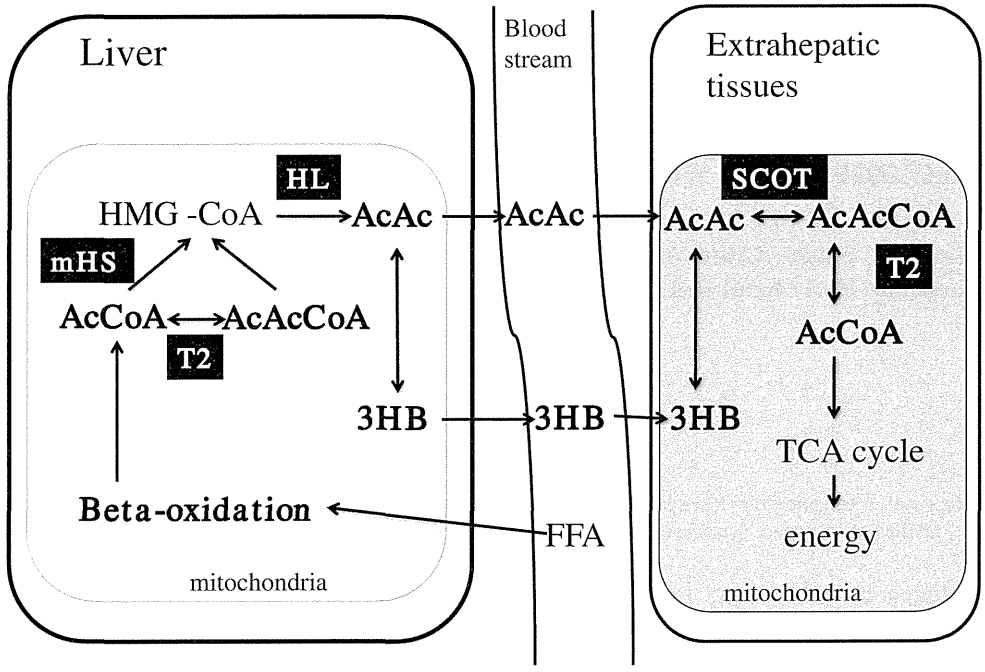

Fig. 1 Summary of ketone body metabolism. *left* Ketogenesis in liver. The HMG-CoA pathway of ketone body formation is much more active in liver than elsewhere. *center* The ketone bodies, 3HB and AcAc, diffuse from liver mitochondria to the circulation and then to extrahepatic tissues including brain. *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

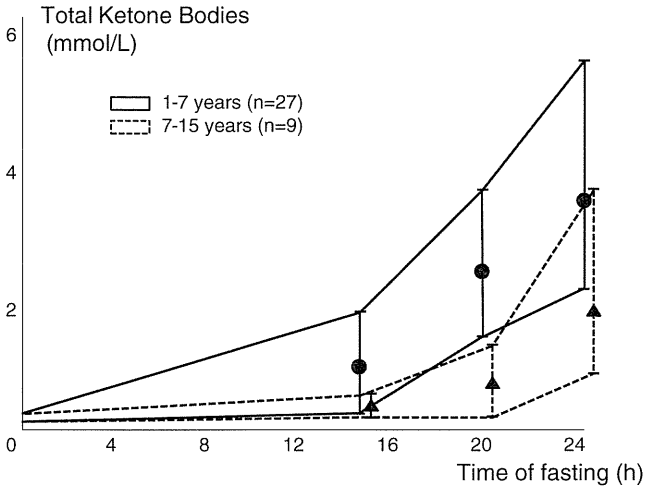

Fig. 2 Plasma total ketone body (TKB) levels as a function of fasting time and age, in groups of children aged 1–7 years and 7–15 years. Results are expressed as 10–90 percentiles with mean values. Redrawn from the data of Bonnefont et al 1990

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

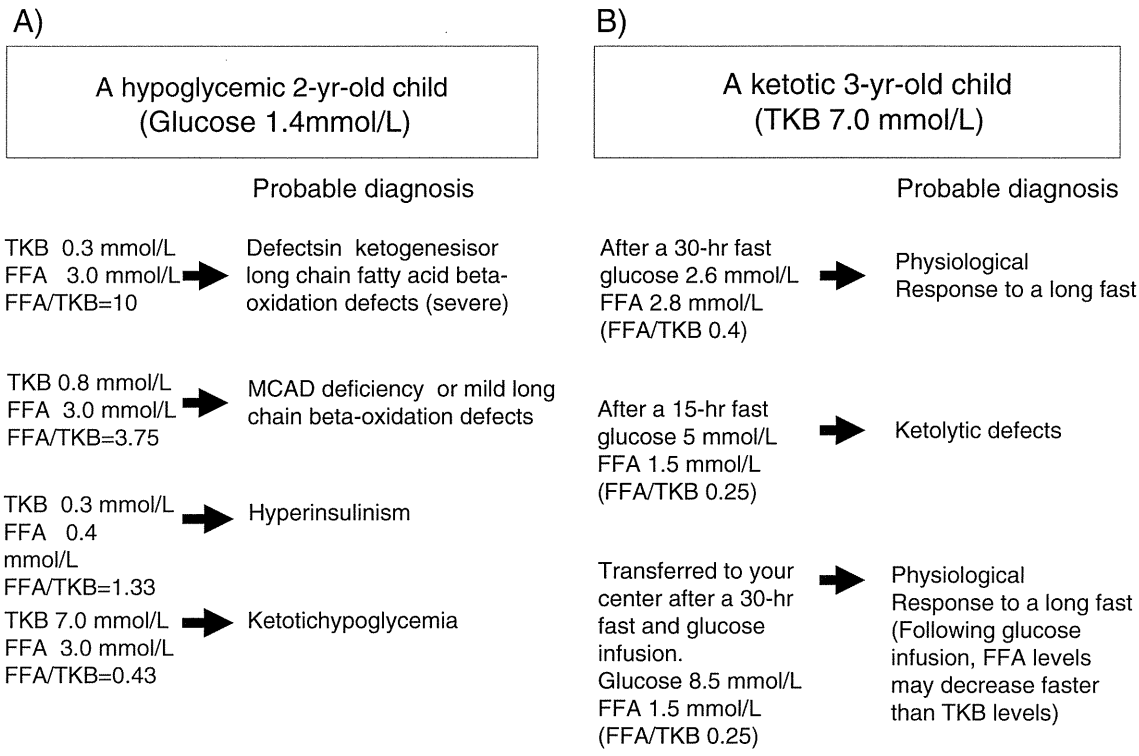

Fig. 3 Examples of the clinical evaluation of ketone body metabolism in acutely ill children. **a** A 2-year-old child has hypoglycemia (glucose 1.4 mmol/L). Possible diagnoses are shown if he has TKB and FFA levels as indicated. **b** A 3-year-old child has hyperketonemia (TKB 7.0 mmol/L). Possible 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

Table 1 Four disorders affecting ketone body metabolsim

	Enzyme abbreviation in this paper	OMIM number	Inheritance	Reported cases	Gene symbol	Locus	Detection in NBS	Blood spots acylcarnitine	Typical urinary organic acid profile
HMG-CoA synthase deficiency	mHS	605911, 600234	AR	>12	*HMGCS2*	1p13-12	NO	C2 elevated in crises, non-specific	Non-specific hopoketotic dicarboxylic aciduria
HMG-CoA lyase deficiency	HL	246450, 613898	AR	>100	*HMGCL*	1p36.11	Possible	C5-OH↑, C6DC↑	3-hydroxyisovalerate, 3-methylglutaconate, 3-hydroxy-3-methylglutarate methylcrotonylglycine
Succinyl-CoA:3-oxioacid CoA transferase deficiency	SCOT	245050, 601424	AR	>20	*OXCT1*	5p113.1	NO	Non-specific	Non-specific ketotic dicarboxylic aciuria
Beta-ketothiolase deficiency	T2	203750, 607809	AR	>100	*ACAT1*	11q22.3-23.1	Unreliable	C5:1 ↑,C5-OH↑	tiglylglycine, 2-methyl-3-hydroxybutyrate, 2-methylacetoacetate

NBS newborn screening

with permanent ketosis (Sakazaki et al 1995; Fukao et al 1996). Their urine is always ketone positive and blood TKB are always high. They are compound heterozygotes for two mutations in the *OXCT1* gene which retain no residual activity in a transient expression analysis of mutant OXCT cDNAs. On the other hand, others do not have permanent ketosis (Fukao et al 2004b, 2010b). Their urine is usually ketone negative. In our cases, they are homozygotes of c.1304C>A (p.T435N), a mutation which retains significant residual SCOT activity. Interestingly, during crises, the severity of ketoacidosis is similar between the two groups, those with and without permanent ketosis. Fasting tests are usually unnecessary for diagnosis but may be useful for assessing fasting intolerance.

Recently, SCOT knockout mice have been reported (Cotter et al 2011, 2013). SCOT knockout mice developed very severe ketoacidosis within 24 h and died within 48 h after birth (Cotter et al 2011). Moreover, SCOT heterozygous mice showed significant elevations of blood ketone body level, especially after a 24 h fast (Cotter et al 2013). Our preliminary data (unpublished, TF) also suggest that heterozygosity for SCOT deficiency may be a risk factor for severe ketoacidosis in humans. More data on heterozygous human carriers are needed to confirm this suggestion.

Beta-ketothiolase deficiency (T2 deficiency)

T2 deficiency was first described in 1971 (Daum et al 1971) and more than 100 patients are known, (e.g., Fukao et al 2001, 2002, 2003a, b, 2007b, 2008, 2010a, c, 2012; Nakamura et al 2001; Zhang et al 2004, 2006; Mrazova et al 2005; Sakurai et al 2007; Thummler et al 2010; Sarafoglou et al 2011; Buhas et al 2013). This disorder is clinically characterized by intermittent ketoacidotic episodes but patients are generally asymptomatic between episodes. Neonatal onset is rare in T2 deficiency. In contrast to its reaction with acetoacetyl-CoA, which can also be performed by medium-chain 3-ketoacyl-CoA thiolase, T2 is the only known enzyme that catalyzes the cleavage of 2-methylacetoacetyl-CoA, a step of isoleucine metabolism (Middleton and Bartlett 1983). As in HMG-CoA lyase deficiency, the accumulation of amino acid catabolic intermediates is a key point in the biochemical diagnosis of T2 deficiency. In urine, tiglylglycine, 2-methy-3-hydroxybutyrate (2M3HB), and 2-methylacetoacetate can be detected, although the latter, labile compound is prone to degradation. In blood acylcarnitine analysis, C5:1 acylcarnitine (tiglylcarnitine) and C5OH acylcarnitine (2-methyl-3-hydroxybutyrylcarnitine) may be elevated, although this is not a consistent finding. Hence, typical T2 deficiency can be suspected following urinary organic acid analysis and blood acylcarnitine analysis.

An important biochemical differential diagnosis is 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD)

Table 2 mHS deficient patients

Case	Onset	Preceding disorders	Metabolic crises			Frequency of crises	Prognosis	*HMGCS2* mutations	Publication
			Symptoms	Hepatomegaly	Glucose at crises (mM)				
Case 1	6 years	AGE(2 days)	coma, convulsion		0.5	1	11y normal	[c.520T>C (p.F174L)]+{=}	Thompson et al 1997
Case 2	1 year 4 months	AGE	coma,	+			4y normal	[c.1270C>T (p.R424*)]+[?]	Morris et al 1998
Case 3	11 months	AGE(2 days)	coma, apnea	+	1.2	1	4y normal	[c.634G>A (p.G212R)]+[c.1499G>A (p.R500H)]	Aledo et al 2001
Case 4	9 months	AGE (a few days)	coma, hepatomegaly	+	2.3	2	4y normal	[c.634G>A (p.G212R)]+[IVS5+1 g>a]	Zschocke et al 2002
Case 5	4 years 6 months	Rota AGE (2 days)	shock (collapse)	+	<1	1	normal	[c.160G>A (p.V54M)]+[c.500A>G (p.Y167C)]	Wolf et al 2003
Case 6 (sib of 5)	1 year 7 months			+		1	normal	the same as case 5	Wolf et al 2003
Case 7	7 months	Appetite loss for 4 days	encephalopathy,	+	<1	1	5y normal	[c.614G>A(p.R188H)]+[c.971T>C(p.M307T)]	Aledo et al 2006
Case 8 (sib of 7)	1 year	Vomiting	extreme lethargy	+		1	>5y normal	the same as case 7	Aledo et al 2006
Case 9	1 year 3 months	AGE	vey unwell	+	<1.6	1	normal	[c.1162G>A (p.G388R)]+[c.1270C>T (p.R424*)]	Ramos et al 2013
Case 10	10 months	infection	hyperpnea and encephalopathy	+	hypoglycemia	1	death at 10 m	[c634A>G(p.G212R)]+[=]	Sass et al 2013
Case 11 (sib of 10)	12 months	infection	hyperpnea and encephalopathy	+	hypoglycemia	1	normal	the same as case 10	Sass et al 2013
Case 12	13 months	AGE (norovirus)	unrousable		0.1	1	death at 18 m	[c.533T>C (p.Y185R)]+[c.1508A>G (p.Y503C)]	Loughrey et al 2013

AGE acute gastroenteritis

Table 3 FFA and ketone body levels in monitored fasting tests or acute crises in mHS patients

	Age	Fasting time	Glucose (mM)	FFA (mM)	TKB (mM)	3HB (mM)	FFA/TKB	FFA/3HB
Case 1	7 years	22 h	2.8			0.2		
Case 2		18 h	2.3	3.96	0.05	0.02	79.2	199
Case 3		12 h	2.3	3.29	0.17		18.9	
Case 4	1 year 8 months	2nd crisis	1.8	3.3		0.064		52
Case 5		19 h	2.9	3.4		<0.05		
Case 6		19 h		2.3		<0.05		
Case 12	1 year 1 months	1st crisis	0.1	3.6		0.18		20

Case numbers are the same as those in Table 1

deficiency (17β-hydroxysteroid dehydrogenase type 10 deficiency, also known as HSD10 disease), a rare disorder with a defect of the enzyme preceding T2 in the isoleucine pathway (Zschocke et al 2000; Zschocke 2012). The pattern of urinary excretion of pathologic metabolites in these two disorders is identical except for the consistent absence of 2-methylacetoacetate in 2M3HBD deficiency. 2M3HBD protein is a moonlight protein which is identical to 17β-hydroxysteroid dehydrogenase type 10 and also one of three components of mitochondrial RNase P (Holzmann et al 2008; Yang et al 2009; Rauschenberger et al 2010). 2M3HBD deficiency is a neurodegenerative disorder with a wide clinical heterogeneity and is clinically different from T2 deficiency. However, we recently experienced a 2M3HBD deficient patient whose initial presentation was a severe ketoacidotic attack, similar with T2 deficiency (Fukao et al unpublished observation), and the first described case with this disease presented with a postnatal metabolic decompensation including ketonuria (Zschocke et al 2000) .

In the eight T2-deficient patients that have been identified in Japan, we compared the metabolite profile with the mutations in the *ACAT1* gene (Table 5). Seven patients had a "mild" genotype, defined here as having at least one mutation with detectable residual activity in the in vitro expression assay. Of note, the patients with mild genotypes developed ketoacidotic crises that were as severe as those of patient GK01, who has two severe mutations with no detectable residual activity (Yamaguchi et al 1988; Fukao et al 1998). Regarding metabolite profiles, GK01 showed the classical urinary organic acids, both during acute crises and under stable conditions. However, in four of six patients with mild genotype, tiglylglycine was not detected, even during acute crises (Fukao et al 2003b, 2010a, 2012; Zhang et al 2004). Under stable conditions, 2M3HB was only faintly detected in patients with mild genotype. Even in acute crisis, C5:1 and C5OH acylcarnitine levels were within control ranges in GK77 and his affected twin sibling GK77b (Fukao et al 2012). Quantitative data for urinary organic acid analysis and acylcarnitine analysis were reported (Fukao et al 2012).

Based on these data in Japanese T2 deficient patients, T2-deficient patients with a mild genotype develop classical severe ketoacidotic crises just as classical T2 deficient patients with complete enzymatic deficiency. In addition, metabolites from isoleucine catabolism are much lower in urinary organic acid and blood acylcarnitine analysis. Even during

Table 4 SCOT deficient patients identified in Japan

GSNumber	Residence	Onset	Frequency of ketoacidotic crises	Typical crisis			Good condition		*OXCT1* mutation
				Blood gas		TKB	Urinary ketone	TKB	
				pH	HCO3 (mM)	(mM)		(mM)	
GS02	Osaka	6 m	3	7.08	5.1	12200	Always positive	858	[c.398T>A (p.V133E)]
GS02s	Osaka	prenatal diag	1	7.29		11400		893	+c.1367G>T (p.C456F)]
GS08	Amami Is.	1y5m	3	7.12	3.7	18500	Usually negative	164	[c.1304C>A (p.T435N)]+[=]
GS09	Amami Is.	10 m	Several	7.00	5.8			341	
GS09b	Amami Is.	10 m	4	7.09	5.4			285	

GS02s is an affected sister of GS02 and GS09b is an affected brother of GS09

HCO3 and TKB mmol/L

Table 5 Japanese T2 deficient patients

Case	*ACAT1* mutations	Onset	1st episode		Number of crises	Present age	MR	Urinary organic acids				Dried blood acylcarnitine			Stable	
								Acute		Stable		Acute				
			pH	HCO3				TIG	2M3HB	TIG	2M3HB	C5:1	C5OH	C5:1	C5OH
GK01	[c.997G>C(p.A333P)]+[c.149delC]	20 m	7.15	4.2	1	28	+	D	D	D	D			D	D
GK19	[c.935C>T (p.I312T)]+[c.278A>G (p.N93S)]	23 m	7.17	3.8	1	17	–	ND	D	ND	FD			ND	FD
GK30	[c.2T>C]+[c.149delC]	9 m	7.01	3.3	3	13	–	D	D	ND	FD			ND	FD
GK31	[c.935C>T (p.I312T)]+c.149delC	18 m	7.07	2.9	1	13	–	D	D	ND	FD			ND	FD
GK64	[c.556G>T (p.D186Y)]+[c.951C>T]	7 m	7.00	8.0	1	4	–	ND	D	ND	FD			ND	ND
GK69	[c.431A>C (p.H144P)]+[c.1168T>C (p.S390P)]	9 m	7.08	4.6	2	25	–			ND	FD			ND	ND
GK77	[c.431A>C (p.H144P)]+[=]	3Y	7.14	6.3	1	4	–	ND	D	ND	FD	ND	ND	FD	ND
GK77b	[c.431A>C (p.H144P)]+[=]	3Y	6.88	1.1	1	3	Died	ND	D			ND	ND	ND	ND

p.A333P, c.149delC, p. D186Y, p.S390P are mutations which retained no resisual activity and the other mutations are mild mutations which retained some residual activity

GK01 is a typical T2 deficient patient and others are patients with "mild" mutations at least on one of two mutant alleles

MR mental retardation; *TIG* tiglylglycine; *2M3HB* 2-methyl3-hydroxybutyrate; *D* detected; *FD* faintly detected; *ND* not detected or within a normal range

ketoacidotic crises, C5:1 and C5OH acylcarnitine levels may be normal.

These results have implications for newborn screening, suggesting that it is probably difficult to reliably detect T2-deficient patients. In support of this, newborn screening did not identify either of two T2-deficient siblings from the USA (Sarafoglou et al 2011). The elder boy had normal screening results but developed a severe ketoacidotic crisis at 10 months of age. He was later diagnosed as having T2 deficiency by enzyme assay and mutation analysis of the *ACAT1* gene. His younger sister, also judged normal by newborn screening, was subsequently shown to have T2 deficiency as well. Although many T2-deficient patients will be detected by newborn acylcarnitine screening, the diagnosis of T2 deficiency cannot be excluded only on the basis of neonatal screening results.

Pregnancy in patients with defects of ketone body metabolism

Pregnancy holds clear risks for individuals with inborn errors of ketogenesis or ketolysis. Normal pregnancy results in an increased metabolic rate and mild ketosis (Mitchell and Fukao 2001). In the first trimester, nausea and vomiting are common, and exacerbate ketosis. One SCOT-deficient woman delivered a healthy baby after a pregnancy with careful metabolic management (Merron and Akhtar 2009). Three T2-deficient women delivered a total of five healthy children without any complications (Sewell et al 1998; Fukao et al 2012). Pregnancies in two women with HL deficiency have been reported (Langendonk et al 2012). Although successful pregnancy is possible, severe complications have been reported in the latter disease. One woman died during a decompensation at 9 weeks of her second pregnancy. Another woman had a miscarriage following a severe decompensation at 10 weeks of pregnancy. As far as conclusions can be drawn from just four pregnancies in two women, pregnancy may be of considerable risk for a patient and her fetus in this disease.

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References

Aledo R, Mir C, Dalton RN et al (2006) Refining the diagnosis of mitochondrial HMG-CoA synthase deficiency. J Inherit Metab Dis 29(1):207–211

Aledo R, Zschocke J, Pie J et al (2001) Genetic basis of mitochondrial HMG-CoA synthase deficiency. Hum Genet 109(1):19–23

Baric I, Sarnavka V, Fumic K et al (2001) A new case of succinyl-CoA: acetoacetate transferase deficiency: favourable course despite very low residual activity. J Inherit Metab Dis 24(1):81–82

Berry GT, Fukao T, Mitchell GA et al (2001) Neonatal hypoglycaemia in severe succinyl-CoA: 3-oxoacid CoA-transferase deficiency. J Inherit Metab Dis 24(5):587–595

Bischof F, Nägele T, Wanders RJ, Trefz FK, Melms A (2004) 3-hydroxy-3-methylglutaryl-CoA lyase deficiency in an adult with leukoencephalopathy. Ann Neurol 56(5):727–730

Bonnefont JP, Specola NB, Vassault A et al (1990) The fasting test in paediatrics: application to the diagnosis of pathological hypo- and hyperketotic states. Eur J Pediatr 150(2):80–85

Bouchard L, Robert MF, Vinarov D et al (2001) Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency: clinical course and description of causal mutations in two patients. Pediatr Res 49(3):326–331

Buhas D, Bernard G, Fukao T, Decarie JC, Chouinard S, Mitchell GA (2013) A treatable new cause of chorea: beta-ketothiolase deficiency. Mov Disord 28(8):1054–1056

Carpenter K, Bhattacharya K, Ellaway C, Zschocke J, Pitt J (2010) Improved sensitivity for HMG CoA synthase detection using key markers on organic acid screen. J Inherit Metab Dis 33:S62

Cornblath M, Gingell RL, Fleming GA, Tildon JT, Leffler AT, Wapnir RA (1971) A new syndrome of ketoacidosis in infancy. J Pediatr 79(3):413–418

Cotter DG, d'Avignon DA, Wentz AE, Weber ML, Crawford PA (2011) Obligate role for ketone body oxidation in neonatal metabolic homeostasis. J Biol Chem 286(9):6902–6910

Cotter DG, Schugar RC, Wentz AE, d'Avignon DA, Crawford PA (2013) Successful adaptation to ketosis by mice with tissue-specific deficiency of ketone body oxidation. Am J Physiol Endocrinol Metab 304(4):E363–E374

Daum RS, Lamm PH, Mamer OA, Scriver CR (1971) A "new" disorder of isoleucine catabolism. Lancet 2(7737):1289–1290

Daum RS, Scriver CR, Mamer OA, Delvin E, Lamm P, Goldman H (1973) An inherited disorder of isoleucine catabolism causing accumulation of alpha-methylacetoacetate and alpha-methyl-beta - hydroxybutyrate, and intermittent metabolic acidosis. Pediatr Res 7(3):149–160

Eckert R (1988) Chapter 16 Animal energetics and temperature relations. In Animal physiology Mechanism and adaptations (3rd edn). Freeman, New York, pp 555–605

Falk RE, Cederbaum SD, Blass JP, Gibson GE, Kark RA, Carrel RE (1976) Ketonic diet in the management of pyruvate dehydrogenase deficiency. Pediatrics 58(5):713–721

Faull K, Bolton P, Halpern B et al (1976a) Letter: patient with defect in leucine metabolism. N Engl J Med 294(18):1013

Faull KF, Bolton PD, Halpern B, Hammond J, Danks DM (1976b) The urinary organic acid profile associated with 3-hydroxy-3-methylglutaric aciduria. Clin Chim Acta 73(3):553–559

Fukao T, Boneh A, Aoki Y, Kondo N (2008) A novel single-base substitution (c.1124A>G) that activates a 5-base upstream cryptic splice donor site within exon 11 in the human mitochondrial acetoacetyl-CoA thiolase gene. Mol Genet Metab 94(4):417–421

Fukao T, Horikawa R, Naiki Y et al (2010a) A novel mutation (c.951C>T) in an exonic splicing enhancer results in exon 10 skipping in the human mitochondrial acetoacetyl-CoA thiolase gene. Mol Genet Metab 100(4):339–344

Fukao T, Ishii T, Amano N et al (2010b) A neonatal-onset succinyl-CoA: 3-ketoacid CoA transferase (SCOT)-deficient patient with T435N and c.658-666dupAACGTGATT p.N220_I222dup mutations in the OXCT1 gene. J Inherit Metab Dis Suppl 3:S307–S313

Fukao T, Kursula P, Owen EP, Kondo N (2007a) Identification and characterization of a temperature-sensitive R268H mutation in the human succinyl-CoA:3-ketoacid CoA transferase (SCOT) gene. Mol Genet Metab 92(3):216–221

Fukao T, Lopaschuk GD, Mitchell GA (2004a) Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. Prostaglandins Leukot Essent Fat Acids 70(3):243–251

Fukao T, Maruyama S, Ohura T et al (2012) Three Japanese Patients with beta-ketothiolase deficiency who share a mutation, c.431A>C (H144P) in ACAT1: subtle abnormality in urinary organic Acid analysis and blood acylcarnitine analysis using tandem mass spectrometry. JIMD Rep 3:107–115

Fukao T, Matsuo N, Zhang GX et al (2003a) Single base substitutions at the initiator codon in the mitochondrial acetoacetyl-CoA thiolase (ACAT1/T2) gene result in production of varying amounts of wild-type T2 polypeptide. Hum Mutat 21(6):587–592

Fukao T, Mitchell GA, Song XQ et al (2000) Succinyl-CoA:3-ketoacid CoA transferase (SCOT): cloning of the human SCOT gene, tertiary structural modeling of the human SCOT monomer, and characterization of three pathogenic mutations. Genomics 68(2):144–151

Fukao T, Nakamura H, Nakamura K et al (2002) Characterization of six mutations in five Spanish patients with mitochondrial acetoacetyl-CoA thiolase deficiency: effects of amino acid substitutions on tertiary structure. Mol Genet Metab 75(3):235–243

Fukao T, Nakamura H, Song XQ et al (1998) Characterization of N93S, I312T, and A333P missense mutations in two Japanese families with mitochondrial acetoacetyl-CoA thiolase deficiency. Hum Mutat 12(4):245–254

Fukao T, Nguyen HT, Nguyen NT et al (2010c) A common mutation, R208X, identified in Vietnamese patients with mitochondrial acetoacetyl-CoA thiolase (T2) deficiency. Mol Genet Metab 100(1):37–41

Fukao T, Sakurai S, Rolland MO et al (2006) A 6-bp deletion at the splice donor site of the first intron resulted in aberrant splicing using a cryptic splice site within exon 1 in a patient with succinyl-CoA: 3-Ketoacid CoA transferase (SCOT) deficiency. Mol Genet Metab 89(3):280–282

Fukao T, Sass JO, Kursula P et al (2011) Clinical and molecular characterization of five patients with succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency. Biochim Biophys Acta 1812(5):619–624

Fukao T, Scriver CR, Kondo N (2001) 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(2):109–114

Fukao T, Shintaku H, Kusubae R et al (2004b) Patients homozygous for the T435N mutation of succinyl-CoA:3-ketoacid CoA transferase (SCOT) do not show permanent ketosis. Pediatr Res 56(6):858–863

Fukao T, Song XQ, Watanabe H et al (1996) Prenatal diagnosis of succinyl-coenzyme A:3-ketoacid coenzyme A transferase deficiency. Prenat Diagn 16(5):471–474

Fukao T, Zhang G, Aoki Y et al (2007b) Identification of Alu-mediated, large deletion-spanning introns 19–26 in PHKA2 in a patient with X-linked liver glycogenosis (hepatic phosphorylase kinase deficiency). Mol Genet Metab 92(1–2):179–182

Fukao T, Zhang GX, Sakura N et al (2003b) The mitochondrial acetoacetyl-CoA thiolase (T2) deficiency in Japanese patients: urinary organic acid and blood acylcarnitine profiles under stable conditions have subtle abnormalities in T2-deficient patients with some residual T2 activity. J Inherit Metab Dis 26(5):423–431

Hogg S, Pierre G, Buck J, Thalange N, Champion M, Calvin J (2012) 4-Hyroxy-6-methyl-2-pyrone and ketonuria in HMG-CoA synthase deficiency. J Inherit Metab Dis 35:S60

Holzmann J, Frank P, Loffler E, Bennett KL, Gerner C, Rossmanith W (2008) RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. Cell 135(3):462–474

Kassovska-Bratinova S, Fukao T, Song XQ et al (1996) Succinyl CoA: 3-oxoacid CoA transferase (SCOT): human cDNA cloning, human chromosomal mapping to 5p13, and mutation detection in a SCOT-deficient patient. Am J Hum Genet 59(3):519–528

Klepper J, Leiendecker B, Bredahl R et al (2002) Introduction of a ketogenic diet in young infants. J Inherit Metab Dis 25(6):449–460

Klepper J, Voit T (2002) Facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome: impaired glucose transport into brain– a review. Eur J Pediatr 161(6):295–304

Langendonk JG, Roos JC, Angus L et al (2012) A series of pregnancies in women with inherited metabolic disease. J Inherit Metab Dis 35(3): 419–424

Leung AA, Chan AK, Ezekowitz JA, Leung AK (2009) A Case of Dilated Cardiomyopathy Associated with 3-Hydroxy-3-Methylglutaryl-Coenzyme A (HMG CoA) Lyase Deficiency. Case Rep Med 2009:183125

Longo N, Fukao T, Singh R et al (2004) Succinyl-CoA:3-ketoacid transferase (SCOT) deficiency in a new patient homozygous for an R217X mutation. J Inherit Metab Dis 27(5):691–692

Loughrey CM, Cundick J, Olpin S, Manning N, Cardy D, Zschocke J (2013) Poor outcome in 3-hydroxy 3-methylglutaryl-CoA (HMG-CoA) synthase deficiency. J Inherit Metab Dis 36(Suppl 2):S197

Merron S, Akhtar R (2009) Management and communication problems in a patient with succinyl-CoA transferase deficiency in pregnancy and labour. Int J Obstet Anesth 18(3):280–283

Middleton B (1973) The oxoacyl-coenzyme A thiolases of animal tissues. Biochem J 132(4):717–730

Middleton B, Bartlett K (1983) The synthesis and characterisation of 2-methylacetoacetyl coenzyme A and its use in the identification of the site of the defect in 2-methylacetoacetic and 2-methyl-3-hydroxybutyric aciduria. Clin Chim Acta 128(2–3):291–305

Mitchell GA, Fukao T (2001) Inborn errors of ketone body metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic & molecular basis of inherited disease. McGraw-Hill, New York, pp 2327–2356

Morris AA (2005) Cerebral ketone body metabolism. J Inherit Metab Dis 28(2):109–121

Morris AA, Lascelles CV, Olpin SE, Lake BD, Leonard JV, Quant PA (1998) Hepatic mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme a synthase deficiency. Pediatr Res 44(3):392–396

Mrazova L, Fukao T, Halovd K et al (2005) Two novel mutations in mitochondrial acetoacetyl-CoA thiolase deficiency. J Inherit Metab Dis 28(2):235–236

Muroi J, Yorifuji T, Uematsu A, Nakahata T (2000a) Cerebral infarction and pancreatitis: possible complications of patients with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. J Inherit Metab Dis 23(6): 636–637

Muroi J, Yorifuji T, Uematsu A et al (2000b) Molecular and clinical analysis of Japanese patients with 3-hydroxy-3-methylglutaryl CoA lyase (HL) deficiency. Hum Genet 107(4):320–326

Nakamura K, Fukao T, Perez-Cerda C et al (2001) A novel single-base substitution (380C>T) that activates a 5-base downstream cryptic splice-acceptor site within exon 5 in almost all transcripts in the human mitochondrial acetoacetyl-CoA thiolase gene. Mol Genet Metab 72(2):115–121

Neal EG, Chaffe H, Schwartz RH et al (2008) The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. Lancet Neurol 7(6):500–506

Niezen-Koning KE, Wanders RJ, Ruiter JP et al (1997) Succinyl-CoA: acetoacetate transferase deficiency: identification of a new patient with a neonatal onset and review of the literature. Eur J Pediatr 156(11):870–873

Perez-Cerda C, Merinero B, Sanz P et al (1992) A new case of succinyl-CoA: acetoacetate transferase deficiency. J Inherit Metab Dis 15(3): 371–373

Pitt J, Peters H, Mishra A et al (2009) Mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase deficiency may be recognized by increased concentration of 4-hydroxy-6-methyl-2-pyrone in urinary organic acid analysis. Mol Genet Metab 98(1–2):51

Plecko B, Stoeckler-Ipsiroglu S, Schober E et al (2002) Oral beta-hydroxybutyrate supplementation in two patients with hyperinsulinemic hypoglycemia: monitoring of beta-hydroxybutyrate levels in blood and cerebrospinal fluid, and in the brain by in vivo magnetic resonance spectroscopy. Pediatr Res 52(2):301–306

Pretorius CJ, Loy Son GG, Bonnici F, Harley EH (1996) Two siblings with episodic ketoacidosis and decreased activity of succinyl-CoA: 3-ketoacid CoA-transferase in cultured fibroblasts. J Inherit Metab Dis 19(3):296–300

Ramos M, Menao S, Arnedo M et al (2013) New case of mitochondrial HMG-CoA synthase deficiency. Functional analysis of eight mutations. Eur J Med Genet 56(8):411–415

Rauschenberger K, Scholer K, Sass JO et al (2010) A non-enzymatic function of 17beta-hydroxysteroid dehydrogenase type 10 is required for mitochondrial integrity and cell survival. EMBO Mol Med 2(2):51–62

Reimao S, Morgado C, Almeida IT, Silva M, Real HC, Campos J (2009) 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: Initial presentation in a young adult. J Inherit Metab Dis 32(Suppl 1):S49–S52

Rolland MO, Guffon N, Mandon G, Divry P (1998) Succinyl-CoA: acetoacetate transferase deficiency. Identification of a new case; prenatal exclusion in three further pregnancies. J Inherit Metab Dis 21(6):687–688

Sakazaki H, Hirayama K, Murakami S et al (1995) A new Japanese case of succinyl-CoA: 3-ketoacid CoA-transferase deficiency. J Inherit Metab Dis 18(3):323–325

Sakurai S, Fukao T, Haapalainen AM et al (2007) Kinetic and expression analyses of seven novel mutations in mitochondrial acetoacetyl-CoA thiolase (T2): identification of a Km mutant and an analysis of the mutational sites in the structure. Mol Genet Metab 90(4):370–378

Sass JO (2012) Inborn errors of ketogenesis and ketone body utilization. J Inherit Metab Dis 35(1):23–28

Sass JO, Kühlwein E, Klauwer D, Rohrbach M, Baumgartner MR (2013) Hemodiafiltration in mitochondrial 3-hydroxy-3-methylglutaryl coenzyme A synthase (HMG-CoA synthase) deficiency. J Inherit Metab Dis 36(Suppl 2):S189

Sarafoglou K, Matern D, Redlinger-Grosse K et al (2011) Siblings with mitochondrial acetoacetyl-CoA thiolase deficiency not identified by newborn screening. Pediatrics 128(1):e246–e250

Sewell AC, Herwig J, Wiegratz I et al (1998) Mitochondrial acetoacetyl-CoA thiolase (beta-ketothiolase) deficiency and pregnancy. J Inherit Metab Dis 21(4):441–442

Shafqat N, Kavanagh KL, Sass JO et al (2013) A structural mapping of mutations causing succinyl-CoA: 3-ketoacid CoA transferase (SCOT) deficiency. J Inherit Metab Dis 36(6):983–987

Snyderman SE, Sansaricq C, Middleton B (1998) Succinyl-CoA: 3-ketoacid CoA-transferase deficiency. Pediatrics 101(4 Pt 1): 709–711

Song XQ, Fukao T, Watanabe H et al (1998) Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency: two pathogenic mutations, V133E and C456F, in Japanese siblings. Hum Mutat 12(2):83–88

Thompson GN, Hsu BY, Pitt JJ, Treacy E, Stanley CA (1997) Fasting hypoketotic coma in a child with deficiency of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase. N Engl J Med 337(17): 1203–1207

Thummler S, Dupont D, Acquaviva C, Fukao T, de Ricaud D (2010) Different clinical presentation in siblings with mitochondrial

acetoacetyl-CoA thiolase deficiency and identification of two novel mutations. Tohoku J Exp Med 220(1):27–31

Tildon JT, Cornblath M (1972) Succinyl-CoA: 3-ketoacid CoA-transferase deficiency. A cause for ketoacidosis in infancy. J Clin Invest 51(3):493–498

Van Hove JL, Grunewald S, Jaeken J et al (2003) D, L-3-hydroxybutyrate treatment of multiple acyl-CoA dehydrogenase deficiency (MADD). Lancet 361(9367):1433–1435

Wolf NI, Rahman S, Clayton PT, Zschocke J (2003) Mitochondrial HMG-CoA synthase deficiency: identification of two further patients carrying two novel mutations. Eur J Pediatr 162(4): 279–280

Yamada K, Fukao T, Zhang G et al (2007) Single-base substitution at the last nucleotide of exon 6 (c.671G>A), resulting in the skipping of exon 6, and exons 6 and 7 in human succinyl-CoA:3-ketoacid CoA transferase (SCOT) gene. Mol Genet Metab 90(3):291–297

Yamaguchi S, Orii T, Sakura N, Miyazawa S, Hashimoto T (1988) Defect in biosynthesis of mitochondrial acetoacetyl-coenzyme A thiolase in cultured fibroblasts from a boy with 3-ketothiolase deficiency. J Clin Invest 81(3):813–817

Yang SY, He XY, Olpin SE et al (2009) Mental retardation linked to mutations in the HSD17B10 gene interfering with neurosteroid and isoleucine metabolism. Proc Natl Acad Sci U S A 106(35):14820–14824

Zhang G, Fukao T, Sakurai S, Yamada K, Gibson MK, Kondo N (2006) Identification of Alu-mediated, large deletion-spanning exons 2–4 in a patient with mitochondrial acetoacetyl-CoA thiolase deficiency. Mol Genet Metab 89(3):222–226

Zhang GX, Fukao T, Rolland MO et al (2004) Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency: T2-deficient patients with "mild" mutation(s) were previously misinterpreted as normal by the coupled assay with tiglyl-CoA. Pediatr Res 56(1):60–64

Zschocke J (2012) HSD10 disease: clinical consequences of mutations in the HSD17B10 gene. J Inherit Metab Dis 35(1):81–89

Zschocke J, Penzien JM, Bielen R et al (2002) The diagnosis of mitochondrial HMG-CoA synthase deficiency. J Pediatr 140(6):778–780

Zschocke J, Ruiter JP, Brand J et al (2000) 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(6):852–855

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Review Article

Inborn errors of ketone body utilization

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Abstract Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency and mitochondrial acetoacetyl-CoA thiolase (beta-ketothiolase or T2) deficiency are classified as autosomal recessive disorders of ketone body utilization characterized by intermittent ketoacidosis. Patients with mutations retaining no residual activity on analysis of expression of mutant cDNA are designated as severe genotype, and patients with at least one mutation retaining significant residual activity, as mild genotype. Permanent ketosis is a pathognomonic characteristic of SCOT-deficient patients with severe genotype. Patients with mild genotype, however, may not have permanent ketosis, although they may develop severe ketoacidotic episodes similar to patients with severe genotype. Permanent ketosis has not been reported in T2 deficiency. In T2-deficient patients with severe genotype, biochemical diagnosis is done on urinary organic acid analysis and blood acylcarnitine analysis to observe characteristic findings during both ketoacidosis and non-episodic conditions. In Japan, however, it was found that T2-deficient patients with mild genotype are common, and typical profiles were not identified on these analyses. Based on a clinical study of ketone body utilization disorders both in Japan and worldwide, we have developed guidelines for disease diagnosis and treatment. These diseases are treatable by avoiding fasting and by providing early infusion of glucose, which enable the patients to grow without sequelae.

Key words inborn errors of ketone body utilization, ketone body, ketone body metabolism, mitochondrial acetoacetyl-CoA thiolase (beta-ketothiolase/T2) deficiency, succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency.

Ketone bodies are important alternative energy sources for maintaining blood glucose level. Ketone bodies, however, are acids and cause ketoacidosis when accumulated, which is hazardous to life.[1] Among patients with severe ketoacidosis, some are affected with inborn errors of ketone body metabolism. Succinyl-CoA:3-ketoacid CoA transferase (SCOT, gene symbol *OXCT1*) deficiency and mitochondrial acetoacetyl-CoA thiolase (beta-ketothiolase or T2, gene symbol *ACAT1*) deficiency are rare inherited metabolic disorders. Approximately 130 cases worldwide and 10 families in Japan have been reported to present with these two disorders.[2–44] Both SCOT and T2 deficiencies are autosomal recessive disorders, characterized by intermittent ketoacidosis. Some patients who experience severe ketoacidosis develop psychomotor retardation or even die. If, however, patients are properly diagnosed, inexpensive preventive measures can be effective, and normal development is expected.

We have been studying the pathophysiology of these disorders and have diagnosed both Japanese patients and those of other nationalities with these disorders. We found that patients with mutations that retain some residual activity are biochemically different from typical patients with these disorders, based on analysis of Japanese patients.[5,6,16,22,26,27,32,38,43]

In this review, we provide an overview of ketone body metabolism and summarize cases of SCOT deficiency and T2 deficiency in Japan. Furthermore, we provide an English-language version of recent guidelines for the diagnosis and treatment of these diseases, originally developed in Japanese in 2012 with support from Health and Labour Science Research Grants for Research on Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan.

Ketone body metabolism

Acetoacetate (AcAc) and 3-hydroxybutyrate (3HB) are the two main ketone bodies. They are 4-carbon carboxylic acids, hence accumulation in excess results in ketoacidosis. Ketone bodies, however, play an important role as vectors of energy transport from the liver to extrahepatic tissues, especially during shortages of glucose. It should be noted that the brain uses ketone bodies as an energy source.[1]

An overview of ketone body metabolism is given in Figure 1. Free fatty acids (FFA) are supplied from adipose tissues. In hepatocytes, beta-oxidation produces large amounts of

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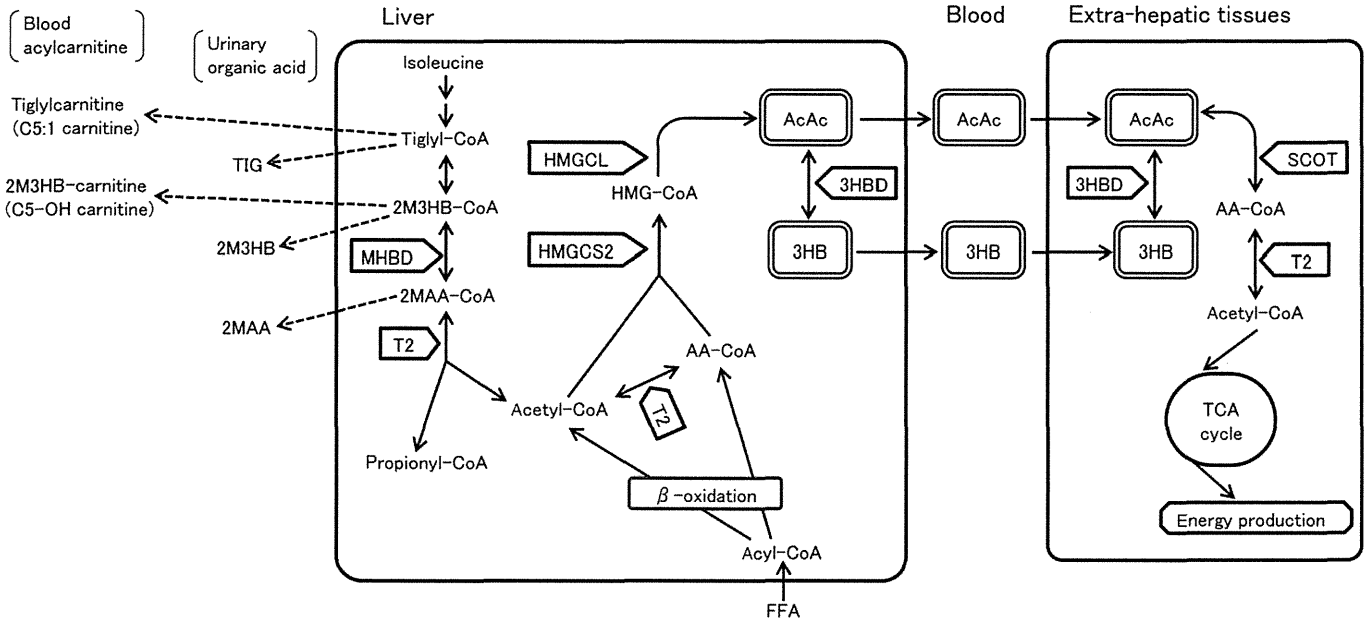

Fig. 1 Overview of ketone body metabolism. 2M3HB, 2-methyl-3-hydroxybutyrate; 2M3HB-, 2-methyl-3-hydroxybutyryl-; 2MAA, 2-methylacetacetate; 2MAA-, 2-methylacetoacetyl-; 3HB, 3-hydroxybutyrate; 3HBD, 3-hydroxybutyrate dehydrogenase; AA-, acetoacetyl-; AcAc, acetoacetate; FFA, free fatty acids; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGCL, HMG-CoA lyase; HMGCS2, mitochondrial HMG-CoA synthase; MHBD, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase; SCOT, succinyl-CoA:3-ketoacid CoA transferase; T2, mitochondrial acetoacetyl-CoA thiolase; TIG, tiglylglycine.

acetyl-CoA and acetoacetyl-CoA. They are condensed to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by mitochondrial HMG-CoA synthase (HMGCS2). AcAc is produced from HMG-CoA by HMG-CoA lyase (HMGCL). AcAc is in part converted into 3HB and then both are transferred to extrahepatic tissues via the bloodstream. There, 3HB is again converted into AcAc and activated into acetoacetyl-CoA by SCOT. Acetoacetyl-CoA is cleaved to acetyl-CoA by T2. These steps are essential for energy production from ketone bodies in extrahepatic tissues.[1]

Ketone body utilization disorders

Clinical symptoms and laboratory findings

SCOT deficiency and T2 deficiency are classified as disorders of ketone body utilization. SCOT deficiency was first described in 1972,[2] since when more than 30 patients have been reported.[2–24] T2 deficiency was first described in 1971,[25] and more than 100 patients have been reported.[25–44] Patients develop ketoacidosis during ketogenic stress such as starvation, febrile conditions and physical stresses because ketone bodies produced in the liver accumulate due to defective utilization in extrahepatic tissues. In physiological ketosis in normal children, both FFA and total ketone bodies (TKB) are elevated proportionally in the blood. TKB, however, are much higher compared with FFA, and the FFA/TKB ratio falls below 0.3 early in fasting patients with these disorders. Generally, ketoacidosis presents prior to hypoglycemia and there are reports of a few cases of hypoglycemia during ketoacidosis. Patients with these disorders have no clinical signs and symptoms during non-ketoacidotic periods if they do not have neurological sequelae of severe ketoacidotic events.

Because deficient use of ketone bodies in extrahepatic tissues is the main pathogenic trait of both diseases, they cannot be distinguished by clinical signs, symptoms or routine laboratory findings, but there are some differences (Table 1).

The first ketoacidotic crisis occurs during the neonatal period in approximately half of SCOT-deficient patients and between 5 months and 2 years of age in the other half. Neonatal onset, however, is very rare in T2 deficiency. Only one case has been diagnosed due to mild ketoacidosis in the neonatal period.[28] The first ketoacidotic event in T2 deficiency is triggered by infection or starvation between approximately 5 months and 2 years of age in almost all cases.

In this review, the term "severe genotype" is used to describe patients whose mutations retain no residual activity on analysis of mutant cDNA expression, and patients with at least one mutation that retains significant residual activity are designated as having the mild genotype. Permanent ketosis has been reported to be a pathognomonic feature of SCOT deficiency. Thus, a patient's urine is almost always ketone positive even when they are well. We did not observe permanent ketosis, however, in Japanese SCOT-deficient patients with mild genotype. Permanent ketosis has not been reported in patients with T2 deficiency, even with severe genotype.

In ketone body utilization, SCOT is the only enzyme to catalyze acetoacetyl-CoA formation in mitochondria, but the T2 catalytic step of acetoacetyl-CoA cleavage can be catalyzed by another thiolase, mitochondrial medium-chain 3-ketoacyl-CoA

Table 1 Clinical profile of ketone body utilization disorders

	SCOT deficiency		T2 deficiency	
	Genotype		Genotype	
	Severe	Mild	Severe	Mild
Onset	Between neonatal period and 2 years old		Between approx. 5 months and 2 years (rarely during neonatal period)	
Permanent ketosis	Observed	Not observed	Not observed	Not observed
Urinary organic acid analysis	No characteristic finding	No characteristic finding	Elevated TIG, 2M3HB and 2MAA	During ketoacidosis, no or slightly elevated TIG, elevated 2M3HB and 2MAA. During non-episodic conditions, slightly elevated 2M3HB or no characteristic finding.
Blood acylcarnitine analysis	No characteristic finding	No characteristic finding	Elevated tiglylcarnitine and 2M3HB carnitine	During ketoacidosis, slightly elevated tiglylcarnitine and 2M3HB carnitine or no characteristic finding.
No. patients worldwide	>30 patients		>100 patients	
No. patients in Japan	2 cases in 1 family	4 cases in 3 families	1 case	7 cases in 6 families

2MAA, 2-methylacetoacetyl; 2M3HB, 2-methyl-3-hydroxybutyryl; SCOT, succinyl-CoA:3-ketoacid CoA transferase; T2, mitochondrial acetoacetyl-CoA thiolase; TIG, tiglylglycine.

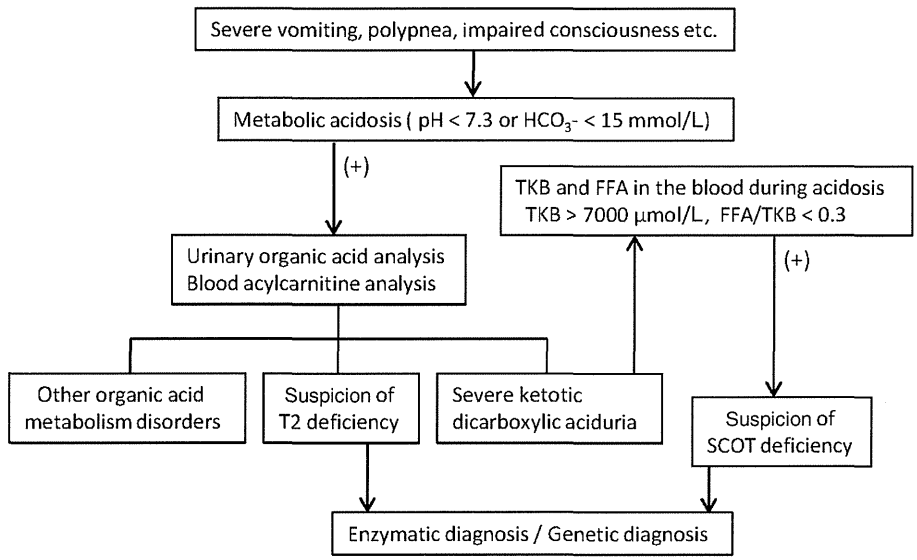

Fig. 2 Diagnostic flow chart of ketone body utilization disorders. FFA, free fatty acids; SCOT, succinyl-CoA:3-ketoacid CoA transferase; TKB, total ketone bodies; T2, mitochondrial acetoacetyl-CoA thiolase.

thiolase.[1] Therefore, complete SCOT deficiency may encompass a complete defect of ketone body utilization in extrahepatic tissues; even in complete T2 deficiency, however, ketone bodies can be used to some extent in extrahepatic tissues. This may explain why permanent ketosis is present in complete SCOT deficiency but not in complete T2 deficiency.

The SCOT step is used only for ketone body utilization, and there are no characteristic metabolites except for large amounts of ketone bodies. In contrast, T2 catalyzes acetoacetyl-CoA cleavage in ketone body utilization and 2-methylacetoacetyl-CoA (2MAA-CoA) cleavage in the isoleucine catabolic pathway (Fig. 1). Hence, T2 deficiency is characterized by accumulated metabolites in isoleucine catabolism. In urinary organic acid analysis, excretion of tiglylglycine (TIG), 2-methyl-3-hydroxybutyrate (2M3HB) and 2-methylacetacetate (2MAA) are characteristic of T2 deficiency. In blood acylcarnitine analysis, elevated blood tiglylcarnitine (C5:1 carnitine) and 2-methyl-3-hydroxybutyrylcarnitine (2M3HB-carnitine/C5-OH carnitine) are observed. T2-deficient patients with mild genotype, however, do not show typical profiles in these analyses.

Enzyme assay and/or molecular diagnosis are essential for confirming diagnosis because (i) SCOT deficiency cannot be diagnosed on metabolite analysis such as urinary organic acid analysis or acylcarnitine analysis; and (ii) some T2-deficient patients do not have typical metabolic profiles.

SCOT deficiency in Japan

Five cases of SCOT deficiency from three families in Japan are summarized in Table 2. Patient GS02 and the younger sister (GS02s) were typical SCOT-deficient patients presenting

Table 2 Japanese SCOT-deficient patients

Case	Onset	No. ketoacidotic crises	Typical crisis			Well condition		Prognosis		*OXCT1* mutation	References
			Blood gas		TKB (μmol/L)	Urinary ketone	TKB (μmol/L)	Age in 2014 (years)	MR		
			pH	HCO_3^- (mmol/L)							
GS02	6 months	3	7.08	5.1	12 200	Always positive	858	10	(–)	[c.398T>A (p.V133E)]+ [c.1367G>T (p.C456F)]	5,6
GS02s	Prenatal diagnosis	1	7.29	ND	11 400		893	8	(–)		
GS08	1 year 5 months	3	7.12	3.7	18 500	Usually negative	164	16	(–)	[c.1304C>A (p.T435N)]+[=]	16
GS09	10 months	Several	7.00	5.8	ND		341	14	(–)		
GS09b	10 months	4	7.09	5.4	ND		285	18	(–)		
GS21	2 d	1	7.07	5.8	ND		240	8	(–)	[c.1304C>A (p.T435N)]+[c.658_666dup9bp]	22

GS02s, affected sister of GS02; GS09b, affected brother of GS09; MR, mental retardation; ND, not determined; *OXCT1*, SCOT gene symbol; SCOT, succinyl-CoA:3-ketoacid CoA transferase; TKB, total ketone bodies.

Table 3 Japanese T2-deficient patients

Case	Onset	No. ketoacidotic crises	Typical crisis		Prognosis		Urinary organic acids				Acylcarnitines				*ACAT1* mutation	Refs.
			Blood gas		Age in 2014 (years)	MR	Acute phase		Stable phase		Acute phase		Stable phase			
			pH	HCO_3^- (mmol/L)			TIG	2M3HB	TIG	2M3HB	C5:1	C5OH	C5:1	C5OH		
GK01	20 months	1	7.15	4.2	33	(+)	D	D	D	D	–	–	D	D	[c.997G>C(p.A333P)]+ [c.149delC]	26,27,32
GK19	23 months	1	7.17	3.8	22	(–)	ND	D	ND	FD	–	–	ND	FD	[c.935C>T (p.I312T)]+[c.278A>G (p.N93S)]	27,32
GK30	9 months	3	7.01	3.3	18	(–)	D	D	ND	FD	–	–	ND	FD	[c.2T>C] + [c.149delC]	32
GK31	18 months	1	7.07	2.9	18	(–)	D	D	ND	FD	–	–	ND	FD	[c.935C>T (p.I312T)]+[c.149delC]	32
GK64	7 months	1	7.00	8.0	9	(–)	ND	D	ND	FD	–	–	ND	ND	[c.556G>T (p.D186Y)]+[c.951C>T]	38
GK69	9 months	2	7.075	4.6	31	(–)	–	–	ND	FD	–	–	ND	ND	[c.431A>C (p.H144P)]+[c.1168T>C (p.S390P)]	43
GK77	3 years	1	7.135	6.3	9	(–)	ND	D	ND	FD	ND	ND	FD	ND	[c.431A>C (p.H144P)]+[=]	43
GK77b	3 years	1	6.88	1.1	3	Died	ND	D	–	–	ND	ND	ND	ND		

Underline, retaining some residual enzyme activity. p.A333P, c.149delC, p.D186Y, p.S390P retained no residual activity. GK77 and GK77b, identical twin siblings who developed ketoacidotic crises at 3 years of age. GK01, typical T2-deficient patient and others are patients with mild mutations on at least one of two mutant alleles. –, not tested; 2M3HB, 2-methyl3-hydroxybutyrate; *ACAT1*, beta-ketothiolase or T2 gene symbol; D, detected; FD, faintly detected; MR, mental retardation; ND, not detected; T2, mitochondrial acetoacetyl-CoA thiolase; TIG, tiglylglycine.

Table 4 Guidelines for diagnosis of ketone body utilization disorders

Characteristics of clinical symptoms
Ketone body utilization disorders should be suspected in the following patients:
(1) Neonates with symptoms including poor sucking, vomiting, hypotonia of muscles and so-called "not doing well" with severe metabolic acidosis
(2) Patients with symptoms including severe vomiting, polypnea and impaired consciousness with severe metabolic acidosis during acute respiratory infection or acute gastroenteritis during infancy (especially after 5 months of age) or early childhood
(3) Patients with more severe ketosis than expected with a clinical history of fasting and/or febrile condition
(4) Patients with urinary ketone positive repeatedly despite being well or symptomless
Ketone bodies accumulate owing to ketogenic stresses such as fasting, febrile conditions or infection at higher levels in patients with ketone body utilization disorders compared with healthy controls, and ketoacidosis occurs. Patients are symptomless during non-ketoacidotic conditions.
Tests for diagnosis
(1) First-line screening tests: blood gas, blood glucose, ammonia etc.
Patients usually show severe ketoacidosis (pH <7.3 or HCO_3^- < 15 mmol/L) during crises. Severe ketoacidosis is observed in patients with other organic acidurias; therefore, urinary organic acid analysis should be performed for differential diagnosis. It is necessary to judge whether the severity of acidosis is beyond physiological metabolic acidosis caused by fasting ketosis etc. In the case of ketone body utilization defects, blood pH is usually very low (6.8–7.2) during crises. Blood ammonia during ketoacidosis is normal or slightly elevated (up to approx. 200–400 μg/dL) and hemodialysis is usually unnecessary. Blood glucose is usually normal, but mild hyperglycemia is also observed. Hypoglycemia may be observed during ketoacidotic episodes during neonatal periods or infancy.
(2) TKB and FFA in the blood
It is critical for the evaluation of ketone body metabolism to measure both TKB and FFA simultaneously during ketoacidosis. TKB is ≥7000 μmol/L or over (often >10 000 μmol/L) during acidosis. Both fasting and postprandial high TKB may suggest defects in ketone body utilization. In cases of physiological ketosis, FFA are also proportionally high, but for ketone body utilization defects, TKB are disproportionally higher than FFA, with an FFA/TKB ratio ≤0.3 in early stages of fasting. Fasting tests are not recommended for all patients and should be conducted at a specialized medical facility under careful control. Some patients may develop ketoacidosis after only 15 h of fasting.
(3) Urinary organic acid analysis
In patients with SCOT deficiency, the characteristic profile of urinary organic acid analysis is not present except for large amounts of 3-hyroxybutyrate and acetoacetate (ketotic dicarboxylic aciduria) even during ketoacidosis.
In typical patients with T2 deficiency, elevated TIG, 2M3HB and 2MAA are found on urinary organic acid analysis during ketoacidosis as well as during non-episodic conditions, which makes biochemical diagnosis possible. In T2-deficient patients with mutations that retain some residual activity, however, the characteristic profile of urinary organic acid analysis may not be observed even during ketoacidosis, and only subtle elevation of 2M3HB may be observed during non-episodic normal conditions. Thus, it is sometimes difficult to suspect T2 deficiency on urinary organic acid analysis during non-episodic conditions. Elevated TIG and 2M3HB in the absence of 2MAA might indicate HSD10 disease rather than T2 deficiency, although 2MAA is unstable and is difficult to detect in some laboratories.
Urinary organic acid analysis during ketoacidosis is necessary to exclude other organic acidemia.
(4) Acylcarnitine analysis
In typical patients with T2 deficiency, elevated C5:1 and C5-OH are found on blood acylcarnitine analysis using tandem mass spectrometry during ketoacidosis as well as during non-episodic conditions. Serum is more informative than blood spots. Additional to urinary organic acid analysis, however, T2-deficient patients with mutations that retain some residual activity cannot be detected on acylcarnitine analysis. SCOT deficiency cannot be identified using this method.
(5) Enzymatic and genetic diagnosis
Enzyme assay and mutation analysis are tests for definitive diagnosis.
Enzyme assay using blood mononuclear cells is sometimes difficult for the correct evaluation of T2 activity. Enzyme assay using fibroblasts is recommended to confirm T2 deficiency.

2M3HB, 2-methyl-3-hydroxybutyrate; 2MAA, 2-methylacetacetate; FFA, free fatty acids; HSD10, 17β-hydroxysteroid dehydrogenase type 10; SCOT, succinyl-CoA:3-ketoacid CoA transferase; T2, mitochondrial acetoacetyl-CoA thiolase; TIG, tiglylglycine; TKB, total ketone bodies.

permanent ketosis. They were compound heterozygotes of two mutations that retained no residual activity, judged to be SCOT-deficient patients with severe genotype.[5,6] In contrast, three patients from two other families (GS08, GS09 and GS09s) had no permanent ketosis and were homozygotes of the T435N mutation that retained significant residual SCOT activity.[16,22] They all developed severe ketoacidotic episodes.

The siblings with severe genotype were reexamined at the age of 9 years (elder brother) and 7 years (younger sister). Urinary ketone was examined for 5 days and all urine samples tested were ketone positive. After 14–15 h of fasting, blood TKB levels exceeded 2 mmol/L and even 2 h after eating, TKB remained at 2 mmol/L, although blood FFA decreased to <0.25 mmol/L after eating. The siblings have not developed severe metabolic acidosis after confirmation of diagnosis.[16]

The siblings with mild genotype have not developed severe metabolic acidosis after confirmation of diagnosis. They were reexamined at the age of 8 years (older brother) and 4 years (younger brother). Urinary ketone was only positive after a 15 h fast, when blood TKB was <0.5 mmol/L in the older brother and <1 mmol/L in the younger brother. TKB 2 h after eating in both patients decreased to half of the fasting levels.[16]

A guarded fasting test in patient GS08 with mild genotype was performed at 2 years of age. TKB exceeded 2, 6, and 9 mmol/L at 12, 14, and 16 h of fasting, respectively. Because TKB exceeded 10 mmol/L at 17 h of fasting, sodium bicarbonate and

Table 5 Guidelines for treatment of ketone body utilization disorders

Treatment in acute episodes
(1) Treatment of hypoglycemia and suppression of ketone body synthesis
It is important to avoid fasting in ketone body utilization disorders. Hypoglycemia should be treated by i.v. injection of 2 mL/kg (1.1 mmol/kg) 10% glucose, followed by continuous infusion of 10% glucose and an appropriate concentration of electrolyte. Target blood glucose level is the upper limit of normal. Even if hypoglycemia is not observed during ketoacidosis, ketoacidosis will not be improved without sufficient glucose supply.
(2) Treatment of acidosis
To treat ketoacidosis, a sufficient glucose supply to suppress ketone body synthesis is important. Sufficient glucose infusion often improves acidosis in several hours.
Although there are various opinions regarding treatment for severe acidosis, the minimum consensus is as follows: when blood pH is <7.1, the patient has no circulatory failure or respiratory failure and is conscious, 1 mmol/kg sodium bicarbonate is injected i.v. over 10 min, followed by continuous infusion of sodium bicarbonate. pH >7.1, PCO_2 >20 mmHg and HCO_3^- > 10 mmol/L are targeted. When blood test data are improved, infusion of sodium bicarbonate is tapered promptly and stopped. There is a report that overdose of sodium bicarbonate may cause hypernatremia and cerebral hemorrhage; thus, attention to dosing is required. Although dialysis is useful to control acidosis, it is not often required.
Treatment in non-episodic conditions
(1) Prevention of severe ketoacidotic episodes
Patients diagnosed with ketone body utilization disorders should avoid long fasting as much as possible. They should have meals rich in carbohydrates frequently during physical stress such as mild infection. In cases of poor feeding due to gastroenteritis or catabolic conditions, glucose infusion should be performed without hesitation. It is useful for patients to monitor urinary ketones using test strips at home.
(2) Restriction of fat intake
Patients diagnosed with ketone body utilization disorders should restrict excess fat intake. A ketogenic diet is contraindicated for them. Fat restriction is usually unnecessary for normal Japanese-style meals, while fat restriction is advisable for Western-style meals.
(3) Restriction of protein intake
Mild restriction of protein intake is a reasonable measure for patients with SCOT deficiency to avoid ketogenic amino acid load, but the long-term effects are unclear. In patients with T2 deficiency, 1.5–2.0 g/kg/day protein intake may be applicable.
(4) Carnitine supplementation
For patients with ketone body utilization disorders who have low blood carnitine, carnitine supplementation is considered.

SCOT, succinyl-CoA:3-ketoacid CoA transferase; T2, mitochondrial acetoacetyl-CoA thiolase.

glucose were injected i.v. to terminate the test. The FFA/TKB ratio fell below 0.3 at 14 h of fasting.[16]

Based on these results, the following are suggested. Patients with severe genotype have typical SCOT deficiency and may have permanent ketosis. Therefore, SCOT deficiency is likely suspected. Patients with mild genotype may develop severe ketoacidotic episodes similar to patients with severe genotype but may not develop permanent ketosis. In general, the long fasting test is not recommended for diagnosis, but the fasting test at 15–20 h is useful for evaluation of fasting tolerance, and should be conducted under careful control because of a risk of acute ketoacidosis.

T2 deficiency in Japan

The cases of eight patients from seven families of T2 deficiency in Japan are summarized in Table 3. Patient GK01 is the only one with severe genotype in Japan. In GK01, elevated TIG and 2M3HB were persistently observed in urine samples during non-episodic normal conditions as well as at the time of ketoacidosis.[26,27,32] In contrast, the other seven patients have mild genotype. TIG was not detected in four of six patients at the time of ketoacidosis, and was not detected in any patients during non-episodic normal conditions. 2M3HB was definitely detected in all patients during ketoacidosis, but was only faintly detected in all cases during non-episodic normal conditions. On blood acylcarnitine analysis, C5:1 carnitine and C5-OH carnitine were elevated in GK01 even during non-episodic normal conditions, but were not detected or were faintly detected in the other six patients with mild genotype.[27,32,38,43] It should be emphasized that in the siblings with mild genotype (GK77 and 77b), these characteristic acylcarnitine levels were not elevated, even during acute episodes.

T2 deficiency is a target disease for newborn mass screening tests using tandem mass spectrometry. There are some reports that asymptomatic T2 deficiency has been diagnosed on newborn mass screening tests.[41,42] In the USA, siblings not positively detected on newborn screening testing were later diagnosed with T2 deficiency.[42] As stated here, patients with mild genotype may be common in Japan, therefore some T2-deficient patients cannot be identified on newborn screening testing.

In the differential diagnosis for T2 deficiency, we should consider 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) deficiency, also known as 17β-hydroxysteroid dehydrogenase type 10 (HSD10) disease, which is an X-linked recessive disorder with clinical symptoms including rapidly progressive retardation of psychomotor performance, convulsion, ablepsia and progressive cardiomyopathy.[45–60] MHBD catalysis is one step upstream from the T2 step in isoleucine catabolism. The results of urinary organic acid analysis and blood acylcarnitine analysis in HSD10 disease are the same as those in T2 deficiency except that 2MAA is not detected in HSD10 disease (Fig. 1).[1] Approximately 20 cases of HSD10 disease have been reported, but clinical heterogeneity is noted. We recently identified the first case of HSD10 disease in Japan. The patient was initially suspected to have T2 deficiency on urinary organic acid analysis, and was then confirmed to have

HSD10 disease on enzyme assay and mutation analysis. He had no neurological regression until 6 years of age, thus a much milder phenotype compared with the previously reported cases.[61]

Guidelines for diagnosis and treatment

Based on clinical information of patients with ketone body utilization disorders in Japan and worldwide, we developed guidelines for its diagnosis (Table 4; Fig. 2) and treatment (Table 5). These diseases are treatable by avoiding fasting and by providing early infusion of glucose, which enable patients to grow without sequelae.

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References

1 Mitchell GA, Fukao T. Inborn errors of ketone body metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Basis of Inherited Disease*. McGraw-Hill, New York, NY, 2001; 2327–56.

2 Tildon JT, Cornblath M. Succinyl-CoA:3-ketoacid CoA-transferase deficiency. A cause for ketoacidosis in infancy. *J. Clin. Invest.* 1972; **51**: 493–8.

3 Cornblath M, Gingell RL, Fleming GA, Tildon JT, Leffler AT, Wapnir RA. A new syndrome of ketoacidosis in infancy. *J. Pediatr.* 1971; **79**: 413–18.

4 Perez-Cerda C, Merinero B, Sanz P *et al.* A new case of succinyl-CoA:acetoacetate transferase deficiency. *J. Inherit. Metab. Dis.* 1992; **15**: 371–3.

5 Sakazaki H, Hirayama K, Murakami S *et al.* A new Japanese case of succinyl-CoA:3-ketoacid CoA-transferase deficiency. *J. Inherit. Metab. Dis.* 1995; **18**: 323–5.

6 Fukao T, Song XQ, Watanabe H *et al.* Prenatal diagnosis of succinyl-coenzyme A:3-ketoacid coenzyme A transferase deficiency. *Prenat. Diagn.* 1996; **16**: 471–4.

7 Kassovska-Bratinova S, Fukao T, Song XQ *et al.* Succinyl CoA:3-oxoacid CoA transferase (SCOT): Human cDNA cloning, human chromosomal mapping to 5p13, and mutation detection in a SCOT-deficient patient. *Am. J. Hum. Genet.* 1996; **59**: 519–28.

8 Pretorius CJ, Loy Son GG, Bonnici F, Harley EH. Two siblings with episodic ketoacidosis and decreased activity of succinyl-CoA:3-ketoacid CoA-transferase in cultured fibroblasts. *J. Inherit. Metab. Dis.* 1996; **19**: 296–300.

9 Niezen-Koning KE, Wanders RJ, Ruiter JP *et al.* Succinyl-CoA:acetoacetate transferase deficiency: Identification of a new patient with a neonatal onset and review of the literature. *Eur. J. Pediatr.* 1997; **156**: 870–73.

10 Rolland MO, Guffon N, Mandon G, Divry P. Succinyl-CoA:acetoacetate transferase deficiency. Identification of a new case; prenatal exclusion in three further pregnancies. *J. Inherit. Metab. Dis.* 1998; **21**: 687–8.

11 Snyderman SE, Sansaricq C, Middleton B. Succinyl-CoA:3-ketoacid CoA-transferase deficiency. *Pediatrics* 1998; **101**: 709–11.

12 Song XQ, Fukao T, Watanabe H *et al.* Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency: Two pathogenic mutations, V133E and C456F, in Japanese siblings. *Hum. Mutat.* 1998; **12**: 83–8.

13 Fukao T, Mitchell GA, Song XQ *et al.* Succinyl-CoA:3-ketoacid CoA transferase (SCOT): Cloning of the human SCOT gene, tertiary structural modeling of the human SCOT monomer, and characterization of three pathogenic mutations. *Genomics* 2000; **68**: 144–51.

14 Baric I, Sarnavka V, Fumic K *et al.* A new case of succinyl-CoA:acetoacetate transferase deficiency: Favourable course despite very low residual activity. *J. Inherit. Metab. Dis.* 2001; **24**: 81–2.

15 Berry GT, Fukao T, Mitchell GA *et al.* Neonatal hypoglycaemia in severe succinyl-CoA:3-oxoacid CoA-transferase deficiency. *J. Inherit. Metab. Dis.* 2001; **24**: 587–95.

16 Fukao T, Shintaku H, Kusubae R *et al.* Patients homozygous for the T435N mutation of succinyl-CoA:3-ketoacid CoA transferase (SCOT) do not show permanent ketosis. *Pediatr. Res.* 2004; **56**: 858–63.

17 Longo N, Fukao T, Singh R *et al.* Succinyl-CoA:3-ketoacid transferase (SCOT) deficiency in a new patient homozygous for an R217X mutation. *J. Inherit. Metab. Dis.* 2004; **27**: 691–2.

18 Fukao T, Sakurai S, Rolland MO *et al.* A 6-bp deletion at the splice donor site of the first intron resulted in aberrant splicing using a cryptic splice site within exon 1 in a patient with succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency. *Mol. Genet. Metab.* 2006; **89**: 280–82.

19 Fukao T, Kursula P, Owen EP, Kondo N. Identification and characterization of a temperature-sensitive R268H mutation in the human succinyl-CoA:3-ketoacid CoA transferase (SCOT) gene. *Mol. Genet. Metab.* 2007; **92**: 216–21.

20 Yamada K, Fukao T, Zhang G *et al.* Single-base substitution at the last nucleotide of exon 6 (c.671G>A), resulting in the skipping of exon 6, and exons 6 and 7 in human succinyl-CoA:3-ketoacid CoA transferase (SCOT) gene. *Mol. Genet. Metab.* 2007; **90**: 291–7.

21 Merron S, Akhtar R. Management and communication problems in a patient with succinyl-CoA transferase deficiency in pregnancy and labour. *Int. J. Obstet. Anesth.* 2009; **18**: 280–83.

22 Fukao T, Ishii T, Amano N *et al.* A neonatal-onset succinyl-CoA:3-ketoacid CoA transferase (SCOT)-deficient patient with T435N and c.658-666dupAACGTGATT p.N220_I222dup mutations in the OXCT1 gene. *J. Inherit. Metab. Dis.* 2010; **33**: 307–13.

23 Fukao T, Sass JO, Kursula P *et al.* Clinical and molecular characterization of five patients with succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency. *Biochim. Biophys. Acta* 2011; **1812**: 619–24.

24 Hori T, Fukao T, Murase K, Sakaguchi N, Harding CO, Kondo N. Molecular basis of two-exon skipping (exons 12 and 13) by c.1248+5g>a in *OXCT1* gene: Study on intermediates of *OXCT1* transcripts in fibroblasts. *Hum. Mutat.* 2013; **34**: 473–80.

25 Daum RS, Lamm PH, Mamer OA, Scriver CR. A "new" disorder of isoleucine catabolism. *Lancet* 1971; **2**: 1289–90.

26 Yamaguchi S, Orii T, Sakura N, Miyazawa S, Hashimoto T. Defect in biosynthesis of mitochondrial acetoacetyl-coenzyme A thiolase in cultured fibroblasts from a boy with 3-ketothiolase deficiency. *J. Clin. Invest.* 1988; **81**: 813–17.

27 Fukao T, Nakamura H, Song XQ *et al.* Characterization of N93S, I312T, and A333P missense mutations in two Japanese families with mitochondrial acetoacetyl-CoA thiolase deficiency. *Hum. Mutat.* 1998; **12**: 245–54.

28 Fukao T, Scriver CR, 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.* 2001; **72**: 109–14.

29 Nakamura K, Fukao T, Perez-Cerda C *et al.* A novel single-base substitution (380C>T) that activates a 5-base downstream cryptic splice-acceptor site within exon 5 in almost all transcripts in the human mitochondrial acetoacetyl-CoA thiolase gene. *Mol. Genet. Metab.* 2001; **72**: 115–21.