Ketone body metabolism and its defects

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

Journal of Inherited Metabolic Disease
Official Journal of the Society for the Study of Inborn Errors of Metabolism

ISSN 0141-8955

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

Springer

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

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

Published online: 08 April 2014

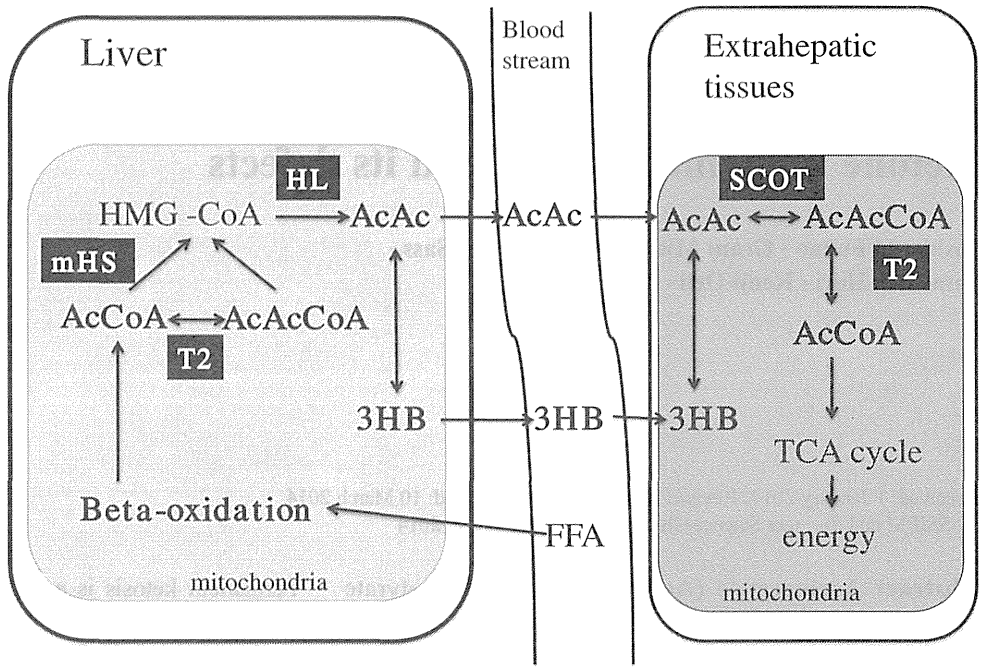


Fig. 1 Summary of ketone body metabolism. *left* Ketogenesis in liver. The HMG-CoA pathway of ketone body formation is much more active in liver than elsewhere. *center* The ketone bodies, 3HB and AcAc, diffuse from liver mitochondria to the circulation and then to extrahepatic tissues including brain.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

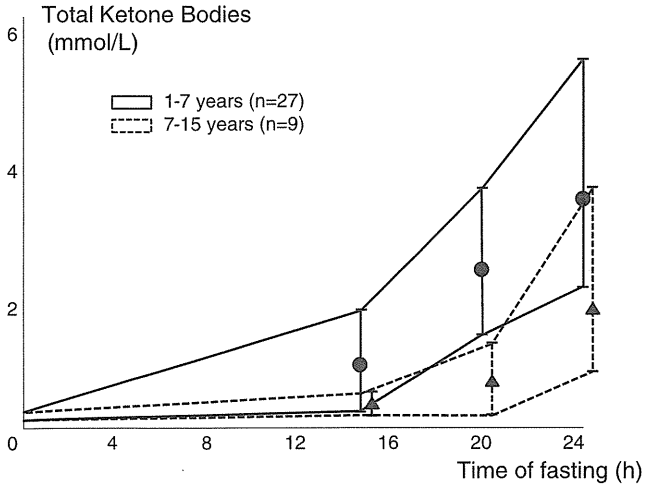


Fig. 2 Plasma total ketone body (TKB) levels as a function of fasting time and age, in groups of children aged 1–7 years and 7–15 years. Results are expressed as 10–90 percentiles with mean values. Redrawn from the data of Bonnefont et al 1990

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

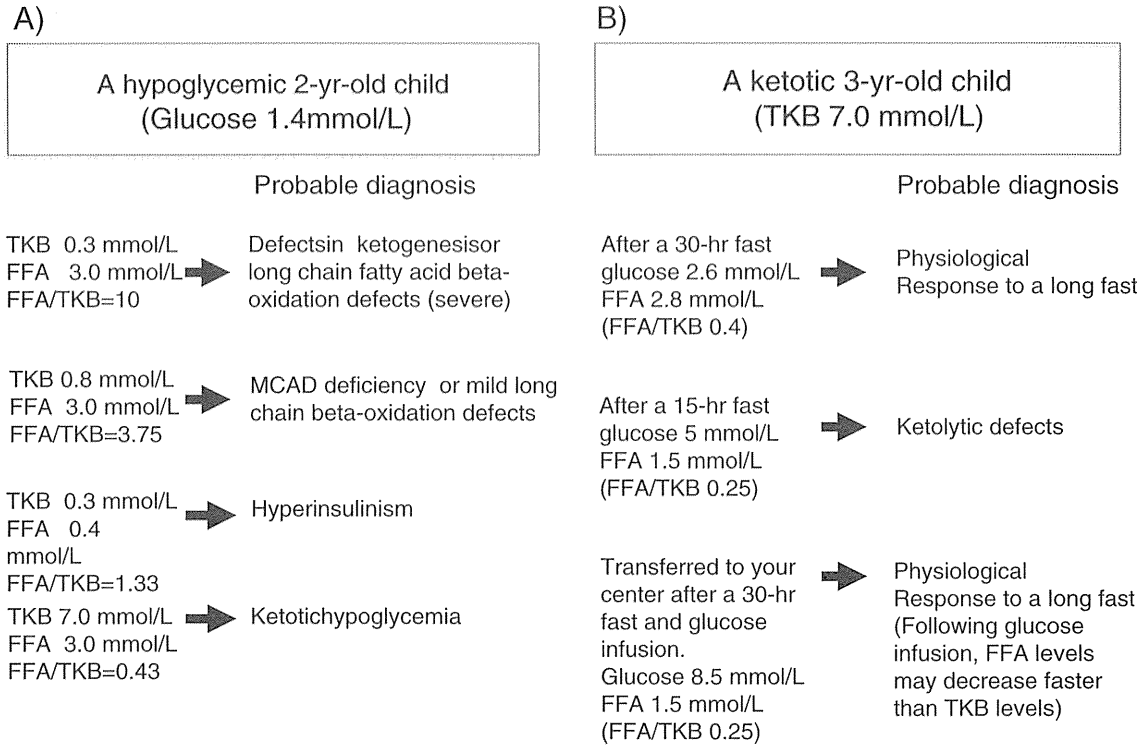


Fig. 3 Examples of the clinical evaluation of ketone body metabolism in acutely ill children. **a** A 2-year-old child has hypoglycemia (glucose 1.4 mmol/L). Possible diagnoses are shown if he has TKB and FFA levels as indicated. **b** A 3-year-old child has hyperketonemia (TKB 7.0 mmol/L). Possible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

	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.

Acknowledgments We thank the many physicians who have referred samples and shared clinical data of patients suspected to be deficient in T2 or SCOT. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Health and Labor Science Research Grants for Research on Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan.

Compliance with Ethics Guidelines

Conflict of Interest None.

Human and Animal Rights and Informed Consent This is a review article, hence, this article does not contain any studies with human or animal subjects performed by any of the authors.

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

Journal of the Neurological Sciences 346 (2014) 350–352

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Letter to the editor

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

Keywords:
Glutaric acidemia type 2
Acylcarnitine profile
Adult onset
Bezafibrate

1. Introduction

Multiple acyl-coenzyme A dehydrogenase deficiency (MADD), also known as glutaric acidemia type 2 (GA2), was first described in 1976 [1]. GA2 is a rare autosomal recessive disorder whose biochemical abnormalities result from a deficiency of one of the two electron transfer flavoproteins (ETF and ETFDH) that transfer electrons from acyl-CoA dehydrogenases to the respiratory chain [2]. The disorder affects multiple metabolic pathways involving branched amino acids, fatty acids, and tryptophan, and results in a variety of distinctive organic acids being discharged. The heterogeneous clinical features of patients with GA2 fall into three subclasses: two neonatal-onset forms (types I/II) and a late-onset form (type III) [3]. The late-onset form is typically characterized by intermittent vomiting, hypoglycemia, hepatomegaly, metabolic acidosis, and/or hyperammonemia, symptoms that are often triggered by general infections or catabolic conditions [4].

Here, we describe the case of a man with lipid-storage myopathy, low muscle carnitine, and an adult-onset form of GA2 with two novel mutations in the *ETFDH* gene. In this case, a combination of a hypolipidemic drug (bezafibrate), riboflavin, and L-carnitine was effective in treating the disease.

2. Case report

A 31-year-old man was referred to our hospital because of muscle weakness and limb fatigability. Nine months earlier, he had gradually developed proximal muscle weakness and fatigability. He exhibited normal psychomotor development. His relatives had no history of neuromuscular disease. Physical examination on admission showed a normally developed, well-nourished man (185 cm, 73 kg) without hepatosplenomegaly. Neurological examination revealed mild muscle weakness in his left iliopsoas muscle (grade 5−). Muscle amyotrophy and myalgia were not noted. The following serum biochemistry markers were elevated: creatine kinase (CK), 689 U/L (normal <230); creatine kinase-MB, 50 U/L (<10); aldolase, 8.9 IU/L (<5.9); myoglobin, 107 ng/mL (<72.0); and triglycerides, 315 mg/dL (<149). The full blood count, blood glucose, renal and thyroid function, immunoglobulins, inflammatory markers, and antinuclear antibodies were normal. Echocardiography, pulmonary function tests, and a brain MRI were normal. Abdominal echography revealed only the fatty liver. A muscle MRI showed a high-density area in the bilateral lower limb muscles in short-T1 inversion recovery (STIR) (Fig. 1A). Atrophy of the biceps was suspected based on a muscle CT scan. Electromyography of the left vastus lateralis muscle and the tibialis anterior muscle displayed myopathic patterns. In the muscle biopsy specimen from the biceps brachii, neither lymphocytic infiltration nor endomysial fibrosis was observed (Fig. 1B), although some fibers contained many vacuoles. These were positively stained with Oil Red O, suggesting a lipid storage myopathy (Fig. 1C).

Total and free carnitine concentrations in muscle specimens were severely decreased at 3.5 (control 15.7 ± 2.8) and 1.7 (12.9 ± 3.7) nmol/mg non-collagen protein (NCP), respectively. Activity of acyl-CoA dehydrogenases was normal. Analysis of urinary organic acids showed increased 2-OH-glutarate, ethylmalonate, and 3-OH-propionate. The acylcarnitine profile of the patient's serum showed a broad-range elevation of acylcarnitines, but no abnormalities were observed in the amino acid profile. This indicated a multiple-dehydrogenation abnormality, which is consistent with GA2. After receiving informed consent, the patient's skin fibroblasts were isolated and cultured, as described previously [5]. Genetic analysis identified novel, compound heterozygous missense mutations in the *ETFDH* gene (890G > T/W297L and 950C > G/P317R). Western blot analysis showed decreased production of ETFDH in the patient's fibroblasts (Fig. 1D). This indicated that the mutations would be pathogenic.

Following treatment with L-carnitine alone, the patient's serum CK reached nearly normal levels. However, his serum acylcarnitine profile remained abnormal (Fig. 1E, left panel). The L-carnitine treatment was then supplemented with riboflavin at 105 mg/day or with bezafibrate (BEZ; 600 mg/day) because the patient showed mild hyperlipidemia, and because this hypolipidemic drug was effective for adolescent GA2 patients [5]. However, the combined treatment of L-carnitine and riboflavin, or L-carnitine and BEZ, failed to improve the acylcarnitine profile (Fig. 1E, left panel) and the patient's symptoms remained stable. For the next 7 months, the patient was treated with L-carnitine alone. During this period, he felt fatigability and his serum CK increased mildly. BEZ was again added to his treatment regimen. His serum acylcarnitine profile improved, but his serum CK remained high and he occasionally complained of fatigue (Fig. 1E, right panel). After 15 months, riboflavin was added to the L-carnitine and BEZ. His serum CK and acylcarnitine profile returned to normal within one month, and his symptoms completely disappeared. This amelioration has continued beyond 6 months.

3. Discussion

We diagnosed a patient with GA2 based on observations of the muscle pathology, acylcarnitine analysis, and *ETFDH* gene mutations. In the

http://dx.doi.org/10.1016/j.jns.2014.08.040

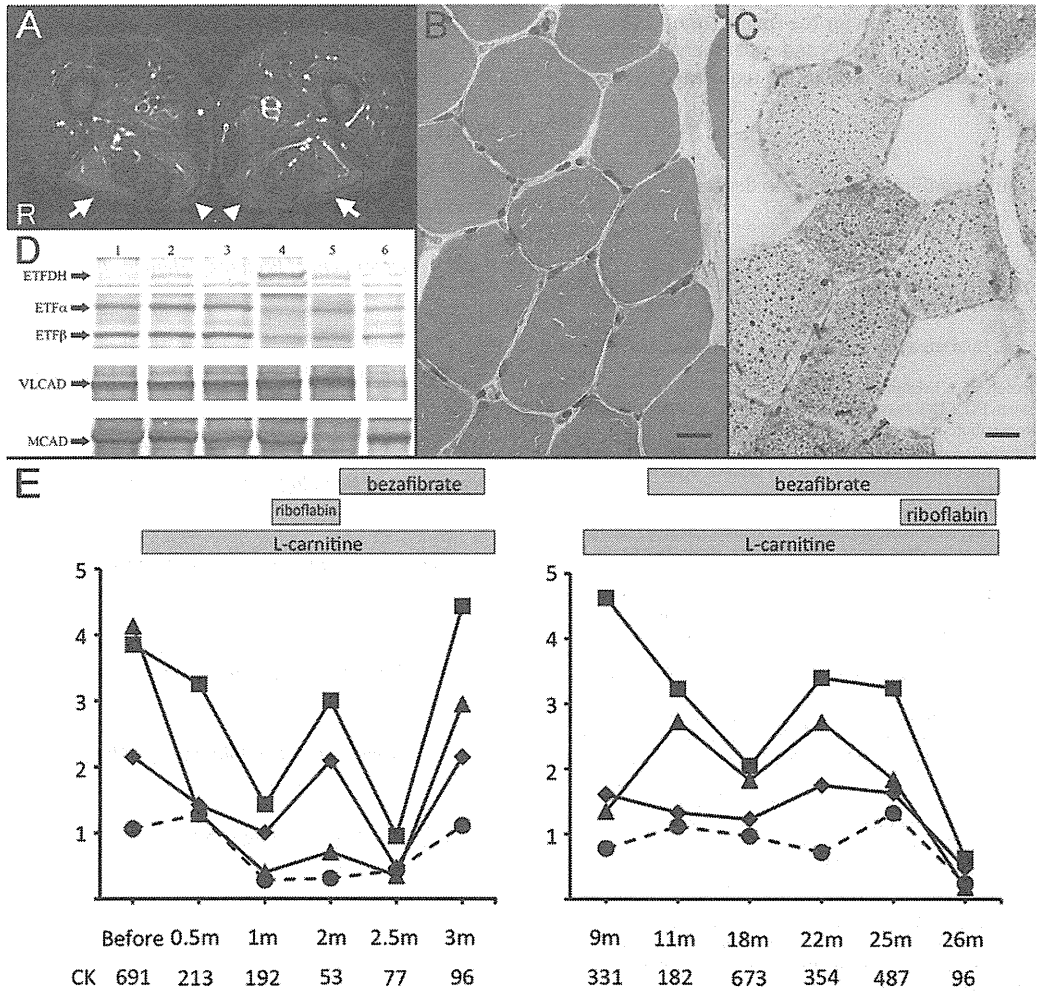


Fig. 1. A. A muscle MRI showed an area of high intensity in the bilateral biceps femoris muscle (arrow) and semimembranous muscle (arrowheads) in short-T1 inversion recovery (STIR). This indicated that increased water content in these muscles due to cellular lysis or fluid accumulation secondary to inflammation [10]. B, C. Biopsy of the patient's right biceps muscle. (B) Hematoxylin and eosin staining showed multiple optically empty vacuoles. (C) Oil Red O staining revealed excessive lipid droplets. The scale bar represents 20 μm. D. Western blot analyses of proteins in the patient's fibroblasts. The patient's fibroblasts were prepared as described previously [5,11]. For analysis of ETFDH, ETFα, and ETFβ, 25 μg of protein was applied to the gel. For analysis of very long-chain acyl-CoA dehydrogenase (VLCAD) and medium-chain acyl-CoA dehydrogenase (MCAD), 10 μg of protein was applied to the gel. Lane 1, patient's fibroblasts; lane 2, control (normal) fibroblasts; lane 3, ETFDH-defective fibroblasts; lane 4, ETFβ-defective fibroblasts; lane 5, MCAD-defective fibroblasts; lane 6, VLCAD-defective fibroblasts. Note that lane 1 from this patient, and lane 3 from the negative control, lack the band corresponding to ETFDH. This indicates that this patient had no ETFDH protein. Compared to control, the patient's fibroblasts showed no change in the expression of ETFα, ETFβ, VLCAD, or MCAD proteins. E. Changes in blood acylcarnitines with various treatments. The acylcarnitine profile of the patient's serum before treatment showed a broad-range elevation of acylcarnitines, including C6, C8, C10, C12, C14, and C16 acylcarnitine at 1.06 nmol/mL (normal <0.46), 2.15 (<1), 3.84 (<0.8), 4.13 (<0.4), 2.81 (<0.3), and 2.22 (<0.5), respectively. In the left panel, BEZ or riboflavin combined with L-carnitine, partially improved serum CK and serum acylcarnitine levels. Combining all three agents completely restored to normal the patient's acylcarnitine profile (right panel). During the seven-month period between the results shown in panels E and F, the patient was treated with L-carnitine alone. Units for acylcarnitine are nmol/mL and for CK are U/L. "m" indicates month. ●, C4; ◆, C8; ■, C10; ▲, C12.

adult myopathic form of GA2, patients sometimes do not show rhabdomyolysis, and there is no typical biochemical examination that can help us to consider the presence of a fatty acid oxidation disorder (FAO), as was observed here. Muscle biopsy and acylcarnitine analysis provide useful information and should be employed without hesitation.

Intake of L-carnitine has been reported to either exacerbate symptoms or to be effective for GA2 patients [6,7]. In the present case, oral carnitine alone leads to only partial improvement based on amelioration of the patient's muscle weakness and decreases in his serum CK and acyl-CoA. Riboflavin supplementation produces improvements in the symptoms and metabolic profiles of GA2 patients with *ETFDH* mutations, and the late-onset form [2]. BEZ is a hypolipidemic drug that is as an agonist of the peroxisome proliferating activator receptor, and was found to be beneficial in Japanese children with *ETFDH* gene mutations exhibiting GA2 [5]. Several mechanisms for the effectiveness of BEZ for FAO have been reported including upregulating mRNA and the activity of several FAO enzymes [8,9]. In the present case, BEZ, L-carnitine, and riboflavin each showed partial effectiveness and produced partial remission in a patient with GA2. In children, BEZ has been administered at doses from 17 to 25 mg/kg/day [5]. In the current patient, 600 mg/day of BEZ was administered, corresponding to only 8.2 mg/kg/day. This low dose was used because of the limitations of BEZ as a hypolipidemic drug and may explain the limited effectiveness of BEZ for our patient. A combination of BEZ, riboflavin, and L-carnitine produced complete remission in this patient, not only of his symptoms and serum CK, but also of his defect in fatty acid metabolism.

This case supports a new option for the treatment of GA2 patients, even in adults. Additional clinical studies and experimental investigation of the mechanisms of action of these drugs are required.

Conflict of interest

The authors have no conflicts of interest to declare.

References

[1] Przyrembel H, Wendel U, Becker K, Bremer HJ, Bruinvis L, Ketting D, et al. Glutaric aciduria type II: report on a previously undescribed metabolic disorder. Clin Chim Acta 1976;66:227–39.
[2] Liang WC, Nishino I. Lipid storage myopathy. Curr Neurol Neurosci Rep 2011;11: 97–103.
[3] Olsen RKJ, Andresen BS, Christensen E, Bross P, Skovby F, Gregersen N. Clear relationship between ETF/ETFDH genotype and phenotype in patients with multiple acyl-CoA dehydrogenation deficiency. Hum Mutat 2003;22:12–23.
[4] Yotsumoto Y, Hasegawa Y, Fukuda S, Kobayashi H, Endo M, Fukao T, et al. Clinical and molecular investigations of Japanese cases of glutaric acidemia type 2. Mol Genet Metab 2008;94:61–7.
[5] Yamaguchi S, Li H, Purevsuren J, Yamada K, Furui M, Takahashi T, et al. Bezafibrate can be a new treatment option for mitochondrial fatty acid oxidation disorders: evaluation by in vitro probe acylcarnitine assay. Mol Genet Metab 2012;107:87–91.
[6] Green A, Preece MA, De Sousa C, Pollitt RJ. Possible deleterious effect of L-carnitine supplementation in a patient with mild multiple acyl-CoA dehydrogenation deficiency (ethylmalonic-adipic aciduria). J Inherit Metab Dis 1991;14:691–7.
[7] Donato SD, Frerman FE, Rimoldi M, Rinaldo P, Taroni F, Wiesmann UN. Systemic carnitine deficiency due to lack of electron transfer flavoprotein: ubiquinone oxidoreductase. Neurology 1986;36:957–63.
[8] Bonnefont JPJ, Bastin JJ, Laforêt PP, Aubey FF, Mogenet AA, Romano SS, et al. Long-term follow-up of bezafibrate treatment in patients with the myopathic form of carnitine palmitoyltransferase 2 deficiency. Clin Pharmacol Ther 2010; 88:101–8.
[9] Djouadi F. Bezafibrate increases very-long-chain acyl-CoA dehydrogenase protein and mRNA expression in deficient fibroblasts and is a potential therapy for fatty acid oxidation disorders. Hum Mol Genet 2005;14:2695–703.
[10] Diekman EF, van der Pol WL, Nievelstein RAJ, Houten SM, Wijburg FA, Visser G. Muscle MRI in patients with long-chain fatty acid oxidation disorders. J Inherit Metab Dis 2013;37:405–13.
[11] Endo M, Hasegawa Y, Fukuda S, Kobayashi H, Yotsumoto Y, Mushimoto Y, et al. In vitro probe acylcarnitine profiling assay using cultured fibroblasts and electrospray ionization tandem mass spectrometry predicts severity of patients with glutaric aciduria type 2. J Chromatogr B 2010;878:1673–6.

Ayako Shioya
Hiroshi Takuma*
Department of Neurology, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan
*Corresponding author at: 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8575, Japan. Tel./fax: +81 29 853 3224.
E-mail address: htakuma@md.tsukuba.ac.jp (H. Takuma).

Seiji Yamaguchi
Department of Pediatrics, Shimane University School of Medicine, Shimane, Japan

Akiko Ishii
Department of Neurology, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan

Masahiko Hiroki
Department of Neurology, Tsukuba Medical Center Hospital, Ibaraki, Japan

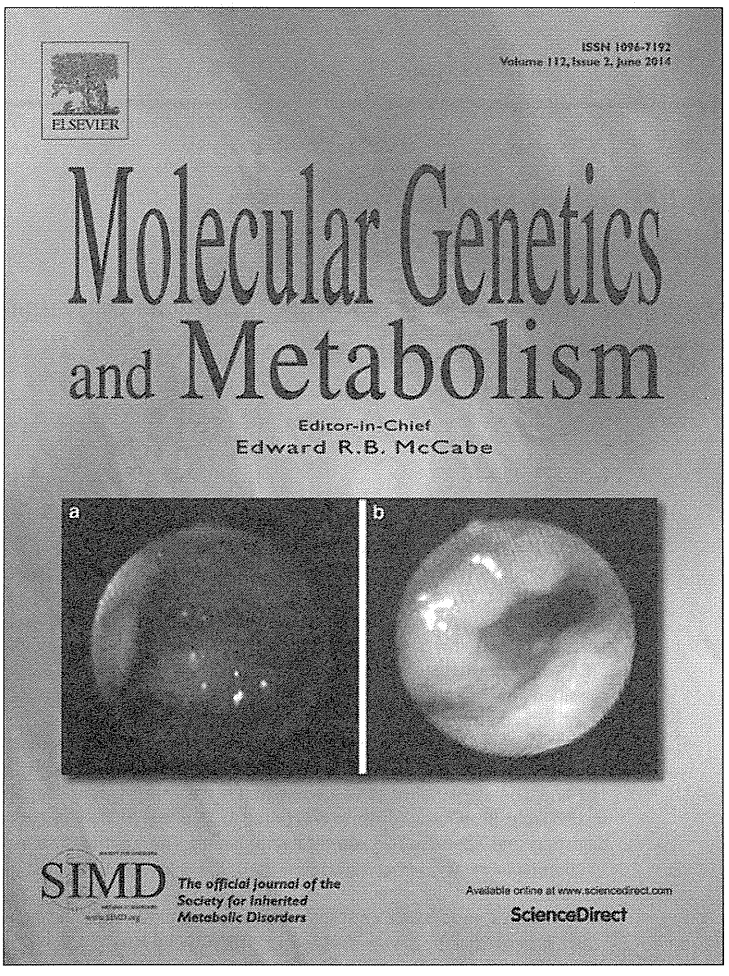
Tokiko Fukuda
Department of Pediatrics, Hamamatsu University School of Medicine, Shizuoka, Japan

Hideo Sugie
Department of Pediatrics, Jichi Medical University, Tochigi, Japan

Yosuke Shigematsu
Department of Health Science, University of Fukui, Fukui, Japan

Akira Tamaoka
Department of Neurology, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan

24 June 2014



Molecular Genetics and Metabolism 112 (2014) 154–159

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

CT and endoscopic evaluation of larynx and trachea in mucopolysaccharidoses

Noriko Morimoto [a,*], Masayuki Kitamura [b], Motomichi Kosuga [c], Torayuki Okuyama [c]

[a] Department of Otolaryngology, National Center for Child Health and Development, Japan
[b] Department of Radiology, National Center for Child Health and Development, Japan
[c] Center for Lysosomal storage diseases, National Center for Child Health and Development, Japan

ARTICLE INFO

Article history:
Received 25 January 2014
Received in revised form 27 March 2014
Accepted 27 March 2014
Available online 4 April 2014

Keywords:
GAG deposit
Morphology
Endoscopy
CT
Tracheal cross-sectional surface area

ABSTRACT

Background: Mucopolysaccharidoses (MPSs) are lysosomal storage disorders caused by lysosomal enzyme deficiencies that result in systemic accumulation of glycosaminoglycans (GAGs). Accumulation of GAGs in the upper airway can lead to respiratory failure. The aim of this study was to investigate changes of the airway by flexible endoscopy and CT.

Methods: Thirty-five patients aging from 2 to 16 years (mean: 9.2 ± 4.4 years) participated in this study. The majority had MPS I (n = 5) or MPS II (n = 25). The shape of the trachea and the cross-sectional trachea surface area (TSA) was determined at the Th1 and Th2 levels. Airway obstruction was evaluated from endoscopic findings and classified into 3 grades (Grades 0, 1, and 2). Forty-five patients in the control group who underwent tracheal CT for other conditions were retrospectively selected from the database.

Results: Tracheal morphology was abnormal in 50–60%, which showed a transversely collapsing narrow trachea. Tracheal deformity was severe in MPS II and MPS IV. The mean TSA of the MPS patients was 55.5 ± 29.0 mm^2 at Th1 and 61.4 ± 29.0 mm^2 at Th2, while that of the control group was 90.1 ± 41.9 mm^2 and 87.9 ± 39.3 mm^2, respectively. Respiratory distress was noted in 15 of the 35 patients, among whom 7 patients showed tracheal deformity and 7 patients had laryngeal redundancy. Three patients had no abnormalities of the larynx or trachea, so other factors such as pharyngeal stenosis or lower airway stenosis might have contributed to their respiratory distress.

Conclusion: CT and flexible endoscopy allow quantitative and morphological evaluation of airway narrowing, which is beneficial for airway management in MPS children.

1. Introduction

Mucopolysaccharidoses (MPSs) are lysosomal storage disorders caused by lysosomal enzyme deficiencies that result in the accumulation of glycosaminoglycans (GAGs) in various organs and tissues. Infiltration of GAGs into the oropharynx, joints, and connective tissues can lead to significant upper airway abnormalities, which increase the risk of anesthesia and can cause respiratory distress [1]. Narrowing of the upper airway occurs due to enlargement of the tongue and adenotonsillar hypertrophy, while MPS patients also develop a short immobile neck, thickening of the supraglottic region, and diffuse thickening of the tracheobronchial tree. In addition, respiratory distress is exacerbated by thoracic cage deformity and tracheobronchial abnormalities due to GAG deposition in the soft tissues [2,3]. It was reported that 25% of children with MPS have anatomical airway abnormalities which lead to difficulty with intubation [4]. In some cases, tracheal narrowing has been attributed to complications of endotracheal intubation or tracheotomy and there have also been many reports of sleep-disordered breathing or difficulty with anesthesia. Respiratory distress due to upper airway obstruction and tracheal stenosis is a severe problem that can result in death.

Thus, evaluation of the airway is of primary importance in children with MPS, especially before general anesthesia. Ingelmo demonstrated that performing MDCT of the airways with 3D reconstruction is useful for preoperative evaluation and planning of airway management in MPS patients [5]. However, this method is time-consuming and requires considerable expertise, so it is not suitable for airway screening at all institutions. On the other hand, flexible endoscopy can provide useful information about upper airway problems and is a safe and effective screening method for both preoperative and postoperative uses [6].

The aim of the present study was to investigate changes of the respiratory tract in children with MPS by helical CT and flexible endoscopy.

* Corresponding author at: Department of Otolaryngology, National Center for Child Health and Development, Okura 2-10-1, Setagaya, Tokyo 1578535, Japan. Fax: +81 3 5494 7909.
E-mail address: morimoto-n@ncchd.go.jp (N. Morimoto).

http://dx.doi.org/10.1016/j.ymgme.2014.03.013
1096-7192/

This study represents the first multidisciplinary assessment of both the trachea and the larynx in children with MPS.

2. Materials and methods

2.1. Patients

Thirty-five MPS patients were enrolled in this study. They were aged 2–16 years (mean age: 9.2 ± 4.4 years), their mean height was 117.4 ± 19.1 cm, and their mean BMI was 19.4 ± 3.5. They included 5 patients with Hurler syndrome (MPS I), 25 patients with Hunter syndrome (MPS II), 2 patients with Sanfilippo syndrome (MPS III), 2 patients with Morquio syndrome (MPS IV), and 1 patient with Maroteaux–Lamy syndrome (MPS VI). Patients with prior tracheotomy or congenital thoracic anomalies were excluded. All of the children with MPS I, II, and IV were evaluated prior to initiation of enzyme replacement therapy or BMT.

Forty-five of the control group were retrospectively selected from a database of patients who underwent upper and lower airway CTs for possible pulmonary metastases or other conditions, and they were matched for age (mean age: 8.2 ± 4.5 years; range: 2–15 years) and stature (mean height: 124.0 ± 26.4 cm). Endoscopic evaluation and sleep studies were not performed in the control subjects.

2.2. Tracheal CT

Helical CT was performed by using a high speed CT scanner without contrast enhancement (GE, Discovery 750HD) for both groups. Contiguous 5-mm slices were obtained with the subject in the supine position. Images were obtained during breath holding in expiration as far as possible. The duration of image acquisition from neck to chest was 3 s, which meant that a stable respiratory phase could be maintained.

Tracheal morphology was evaluated at the Th1 and Th2 levels, and was classified into the following 4 categories [7,8]: D-shaped (the transverse diameter is larger than the anteroposterior diameter due to collapse in the latter direction), W-shaped (an elliptical trachea with a larger anteroposterior diameter than transverse diameter due to transverse collapse), O-shaped (slight deformity with a small posterior membranous region), and normal (C-shaped with equal transverse and anteroposterior diameters) (Fig. 1). Single images obtained at the Th1 level and Th2 level were approximated with the mediastinal window in both MPS patients and controls [2], and then the selected images were digitized. Each image was traced twice using an Advantage workstation (GE, Tokyo), and the tracheal cross-sectional surface area (TSA) was calculated at the Th1 and Th2 levels.

2.3. Endoscopic evaluation

The MPS patients underwent endoscopic examination (Pentax, Tokyo, Japan) during spontaneous ventilation and images were captured with a video monitor. The airway was assessed at the level of the epiglottis, the cricoid, and the subglottic region, and findings were graded as follows (Table 1). Grade 0 was a normal airway. Grade 1 meant edema and swelling of the epiglottis or cricoid without redundant mucosa. The false vocal cords showed edema and hypertrophy, partly obscuring the true vocal cords. Grade 2 meant that the redundant mucosa of the epiglottis and cricoid caused inspiratory obstruction, while the true vocal cords were obscured due to hypertrophy of the false cords.

2.3.1. Sleep study

All MPS patients underwent overnight pulse oximetry as a screening test and polysomnography (PSG) was performed as part of the preoperative workup in some patients. Obstructive sleep apnea (OSAS) was

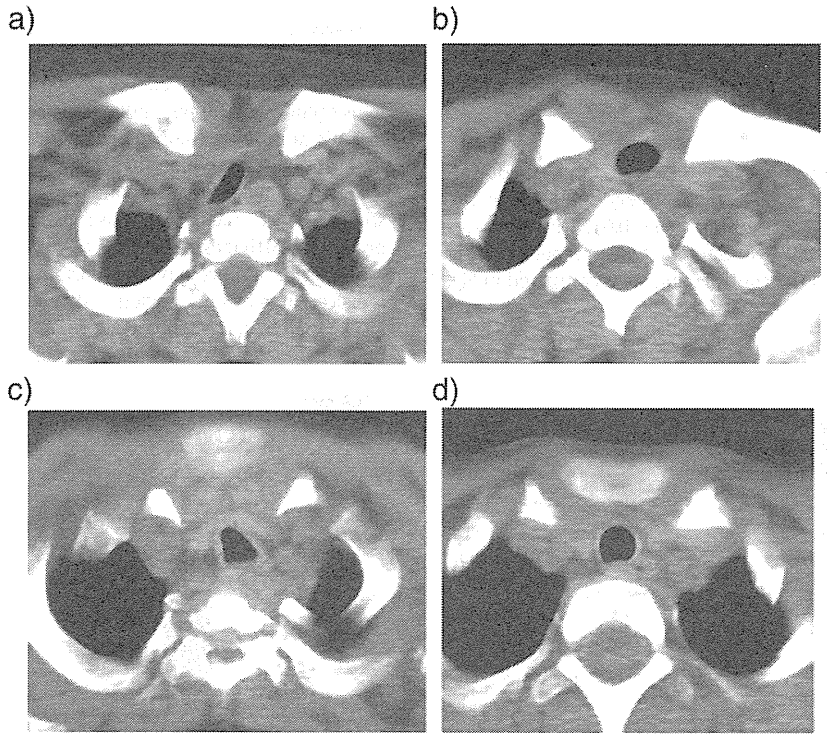


Fig. 1. Morphological change at Th2 level of the trachea. a) W type shows transverse collapse. b) D type shows antero-posterior collapse. c) O type shows deformed trachea slightly. d) Normal trachea.

Table 1
Endoscopic classification of the larynx.

Grade 0	Normal laryngeal structure	
Grade 1	Mucosa is edematous and hypertrophic, but vocal cords are clearly visible.	
Grade 2	Mucosa is edematous and redundant flaccid mucosa cause inspiratory obstruction. Vocal cords are clearly invisible.	

defined as an oxygen desaturation index (ODI) ≥4, which meant that the SpO_2 was at least 4 points lower than baseline ≥4 times per hour.

3. Results

3.1. Morphological analysis of the trachea

Assessment of morphological changes of the tracheal lumen at the Th1 and Th2 levels revealed abnormal morphology at the Th1 level in 17 of the 35 MPS patients (50%), with 2 (6%), 13 (37%), and 2 (6%) patients having a D-shaped, O-shaped, and W-shaped deformity, respectively. When the tracheal lumen was assessed at the Th2 level, morphological abnormality was found in 21 of the 35 patients (60%), with 6 (17%), 7 (20%), and 8 (23%) patients having a D-shaped, O-shaped, and W-shaped deformity, respectively. The characteristics of the tracheal lumen in each type of MPS are shown in Fig. 2. A W-shaped or D-shaped deformity was seen in 11/25 patients with MPS II and 2/2 patients with MPS IV.

3.2. Cross-sectional area of the trachea

In the MPS patients, the mean TSA was 55.5 ± 29.0 mm^2 and 61.4 ± 29.0 mm^2 at the Th1 and Th2 levels, respectively, while the control group had a mean TSA of 90.1 ± 41.9 mm^2 ($p < 0.01$, Student's t-test) and 87.9 ± 39.3 mm^2 ($p < 0.05$, Student's t-test), respectively. The mean TSA of the patients and the control subjects is compared in Fig. 3.

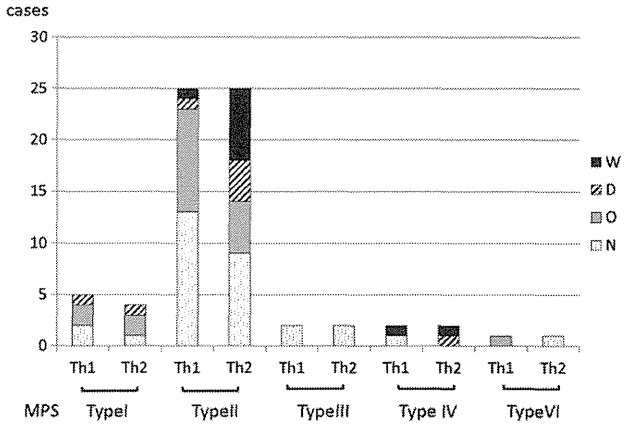


Fig. 2. Tracheal ring deformity at Th1 and Th2 levels. Severe tracheal stenosis was found in Type II and Type IV.

3.3. Endoscopic findings

Endoscopic examination revealed deformity of the laryngeal architecture due to GAG deposition in 23 of the 35 MPS patients (66%). Grade 2 deformity of the arytenoid mucosa was found in 9 of the 35 patients (26%) (Fig. 4a). They had inspiratory stridor and occasional feeding problems associated with coughing and regurgitation, while 5 of them also had sleep apnea. Grade 2 deformity of the false vocal cords caused narrowing of the tracheal airway in 9 patients (Fig. 4b). Grade 2 deformity of the epiglottis was only seen in 2 patients, and was associated with prolapse into the laryngeal inlet.

The clinical respiratory features of the subjects are summarized in Table 2.

3.4. Respiratory features versus anatomical changes

Dysphagia was present in 5 of the 35 patients and required a gastric feeding tube. Four of these 5 patients had MPS II and one had MPS III. All 5 patients showed arytenoid distraction and 3 had a W-shaped trachea. Thirteen of 35 patients had previously undergone adenotonsillectomy because of nasopharyngeal obstruction.

Overnight pulse oximetry showed obstructive sleep apnea (OSA) with a ODI4 > 4 in 15 of the 35 patients, including 2 patients, 12 patients, and 1 patient with MPS I, MPS II, and MPS IV, respectively. Among the 15 patients with OSA, deformation of the arytenoid mucosa and false vocal cords was seen in 10 and 5 patients, respectively. Tracheal deformity was found in 7 of the 15 patients, who had a W-shaped or D-shaped trachea. On the other hand, three of the 15 patients with OSA did not show any abnormalities of the larynx and trachea. Four of the 15 patients with OSA were overweight and had a BMI > 20. In addition, six patients gradually developed upper airway obstruction even after adenotonsillectomy. Three of these six patients had Grade 2 arytenoid deformity and 2 showed tracheal narrowing, but one patient had no abnormalities of the larynx or trachea and the BMI was below 20.

4. Discussion

In patients with mucopolysaccharidoses (MPSs), respiratory problems occur due to upper and lower airway abnormalities. Accumulation of GAGs causes distension of the tongue and pharyngeal mucosa resulting in narrowing of the pharyngeal space [9,1,10,11]. GAG deposition also leads to redundant tissue around the larynx, which prolapses into the respiratory tract. Tracheal stenosis is due to GAG deposition in the tracheobronchial cartilages, which may lead to tracheomalacia [3,2] and can influence both morbidity and mortality [12]. Upper airway obstruction is exacerbated by neck flexion [13], while extension of the neck increases airway patency in MPS IV. GAG deposits cause alveolar and interstitial pulmonary involvement, impairing pulmonary function [14].

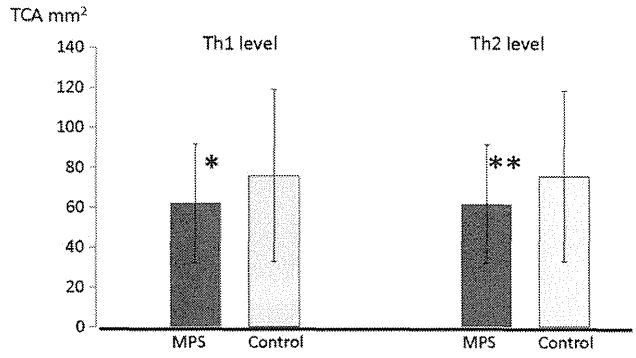


Fig. 3. Tracheal cross-sectional surface area (TSA) at Th1 and Th2. (*$p < 0.01$ and **$p < 0.05$, Student t-test.).