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ORIGINAL ARTICLE

p.D645E of Acid α-Glucosidase Is the Most Common Mutation in Thai Patients with Infantile-Onset Pompe Disease

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Aim: To describe genetic features of five unrelated Thai families with infantile-onset Pompe disease caused by mutations in the acid α-glucosidase (*GAA*) gene. *Methods:* Total RNA and genomic DNA were extracted from peripheral blood leukocytes, and mutation analysis of the entire coding regions of the *GAA* gene was performed in our first patient. Polymerase chain reaction-restriction fragment length polymorphism analysis was also used for a particular mutation in subsequent patients. *Results:* The mutation analysis revealed that all patients harbored the same mutation, c.1935C > A (p.D645E), with three being homozygotes. The p.D645E, therefore, accounted for 80% (8 out of 10 alleles) of the mutations. *Conclusions:* We identified five unrelated Thai patients with infantile-onset Pompe disease with no history of consanguinity. Finding of the most common mutation, p.D645E, in this study will help facilitate prenatal diagnosis of their family members and molecular diagnosis of future suspected patients. Analysis of common mutations could be the most effective strategy in identifying *GAA* mutations responsible for Pompe disease in the Thai population.

Introduction

Pompe disease or glycogen storage disease type II or acid maltase deficiency (OMIM# 232300) is an autosomal recessive lysosomal storage disorder caused by a deficiency of the lysosomal enzyme acid α-glucosidase (GAA) (EC. 3.2.1.20). Deficiency of this enzyme that degrades glycogen results in an accumulation of glycogen inside the lysosomes in multiple tissues with the most markedly affected being cardiac, skeletal, and smooth muscles (Hirschhorn and Reuser, 2001). Its clinical severity ranges from a rapidly progressive infantile-onset disease with severe hypotonia, generalized muscle weakness, and hypertrophic cardiomyopathy to a milder late-onset disease with gradually progressive myopathy and respiratory problems without cardiac involvement (Hirschhorn and Reuser, 2001).

Lysosomal GAA is encoded by the *GAA* gene, which is localized to human chromosome 17q25.2-q25.3. It consists of 20 exons and translates into a protein of 952 amino acids (Hoefsloot *et al.*, 1990; Kuo *et al.*, 1996). At least 290 different sequence variants in the *GAA* gene have been described, with the majority being missense/nonsense mutations (www.hgmd.cf.ac.uk, accessed February 2010; www.pompecenter.nl). It has been demonstrated that some of these mutations are more common in some ethic groups due to founder effects. For example, the p.R854X and p.D645E mutations have been identified in the majority of African American and Chinese cases, respectively (Becker *et al.*, 1998; Shieh and Lin, 1998; Montalvo *et al.*, 2006).

In this study, we report clinical and molecular characterization of five Thai patients with infantile-onset Pompe disease. Mutation analysis of the *GAA* gene revealed the p.D645E in all patients, suggesting it as the most common mutation in the Thai population.

Materials and Methods

Patients

Five patients from unrelated families were clinically found to have infantile-onset form of Pompe disease at the Genetic Unit, Department of Pediatrics, King Chulalongkorn Memorial Hospital, and were included in the study. Selection criteria were based on clinical presentations. There was no history of consanguinity in any of the families.

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Mutation analysis

Peripheral blood samples were obtained from the probands and their available parents after written informed consent. Total RNA and genomic DNA were extracted from peripheral blood leukocytes using Qiagen RNA and DNA blood mini kits according to manufacturer's instructions, respectively (Qiagen, Valencia, CA). Reverse transcription was performed using ImProm-II™ reverse transcriptase (Promega, Madison, WI), according to the manufacturer's instructions. For our first patient, sequencing of the entire coding regions using genomic DNA as template was performed by a private company in the United Kingdom. For subsequent patients, we first used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis with the *Bsa*HI restriction enzyme to screen for the p.D645E. For patients who were not homozygous for the p.D645E, the entire coding regions were PCR-sequenced using complementary DNA as template. RFLP was also used to screen for the presence of the p.D645E mutation in 114 control chromosomes from unaffected ethnic-matched individuals.

PCR-RFLP analysis with the *Mbo*II restriction enzyme was performed to screen for the intronic mutation, c.-32-13T > G (IVS1-13T > G), in four Pompe patients who did not have sequencing of the entire coding regions of the *GAA* gene. The digested products were electrophoresed on a 1.5% agarose gel stained with ethidium bromide.

Results

All patients had clinical features consistent with infantile-onset Pompe disease. Mutation analysis of the *GAA* gene successfully identified mutations in all of them. Three patients were homozygous for the c.1935C > A (p.D645E) mutation that was inherited from each of their parents (Table 1; Fig. 1). The other two were compound heterozygotes, with one being c.1726G > A (p.G576S)/c.1935C > A (p.D645E). The p.G576S and p.D645E mutations were inherited from the father and the mother, respectively (Table 1). The other patient was also found to be heterozygous for the p.D645E mutation (Table 1), but the other mutant allele was unidentified. The presence of the p.D645E mutation was also screened in 114 ethnic-matched control chromosomes and none of them harbored this mutation (data not shown).

The common late-onset intronic mutation, c.-32-13T > G (IVS1-13T > G), frequently identified in the Caucasian population (Hirschhorn and Reuser, 2001) was also evaluated in this cohort. None was found to carry the IVS1-13T > G mutation (data not shown).

Table 1. Results of Acid α-Glucosidase Mutation Analysis in Five Unrelated Thai Families

Patient	*Mutation*	*Father*	*Mother*
Patient 1	p.D645E/p.D645E	p.D645E/N	p.D645E/N
Patient 2	p.D645E/p.D645E	p.D645E/N	p.D645E/N
Patient 3	p.D645E/p.D645E	p.D645E/N	p.D645E/N
Patient 4	p.D645E/p.G576S	p.G576S/N	p.D645E/N
Patient 5	p.D645E/U	p.D645E/N	U

All patients were women and had no history of consanguinity. They all had hypotonia, cardiomegaly, short PR interval, and biventricular hypertrophy.

U, unidentified.

Discussion

We described five unrelated Thai patients with clinical features consistent with Pompe disease. All were found to have the infantile-onset form. The p.D645E mutation was identified in all patients with three being homozygotes. Of the nine mutant alleles identified, eight were c.1935C > A (p.D645E) and the other was c.1726G > A (p.G576S). The finding of p.D645E in all five Pompe patients (100%) and in 8 out of 10 alleles (80%) strongly suggests it as the most common mutation in the Thai population. Its presence was also evaluated in 114 ethnic-matched control chromosomes, and none was found to carry this pathogenic mutation. This finding indicated that the frequency of this allele in our population should be <1%.

Interestingly, this mutation has been found to be a frequent mutation in Chinese patients with infantile-onset Pompe disease due to a founder effect (Shieh and Lin, 1998). In addition, the IVS1-13T > G, which has been frequently identified in Caucasian individuals with Pompe disease, was not detected in our Thai cohort.

The c.1726G > A (p.G576S) mutation was detected in one of our patients. She was compound heterozygous for the p.G576S/p.D645E. The p.G576S was first described in a Pakistani patient with infantile-onset Pompe disease and subsequently found in the Chinese and Japanese populations (Suzuki *et al.*, 1988; Pipo *et al.*, 2003; Kroos *et al.*, 2008). The *GAA* gene is relatively large and its identified mutations are distributed all over the gene. Knowing the most common mutation and having a rapid, reliable, and inexpensive method to identify it will definitely facilitate diagnosis of future suspected patients of the Thai ethnicity. After our first patient, a product of nonconsanguineous parents, was found to be homozygous for the p.D645E, we hypothesized that this mutation might be common in our population. The PCR-RFLP to detect p.D645E was therefore chosen as a screening test for molecular confirmation of Pompe disease. Being able

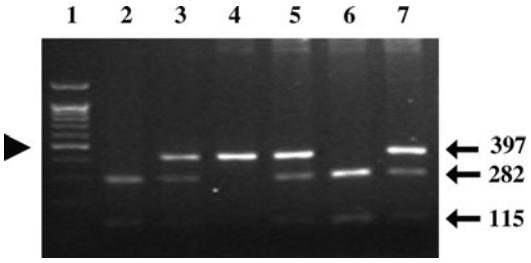

FIG. 1. Polymerase chain reaction-restriction fragment length polymorphism analysis of *GAA* c.1935C > A. Lane 1, 100-bp marker; lane 2, unaffected control; lane 3, heterozygous carrier; lane 4, patient; lane 5, mother; lane 6, unaffected brother; lane 7, father. In the second lane, *Bsa*HI digested the wild-type allele of the control into 282- and 115-bp products (arrows). The c.1935C > A mutation in the patient eliminates the restriction site, leaving the uncut 397-bp product. The analysis showed that the patient was homozygous for the mutation, whereas the parents were heterozygotes. The 500-bp band was indicated by an arrowhead.

to identify the mutation in all four subsequent patients, the method has been proved to be efficient.

In summary, we reported five unrelated Thai patients with infantile-onset Pompe disease. All harbored the c.1935C > A (p.D645E) mutation, with three being homozygotes. That p.D645E has been identified in 8 out of 10 alleles (80%) strongly suggests it as the most common mutation in the Thai population. Knowing the common disease-causing mutation in the *GAA* gene will help facilitate genetic testing leading to rapid and reliable diagnosis and proper genetic counseling of Pompe disease in the Thai population.

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Disclosure Statement

No competing financial interests exist.

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