Beta-ketothiolase deficiency

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Abstract
Beta-ketothiolase deficiency is a defect of mitochondrial acetoacetyl-CoA thiolase (T2) involving ketone body metabolism and isoleucine catabolism. This new rare disorder is characterized by normal early development followed by a progressive loss of mental and motor skills, it is clinically characterized by intermittent ketoacidotic episodes, with however, no clinical symptoms between episodes. Ketoacidotic episodes are usually severe and sometimes accompanied by lethargy/coma. Some patients may have neurological impairments as a sequela of these episodes. T2 deficiency is characterized by urinary excretion of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate, and tiglylglycine but its extent is variable. Acylcarnitine analysis is also useful for the diagnosis. However, the diagnosis should be confirmed by enzyme assay. Treatments of acute episodes include infusion of sufficient glucose and correction of acidosis. Fundamental management includes mild protein restriction and prophylactic glucose infusion during mild illness. This disorder has usually a favorable outcome. Clinical consequences can be avoided by early diagnosis, appropriate management of ketoacidosis, and modest protein restriction.

Keywords
mitochondrial acetoacetyl-CoA thiolase, beta-ketothiolase, 2-methylacetoacetyl-CoA thiolase, 2-methylacetoacetate, 2-methyl-3-hydroxybutyrare, tiglylglycine, tiglylcarnitine, ketoacidosis, ketosis, ketolytic defect, isoleucine catabolism, ketone body.

Disease name and synonyms

At least 6 thiolases can be found in human cells and all of them can be called beta-ketothiolases. However, the name "beta-ketothiolase deficiency" is exclusively used for the defect of mitochondrial acetoacetyl-CoA thiolase (EC 2.3.1.9). The specific term "mitochondrial acetoacetyl-CoA thiolase deficiency" is preferable to "beta-ketothiolase deficiency". The following terms are synonyms for beta-ketothiolase deficiency. In this text, mitochondrial acetoacetyl-CoA thiolase is abbreviated as T2.

Synonyms
2-methyl-3-hydroxybutyric acidemia,
Beta-ketothiolase deficiency
Mitochondrial acetoacetyl-CoA thiolase deficiency
3-oxothiolase deficiency,
3-ketothiolase deficiency,
2-methylacetoacetyl-CoA thiolase deficiency

Diagnostic criteria/Definition
Mitochondrial acetoacetyl-CoA thiolase is responsible for cleavage of 2-methylacetoacetyl-CoA in isoleucine metabolism, acetoacetyl-CoA formation in ketogenesis and acetoacetyl-CoA cleavage in ketolysis (1). Hence ketone body metabolism and isoleucine catabolism are disturbed in T2 deficiency. This disorder is clinically characterized by intermittent ketoacidotic episodes, sometimes accompanied by unconsciousness. Ketoacidotic episodes usually follow gastroenteritis, other infections, fasting or other stresses, which are the causes of physiological ketosis in normal children. Hence, the diagnosis should be considered if a patient develops or has previously experienced ketoacidosis more severe than the physiological response to stress.
Urinary organic acid analysis, even between ketoacidotic episodes, usually enables physicians to make the diagnosis of T2 deficiency. Excessive excretion of 2-methyl-3-hydroxybutyrate, tiglylglycine, and 2-methylacetoacetate is a typical organic acid profile of this disorder in urine both during or between ketoacidotic episodes. However, it should be stressed that the absence of tiglylglycine or 2-methylacetoacetate cannot exclude the diagnosis (see diagnostic methods). The most reliable marker is the excessive excretion of 2-methyl-3-hydroxybutyrate. The deficiency in 2-methyl-3-hydroxyacyl-CoA dehydrogenase which catalyzes the step just upstream to the T2 step in isoleucine catabolism was recently discovered in a patient who was primarily suspected of presenting with T2 deficiency (2). This new disorder is characterized by normal early development followed by a progressive loss of mental and motor skills. This disorder should be considered if patients with urinary excretion of tiglylglycine and 2-methyl-3-hydroxybutyrate appear to have normal T2 activity (see the paragraphs for differential diagnosis).
Enzymatic diagnosis is essential to confirm T2 deficiency. If a patient is clinically suspected of presenting with T2 deficiency or succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency , and even if 2-methyl-3-hydroxybutyrate is not identified in urine, enzymatic confirmation for both disorders is highly recommended (See diagnostic methods)

Prevalence
Over 40 cases have been dealt with in publications and more than 20 other patients have been reported (3-44). There is no particular ethnic predisposition: patients have been reported in the Netherlands, Germany, France, Switzerland, Spain, Italy, the UK, Norway, Canada, the USA, Brazil, Argentina, Chile, Australia, Saudi Arabia, Israel, Yemen, Tunisia, Laos, Vietnam, China, and Japan.

Clinical description
T2 deficiency is clinically characterized by intermittent ketoacidotic crises with however no clinical symptoms between these episodes. This disorder is clinically heterogeneous (1, 45, 46). In the first part, the typical clinical course of T2 deficiency will be summarized and then atypical clinical features described.
T2 deficient patients usually have no clinical symptoms in the neonatal period and early infancy. In most cases the first ketoacidotic attack occurs when they are between 5 months and 2 years old. Ketoacidotic events usually follow gastroenteritis and/or febrile illness such as upper respiratory infection, measles, or otitis media. Clinical symptoms of ketoacidotic crisis include vomiting, dehydration, polypnea and/or dyspnea, hypotonus, lethargy which is sometimes followed by coma. Some patients develop convulsion. A febrile state followed by unconsciousness and convulsion mimics encephalitis/ encephalopathy. Blood gas analysis reveals severe metabolic acidosis with partial respiratory compensation. Blood PH is usually less than 7.1, bicarbonate less than 7 mmol/L, and base excess less than -20mmol/L. Blood glucose level is generally normal, however, it varies from 0.6 mmol/L (hypoglycemia) to 12.7 mmol/L (hyperglycemia), the former mimics ketotic hypoglycemia (18) and the latter mimics diabetic ketoacidosis (33, 38). Hyperammonemia is rare but was noted (Max 307 μmol/L) in some patients during crises. However, hyperammonemia in T2 deficiency appears to be less severe than in isovaleric acidemia and propionic acidemia. Intravenous fluid therapy with glucose and alkali such as sodium bicarbonate improves the general condition of the patient. Among 39 reported cases, 4 patients died during ketoacidotic attacks or died of sequelae of the attacks. Moreover, it is noteworthy that 9 siblings of these patients died when they were less than two years of age, possibly due to ketoacidotic attacks. About one third of the patients showed

psychomotor or mental developmental delay, truncal ataxia, or hypotonia as a sequela of ketoacidotic attacks whereas the others recovered completely.
Fukao *et al.* (46) recently summarized the clinical phenotypes and outcome of T2 deficiency in 26 enzymatically proven and mutation-defined patients (complete data are available at http://www.gifu-u.ac.jp/~fukao2/T2Dtable1.htm). The follow-up observations showed that in general T2 deficiency has a favorable outcome and that three patients had impaired cognitive development, one had ataxia, and one had died. These consequences seemed to be sequelae of severe ketoacidotic crises. About half of the patients were diagnosed as T2-deficient at or after the first ketoacidotic crisis and experienced no further crises. Three of the 26 patients were diagnosed as T2-deficient in familial analyses and have never experienced ketoacidotic crisis. Hence, clinical consequences of T2 deficiency can be avoided by early diagnosis, appropriate management of ketoacidosis, and modest protein restriction.
A T2-deficient woman was successfully pregnant and delivered a baby (43). The patient excreted typical urinary organic acids during her pregnancy with a low level of serum carnitine (free 7.9 μM/L, total 17.2 μM/L). She was receiving carnitine supplementation and delivered her baby who was a heterozygote carrier of T2 deficiency and had had no clinical symptoms so far.
Neonatal onset is rare in T2 deficiency. One out of the 26 patients presented with mild neonatal ketoacidosis (46) .
Dilated cardiomyopathy was reported in an atypical T2-deficient patient. However, her fibroblasts showed reduced CO_2 production from isoleucine but T2 activity was not assayed (6).
Three patients from Saudi Arabia and Yemen (32) were different from typical T2- deficient patients:
1) They all showed developmental delay before the first ketoacidotic attacks.
2) Brain MRI of these patients showed bilateral external capsule lesions which increase T2 intensity laterally within the posterior half of the putamen, bilaterally continuing up into the lower part of the corona radiata.
Similar MRI findings were noted in one of the 26 patients whose T2 gene mutation was confirmed (23, 46). Tunisian patients were also reported to have neurological involvement (44).

Differential diagnosis
Differential diagnosis includes all the disorders causing ketoacidosis (ketosis) (1). Ketosis should be assessed together with blood glucose levels. Although the presence of hypo/hyperglycemia can not exclude T2 deficiency, normoglycemia is common in this disorder. Extreme hyperglycinemia with ketosis suggests the presence of diabetes. In the case of hypoglycemia accompanied by ketosis, we should consider several conditions and disorders:
1) hormonal defects such as glucocorticoid deficiency and growth hormone deficiency;
2) defects in glucose and glycogen metabolism including glycogen synthase deficiency;
3) ketotic hypoglycemia
During an acute ketotic/ketoacidotic episode, at least levels of blood gas, blood glucose, lactate, pyruvate, ammonia, and urinary organic acids should be examined. These data can exclude congenital lactic acidosis, other organic types of aciduria such as methylmalonic, propionic, isovaleric acidemias. Salicylate poisoning can cause ketoacidosis and ketone bodies can produce a false positive result in some screening tests for salicylate.

Two major differential diagnoses must be considered.
From the point of view of clinical similarity,
Succinyl-CoA:3-ketoacid CoA transferase (SCOT) deficiency
Ketotic hypoglycemia
Recurrent ketosis of childhood
Ketotic hypoglycemia and recurrent ketosis of childhood are common causes of ketosis in childhood. So, if ketosis is more severe than that in typical hypoketotic hypoglycemia or recurrent ketosis of childhood, T2 deficiency and SCOT deficiency should be considered. T2 deficiency can be suspected by the urinary organic acid profile but SCOT deficiency has no characteristic organic acid profile. However, as suggested above, enzymatic confirmation for both T2 deficiency and SCOT deficiency should be preferred.
SCOT deficiency is also characterized by intermittent ketoacidotic episodes with however no clinical symptom between these episodes. About half of SCOT-deficient patients have neonatal onset although neonatal onset of T2 deficiency is rare. Permanent ketosis is the most prominent feature of SCOT deficiency. Even in the postprandial period, patients usually have a high blood ketone body level. But a recent study showed that permanent ketosis is not always present in all SCOT-deficient patients (for further information, see the text on succinyl-CoA:3-ketoacid CoA transferase deficiency).

From the point of view of similarity in organic acid profile,

2-methyl-3-hydroxyacyl-CoA dehydrogenase (MHBD) deficiency

2-methyl-3-hydroxyacyl-CoA dehydrogenase deficiency was discovered very recently in a patient who was initially suspected as T2-deficient but whose fibroblasts had normal T2 activity (2). From the literature, urinary organic acid analysis showed marked excretion of tiglylglycine and 2-methyl-3-hydroxybutyrate. Even after an oral isoleucine challenge with 100 mg/kg body weight, there was no elevation in 2-methylacetoacetate. These data suggested that this patient had a block at step from 2-methyl-3-hydroxybutyryl-CoA to 2-methylacetoacetyl-CoA, one step upstream to the defect in T2 deficiency. The patient developed metabolic acidosis with severe hypoglycemia, had elevated lactate level, mild hyperammonemia, and ketonuria on the second day of life. No further acidotic episode occurred. The patient was severely retarded with marked restless, choreathetoid movements, marked hypotonia. In these 2 years, several patients with this disorder have been reported (70-75). MHBD deficiency is characterized by normal early development followed by a progressive loss of mental and motor skills. Although the first patient developed metabolic acidosis, clinical manifestation of MHBD deficiency is different from that of T2 deficiency. Recently MHBD deficiency revealed to be a defect in a X-chromosomal gene, HADH2 (75). MHBD deficiency has an X chromosome-linked recessive trait. At present, if patients with the above urinary organic acid profile appear to have normal T2 activity, this disorder is a candidate for possible diagnosis.

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Management including treatment

General management

It is important to avoid development of severe ketoacidosis.

Diet

Mild restriction of protein intake (1.5-2.0 g/kg/day) is a fundamental and reasonable treatment to reduce isoleucine load. Fat-rich diet induces ketogenesis, hence, should be avoided. There is no clear answer as to when protein restriction can be safely stopped. If patients have been used to the diet, there seems to be no reason to stop the diet. Some T2-deficient cases whose T2 activity was null tolerated normal protein intake without ketoacidotic events (9, 20, 39). However, this does not mean that mild protein restriction in T2 deficiency is not required. Our survey of 26 T2-deficient patients showed that the latest ketoacidotic episode occurred at the age of 10 years in one patient, but most ketoacidotic episodes occurred by 5-6 years of age (46). This information may serve as a reference. At least protein and fat-rich diets are not recommended in even adult patients.

Prophylactic glucose intake/infusion

Prolonged fasting should be avoided. If the patient is febrile or vomits, intravenous glucose infusion should be considered. It is a good idea to check the patient's urinary ketone body using a ketostick at home. If ketone tests are weakly positive in milder illness, some carbohydrate-rich snack or drink should be provided; If ketonuria is moderate, medical help should be considered.

Carnitine supplementation

L-Carnitine supplementation (50-200 mg/kg/day) may be considered, particularly if patients have low carnitine levels. The total carnitine level was reported to be decreased in some T2-deficient patients (36, 38, 40).

Management of acute episodes

It is important to suppress ketogenesis and to correct acidosis during acute episodes. Although the diagnosis is not usually confirmed during the first ketoacidotic crisis, the treatment of T2-deficient ketoacidosis is basically treated like ketoacidosis. Physicians recognize the patient to be in an emergency state by symptoms of vomiting, polypnea or dyspnea, hytotonus, unconsciousness. Routine laboratory tests (electrolytes, blood gas, glucose, ammonia, urinalysis, etc) may indicate severe metabolic acidosis, dehydration, with/without hyperammonemia, with massive ketonuria. For further evaluation, sample collection of lactic and pyruvic acids, amino acid analysis, urinary organic acid analysis at initial stage are usually important.

Even in the patient showing normoglycemia, sufficient glucose infusion to maintain blood glucose levels in the upper normal is important to suppress ketogenesis. Intravenous fluid with glucose and appropriate electrolytes should be administered to keep sufficient urine output. The treatment of metabolic acidosis is controversial. Initially a slow bolus of bicarbonate (1 mmol/kg over 10 min) may be given followed by continuous infusion. The level of blood gas and electrolytes should be frequently checked to avoid a rapid correction and hypernatremia. Aggressive alkalization maybe deleterious and can cause hypernatremia, hyperosmolarity, and paradoxycal central nervous system acidosis.

Peritoneal dialysis was not performed in the reported cases except for one patient who was treated successfully with bicarbonate-based peritoneal dialysis. Invasive methods like dialysis are effective but rarely necessary. Carnitine supplementation may be considered to facilitate excretion of accumulated acyl-CoAs as acylcarnitines to urine. Some patients needed supportive therapy like mechanical ventilation for dyspnea or unconsciousness.

Etiology

T2 role in ketone body metabolism and Isoleucine catabolism

T2 is an important enzyme for ketogenesis in the liver and for ketolysis in extrahepatic tissues as well as for isoleucine catabolism.

Ketone body metabolism

Ketone bodies are important vectors of energy from the liver to extrahepatic tissues, especially during shortage of glucose. In hepatic mitochondria, ketone bodies are produced using excess of acetyl-CoA mainly derived from accelerated fatty acid beta-oxidation. T2 plays a role in the formation of acetoacetyl-CoA from acetyl-CoA. The reaction is followed by acetoacetate production via mitochondrial HMG-CoA by HMG-CoA synthase and HMG-CoA lyase. In extrahepatic tissues, acetoacetate is activated as acetoacetyl-CoA by SCOT, then T2 mediates thiolysis from acetoacetyl-CoA to acetyl-CoA. Acetyl-CoA is then used as an energy source via the TCA cycle. Hence T2 plays a role in both ketogenesis in the liver and ketolysis in extrahepatic tissues. Since ketoacidosis is a main feature of T2 deficieny, T2 seems to be more important for ketolysis than ketogenesis. Another mitochondrial medium-chain 3-ketoacyl-CoA thiolase, which plays a role mainly in the beta-oxidation spiral, can also catalize acetoacetyl-CoA formation. This enzyme can compensate for the T2 defect in ketogenesis. In ketolysis in extrahepatic tissues, this enzyme may also compensate in part for the lack of T2 in acetoacetyl-CoA thiolysis.

Isoleucine catabolism

T2 also catalyzes thiolysis of 2-methylacetoacetyl-CoA in isoleucine catabolism. T2 is believed to catalyze this reaction exclusively. Hence, 2-methylacetoacetyl-CoA is accumulated in T2 deficiency. Since the two reactions usptream to T2 reaction are reversible, 2-methyl-3-hydroxybutyryl-CoA and tiglyl-CoA are also accumulated. The characteristic urinary organic acids are derived from these compounds. Since 2-methylacetoacetate and its decarboxylated form, 2-butanone, are volatile, they cannot always be detected. Mitochondrial medium-chain 3-ketoacyl-CoA thiolase is abundant in the liver and has a very low activity toward 2-methylacetoacetyl-CoA (10). T2 and this medium-chain thiolase may contribute to 92% and 8%, respectively, of total 2-methylacetoacetyl-CoA cleavage in rat liver. If medium-chain 3-ketoacyl-CoA thiolase in humans has similar conditions, this might mean that patients with complete T2 deficiency still have some residual aceacetyl-CoA and 2-methylacetoacetyl-CoA thiolase activity by the medium-chain 3-ketoacyl-CoA thiolase.

T2 protein and gene

Human T2 is a homotetramer of the 41-kDa subunit (47). T2 protein is widely expressed and abundant in the liver, kidney, heart, and adrenal gland in humans (48). Human T2 cDNA is about 1.5 kb long and encodes for a precursor of 427 amino acids, including a 33-amino acid leader polypeptide (47). The human T2 gene spans approximately 27 kb, contains 12 exons and 11 introns (49), and is located at 11q22.3-q23.1(gene locus ACAT; 50). The 5' flanking region lacks a conventional TATA box but is GC-rich and contains two CAAT boxes, suggesting that T2 gene is a housekeeping gene. Mutations in T2-deficient patients are heterogeneous and there is no common mutation as far as we can determine. A table for T2 gene mutations in the 26 T2-deficient patients is available (http://www.gifu-u.ac.jp/~fukao2/T2Dtable2.html)

Diagnostic methods

Metabolite

Urinary organic acid analysis or blood acyl-carnitine analysis gives a diagnostic clue for T2 deficiency.

Urinary organic acid analysis

A typical organic acid profile of an acute episode is massive excretion of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate, and tiglylglycine with massive excretion of ketone bodies and dicarboxylic acids (1, 51, 52). Even in the urine between crises, 2-methyl3-hydroxybutyrate and tiglylglycine with/without 2-methylacetoacetate is detected in the case of typical T2 deficient patients.

However, there are considerable variations in the patterns and amounts of abnormal organic acids excreted by T2-deficient patients. It is difficult to specify a typical quantitative profile. Fukao *et al*. also identified sibling cases of the mild form of T2 deficiency (39). The proband has never excreted detectable tiglylglycine even during an acute episode. The elder asymptomatic brother who also excreted only a

small amount of 2-methyl-3-hydroxybutyrate, was confirmed as T2-deficient. Tiglylglycine was not detected in 7 of the 26 T2-deficient patients and 5 of them had mild gene mutations. Hence, the most characteristic elevated urinary metabolite of T2 deficiency is 2-methyl-3-hydroxybutyrate.
Recently, we examined urinary organic acid profiles in 5 Japanese patients during non-episodic condition. T2 deficient patients with null mutations in either mutant allele had typical profiles for the T2 deficiency, however, in T2 deficient patients with mutation(s) retaining some residual T2 activity in at least one of two mutant alleles, tiglylglycine was not or only faintly detected and the 2-methyl-3-hydroxybutyrate levels were less than cutoff value in our system. This indicates that mild form of T2 deficiency can be misdiagnosed as normal if the analysis is performed under non-episodic conditions (68)
As described in the differential diagnosis, 2-methyl-3-hydroxyacyl-CoA dehydrogenase deficiency has a very similar organic aicd profile to T2 deficiency (2), hence it is very difficult to distinguish these disorders by organic acid analysis alone. Propionic acidemia and methylmalonic acidemia also affect isoleucine catabolism downstream to T2 deficiency and 2-methyl-3-hydroxybutyrate and tiglylglycine are also detected in these disorders, however, one can make the difference between T2 deficiency and propionic acidemia and methylmalonic acidemia by the absence of 3-hydroxypropionic acid, methylcitric acid and propionylglycine.

Acylcarnitine analysis
Acylcarnitine analysis in urine or dried blood spotted on Guthrie paper is a very useful diagnostic tool in the various types of organic acidemia and beta-oxidation defects (32, 38). The presence of tiglylcarnitine and 2-methyl-3-hydroxybutyrylcarnitine suggests T2 deficiency. Recently, we examined acylcarnitine profiles from blood spots in 5 Japanese patients during non-episodic condition. T2 deficient patients with null mutations in either mutant allele had typical profiles for the T2 deficiency, however, in T2 deficient patients with mutation(s) retaining some residual T2 activity in at least one of two mutant alleles, their C5:1 carnitine levels were within the normal range and OH-C5 carnitine was detected just around the cutoff value in our newborn screening pilot test. Hence, this analysis under stable conditions is not reliable for diagnosing mild form of T2 deficiency. This indicates that mild form of T2eficiency can be misdiagnosed as normal if the analysis is performed under non-episodic conditions and possibly during the newborn screening for inborn errors of metabolism. (68)
Similar consideration with organic acid analysis can apply to 2-methyl-3-hydroxyacyl-CoA dehydrogenase deficiency and other types of organic aciduria in isoleucine metabolism.

Enzyme assay
As described above, enzyme assay is required for the final diagnosis of T2 deficiency.
There are two kinds of assays available: either direct in vitro assays and or indirect assays.

Direct assays for T2

Potassium-ion dependent acetoacetyl-CoA thiolase assay:
T2 is the only thiolase that is activated in the presence of potassium ions. The difference between acetoacetyl-CoA thiolase activities in the absence and presence of potassium ions is derived from T2 (8). This method is very simple and no specific substrate is necessary, hence, it is most commonly used for evaluation of T2 activity. This assay can also be carried out on peripheral blood mononuclear cells or PBMC (lymphocytes) or polymorphonuclear cells (15). Epstein Barr or EB virus-transformed lymphoblasts have relatively high thiolase activity and reliable source of enzyme assay (39). The ratios of acetoacetyl-CoA thiolase activities in the presence/absence of potassium ions in fibroblasts, PBMCs and EB-transformed lymphoblasts are approximately 1.0 in the case of T2 deficient whereas it is approximately 1.7-2.3 for controls and 1.3-1.9 for heterozygous carriers. However, it is sometimes difficult to make the distinction between carriers and controls by this assay (53).

2-methylacetoacetyl-CoA thiolase activity
a more specific and sensitive assay using a specific substrate for T2, 2-methylacetoacetyl-CoA, was developed by Middleton and Bartlett (10). Although this substrate is not stable and not marketed, residual T2 activity can be evaluated more sensitively than in the method described above. Most T2-deficient patients have activity of 0 to 4 percent of normal in fibroblasts using this substrate.

A coupled assay detecting defects distal to enoyl-CoA hydratase in isoleucine catabolism
Tiglyl-CoA was incubated with $NaH^{14}CO_3$ and measured fixation into [^{14}C]methylmalonyl-CoA through 2-methyl-3-hydroxybutyryl-CoA, 2-methylacetoacetyl-CoA, and propionyl-CoA. In this assay, endogeneous crotonase (eneyl-CoA hydratase), 2-methyl-3-hydroxyacyl-CoA

dehydrogenase, T2, and propionyl-CoA carboxylase are involved. Hence it is useful for screening defects in isoleucine catabolic pathway. However, as discussed above, theoretically, T2 deficiency can not be distinguished from 2-methyl-3-hydroxyacyl-CoA dehydrogenase deficiency. Five patients with T2 deficiency had about 2 percent of normal activity (54). However, one patient with the T2 deficiency (LV, our laboratory number GK03) was reported to have exceptionally high activity (8.7±3.7 pmol/min per mg protein; control values 32±23 pmol/min per mg protein) compared to other T2-deficient patients (0.7±0.5 pmol/min per mg protein) in this assay. Middleton et al. reported that GK03's fibroblasts have low but significant residual T2 activity (6.6 nmol/min/mg protein; control values 89±11 nmol/min/mg protein; typical T2-deficient fibroblasts < 4.0 nmol/min/mg protein), using the T2 specific assay with 2-methylacetoacetyl-CoA (10). Taken together, even very low residual T2 activity may result in much higher residual activity in the coupled assay using tiglyl-CoA.
Recently, we diagnosed two patients (GK45 and GK47) as T2-deficient by the absence of potassium ion-activated acetoacetyl-CoA thiolase activity whereas these patients were previously misinterpreted as normal by the coupled assay with tiglyl-CoA as a substrate. One of them was previously reported as "a new case of 2-methylacetoacetyl-CoA thiolase deficiency?"(65) since the coupled assay resulted in the normal activity. Expression analysis of mutant cDNAs clearly showed that GK45 and GK47 had "mild" mutations (A132G, D339-V340insD) which retained some residual T2 activity, at least one of two mutant alleles. These results raise the possibility that T2-deficient patients with "mild" mutations have been misinterpreted as normal by the coupled assay with tiglyl-CoA(69).
So, now a combination of the specific assay and this assay becomes useful.

Molecular diagnosis
T2 protein detection by immunoblot analysis: If T2 protein cannot be detected or is extremely decreased, this observation re-confirmed T2 deficiency (53,55, 56). However, some mutations may not destabilize T2 protein but affect enzyme activity. Immunoblot analysis has been used in our lab as an complementary diagnostic method after enzyme assay.
Mutation analysis: T2 cDNA is about 1.5 kb long and can be amplified as two overlapping fragments (47). The T2 gene has 12 exons and all primer sequences to amplify T2 cDNA and each exon are now available (57,58). However, in T2 deficiency there is no common mutation and gene mutations are highly heterogeneous among T2-deficient families. We have identified more than 30 different mutations and missense mutations were confirmed as causative mutations in a mutant cDNA expression assay (23,28, 31,34, 35, 42, 43, 46, 57, 59-62). Hence, if mutations identified in a new patient are the same as those previously identified, diagnosis at the gene level can be made by sequencing alone. However, a new missense mutation should be confirmed as causative by cDNA expression analysis. There is no clear phenotype/genotype correlation in T2 deficiency (46).

Genetic counseling and prenatal diagnosis
T2 deficiency is an autosomal recessive disorder. As far as we know, no heterozygous carrier has clinical symptoms. It is important to identify asymptomatic T2-deficient siblings in family analysis to avoid them developing ketoacidotic crises in the future.
Prenatal diagnosis can be made by measurement of T2 activity in cultured amniocytes or chorionic villus cells, since these cells express sufficient T2 activity to analyze (63). Prenatal diagnosis was reported in a family in which mutations had been identified (64). Combination of mutation detection by restriction enzyme assay with restriction fragment length polymorphisms and immunoblot analysis were used for the analysis.
If parents wish to have prenatal diagnosis of T2 deficiency, physicians should give the parents the following observations made in 26 enzymatically-confirmed patients and discuss prenatal diagnosis:
1) neonatal onset is rare in T2 deficiency.
2) acute decompensation can be avoided if the diagnosis is known.
3) Neurological complications seem to be as a sequelae of severe ketoacidotic crises.
My personal opinion is that prenatal diagnosis is not necessary in T2 deficiency.

Unresolved questions

Neurologic impairment: neurological impairment is thought to be a sequela of severe ketoacidotic crises
In fact, most T2-deficient patients achieved normal growth and development. However, some patients in Saudi Arabia, Yemen, and Tunisia had a high incidence of severe neurological impairments, compared with the series of the 26 T2-deficient patients. Some of them had neurological abnormalities before the first crisis. What makes this difference? Do

environmental factors such as climate and foods influence the phenotype of patients with T2 deficiency? Do they have special mutations?

There are patients who are suspected to be T2-deficient relying on clinical features and urinary organic acid profile but whose T2 activity is normal (65)

At least, some of the patients with similar urinary organic acid profile and normal T2 activity may have 2-methyl-3-hydroxyacyl-CoA dehydrogenase deficiency. At present we have no data as to what percentage of such patients have this disorder. There are also a lot of patients who develop severe and non-physiological ketoacidotic crises but whose T2 and succinyl-CoA:3-ketoacid CoA transferase activities are normal. Is there another metabolic disorder triggering intermittent ketoacidotic crises? That remains to be determined.

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