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REVIEW ARTICLE

Recent advances in understanding beta-ketothiolase (mitochondrial acetoacetyl-CoA thiolase, T2) deficiency

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Abstract

Beta-ketothiolase (mitochondrial acetoacetyl-CoA thiolase, T2) deficiency (OMIM #203750, *607809) is an inborn error of metabolism that affects isoleucine catabolism and ketone body metabolism. This disorder is clinically characterized by intermittent ketoacidotic crises under ketogenic stresses. In addition to a previous 26-case series, four series of T2-deficient patients were recently reported from different regions. In these series, most T2-deficient patients developed their first ketoacidotic crises between the ages of 6 months and 3 years. Most patients experienced less than three metabolic crises. Newborn screening (NBS) for T2 deficiency is performed in some countries but some T2-deficient patients have been missed by NBS. Therefore, T2 deficiency should be considered in patients with severe metabolic acidosis, even in regions where NBS for T2 deficiency is performed. Neurological manifestations, especially extrapyramidal manifestations, can occur as sequelae to severe metabolic acidosis; however, this can also occur in patients without any apparent metabolic crisis or before the onset of metabolic crisis.

Introduction

Beta-ketothiolase (mitochondrial acetoacetyl-CoA thiolase, T2, EC 2.3.1.9) deficiency (OMIM #203750, *607809) was first described as an inborn error of isoleucine catabolism in 1971 [1–3]. Later this disorder was revealed to be a defect in mitochondrial acetoacetyl-CoA thiolase activity that affects both isoleucine catabolism and ketolysis [4]. Hence, this disorder is classified as an organic aciduria or as a defect in ketolysis. The disorder is clinically characterized by intermittent ketoacidotic episodes [5–7]. Our group has intensively studied T2 deficiency from both clinical and molecular perspectives [3, 8–45]. A cohort of 26 cases was previously reported in 2001 [25]. In 2017, four more cohorts of 26, 41, 32, and 10 cases from different areas and ethnicities were reported [41, 42, 46, 47]. This number of cases may be sufficient to understand the clinical manifestation of the disorder. Here, we review the clinical manifestation of T2 deficiency, mainly in patients from the five reported cohorts and we discuss what remains to be solved.

We will especially focus on newborn screening (NBS) and neurological manifestation of the disorder. T2 deficiency is included in newborn screening in some countries, although there are several reports of false-negative results [46, 48–51], including in Japanese cases [3, 15, 28]. Neurological manifestation/complication in T2 deficiency has been regarded as a sequela of severe metabolic acidosis; however, several patients have developed neurological problems without apparent severe metabolic acidosis [9, 13, 25, 26, 31, 32, 35, 38, 46, 47, 52].

Pathophysiology

T2 catalyzes the interconversion of acetyl-CoA and acetoacetyl-CoA in mitochondria of both liver and

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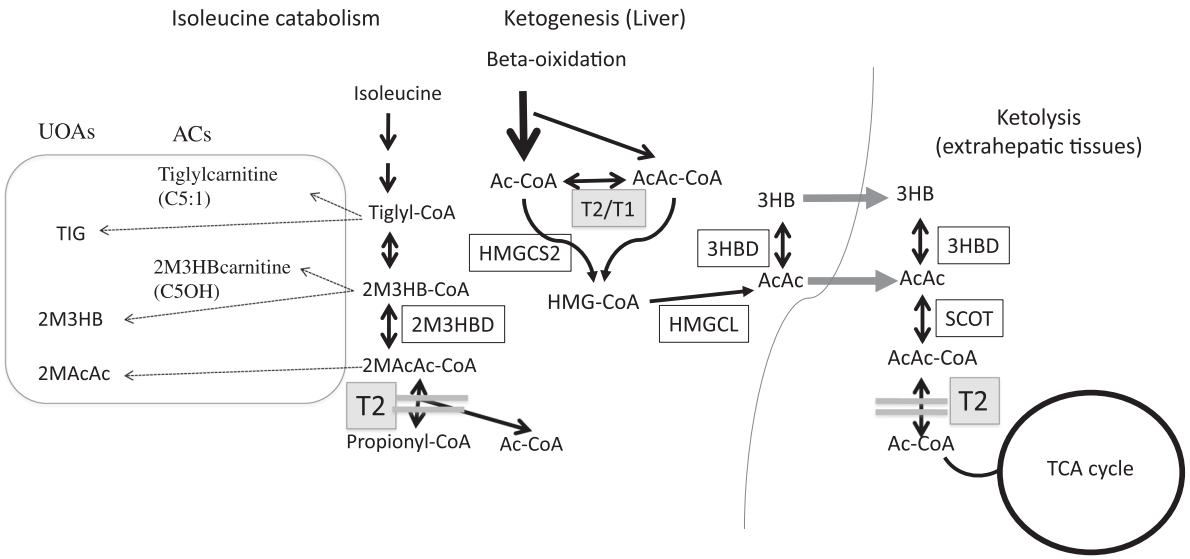

Fig. 1 Overview of ketone body metabolism and isoleucine catabolism. T2 is involved in isoleucine catabolism, ketogenesis in the liver, and ketolysis in extrahepatic tissues. 2M3HB 2-methyl-3-hydroxybutyrate, 2M3HB-CoA 2-methyl-3-hydroxybutyryl-CoA, 2M3HBD 2-methyl-3-hydroxybutyrate dehydrogenase, 2MAcAc 2-methylacetoacetate, 2MAcAc-CoA 2-methylacetoacetyl-CoA, 3HB 3-hydroxybutyrate, 3HBD 3-hydroxybutyrate dehydrogenase, Ac-CoA acetyl-coenzyme A, AcAc Acetoacetate, AcAc-CoA acetoacetyl-CoA, ACs acylcarnitines, CoA coenzyme A, HMG-CoA 3-hydroxy-3-methylglutaryl-CoA, HMGCL HMG-CoA lyase, HMGCS2 mitochondrial HMG-CoA synthase, SCOT succinyl-CoA:3-oxoacid CoA transferase, T1 mitochondrial medium-chain 3-ketoacyl-CoA thiolase, T2 mitochondrial acetoacetyl-CoA thiolase, TCA tricarboxylic acid, TIG tiglylglycine, UOAs urinary organic acids

extrahepatic tissues (Fig. 1) [5]. During hepatic ketogenesis, the T2 reaction in the direction of acetoacetyl-CoA formation from abundant acetyl-CoA is favored. In extrahepatic tissues, T2 catalyzes predominantly acetoacetyl-CoA cleavage to form acetyl-CoA. Because ketoacidosis is the main feature of T2 deficiency, its function in ketolysis is physiologically more important than its role in hepatic ketogenesis. In T2-deficient liver, another thiolase, mitochondrial medium-chain 3-ketoacyl-CoA thiolase (T1), which mainly functions to cleave medium-chain 3-ketoacyl-CoA during fatty acid beta-oxidation, may compensate for T2 deficiency [53].

T2 also catalyzes 2-methylacetoacetyl-CoA cleavage into acetyl-CoA and propionyl-CoA in isoleucine catabolism [54]. T2 deficiency results in accumulation of isoleucine-catabolic intermediates, such as 2-methylacetoacetyl-CoA, 2-methyl-3-hydroxybutyryl-CoA and tiglyl-CoA. Urinary elevated excretion of 2-methylacetoacetate (2MAcAc), 2-methyl-3-hydroxybutyrate (2M3HB), and tiglylglycine (TIG) is characteristic for T2 deficiency, while elevations of C5-OH carnitine (2-methyl-3-hydroxybutyryl-carnitine) and C5:1 carnitine (tiglylcarnitine) are characteristic in blood acylcarnitine analysis. These chemical features are in accord with the fact that T1 has little affinity for 2-methylacetoacetyl-CoA as a substrate. Of note, these chemical features, when present, are highly suggestive of T2 deficiency but their absence, especially during an asymptomatic period, does not absolutely rule out T2 deficiency [3, 28].

The T2 enzyme is a homotetramer of 41 kDa. The T2 gene (*Acetyl Coenzyme A Acetyltransferase 1*, *ACAT1*) is located on chromosome 11q22.3-23.1 and includes 12 exons that span ~27 kb. At Gifu University, we have identified more than 80 T2 gene mutations in more than 120 patients. There are some common mutations in specific populations. For example, p.R208* and c.1006-1g>c were identified in nearly 70 and 20%, respectively, of mutant alleles in the Vietnamese population [41]. p.M193R was identified in nearly 50% of mutant alleles in the Indian population [42]. Other common mutations identified in at least four independent T2-deficient families are p.G152A, p.N158D, p.N158S, p.G183R, p.Q272X, c.828+1g>t, p.T297M, and c.1163+2t>c. There is a significant biochemical-genotype correlation, but no apparent phenotype–genotype correlation. Transient expression analysis of mutant cDNAs showed some mutations retain significant residual T2 activities. Such mutations are designated as "mild" mutations. In this review, patients with mild genotypes are defined as patients who have "mild" mutations in at least one of two mutant alleles.

Clinical manifestation

A series of 26 cases was previously reported in 2001 [25]. In 2017, other series of 26 cases from France [47], 41 cases from Vietnam [41], 32 mostly from Turkey, The Netherlands, and Germany [46], and 10 from India [42] were reported. Hence in

total, 135 T2-deficient patients are described in these five reports. The study of 2001 clearly described the clinical manifestation of T2-deficient patients and is still the most informative in some aspects (Table 1). The latest four reports provide some unique information. The series from Vietnam provides typical clinical information for patients who have two null mutations (severe genotype), because most patients had a combination of two null mutations, R208X and c.1006-1g>c [41]. The series from France focused on neurological complications in T2 deficiency [47]. We will discuss neurological manifestations together with these data later. The series from Turkey, The Netherlands and Germany [46], and from France [47] provide information of patients who were diagnosed in early childhood and who were more than 10 years-old at the time of study.

Onset

Figure 2a shows the onset of symptoms for the 135 patients listed in the five series. Most patients developed their first ketoacidotic crisis between 6 and 36 months of age with the peak onset at 6–11 months of age. Neonatal onset of T2 deficiency is very rare and only two patients had neonatal onset. The early neonatal period is one of catabolic conditions and is a major period for the onset peak of typical organic acidemia, such as isovaleric acidemia, propionic acidemia, methylmalonic acidemia, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase deficiency as well as another ketolytic defect, succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency [6]. Only three patients had onset after 6 years of age. Fourteen percent of patients did not have any metabolic episodes; among them 11 patients were diagnosed through NBS or familial analysis in infancy and eight patients were diagnosed after 1 year of age by familial analyses. Figure 2b shows the onset age of 57 and 12 patients with confirmed severe and mild genotypes, respectively. The peak of onset age was 6–11 and 12–17 months in patients with severe and mild genotypes, respectively, although the number of patients with mild genotype was small. In most cases, the first ketoacidotic episode followed an intercurrent illness, especially gastroenteritis or respiratory disease in association with fasting and catabolic conditions. The onset of T2 deficiency was common in late infancy and early childhood because children usually experience their first episodes of infection during this period. In one case, excessive protein intake on the day preceding the episode was reported. In T2 deficiency, catabolic conditions appear to confer a higher risk than that of protein overload.

Metabolic profiles in the first crises

Metabolic profiles are available for the Vietnamese series [41]. Most patients belong to the severe genotype; therefore, metabolic profiles are expected to be typical. Metabolic acidosis was in general severe. Blood pH and HCO_3 ranged between 6.80–7.26 (mean 7.02) and 0–10.7 mmol/L (mean 3.2), respectively. In general, during metabolic acidosis, blood glucose levels vary, ranging between 1.7 and 23.3 mmol/L (mean 7.51). Hyperglycemia (>7.8 mmol/L) was observed in 28% of patients and hypoglycemia (<2.5 mmol/L) was present in 8% of patients. Hyperglycemia mimicking diabetic ketoacidosis is sometimes observed in another ketolytic defect (SCOT deficiency) [55] and in other organic acidurias [56–58]. Such hyperglycemia may be because of a stress reaction. Hyperammonemia (>170 μmol/L) presented in 23% of patients. However, the highest level of ammonia was 307 μmol/L. This is quite different from other organic acidemias. Hence, among 39 symptomatic patients only four needed hemodialysis, although mechanical ventilation was used in 16 patients.

Death

Among 135 patients, seven patients died of a severe ketoacidotic crisis or from sequelae of a severe crisis. In the first series, only GK16 died of sequelae of the first crisis [25]. She suffered a brain hemorrhage because of hypernatremia caused by excessive sodium bicarbonate use during the crisis. In the Vietnamese series, five patients died (two of them at the first crisis, two of them at the second crisis, and one patient died because of neurological sequelae following the first crisis) [41]. In the Indian series, one patient (GK108) died at the first crisis [8], and one patient died at the second crisis in the Turkish and German series [46]. The three patients above who died at the second metabolic crisis were diagnosed as T2 deficiency at the first metabolic crises; therefore, death could have been avoided.

Frequency of ketoacidotic crises

From the five series of T2-deficient patients, 39 were followed up for more than 10 years and the frequency of ketoacidotic crises in these patients was recorded (Fig. 3). About 34% of patients experienced only one metabolic crisis, which led to the diagnosis. This means that early diagnosis and management may prevent the development of severe subsequent metabolic crises. Most patients developed ketoacidotic crises only three times or less. Seven patients had no episodes. Information of the latest crises was available in two series. In the Vietnamese series, all metabolic crises occurred within 5 years of age [41], whereas in 26 cases reported in 2001, five patients developed severe metabolic crises between 5 and 10 years of age [25]. It should be noted that four of these five patients were diagnosed as T2 deficiency in their last crisis, when they were older than 5 years. Hence, after confirming the diagnosis of T2 deficiency, most metabolic crises occurred

Table 1 26 T2-deficient patients

Case	Birth year	Sex	Race	Consanguinity	Family history	Onset	Acute episodes (the first attack, or typical attack)									Neurological evaluation	Non-episodic condition			Crisis	Outcome		Mutations	Genotype
							Preceeding event	Blood gas			Urinary organic acids			NH3	Glucose	Unconsciousness		Urinary organic acids			Frequency	Failure to thrive	Mental retardation	[Paternal]: [Maternal]	
								pH	HCO_3	BD	TIG	2M3HB	2MAA				Before 1st crisis	TIG	2M3HB	2MAA					
GK01	1981	M	J	ND	ND	20 m	Asthma	7.15	4.2	23	D	D	D	91	4.5	1d	NP	D	D	D or ND	1	NO	YES	[c.149delC]: [p.A333P]	Severe
GK04	1973	M	C (Ne)	ND	[a]	48 m		7.06	6.8	25	D	D				+	NP				3	NO	NO	[p.G183R]: [c.1006–2a>c]	Severe
GK05	1945	M	C (Ne)	ND	[a]	—				No ketoacidotic episode								D	D	D	0	NO	NO	[p.G183R]: [c.828 +1g>t]	Severe
GK06	1988	M	C (G)	ND	ND	6 m		7.24	11	17	D	D	D	72	3.3	4w	[e]	D	D	D	5	YES	YES	[no mRNA]: [p.A380T]	Mild
GK07	1986	F	C (USA)	ND	ND	9 m	GAS	7.13	3	23	D	D	ND		3.8	2d	NP	D	D	ND	3	NO	NO	[p.Q272X]: [p.G379V]	Severe
GK08	1963	M	C (Can)	plus	[b]	12 m			20.4	5	(D)	(D)	(D)			4d	NP	D	D	D	12	NO	NO	[c.2T>A]: [=]	Mild
GK08s	1967	F	C (Can)	Plus	[b]	12 m	Mumps		22.2	2.7	(D)	(D)	(D)			+	NP	D	D	D	3	NO	NO	[c.2T>A]: [=]	Mild
GK09	1965	M	C (Can)	ND	ND	17 m		6.99	8	24	ND	D	D		3.1	2d	NP	ND or D	D	D	5	NO	NO	[c.1163+2t>c]: [=]	Severe
GK10	1988	M	Viet	ND	[b]	6 m	GAS	7.08	5	22	D	D	D		5.8	–	NP				2	NO	NO	[c.1006-1g>c]: [=]	Severe
GK11	1986	M	C (Can)	ND	ND	25 m	RI	6.99	3.1	28	ND	D	D	64	6	+	NP	ND	D	D	1	NO	NO(AD)	[p.N158D]: [p.T297M]	Mild
GK12	1986	M	Bra	ND	ND	23 m	GAS	7.02	4		D	D	D		**2.3**	2d	[f]	D	D	D	2	NO	NO	[p.Y33X]: [p.G405del]	Severe
GK13	1988	F	C (Sw)	ND	ND	21 m		7.01	3.8		D	D	D		N	2d	NP	D	D	D	1	NO	NO	[c.754insCT]: [c.435+1g>a]	Severe
GK14	1986	M	C (Sw)	ND	ND	21 m		6.96			D	D	D	2.5	N	3d	NP	D	D	D	1	NO	NO	[c.435+1g>a]: [c.83delAT]	Severe
GK15	1990	M	C (Can)	ND	ND	10 m	RI	7.06			D	D	D			+	NP	D	D	D	2	NO	NO	[p.N158D]: [p.Q272X]	Severe
GK16	1990	F	C (USA)	ND	ND	16 m	GAS	7.07	3.3		ND	D	D		5.2	+	NP				1	NO	died	[p.A301P]: [c.828 +1g>t]	Mild
GK17	1989	M	C (Ne)	ND	[c]	5 m	GAS	6.9	5	29	D	D	D			–	NP	D	D	D	1	NO	NO	*c.731–46_c.752del68bp]: [c.1163+2t>c]*	Severe
GK18	1991	F	C (Sp)	ND	ND	12 m		6.76	3.3	33	ND	D	D	N	17	–	NP	ND	D	D	3	NO	NO	[p.K124R]: [=]	Severe
GK19	1992	M	J	Plus	[d]	23 m	RI	7.17	3.8	25.1	ND	D	D		4.2	1d	NP	ND	D	ND	1	NO	NO	[p.I312T]: [p.N93S]	Mild
GK19b	1988	M	J	Plus	[d]					No ketoacidotic episode								ND	D	ND	0	NO	NO	[p.I312T]: [p.N93S]	Mild
GK20	1992	F	C (Can)	ND	ND	22 m		6.98	2.7	28	ND	D	D	33	**Low**		NP	ND	D	D	1	NO	NO	*[p.Y219H]: [p.Q272X]*	Severe
GK21	1969	F	C (G)	ND	ND	4Y	RI	7.04		23	D	D	D			1d	NP	D	D	ND	2	NO	NO	*[p.E345del]: [c.1084insC]*	Severe
GK22	1987	F	C (Sp)	ND	ND	7 m	GAS	7.06	8.1	20	D	D	D	48	**1.6**		NP	D	D	D	1	NO	NO	*[p.G152A]: [p.E354V]*	Severe
GK23	1992	F	C (Sp)	ND	ND	14 m	Asthma	6.89	3.4	20	ND	D	D	35	8.1	1d	NP	ND	D	D	1	NO	NO	[p.Q145E]: [=]	Mild
GK24	1995	F	C (USA)	ND	ND	3d		7.3		13	D	D	D		**1.7**	+	NP				1	NO	NO	*[p.G152A]: [p.D253E]*	Severe
GK25	1985	M	C (Sp)	ND	[d]	5 m	GAS	7.2	7	20	(D)	(D)	(D)	99	N	7–10d	NP	D	D	D	1	NO	NO	[p.A127V]: [=]	Severe

Table 1 (continued)

Case	Birth year	Sex	Race	Consanguinity	Family history	Onset	Acute episodes (the first attack, or typical attack)								Neurological evaluation	Non-episodic condition			Crisis	Outcome		Mutations	Genotype
							Preceding event	Blood gas		Urinary organic acids			NH3	Glucose	Unconsciousness	Urinary organic acids			Frequency	Failure to thrive	Mental retardation	[Paternal]: [Maternal]	
								pH	HCO_3	BD	TIG	2M3HB	2MAA			TIG	2M3HB	2MAA					
GK25s	1980	F	C (Sp)	ND	[d]		No ketoacidotic episode								Before 1st crisis	D	D	D	0	NO	NO	[p.A127V]: [=]	Severe

Bold values are highlighted because the values are abnormal

The information described was at the time of 2000

Mutations (italic) means that we did not determine paternal and maternal alleles

Race(nationality): *J* Japanese, *C* Caucasian, *Ne* Netherland, *G* Germany, *Can* Canada, *Viet* Vietnamese, *Bra* Brazil, *Sw* Switzerland, *Sp* Spain, *ND* not detected or under upper-cutoff level, *D* detected, *(D)* should be detected since it was detected in even good condition, *NP* nothing particular, *GAS* gastroenteritis, *RI* respiratory infection including tonsillitis, croup, and bronchitis

[a]GK05 is the father of GK04

[b]History of infantile death of sibling

[c]GK17 has an affected younger sister who has not developed crisis until 3 years of age

[d]GK19b is GK19's brother, GK25s is GK25's sister

[e]This patient had opisthotonus

[f]This patient had hypotonia, motor delay with seizure

before 5 years of age. Even in a fasting test in normal children, blood ketone body levels are much lower at the age of 7–15 than at the age of 1–7 years [59]. This may mean ketone bodies are less important in older children for the maintenance of blood glucose levels compared with younger children and may explain why ketoacidotic events become less frequent with age in T2 deficiency.

Diagnosis

The main clinical manifestation of T2 deficiency is severe metabolic acidosis; therefore, urinary organic acid analysis is the most applicable second line metabolic analysis. T2 deficiency is chemically diagnosed by the presence of TIG, 2M3HB, and 2MAcAc from the isoleucine-catabolic pathway. 2MAcAc is unstable and can be difficult to detect. A combination of TIG and 2M3HB without 2MAcAc is characteristic for 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (2M3HBD) deficiency [60, 61]. This disorder is now called HSD10 disease and is an important differential diagnosis for T2 deficiency (Fig. 1). TIG was not detected in seven of the 26 patients in Table 1 even during acute crises; six of these patients were classified as mild genotype. Hence, the absence of TIG does not exclude T2 deficiency.

Among eight Japanese patients (Table 2), only one (GK01) had a severe genotype, the others being classified as mild genotype [3, 28]. These patients with mild genotype presented with severe ketoacidosis that was typical for patients with severe genotype, but their urinary organic acid and blood acylcarnitine profiles, especially during asymptomatic conditions, differ from those of typical severe genotype patients. In mild genotype patients, tiglylglycine may not be detected by urine organic acid analysis even during metabolic crisis. Between episodes, 2-methyl-3-hydroxybutyrate may also be only slightly elevated or even undetectable. Similarly, blood acylcarnitine analysis may be normal during non-episodic periods and even during acute episodes of metabolic decompensation in some T2-deficient patients with mild genotype (see Newborn screening for T2 deficiency).

Enzyme assays or mutation analysis is essential to confirm T2 deficiency. There are several kinds of enzyme assay. Measurement of potassium-ion activated acetoacetyl-CoA thiolase activity using fibroblasts is the gold standard method [62, 63]. Lymphocytes (peripheral blood mononuclear cells) can also be used but contamination with red blood cells may affect this assay. A 2-methylacetoacetyl-CoA thiolase specific assay was also reported [54] but this substrate is not commercially available. A coupling assay with tiglyl-CoA [64] cannot distinguish T2 deficiency from 2M3HBD deficiency (HSD10 disease).

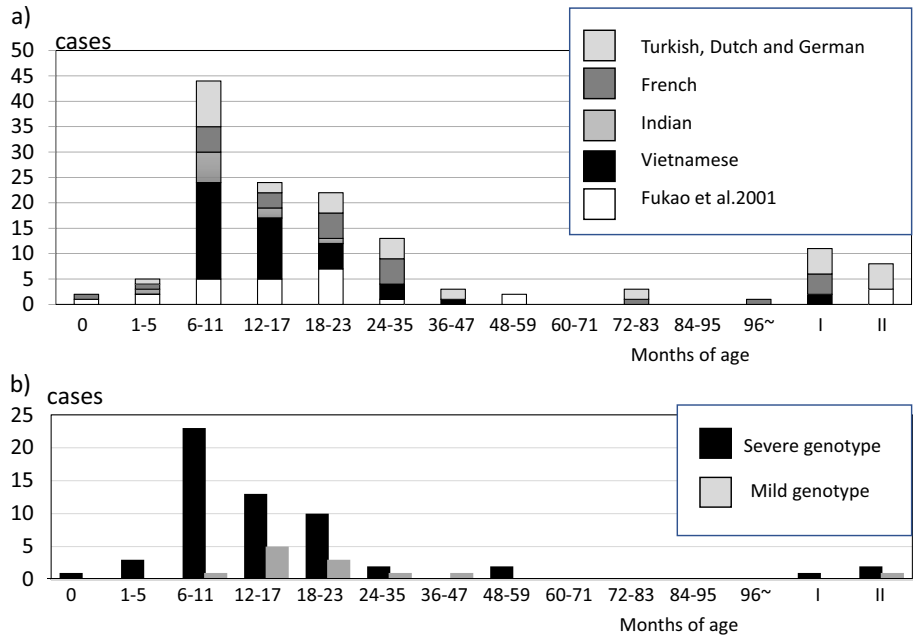

Fig. 2 Onset of first ketoacidotic crises. **a** Onset of first metabolic crisis in all patients in the five series are shown. The vertical and horizontal axes indicate number of patients and their age in months, respectively. I indicates that patients were identified as having T2 deficiency before 1 year of age by newborn screening or familial screening; II indicates that patients were identified as having T2 deficiency after 1 year of age by familial analysis. **b** Onset of first metabolic crisis in 57 and 12 patients with confirmed severe and mild genotypes, respectively. The vertical and horizontal axes indicate number of patients and their age in months, respectively

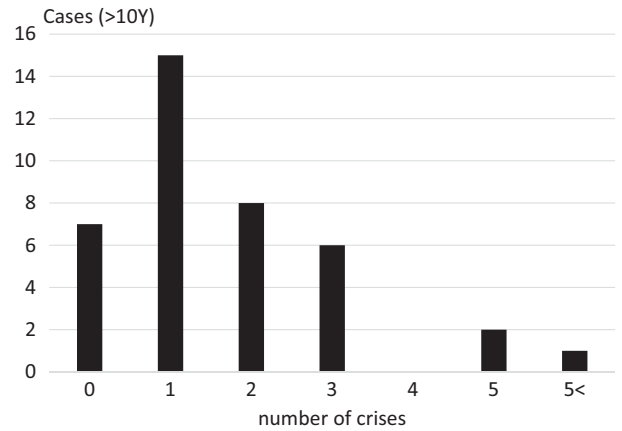

Fig. 3 Frequency of ketoacidotic crises. Frequency of metabolic crises in patients who were more than 10 years of age at last follow-up

Mutation analysis using genomic PCR and direct sequencing is a standard method for diagnosing T2 deficiency [22]. However cDNA analysis, multiplex ligation-dependent probe amplification, mutant cDNA expression analysis followed by enzyme assay, and a mini-gene splicing assay may be necessary to confirm whether an identified novel variant is a causative mutation or not [31, 32, 43].

Management

Management is divided into that for acute crisis and for non-episodic conditions [65].

Treatment of acute crisis

Even when the patient shows normoglycemia, intravenous glucose should be given to maintain blood glucose levels in the upper normal range; this is important to suppress ketogenesis. Insulin, together with sufficient glucose, can be used to further suppress ketosis in severe episodes, with frequent blood glucose monitoring. Treatment of metabolic acidosis using sodium bicarbonate is controversial. Minimal usage of sodium bicarbonate is recommended. In general, hemodialysis is not necessary if ketogenesis is successfully suppressed. Among Vietnamese 41 patients, hemodialysis was performed for only four patients [41].

Management in non-episodic conditions

It is important to avoid severe metabolic crises. For this purpose, fasting avoidance is the basis of long-term management. During ketogenic stressors, such as vomiting, appetite loss, or infection, sufficient oral glucose supplementation is essential to avoid ketoacidotic crises. Episodes associated with moderate or high urine ketones should be treated by intravenous glucose infusion. Mild dietary protein restriction (1.5–2 g/kg/day) is a fundamental and reasonable treatment to reduce isoleucine load. This is discussed later (neurological manifestation). Patients should also restrict excess fat intake; a ketogenic diet is contraindicated. L-carnitine supplementation may be considered, if patients have low free carnitine levels.

Table 2 Eight Japanese T2-deficient patients

Case	Genotypes		1st episode			Urinary organic acids				Blood spots Acs				Number of episodes	Neuro-logical problem
			Onset	Blood gas		Acute crisis		Well condition		Acute Crisis		Well condition			
				pH	HCO_3	TIG	2M3HB	TIG	2M3HB	C5:1	C5OH	C5:1	C5OH		
GK01	p.A333P	c.149delC	20 m	7.15	4.2	D	D	D	D	NA	NA	0.85*	3.61*	1	Dev.Delay
GK19	**p.I312T**	**p.N93S**	23 m	7.17	3.8	ND	D	ND	ND	NA	NA	0.04*	0.50*	1	—
GK30	**c.2T>C**	c.149delC	9 m	7.01	3.3	D	D	ND	ND	NA	NA	0.06*	0.52*	3	—
GK31	**p.I312T**	c.149delC	18 m	7.07	2.9	D	D	ND	ND	NA	NA	0.13*	0.43*	1	—
GK64	p.D186Y	**c.951C>T**	7 m	7.00	8.0	ND	D	ND	ND	NA	NA	0.49*	0.54*	1	—
GK69	**p.H144P**	p.S390P	9 m	7.08	4.6	NA	NA	ND	ND	NA	NA	NA	NA	2	—
GK77	**p.H144P**	**p.H144P**	3Y	7.14	6.3	ND	D	ND	ND	0.01	0.11	NA	NA	1	—
GK77B	**p.H144P**	**p.H144P**	3Y	6.88	1.1	ND	D	NA	NA	0.03	0.11	0.01	0.19	1	Died
								Upper-cutoff*		0.49	0.5				
								Upper-cutoff		0.08	1.00	0.08	1.00		

Mutations in bold letters are mutations that retained significant residual activity

D detected, *ND* not detected or under cutoff level, *NA* not applicable, *Acs* acylcarnitines

In blood Acs, upper-cutoff values with and without * are those for the values with and without *, respectively

Newborn screening for T2 deficiency

T2 deficiency is a suitable disorder for NBS from a clinical perspective. This disorder rarely has neonatal onset and most patients develop ketoacidotic crises during 6 months to 3 years of age. In most patients, the first ketoacidotic crisis is the most severe and is associated with their prognosis. Such severe metabolic crises could be avoided if parents knew how to manage the condition. However, false-negative results appear to be inevitable in NBS for T2 deficiency.

We predicted in 2003 that NBS might miss some T2-deficient patients based on acylcarnitine profiles in Japanese patients [28]. Later, we also reported that levels of C5OH and C5:1 were within normal ranges in acylcarnitine profiles even during acute metabolic crises in two T2-deficient cases, GK77 and his brother GK77b [3]. As shown in Table 2, in Japan, most T2-deficient patients identified thus far, have at least one "mild" mutation, which retains significant residual T2 activity. In these patients, metabolites from isoleucine catabolism in blood acylcarnitine and urinary organic acid analyses were much lower than that in typical T2-deficient patients with two null mutations. Hence, we expected that NBS might miss such patients with mild mutations. This is why T2 deficiency is classified among secondary target diseases that are not necessarily screened in NBS in Japan. However, in our opinion, T2 deficiency should be included in NBS; even if routine NBS misses some T2-deficient patients, some will still be detected.

False-negative results in NBS were reported in eight patients from seven families [48–51]. Table 3 shows false-negative NBS results in these patients. Different cutoff values were used in different laboratories. From July 1997 to July 2005, NBS in North Carolina, USA, identified two T2-deficient patients through elevated C5:1 [51]. During this period, one T2-deficient patient was missed by NBS and later at 15 months of age this patient developed ketoacidosis. In a similar NBS program in Minnesota, USA, from January 2001 to November 2010, one case was identified and two sibling cases were missed [48]. The elder brother developed severe metabolic acidosis at the age of 10 months. He was suspected as having T2 deficiency by urinary organic acid profile and his diagnosis was confirmed by enzyme assay and mutation detection. Even during acute crises, his plasma C5-OH and C5:1 levels were normal, similar with cases GK77 and GK77b [3]. In the NBS program of New South Wales, Australia, from 1998 to 2012, two patients with T2 deficiency were missed using a screening marker of C5-OH [50]. A Boston group also reported two T2-deficient patients missed by NBS [49]. These patients developed normally before their first metabolic crises and manifested neurological complications after the crises. Hence, it is important that T2 deficiency should be considered for a patient with severe metabolic acidosis even in regions where T2 deficiency is included in NBS target diseases.

NBS studies have shown the incidence of T2 deficiency in some regions. The incidence of T2 deficiency in North Carolina, USA, from 1997 to 2005 was nearly 1 per 313,000 newborns [51], whereas that in Minnesota, USA, from January 2001 to November 2010 was 1 per 232,000 newborns [48]. No T2-deficient patients were identified in 3.36 million Japanese newborns up to 2015 by tandem mass spectrometry NBS including pilot NBS [66]. Furthermore, no T2-deficient patients missed by NBS were detected in Japan.

In most newborn screening centers in Japan, screening criteria for T2 deficiency is that both C5-OH and C5:1 are

Table 3 T2-deficient patients who were not positively screened by NBS

Case	NBS		Development before 1st crisis	1st crisis				Outcome	References
	C5-0H (cutoff)	C5:1 (cutoff)		Onset	pH	HCO_3	MRI		
1		Normal (0.39)		15 m					[51]
2	0.16 (0.6)	0.02 (0.25)	Normal	10 m	7.15	4		Full scale IQ 68 (nonverval 83, verbal 57)	[48]
3	0.27 (0.6)	0.06 (0.25)	Normal	No crisis				Normal growth and development	[48]
4	1.2 (1.5)		Normal	14 m				Normal development	[50]
5	1.2 (1.5)		Normal	19 m				Mainstream schooling	[50]
6	0.18 (0.8)	0.04 (0.08)	Normal	28 m	6.87	5	+	Dystonia, athetosis dyskinesia	[49]
7	NA	NA	Normal	6 m	7.07	3	+	Choreoathetose	[49]
8 (O3)	NA	NA	Normal	5 m	NA	NA	NA	Normal development	[46]

Cutoff values were different among studies. Hence cutoff values in each study are shown in parenthesis

MRI+ indicates basal ganglia lesion

Case 1; During metabolic stress, C5:1 level was high

Case 7; Even during 1st crisis, plasma C5:1 and C5-OH levels were normal

NA No data available

elevated to more than 1.0 and 0.05 nmol/mL, respectively. On its own, C5-OH elevated to more than 1.0 nmol/mL is used as a screening marker for methylcrotonyl-CoA carboxylase deficiency, multiple carboxylase deficiency and HMG-CoA lyase deficiency. Differential diagnosis for these conditions is made by urinary organic acid analysis. Hence, if a T2-deficient patient presents with elevated C5-OH, careful detection of 2MAcAc in a urinary organic acid profile leads to the diagnosis of T2 deficiency. Increased C5:1 is a specific marker for T2 deficiency and HSD10 deficiency (see next paragraph). If C5:1 is used independently as a screening marker for T2 deficiency and HSD10 disease with a lower cutoff level than the present value, more T2-deficient patients may be positively screened, although the C5:1 level is associated with some catabolic conditions.

2M3HBD deficiency (HSD10 disease) is the most important differential diagnosis of T2 deficiency in NBS-screened patients. 2M3HBD deficiency is a rare X-linked neurodegenerative condition caused by abnormalities in the *HSD17B10* gene [60, 61, 66]. HSD10 protein is a multifunctional protein and functions as 2M3HBD, mitochondrial 17beta-hydroxysteroid dehydrogenase and is one of the three components of mitochondrial RNase P. The classical infantile form of HSD10 disease is characterized by a progressive neurodegenerative course with retinopathy and cardiomyopathy, although HSD10 disease has broad clinical heterogeneity. Because this disorder affects one step upstream from T2 deficiency in isoleucine catabolism (Fig. 1), a profile of elevated C5:1 and C5-OH is characteristic for both HSD10 disease and T2 deficiency. Hence, theoretically, this disorder is also screened for by the same criteria as T2 deficiency in NBS. To our knowledge, no HSD10 disease patient has been positively screened by NBS thus far. A profile of elevated urinary TIG and 2M3HB is also a shared characteristic for these two disorders. The only difference is the absence of 2MAcAc in HSD10 disease. However, it is sometimes difficult to detect 2MAcAc in T2 deficiency because of 2MAcAc instability. Hence, if a male baby is positively screened for T2 deficiency, this baby should be carefully examined for the possibility of both disorders. In one 2M3HBD deficient patient, pilot NBS at 5 days of age showed C5:1 levels of 0.07 nmol/mL and C5-OH levels of 0.29 nmol/mL. Notably, C5:1 was elevated, whereas C5-OH was within its cutoff limit in this 2M3HB deficient patient [67]. Such high C5:1 levels were not observed in more than 10,000 newborns in Gifu, Japan (unpublished observation). Thus, these data suggest that some T2 deficiency and HSD10 disease could be positively screened by NBS. Further analysis is necessary.

Neurological manifestation

It is well-known that neurological complications, such as developmental delay, ataxia, myoclonus, and other extrapyramidal symptoms are caused as sequelae of severe metabolic crisis in T2 deficiency, often with basal ganglia lesions. However, we should reconsider neurological problems in T2 deficiency. Some patients developed neurological manifestations that did not associate with apparent severe metabolic crises [9, 13, 31, 32, 35, 38, 46, 47, 52, 68]. Here, we focus on neurological manifestation that is not associated with severe metabolic crises.

In 1994, Ozand et al. [52] reported four T2-deficient patients with delayed development before their first acidotic events. Basal ganglia lesions were identified in three of them by MRI imaging after the metabolic episodes. Ozand et al. first reviewed the neurological problem in T2

deficiency and summarized eight patients from previous case reports who had neurological symptoms. Among them, one patient (GK02) also had delayed development in infancy before the first crisis [68].

Before 2013, although neurological manifestation before a first metabolic crisis was presented in some T2-deficient patients (GK06 [9] GK12 [13], GK32 [32], and GK41 [31]), most such patients presented with motor delay and hypotonia, and extrapyramidal manifestation became evident after their metabolic crisis, as shown in Table 4. Little attention was paid to extrapyramidal manifestation before or without severe metabolic crisis. In 2013, we described a peculiar case, GK84, who suffered hypotonia since 4 months of age and continuous involuntary upper extremity movements since 10 months of age [38]. His development was delayed, and at 4 years of age daily activities were difficult because of involuntary movements and MRI showed bilateral T2 hyperintensities in both the putamen and cerebral peduncles. His first metabolic crisis occurred at 5 years of age following gastroenteritis. This case clearly showed that extrapyramidal abnormalities can occur without apparent metabolic crisis in some T2-deficient patients. Retrospectively, extrapyramidal manifestation was also seen in GK36 and GK41. Hypotonia and developmental delay in other patients were also possibly caused by extrapyramidal abnormality. A recent report by Paquay et al. [47] supports our finding. They focused on neurological involvements in the series of 26 French T2-deficient patients. Among them, two patients who had never had an apparent ketoacidotic crisis, showed neurological symptoms, including motor delay, hypotonia, ataxia, and dystonia since infancy or early childhood. Another two patients had motor delay, hypotonia, dyskinesia before their first crisis and another patient developed choreoathetosis and dystonia from the age of 15 years after a long interval from her first and only ketoacidotic crisis. Basal ganglia lesions were identified in all these cases.

Neurological manifestations and basal ganglia lesions are apparently associated with severe metabolic crises in T2 deficiency. However, based on the above findings, neurological manifestations, mainly extrapyramidal manifestations, may occur without severe metabolic decompensation in infancy or early childhood and may be worsened after severe metabolic crises in some T2-deficient patients. It should be stressed that a large number of T2-deficient patients do not develop neurological manifestation even after metabolic crisis.

The pathogenesis of neurological manifestation and basal ganglia lesions remains unclear. In propionic acidemia and methylmalonic acidemia, basal ganglia lesions are also regarded as sequela of severe metabolic decompensation [69–72]. However, extrapyramidal manifestation and basal ganglia lesions without severe metabolic episodes have also been described in these disorders [73, 74]. In HSD10 disease, there is a defect of the step just upstream from T2 in the isoleucine-catabolic pathway and basal ganglia lesions have also been reported [75, 76]. However, patients with SCOT deficiency, another ketolytic defect, also developed severe ketoacidosis but no basal ganglia lesions have been reported in SCOT deficiency. SCOT deficiency affects only ketolysis and T2 deficiency affects both ketolysis and isoleucine catabolism. Hence, it is reasonable that neurological manifestation is attributed to impairment of isoleucine catabolism.

Isoleucine is catabolized within the rat brain and serves as fuel for brain energy metabolism [77]. Hence, blockage of the isoleucine-catabolic pathway may result in accumulation of tiglyl-CoA, 2M3HB-CoA, and 2-methylacetoacetyl-CoA and Coenzyme A sequestration, toxicity or redistribution (CASTOR), as proposed by Mitchell et al. [78]. Does accumulation of 2M3HB and 2AcAc result in basal ganglia lesions, leading to extrapyramidal manifestation? There are only a few reports about a direct influence of these compounds on brain function [79, 80]. They showed that these compounds inhibited aerobic energy metabolism in the TCA cycle and the mitochondrial respiratory chain and induced oxidative stress in rat brain cortex in vitro. The basal ganglia may be vulnerable if these compounds affect the human brain in vivo in a similar way.

However, these findings cannot explain why some patients have such basal ganglia lesions without severe metabolic crises. At present, we cannot predict whether a T2-deficient patient will have neurological manifestation or not. Genotype does not appear to be associated with clinical manifestation including neurological manifestation. It is possible that other genetic or environmental factors, which may enhance the toxic effect of isoleucine metabolites, are associated with basal ganglia lesions. One possible environmental factor is excess isoleucine load. Mild protein restriction (1.5–2.0 g/kg/day) has been recommended from a theoretical standpoint but is not evidence-based [5, 65]; there is no data whether this level of protein restriction results in reduced 2M3HB and 2MAcAc levels or reduced intra-brain isoleucine load. One possible genetic factor is the efficiency of monocarboxylate transporters that function through the mitochondrial and cell membrane. 2M3HB and 2AcAc are monocarboxylates; therefore, such transporters may determine intracellular or intramitochondrial concentration of 2M3HB and 2MAcAc.

Lastly, how to manage T2-deficient babies who are asymptomatic and identified by NBS or familial analysis? Fasting avoidance and sufficient glucose supplementation (including intravenous infusion) under ketogenic stresses are the basis of long-term management. Should we apply mild protein restriction to all T2-deficient newborns? Theoretically, isoleucine overload should be avoided. There is, however, no evidence for effectiveness of this treatment. Paquay et al. compared six patients with neurological manifestation and 20 patients without it and could not find

Table. 4 Neurological involvements that do not appear to be associated with severe metabolic acidosis

Patient	Consanguinity	Age at diagnosis	First acidotic episode	Before 1st crisis						Evolution		References
				Motor delay	Hypotonia	Dyskinesia	Dystonia	Ataxia	Involl. mov.		Brain MRI/CT abnormalities	
OZ1	Y	7 m	7 m	+	+					Improvement	+	[52]
OZ2	Y	FS	6Y	+						Unchanged	+	[52]
OZ3	Y	1 y	8 m	+	+					Improvement		[52]
OZ4	N	8 m	2 m	+						Severely retarded after her 4th crisis at 14 m		[52]
GK02	N	7 y	16 m	+	+					Developmentally retarded, involuntary movement		[26]
GK06	N	6 m	6 m	+					1)	Dystonia, dyskinesia, severely retarded	+	[9]
GK12	N	23 m	23 m	+	+					Improvement		[13]
GK32	Y	9 m	9 m	+						Ataxia, clumsiness, dysarthria, choreoathetoid movement		[32]
GK36 (O14)	N	26 m	NO	+				+		Mild disability		[46]
GK41		14 m	14 m	+	+				2)			[31]
GK84	N	17Y	5Y	+	+				+	At 17Y, dysarthria, generalized choreic movements, and myoclonic jerks	+	[38]
F11	Y	N FS	NO	+	+		+			From the age of 5 m, symptoms occurred	+	[47]
F17	N	3 m FS	NO	+		+	+	+		From the age of 19 m, symptoms occurred	+	[47]
F19	Y	2 m FS	5 m	+	+					Axial hypotonia,dystonia were present at 2.5Y	+	[47]
F4	N	1Y	1Y	+	+	+				Symptoms improved	Normal	[47]
F21	N	11 m	11 m							From the age of 15Y, choreoathetosis and dystonia occurred	+	[47]

1) Mild opisthotonus

2) Jittery movements

FS familial screening

NFS neonatal familial screening

Invol. mov. involuntary movement

any relationship between protein intake and neurological manifestation [47]. To reduce intramitochondrial accumulation of isoleucine metabolites, including their CoA-conjugates, L-Carnitine supplementation is also theoretically reasonable. However, L-Carnitine supplementation does not seem to be effective for prevention of neurological manifestation because L-Carnitine was supplemented in all of the six patients with neurological manifestation in Paquay's series [47]. Because this disorder is so rare, it is difficult to perform a prospective clinical trial to study whether protein restriction and L-Carnitine supplementation are protective against neurological manifestation. We also need to understand metabolic pathophysiology in the brain to elucidate the pathogenesis of neurological manifestation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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