Characterization and outcome of 41 patients with beta-ketothiolase deficiency: 10 years' experience of a medical center in northern Vietnam

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Word counts

Text: 2951

Summary: 221

Number of figures: 2

Number of tables: 1

Abstract

Beta-ketothiolase (T2) deficiency is an inherited disease of isoleucine and ketone body metabolism caused by mutations in the *ACAT1* gene. Between 2005 and 2016, a total of 41 patients with T2 deficiency were identified at a medical center in northern Vietnam, with an estimated incidence of 1 in 190,000 newborns. Most patients manifested ketoacidotic episodes of varying severity between 6 and 18 months of age. Remarkably, 28% of patients showed high blood glucose levels (up to 23.3 mmol/L). Ketoacidotic episodes recurred in 43% of patients. The age of onset, frequency of episodes, and identified genotype did not affect patient outcomes that were generally favorable, with the exception of 7 cases (5 died and 2 had neurological sequelae). Custom-tailored acute and follow-up management was critical for a positive clinical outcome. Two null mutations, c.622C>T (p.Arg208*) and c.1006-1G>C (p.Val336fs), accounted for 66% and 19% of all identified *ACAT1* mutant alleles, respectively. Most patients showed characteristic biochemical abnormalities. A newborn screening program could be expected to have a high yield in Vietnam. Investigation findings of haplotypes linked to the most common *ACAT1* mutation (c.622C>T) are consistent with an ancient common founder of mutation-bearing chromosomes belonging to the Kinh ethnic population. The direct management and long-term follow-up of a large number of T2-deficient patients enabled us to study the natural history of this rare disease.

Keywords: T2 deficiency, patient outcome, *ACAT1*, ketoacidosis, metabolic encephalopathy, founder effect.

Synopsis

Beta-ketothiolase deficiency has a high incidence in Vietnam, where the most common *ACAT1* c.622C>T mutation was likely introduced by an ancient founder belonging to the Kinh ethnic population, and this disease has clinical phenotypes that are markedly variable, even among patients that share an identical genotype and ancestry, as well as similar personal histories and daily life environment.

Compliance with Ethics Guidelines

Conflict of Interest:

Toshiyuki Fukao and Yuka Aoyama received Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan [Nos. 16K09962 and 15K01693, respectively]. Roberto Colombo received a Grant-in-Aid for Scientific Research from Regione Lombardia, Italy (Innovative Research Project 1137-2010).

Khanh Ngoc Nguyen, Elsayed Abdelkreem, Yuki Hasegawa, Ngoc Thi Bich Can, Thao Phuong Bui, Hai Thanh Le, Mai Thi Chi Tran, Hoan Thi Nguyen, Hung Thanh Trinh, Hideo Sasai, Seiji Yamaguchi, and Dung Chi Vu declare that they have no conflicts of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients (or their parents) included in the study.

Authors' Contributions

Khanh Ngoc Nguyen, Ngoc Thi Bich Can, Thao Phuong Bui, Hai Thanh Le, Hoan Thi Nguyen, Hung Thanh Trinh, and Dung Chi Vu were involved in clinical management, follow-up, and the collection of patient data. Roberto Colombo performed haplotype analysis and inference of mutation age. Yuki Hasegawa and Seigi Yamaguichi contributed to chemical diagnosis by urinary organic acid and blood acylcarnitine analyses. Elsayed Abdelkreem, Yuka Aoyama, Hideo Sasai, and Toshiyuki Fukao performed mutation analyses. Tran Thi Chi Mai performed laboratory tests, including urinary organic acid analysis, at Vietnam National Children's

Hospital. Khanh Ngoc Nguyen and Elsayed Abdelkreem drafted the first version of manuscript. Toshiyuki Fukao and Dung Chi Vu supervised the study and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors confirm the absence of previous similar or simultaneous publications.

INTRODUCTION

Deficiency of beta-ketothiolase (alternate name: mitochondrial acetoacetyl-CoA thiolase [T2] deficiency; EC 2.3.1.9) (Online Mendelian Inheritance in Man [OMIM] 203750, 607809) is an inherited disease of isoleucine and ketone body metabolism caused by mutations in the *ACAT1* gene (Fukao et al, 2014; Hori et al, 2015). Since the first report of T2 deficiency by Daum et al (1971), more than 100 patients with this disorder have been identified worldwide, with no ethnic predisposition (Abdelkreem et al, 2016). T2 deficiency is clinically characterized by intermittent ketoacidotic episodes of variable severity; no symptoms exist under stable patient conditions, except for possible consequences of a previous episode. Characteristic laboratory findings include significant ketonuria and increased urinary excretion of isoleucine catabolic intermediates 2-methyl-3-hydroxybutyrate (2M3HB), 2-methylacetoacetate (2MAA), and tiglylglycine (TIG) (Fukao et al, 2014). Blood acylcarnitine analysis commonly reveals elevated levels of C5:1 and C5-OH carnitines. However, atypical clinical and laboratory manifestations have also been reported (Abdelkreem et al, 2016).

Current knowledge about T2 deficiency rests mainly on published individual case reports; only a few studies have described the collective features of patient groups. The two largest published studies depend on either a review of the literature (Søvik, 1993) or questionnaires returned from supervising physicians (Fukao et al, 2001). From 2005 to 2016, we diagnosed a total of 41 Vietnamese patients with T2 deficiency. To our knowledge, this is the largest number of patients from a single country or ethnic group investigated thus far.

In this work, we report the clinical phenotypes, biochemical and molecular characteristics, and outcomes of 41 Vietnamese patients with T2 deficiency. The direct management and long-term follow-up of a large number of patients enabled us to study the natural history of this rare disease. Investigation of haplotypes linked to the most commonly

reported *ACAT1* mutation among patients provided evidence for a founder effect as a plausible explanation of the genetic epidemiology of T2 deficiency in Vietnam.

PARTICIPANTS, MATERIALS, AND METHODS

Participants

We included all (41) patients with T2 deficiency identified at Vietnam National Children's Hospital (Hanoi) during the period between 2005 and 2016. Eight patients had been previously reported (Fukao et al, 2010). Thirty unrelated healthy participants of Kinh ancestry served as controls for haplotype investigation and modeling of linkage disequilibrium (LD) decay over generations. This study was approved by the Ethical Committee of Vietnam National Children's Hospital. Informed consent was obtained from all adult participants and the parents of minors included in the study.

Patient data were retrospectively collected from medical records at Vietnam National Children's Hospital. These included clinical and demographic data reported by physicians on initial presentation and at each follow-up visit (every 6–12 months). An assessment and evaluation of patients' growth and development were conducted using the World Health Organization child growth standards (2006 release) (http://www.cdc.gov/growthcharts/who_charts.htm) and the Denver II developmental screening test (http://denverii.com), respectively. Routine laboratory tests, including blood counts, gases, electrolytes, glucose, ammonia, and urinary ketones levels were performed according to standard methods. Brain imaging was obtained when clinically indicated. Urinary organic acid and blood acylcarnitine analyses for 37 patients were carried out at Shimane University (Izumo, Japan) using dried urine and blood samples absorbed on filter paper, as

previously described (Fu et al, 2000). Fresh urine samples from 7 patients were analyzed for organic acids at Vietnam National Children's Hospital, according to standard methods.

Genotyping

Thirty-two patients from 27 different families were screened for *ACAT1* mutations at Gifu University (Gifu, Japan) using DNA samples purified from patients' blood, as previously described (Fukao et al, 2010). Identified mutations are described according to the latest recommendations, HGVS version 15.11 (http://varnomen.hgvs.org/), (den Dunnen et al, 2016), using *ACAT1* NCBI Reference Sequences: **NM_000019.3** and **NG_009888.1**. Five dinucleotide (D11S1325, D11S1781, D11S1343, D11S4206, and D11S1793) and one tetranucleotide (D11S1391) short tandem repeats (STRs) were genotyped by PCR amplification and capillary electrophoresis at the Center for the Study of Rare Hereditary Diseases, Niguarda Ca' Granda Metropolitan Hospital (Milan, Italy). DNA samples for haplotype investigation and LD analysis were obtained from 16 patients, their parents, and 30 controls. Details of LD analysis and methods for estimation of the age of mutation are provided in Supplementary file 1.

RESULTS

Table 1 summarizes the demographic, clinical, and laboratory features of patients included in our study. Further detailed data on patients are reported (Supplementary files 2 and 3).

Demographic data

All patients (23 males and 18 females) were born between 2002 and 2016. Most patients (*N*=35) resided in northern Vietnam; accordingly, the estimated incidence of T2 deficiency is

1 in 190,000 newborns in this area. All patients were born to nonconsanguineous parents of Kinh ancestry, which is the main Vietnamese ethnic group (86% of population). Patients belonged to 34 different Vietnamese families; 7 participants are sib pairs. Two unrelated families had a history of unexplained sibling death.

Clinical data of first ketoacidotic episode

Three of 41 patients were diagnosed through screening the siblings of patients; to date, 2 siblings have been asymptomatic. In 34 of the 39 symptomatic patients, the age at onset of the first ketoacidotic episode was between 6 and 18 months (Fig. 1). Ketoacidotic episodes were associated with an intercurrent illness (often gastroenteritis and/or respiratory infections) in 38 of 39 patients; in 1 case, excessive protein intake on the day preceding the episode was reported. The clinical status on admission was generally severe. Nearly all patients were dehydrated and suffered from major central nervous system (CNS) manifestations (altered mental status, convulsions); hypotension was evident in 10% of patients (Table 1).

Laboratory data of first ketoacidotic episode

Severe high anion gap metabolic acidosis (pH: 6.8–7.26; HCO_3^-: 1–10.7 mmol/L; base deficit: 17–30 mmol/L) and ketonuria were detected during acute ketoacidotic episodes. Blood glucose levels were normal in 23 cases whereas hyperglycemia (> 7.8 mmol/L) and hypoglycemia (< 2.5 mmol/L) were observed in 11 and 3 cases, respectively. Mild hyperammonemia (> 170 μmol/L) was present in 7 cases. Patients had no significant electrolyte disturbances except for 2 patients who experienced hypokalemia. Brain imaging abnormalities were identified in 4 of 28 cases who underwent brain imaging. Magnetic resonance imaging in 2 patients showed abnormalities affecting the basal ganglia (hyperintensity on T2W and hypointensity on T1W) and cerebral cortex (cerebral atrophy, hyperintensity on T2W, and hypointensity on T1W).

Computed tomography in the other 2 patients showed bilateral hypodensities affecting posterior limb of the internal capsule. Of these 4 patients, 1 died during his second acute episode, and the other 3 patients developed clinically apparent neurodevelopmental impairments (Table 1).

Except for 1 case, all patients who underwent urinary organic acid and/or acylcarnitine analyses showed typical abnormalities; an increase in 2MAA levels could only be detected using fresh urine samples. Interestingly, patient no. 38 (GK118s) showed normal urinary organic acid and acylcarnitine profiles analyzed under stable conditions. Eight *ACAT1* mutations were identified in 32 patients from 27 different families. The c.622C>T (p.Arg208*) mutation was the most common, representing 66% of all mutant alleles, followed by c.1006-1G>C (p.Val336fs), which accounted for 19% of identified mutations (Fig. 2). Details of patients' mutations are reported in Supplementary file 2.

Management

Acute management for ketoacidotic episodes and long-term preventive measures were implemented, as previously described (Hori et al, 2015). Patients received intravenous glucose during acute ketoacidosis for correcting hypoglycemia and suppressing ketogenesis; target blood glucose level was the upper limit of normal. Clinical and laboratory parameters of patients dictated individualized infusion of appropriate fluids and electrolytes. Ketoacidosis gradually improved to sufficient glucose infusion; other measures (e.g., sodium bicarbonate, dialysis) were used for exceptional cases with severe acidosis. Most patients required intensive care management during acute episodes; of the 39 symptomatic patients, mechanical ventilation and hemodialysis were required for 16 and 4 of them, respectively. Avoiding long fasting was the mainstay for preventing severe ketoacidotic episode. Patients were advised to have frequent carbohydrate-rich meals, particularly during ketogenic stresses such as

infections; glucose infusion was given when an intercurrent illness precluded oral intake. Home monitoring of urinary ketones using test strips was useful. Patients were advised to avoid excess fat and protein intake.

Follow-up and outcome

Figure 1 outlines patient ketoacidotic episodic events and outcomes during the course of the study. Sixteen of 37 patients had recurrent ketoacidotic episodes (recurrent episodes: *N*=25; frequency: 1–5 times per patient). A total of 5 patients died: 2 during their first episode of ketoacidosis, 2 during their second episode, and 1 patient died owing to neurological sequelae following the first episode. Patients' growth parameters were appropriate to age; however, significant neurodevelopmental impairment was observed in 2 cases.

Age of the founder mutation

The occurrence of the same *ACAT1* mutation (c.622C>T) in 26 T2-deficient patients belonging to 22 independent families of Kinh ancestry is striking. This raised the question of whether unrelated identical mutations have been transmitted through these families or whether they share a common ancestor who introduced the mutation into the population by a founder effect. We addressed this intriguing question by performing haplotype analysis using 6 STR polymorphisms and the c.622C>T mutation. The presence of an ancestral seven-marker haplotype (3-4-2-T-5-2-6) and a number of related haplotypes derived from recombination events in the mutation-bearing chromosomes are compatible with the hypothesis that the pathogenic variant c.622C>T can be traced to an ancient common founder belonging to the Kinh ethnic population.

The mean (± SD) overall age estimate (in generations [g]) for the most recent common ancestor (MRCA) of the *ACAT1* c.622C>T mutation is 88.7±17.2 g (95% CI: 76.8–100.6 g)

using the algorithm of Risch et al (1995), and 83.7±16.8 g (95% CI: 75.7–91.7 g) by the method of Reich and Goldstein (1999) (Supplementary file 4). From a Bayesian perspective, applying a mean population growth rate of 0.07, a Markov chain Monte Carlo algorithm (Rannala and Reeve, 2001) yielded an age estimate of 96.4 g (95% CI: 68.5–183.1 g).

DISCUSSION

The incidence of T2 deficiency has been estimated to be less than one per million newborns worldwide. Ongoing identification of an increasing number of cases, particularly as a consequence of extended newborn screening programs, has revealed a higher prevalence in some geographic areas, such as 1 in 232,000 newborns in Minnesota, USA (Sarafoglou et al, 2011). Based on our study, the estimated incidence of T2 deficiency could be 1 in 190,000 newborns in northern Vietnam. This may be underestimated because undiagnosed asymptomatic, mildly symptomatic, or deceased cases may have been missed (Abdelkreem et al, 2016). The vast majority of T2-deficient families were outbred, which further supports the hypothesis of higher incidence. These data support newborn screening in Vietnam for T2 deficiency.

As has been previously reported (Fukao et al, 2001; Søvik, 1993), the age at onset of ketoacidotic episodes in Vietnamese T2-deficient patients usually ranges between 6 and 18 months of age. Because mitochondrial medium-chain 3-ketoacyl-CoA thiolase (T1) can partially compensate for T2 deficiency in ketone body utilization, ketogenic triggers such as prolonged fasting, infection, and protein-rich foods play an important role in disrupting this compensation and precipitating acute ketoacidosis (Fukao et al, 2014). Such triggers are less likely to be present in the first 3–6 months after birth because of frequent feeding, protective maternally acquired immunoglobulins, and the relatively lower protein content of breast and

ordinary milk formulas. On the other hand, older children develop ketosis slower than younger children (Bonnefont et al, 1990) thanks to decreased energy demands relative to body weight coupled with increased muscle mass, which provides a protein reservoir for gluconeogenesis (Fukao et al, 2014). Accordingly, ketoacidotic episodes rarely manifest in patients with T2 deficiency during early infancy or after early childhood. In contrast, the frequent neonatal presentation and persistent ketosis in succinyl-CoA:3-oxoacid CoA transferase (SCOT) deficiency, another ketolytic disorder, can be attributed to a lack of any compensatory enzymes for SCOT deficiency in ketone body utilization (Fukao et al, 2014).

Blood glucose is generally normal during ketoacidotic episodes in patients with T2 deficiency; a few cases might show hypoglycemia or mild hyperglycemia (0.6–14.1 mmol/L) (Abdelkreem et al, 2016). Notwithstanding, 28% of the patients investigated in this study had hyperglycemia during acute episodes; 1 case had the highest recorded blood glucose level reported thus far in a T2-deficient child (23.3 mmol/L). Significant hyperglycemia associated with ketoacidosis has been documented in certain organic acidurias such as propionic acidemia, methylmalonic acidemia, and isovaleric acidemia, and also in SCOT deficiency (Erdol et al, 2016). Hyperglycemia may evolve as a reaction to metabolic stress. Given the different approaches to treatment required, under these circumstances, it is crucial to avoid an initial misdiagnosis of diabetic ketoacidosis (Erdol et al, 2016).

Unlike the genetic heterogeneity usually found in patients with T2 deficiency (Hori et al, 2014), only two mutations (c.622C>T and c.1006-1G>C) accounted for the vast majority (85%) of *ACAT1* mutations identified in our Vietnamese patients. Although all patients were born to nonconsanguineous parents, half were homozygous for c.622C>T. This is consistent with a high carrier rate for c.622C>T among the Vietnamese population. However, previous screening of 400 healthy Vietnamese participants could not identify any heterozygotes for c.622C>T (Fukao et al, 2010). Further investigation on a larger scale is warranted. In addition,

it is noteworthy that c.622C>T is not a specific Vietnamese *ACAT1* mutation; it has been reported in Dutch, American, and Chinese patients as well (Fukao et al, 2014; Sarafoglou et al, 2011; Wen et al, 2016).

In Vietnamese patients of the Kinh ethnic group, we identified an ancestral haplotype spanning 3.4 Mb and encompassing the c.622C>T *ACAT1* mutation on chromosome 11q22.3. The decay of LD over generations, modeled using different algorithms, would date the age of the MRCA of the mutation-bearing chromosomes to 1900–2500 years ago, if we assume 25 years per generation. The estimated age is consistent with the historically plausible scenario in which a founder effect originated in northern Vietnam during the last centuries BCE or at the beginning of the CE. The effect may have arisen among a successful group of conquerors or migrants who arrived in the region and underwent demographic expansion in the colonized lands before spreading over the area and intermixing with the pre-existing population or other groups of migrants. This mainly occurred when Âu Việt peoples migrated from southern China to the Red River Delta and mixed with the indigenous Văn Lang people (3rd century BCE) or around 111 BCE when Han troops invaded and annexed the kingdom of Nam Việt. After that, a large number of Chinese people including ordinary people, mandarins, and scholars moved to the conquered lands and settled there (Nguyễn, 2012; Taylor, 1983).

The unique genetic characterization among T2-deficient Vietnamese patients has a remarkable impact on patients' biochemical profiles. Almost all patients demonstrated the characteristic increased urinary excretion of isoleucine catabolic intermediates, resulting from the presence of homozygous or compound heterozygous null mutations (c.622C>T and c.1006-1G>C) and the consequent loss of T2 enzyme activity. Because 2MAA is readily decarboxylated non-enzymatically to 2-butanone (Aramaki et al, 1991), it could be detected in our patients only by analyzing fresh urine samples. On the other hand, the only patient who showed no increased urinary excretion of 2MAA, 2M3HB, or TIG was homozygous for a mild

mutation (c.1A>G) with residual (11%) T2 enzyme activity (Fukao et al, 2003). T2-deficient patients with mild mutations may have atypical or subtle biochemical profiles not only under metabolically stable conditions but also during acute episodes of decompensation (Fukao et al, 2012). Therefore, a prospective newborn screening program may have a higher yield in Vietnam than in other countries where mild mutations may be more common. Indeed, typical abnormalities were identified in nearly all (38/39) T2 deficient Vietnamese patients who underwent blood acylcarnitine analysis. Nevertheless, it should be pointed out that a normal newborn screening result does not absolutely exclude a diagnosis of T2 deficiency (Fukao et al, 2012).

Symptomatic patients in our study manifested relatively severe episodes of ketoacidosis associated with dehydration and CNS dysfunction. The brain lesions identified in 4 Vietnamese patients after ketoacidotic episodes may be attributed to a potential nonspecific consequence of severe acidosis; this may also complicate mitochondrial respiratory chain disorders and certain organic acidurias (Akella et al, 2014; O'Neill et al, 2014). Nevertheless, some reported cases of T2 deficiency had brain imaging abnormalities with clinical neurological impairment, even in the absence of a previous episode of ketoacidosis (Ozand et al, 1994; Buhas et al, 2013). Accumulated isoleucine catabolic intermediates may have a direct neurotoxic effect even in the absence of frank metabolic decompensation (Buhas et al, 2013, Leipnitz et al, 2010). T2 deficiency is a potentially serious disease. In addition to other intensive care measures, mechanical ventilation was required for management of acute ketoacidotic episodes in 41% of patients. Seven of 41 patients had unfavorable outcomes. Most adverse consequences were related to the first ketoacidotic episode, when the diagnosis had not yet been confirmed. This is also quite supportive for a newborn screening program in Vietnam for T2 deficiency. Neither the age at onset nor frequency of ketoacidotic episodes affected the eventual outcome. Vietnamese T2-deficient patients had ketoacidotic episodes of variable onset, severity, and

outcome, despite sharing not only a common genotype but also the same ancestry and living conditions. This underscores a lack of genotype–phenotype correlation in T2 deficiency. Appropriate acute and preventive management appear to be critical for a favorable outcome (Hori et al, 2015; Fukao et al, 2001).

In conclusion, T2 deficiency likely has a high incidence in Vietnam where c.622C>T mutation seems to have an ancient common founder of Kinh ancestry. This disease has variable clinical phenotypes even when patients share a common genotype and ancestry, as well as similar personal histories and daily life environments. Most patients could have a favorable outcome with early diagnosis and proper management. Looking ahead, a newborn screening program for beta-ketothiolase deficiency may have a high yield in Vietnam.

Acknowledgments

The authors thank Ms. Naomi Sakaguchi (technician; Gifu University) for her invaluable technical assistance.

Funding

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant nos. 16K09962 and 15K01693) and, in part, by Regione Lombardia, Italy (Innovative Research Project 1137-2010).

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Figure Legends

Figure 1 Ketoacidotic episodes by age among 41 Vietnamese patients with beta-ketothiolase deficiency

Vertical axis indicates patients (females are underlined) and horizontal axis indicates age in years. Sib pairs are denoted by b (brother) and s (sister) following patient codes. Black and white circles depict first and recurrent ketoacidotic episodes, respectively. Solid lines show the duration of follow-up, from diagnosis through age at last follow-up. Patient outcomes were favorable unless specified otherwise, as follows: C, complication (neurological); D, died; M, missing. GK number is an internal identifier for patients with beta-ketothiolase deficiency whose *ACAT1* mutations were identified at Gifu University (Gifu, Japan).

Figure 2 Relative frequency of *ACAT1* mutations identified in 32 Vietnamese patients with beta-ketothiolase deficiency

Mutations are described according to the latest HGVS recommendations (version 15.11) using *ACAT1* NCBI Reference Sequences: **NM_000019.3** and **NG_009888.1**.