Mitochondrial acetoacetyl-CoA thiolase (T2) catalyzes 2-methylacetoacetyl-CoA cleavage into acetyl-CoA and propionyl-CoA in isoleucine catabolism and interconversion between acetyl-CoA and acetoacetyl-CoA in ketone body metabolism. T2 deficiency is a rare metabolic disease of autosomal recessive inheritance. The disorder is characterized by intermittent ketoacidotic episodes. The onset of clinical symptoms is in the infant or toddler period. The frequency of episodes declines with age, stopping before adolescence. Here we report two siblings with this disorder. The proband (GK65) is a French girl born from non-consanguineous parents. She presented several ketoacidotic episodes with 5 hospitalizations from age 2 to 4 years, the first of them complicated by ketoacidotic coma. Minor episodes, which are generally provoked by infections or high protein intake, still persist at age of 16 years. Molecular analysis of the T2 gene has revealed the compound heterozygosity of c.578T>C (M193T) and IVS8+5g>1. The latter mutation results in skipping of exon 8. In contrast, the younger brother (GK65b) had a unique ketoacidotic crisis at the age of 6 years that is the oldest-age first crisis among T2-deficient patients reported thus far. Despite the mild phenotype, he carried the same T2 gene mutations as his sister (GK65). Furthermore, T2 catalytic activity and T2 protein were not detected in the fibroblasts derived from GK65 and GK65b. In conclusion, the siblings with the same T2 gene mutations present different clinical severity. Diagnostic testing for asymptomatic siblings is important in the management of T2-deficient families.

Keywords: T2 deficiency/mitochondrial acetoacetyl-CoA thiolase deficiency/β-ketothiolase deficiency/genotype/phenotype correlation/mutation


Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency (OMIM 203750) is an inborn error of metabolism that affects the catabolism of isoleucine and ketone bodies. It is a rare disease of autosomal recessive inheritance with less than 100 patients described in the literature. This disorder, first described by Daum et al. (1971), is characterized by intermittent episodes of metabolic ketoacidosis associated with vomiting and unconsciousness often triggered by infections (Fukao et al. 2001). There are no clinical symptoms between episodes. Typical T2 deficiency (T2D) is easily diagnosed by urinary organic acid analysis, characterized by massive excretion of tiglylglycine, 2-methyl-3-hydroxybutyrate and 2-methylacetoacetate (Fukao et al. 2001, 2003). T2D usually has a favorable outcome (Fukao et al. 2001), but there is a risk of death and neurological sequelae from an acute ketoacidotic episode. Diagnosis is confirmed by measurement of T2 activity on cultured skin fibroblasts (Zhang et al. 2004). T2D is caused by mutations in the ACAT1 (T2) gene located on chromosome 11q22.3-q23.1 (Fukao et al. 1990; Kano et al. 1991). T2D is very heterogeneous at the genotype level with at least 50 different mutations described (Fukao et al. 1995, 2001).

We present here the cases of two siblings diagnosed with T2D who were quite different in terms of onset and frequency of ketoacidotic episodes.

Clinical Report

The proband (GK65) is a French girl born from non-consanguineous parents in 1992. She was well until 25...
months of age when she presented her first ketoacidotic episode following rhinopharyngitis. After a 48-hour period of anorexia and vomiting she was admitted to hospital because of alterations of consciousness and dyspnea. Blood gas analysis revealed severe metabolic acidosis with a pH of 6.98 and HCO₃ of 3.5 mmol/l. Urine dipstick testing showed massive ketonuria. Additional standard blood tests were normal. Clinical improvement was obtained by intravenous glucose and bicarbonate infusion therapy within 36 hours. There have not been any neurological sequelae due to this severe ketoacidotic coma.

Metabolic analyses were performed during the acute episode. Urinary organic acid chromatography revealed massive excretion of tiglylglycine (330 µmol/mmol creatinine), 2-methyl-3-hydroxybutyrate (692 µmol/mmol creatinine) and 2-methylacetacetate (non-quantifiable), characteristic of T2 deficiency. Diagnosis was then confirmed by enzymatic measurement of T2 activity on cultured skin fibroblasts by a coupled assay with tiglyl-CoA as the substrate, as previously reported (Gibson et al. 1992). T2 activity markedly decreased in the cells from our patient (3% of control cells). Under stable conditions after this first episode, the urinary organic acid profile still showed excretion of 2-methyl-3-hydroxybutyrate (148 µmol/l for a normal value < 14), but no significant excretion of tiglylglycine.

There were four further hospitalizations for less severe ketoacidotic episodes which occurred until the age of 4 years, three of them following infections. Mild episodes, mostly induced by infections, once or twice per year, were successfully managed by the patient and his parents at home and still persisted at age 16 years. Attacks were also provoked by high protein intake (such as an egg and a steak during the same meal). Episodes are now easily controlled by sweet liquid, such as coke. Bicarbonate mixed with coke was the treatment of choice before age 10.

The youngest brother (GK65b), born in 2000, presented his first and up to now the only ketoacidotic episode at the age of 6 years following rotavirus gastroenteritis. He suffered from fever, anorexia and vomiting for about 3 days. At hospitalization he presented general alteration and dyspnea. Blood pH was 7.15 with HCO₃ of 9.8 mmol/l. He also presented massive ketonuria. T2 deficiency was suspected because of his sister’s medical history and the characteristic urinary organic acid profile.

The parents had not taken him to metabolic counselling and no urinary organic acid analysis had been performed until this severe episode. The boy didn’t present any ketoacidotic episode earlier in life despite infections like chickenpox and several operations with general anesthesia (hydrocella at the age of 3 years, adenoidectomy at the age of 5 years, transtympanic drainage at the age of 6 years). Another operation performed after the diagnosis of T2D at 7 years of age (transtympanic drainage), as well as high protein intake, didn’t provoke any attack either. Diagnosis of T2D was confirmed by enzymatic analysis on cultured fibroblasts of GK65 and GK65b.

Table 1. Acetoacetyl-CoA thiolase activities in the absence and presence of potassium ions.

<table>
<thead>
<tr>
<th>Fibroblasts</th>
<th>acetoacetyl-CoA thiolase activity</th>
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<tbody>
<tr>
<td></td>
<td>−K⁺</td>
</tr>
<tr>
<td>Present control</td>
<td>3.6</td>
</tr>
<tr>
<td>GK65</td>
<td>3.8</td>
</tr>
<tr>
<td>GK65b</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Enzyme activity is expressed as nmol/min/mg protein. Potassium-ion-activated acetoacetyl-CoA thiolase activity was not detected in the fibroblasts of GK65 and GK65b since the ratio of +K⁺/−K⁺ was 1.0.
skin fibroblasts by measuring T2 activity using acetoacetyl-CoA as the substrate with/without potassium ions (Robinson et al. 1979).

The fibroblasts from GK65 and GK65b were re-examined simultaneously at Gifu University in 2008. The result of a T2 enzyme assay using acetoacetyl-CoA as the substrate with/without potassium ions is shown in Table 1. No potassium ion-activated acetoacetyl-CoA thiolase activity was detected in GK65 or GK65b. Immunoblot analysis showed that T2 protein was not detectable in the samples from GK65 and GK65b, whereas succinyl-CoA:3-ketoacid CoA transferase (SCOT) protein was clearly detected in all samples (Fig. 1). Routine mutation screening at the genomic level (Fukao et al. 2007) revealed that both patients were compound heterozygotes of c.578C>T (M193T) from the mother and IVS8+5g>t from the father. cDNA analysis of the siblings revealed the presence of exon 8 skipping in about half of their cDNA clones. This result indicates that IVS8+5g>t caused the exon skipping.

**Discussion**

We have described two sibling patients affected by T2 deficiency with quite different clinical presentations. The older sister (GK65) presented several episodes of decompensation with 5 hospitalizations from age 2 to 4 years, the first of them complicated by ketoacidotic coma. Minor episodes, which are generally provoked by infections or high protein intake, still persist at age 16 years. The younger boy (GK65b) did not have urinary organic acid analysis, although he was born after the confirmation of his sister’s diagnosis, and presented only one ketoacidotic attack at the age of 6 years.

The case presentations of GK65 and GK65b include important messages. First, the most important management of T2D is the prevention of severe ketoacidotic crises. GK65b did not have any presymptomatic tests despite the diagnostic confirmation of T2D in GK65 before GK65b’s birth. If GK65b had been analyzed by urinary organic acid analysis after his birth, he could have been diagnosed as having T2D and the severe ketoacidotic episode at the age of 6 might have been avoided. A presymptomatic test can accurately detect the existence of an underlying potential T2D condition. Presymptomatic diagnosis of T2D prevents severe ketoacidosis and its risk of sequelae or even death. Second, to our knowledge, the first ketoacidotic crisis at the age of 6 years is the oldest-age first crisis among T2 deficient patients to date. Fukao et al. (2001) described the median onset of symptoms at the age of 15 months (3 days to 48 months) in 26 cases of enzymatically confirmed T2D. GK65b had not experienced any ketoacidotic crisis in spite of infections like chickenpox and several operations with general anesthesia. However, at the age of 6 years, rotavirus gastroenteritis induced severe ketoacidotic crisis. Gastroenteritis is indeed the most frequent pre-existing condition for ketoacidotic crises in T2D subjects. The different clinical courses between these siblings might be based on the possibility that the parents’ attention to the elder sister prevented a severe ketoacidotic crisis in the younger boy beforehand. Third, the elder sister, GK65, still experiences minor episodes at the age of 16 years. Those episodes occurred once or twice a year with urinary ketone-positive manifestations. They were easily managed at home by the patient and her parents. The frequency of attacks in T2D decreases with age, the last being reported at the age of 10 years in a previous follow-up study (Fukao et al. 2001). Nevertheless, we cannot rule out the possibility that some patients in the follow-up study also experienced such mild episodes without reporting them. Though GK65 experienced ketotic events even after the age of 10 years, the fact that such events could be managed at home is also informative.

Molecular analysis revealed that the affected siblings
are compound heterozygotes of M193T from the mother, and IVS8+5g>t from the father. Most reported mutations in T2D are single-based substitutions resulting in nonsense, missense, or splice mutations of the T2 gene (Fukao et al. 1995, 2001). T2 gene deletion and tandem duplication also have been reported (Zhang et al. 2006; Fukao et al. 2007).

The IVS8+5g>t mutation resulted in a drastically reduced Shapiro and Senapathy score (Shapiro and Senapathy 1987) at the authentic splice donor site of intron 8 from 82 (TG/tgtatt) to 68 (TG/gttatt). Exon 8 skipping was identified in almost half of the cDNA clones from GK65 and GK65b and no other nucleotide substitutions in exon 8 or surrounding introns (~100bp) were identified. The importance of G at position +5 was previously well documented. Buratti et al. (2007) summarized 346 aberrant splice donor sites that were activated by mutations in 166 human diseases. Point mutations leading to cryptic splice donor site activation were most common in the first intron nucleotide, followed by the fifth nucleotide. Substitutions at position +5 were exclusively g>a transitions. In our case, no cryptic splice donor site was apparently activated and the substitution was g>t at position 5. We previously identified several mutations, which resulted in exon 8 skipping in the T2 gene. Among them, IVS8+1g>t caused exon 8 skipping in almost all transcripts (Fukao et al. 1992) and c.816C>T (Q272X) caused exon 8 skipping in some transcripts (Fukao et al. 1994). In both cases, no aberrant splicing variants using any other cryptic splice sites were identified. This may indicate no available cryptic splice donor site in intron 8. We concluded that IVS8+5g>t is responsible for exon 8 skipping in GK65 and GK65b.

T2D is very heterogeneous at the genotype level with at least 50 different mutations described (Fukao et al. 1995, 2001). We identified two novel T2 gene mutations in this family. No genotype-phenotype correlation could be identified and mutant siblings can thus present different clinical severity (Fukao et al. 1995, 2001), as these siblings did. T2D usually has a favorable outcome, but there is a risk of death and neurological sequelae due to an acute ketoacidotic episode (Ozand et al. 1994; Fukao et al. 2001). Hence diagnostic testing for asymptomatic siblings is important in the management of T2D families.

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Siblings with β-ketothiolase Deficiency