

# A novel frameshift mutation of *HEXA* gene in the first family with classical infantile Tay-Sachs disease in Thailand

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## Abstract

Tay-Sachs disease (TSD) is an autosomal recessive neurodegenerative disorder caused by mutations in the *HEXA* gene resulting in a deficiency of  $\beta$ -hexosaminidase A (HEX A) enzyme. To our knowledge, TSD has never been reported in Thai population. We describe the first case of classic infantile TSD in a 2-year-old Thai boy who presented with first episode of seizure and neuroregression since 9 months of age. Hyperacusis, progressive macrocephaly and macular cherry red spots were also detected during examination. Brain MRI revealed hyperintensity in the basal ganglion on T1-weighted and partial corpus callosum agenesis. Measurement of  $\beta$ -hexosaminidase activity in the patient leukocytes showed low total  $\beta$ -hexosaminidase (62.6 normal 801 $\pm$ 190 nmol/mg protein/hr) and low %HEX A (7.57 normal 55-72%HEX A) activity compatible with TSD. Mutation analysis of the *HEXA* gene revealed compound heterozygous of a novel frameshift mutation (c.1207delG or p.E403SfsX20) in exon 11 which was inherited from the mother and a previously described missense mutation (c.1510C>T or p.R504C) in exon 13 which was inherited from the father, respectively.

**Conclusion.** We report a clinical, biochemical and molecular analysis in the first case of genetically confirmed classic infantile TSD in Thailand.

## INTRODUCTION

Tay-Sachs disease (TSD) or GM2 gangliosidosis B variant (MIM#272800) is an autosomal recessive neurodegenerative disorder caused by a deficiency of  $\beta$ -hexosaminidase A (HEX A) enzyme. HEX A is a lysosomal enzyme composed of one  $\alpha$ - and one  $\beta$ -subunit which are encoded by the *HEXA* and *HEXB* genes, respectively. TSD is caused by mutations in the *HEXA* gene (MIM\*606869) located on chromosome 15q23-q24.<sup>1,2</sup> The *HEXA* gene consists of 14 exons and contains 1,587 bp of coding sequence encoding a 529 amino acid protein. More than 130 mutations have been identified in the *HEXA* gene database (<http://www.hgmd.cf.ac.uk>, <http://www.hexdb.mcgill.ca/hexadb>), including single base substitutions, small deletions, duplications or insertions, splice-site mutations, large deletions and rare complex rearrangements. Most of these mutations are sporadic and unique to individual families. Some of the mutations have been reported as ethnic specific mutation in certain populations such as three mutations including c.1277\_1278insTATC,

c.1421+1G>C and c.805G>A (p.G269S) which were found in 94-98% of the Ashkenazi Jewish TSD patients.<sup>3,4</sup>

TSD is classified into three different forms including classic infantile, juvenile and adult late-onset. The classic infantile form is the most common and is characterized by early onset of hypotonia, hyperacusis, macular cherry-red spots, intractable seizures and progressive neurological deterioration leading to death in early childhood. Affected children rarely survive beyond five years of age. Juvenile and adult forms are uncommon with a later onset and slower disease course. The overall prevalence of TSD is estimated to be about 1 in 200,000 live births in the general population.<sup>5</sup> However, the prevalence is high in some particular ethnicities such as Ashkenazi Jewish which is 1 in 2,500-3,900 live births.<sup>6</sup> To date, TSD has never been reported in Thai population.

In this study, we report the clinical, biochemical and molecular characterization of a 2-year-old boy affected with classic infantile TSD, the first case of genetically confirmed *HEXA* mutation in Thailand.

## CASE REPORT

T.D. is a Thai boy who is the only child of non-consanguineous parents. He was born at term with uneventful prenatal and perinatal history. He was healthy and normal milestone until 9 months of age. At time, he experienced his first episode of seizure then deteriorated in term of seizures and developmental milestone. His neurological deterioration was rapidly progressive, followed by loss of motor ability and finally bed-ridden by the age of 1 year. He was initially diagnosed as myoclonic seizures with encephalopathy and spastic cerebral palsy. He was admitted to the hospital several times according to gastro-esophageal reflux disease and recurrent pneumonia. His parents noticed that he also had hyperacusis to noise and tactile stimuli. He was evaluated by pediatric neurologist and geneticist at the age of 2 years when progressive macrocephaly was detected. There were no other dysmorphic features. On neurological examination, he had axial hypotonia and increased deep tendon reflexes all extremities. Ophthalmological examination revealed bilateral macular cherry red spots. Other examinations were unremarkable. Routine hematological and biochemical investigations were normal. Investigation for inborn error of metabolisms (IEMs) revealed normal serum ammonia, serum lactate, plasma amino acids and urine organic acids. A brain magnetic resonance imaging (MRI) exhibited hyperintensity in the basal ganglion on T1-weighted images and partial agenesis of the corpus callosum. The diagnosis was established by measurement of leukocyte  $\beta$ -hexosaminidase activity including total  $\beta$ -hexosaminidase and the percentage of HEX A (%HEX A) activity. Total  $\beta$ -hexosaminidase activity (62.60 normal 801 $\pm$ 190 nmol/mg protein/hr) was markedly below the normal range and a low percentage of HEX A activity (7.57% normal 55-72%HEX A) was found, compatible with the diagnosis of TSD.

### Molecular analysis of the *HEXA* gene

#### Methods

After informed consent was obtained, peripheral blood EDTA samples from the patient and the parents were collected. Genomic DNA was extracted from peripheral blood lymphocytes using commercial available kits according to manufacturer's protocol. Mutation analysis was characterized by direct DNA sequencing of all 14 coding exons and exon-intron boundaries of *HEXA*

gene according to protocols described previously.<sup>2</sup> The nucleotides and amino acids were numbered according to the RefSeq NM\_000520.4 and NP\_000511.2, respectively. Putative mutations were confirmed in two separate PCR from the patient DNA.

#### Results

Mutation analysis by direct DNA sequencing of all 14 coding exons and exon-intron junction of the *HEXA* gene revealed compound heterozygous of one frameshift and one missense mutation in the patient DNA. The heterozygous frameshift mutation was deletion of G at nucleotide 1207 (c.1207delG) which is located in exon 11 (Figure 1A). This frameshift mutation resulted in a premature termination codon at the codon 422 (p.E403SfsX20) and was predicted to encode a truncated  $\alpha$ -subunit of HEX A. The heterozygous missense mutation was C to T change at nucleotide 1510 (c.1510C>T), resulted in the conversion of amino acid from arginine to cysteine at codon 504 (p.R504C) in exon 13 (Figure 1B). Molecular analysis of both parental DNA revealed that the heterozygous frameshift mutation (c.1207delG) was inherited from the mother, whereas the heterozygous missense mutation (c.1510C>T) was inherited from the father (Figure 1C and 1D).

## DISCUSSION

TSD is an autosomal recessive lysosomal storage diseases caused by deficiency of HEX A enzyme. HEX A is a heterodimeric protein composed of  $\alpha$ - and  $\beta$ -subunits. Mutations in the *HEXA* gene which encodes the  $\alpha$ -subunit cause TSD, whereas mutations in the *HEXB* gene which encodes the  $\beta$ -subunit are causative of Sandhoff disease (MIM#268800). In Asia, TSD has been occasionally reported in central and south Asian populations including Chinese, Indian, Japanese, South Korean and Malaysian.<sup>7-11</sup> To date, only Sandhoff disease has been reported in Thai population.<sup>12</sup> Our patient described in this study is the first case of classic infantile TSD in Thai population. The clinical and neuroimaging findings of the patient are almost similar to other reported infantile-onset TSD patients from different ethnic groups.<sup>7-9,11,13-15</sup> The diagnosis of TSD was confirmed by determination of leukocyte  $\beta$ -hexosaminidase activity. Both total  $\beta$ -hexosaminidase and the percentage of HEX A (%HEX A) activity were low in our patient leukocytes. The enzyme deficiency results in the neuronal accumulation of GM2 ganglioside

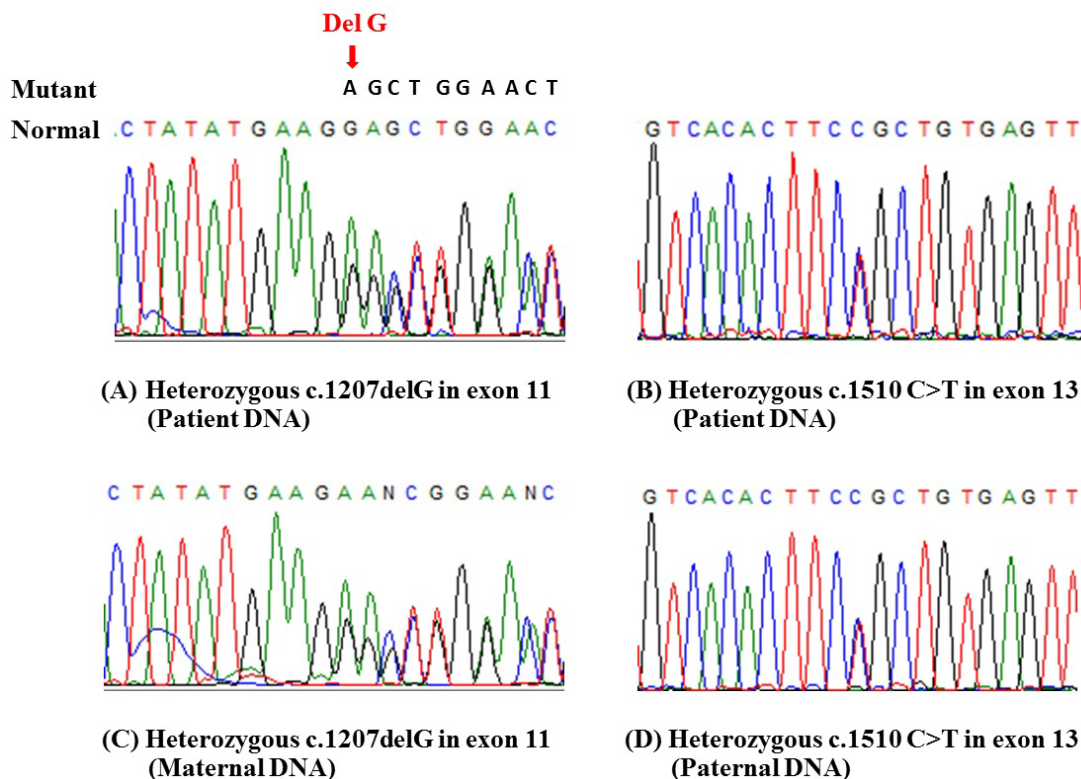


Fig. 1: Sequence analysis of the *HEXA* gene in the patient DNA revealed a heterozygous c.1207delG mutation in exon 11 (A) which is inherited from the mother (C) and a heterozygous c.1510C>T mutation in exon 13 (B) which is inherited from the father (D).

leading to the clinical characteristic of TSD as demonstrated in our patient. According to the unavailability of skin fibroblast culture in our institution, direct mutation analysis of the *HEXA* gene was used to confirm the diagnosis of TSD in our patient.

After molecular analysis of the *HEXA* gene was performed in our patient DNA, compound heterozygous of one previously described and one novel mutation have been identified. The first mutation is missense mutation c.1510C>T (p.R504C) in exon 13. This mutation was first described in German siblings affected with chronic GM2 gangliosidosis.<sup>16</sup> The substitution of R504 to C causes a defective in  $\alpha$ - and  $\beta$ -subunit dimerization which results in no enzymatic activity.<sup>16-17</sup> The high frequency of this mutation is probably caused by the presence of CpG dinucleotides which are known as mutagenic hot spots at this position.<sup>15,16-19</sup> Other reported mutations in codon 504 were R504H, R504L and deletion of cytosine in this codon.<sup>14,20-21</sup> The second mutation is a novel heterozygous frameshift mutation c.1207delG (p.E403SfsX20) in exon 11.

This mutation is predicted to result in a premature termination at the codon 422 causing a truncation of  $\alpha$  subunit of HEX A enzyme. To date, more than 130 different *HEXA* mutations have been identified including various types of mutation. Most of the mutations are either nonsense or frameshift which are located throughout the gene, whereas most of the missense mutations are clustered in highly conserved amino acids in ligand-like binding domain. As in our patient who is the first case of genetically confirmed *HEXA* mutation in Thailand, the missense mutation is located in exon 13 which is located in the extrahelix in domain II.<sup>17</sup> Compound heterozygous of these two null alleles explained the clinical severity and infantile-onset of TSD in our patient.

Molecular genetic testing is clinically important not only for diagnostic confirmation but it can sometimes be used to prognostic predictions and can contribute to the management of the patient and family. Classic infantile TSD is the most common form. Affected infants typically appear normal until the age of 3 to 6 months when progressive loss of neurological function

starts and is usually fatal by age 4 or 5 years.<sup>5</sup> Our patient presented with early infantile-onset of intractable seizures and progressive neurological deterioration leading to death at the age of 4 years which is compatible with classical acute infantile form of TSD. Even though the %HEX A activity in the patient leukocytes was slightly higher (7%) than in the individuals with acute infantile form (0-5%), mutation analysis revealed compound heterozygous of two null alleles which were predicted to result in no or extremely low enzymatic activity. Therefore, the residual enzymatic activity alone may not be the good indicator for the severity of the disease. Once our index case has been identified, familial investigation must be required according to the possibility of autosomal recessive transmission. Mutation analysis of both parents was performed. The missense mutation c.1510C>T (p.R504C) was identified in the paternal DNA while the novel frameshift mutation c.1207delG (p.E403SfsX20) was detected in the maternal DNA, indicating that our proband's parents are both carriers for TSD. Since there is currently no effective treatment for TSD, genetic counseling and prenatal diagnosis in subsequent pregnancies should be recommended to this family due to high recurrence risk.

In conclusion, we report a compound heterozygous of a novel frameshift and a previous described missense mutations in the first Thai family with classic infantile TSD which has never been reported in Thailand.

## ACKNOWLEDGEMENT

The authors would like to thank Professor W-L Hwu from Department of Pediatrics and Medical Genetics, National Taiwan University Hospital for the support of biochemical diagnosis (measurement of  $\beta$ -hexosaminidase activity) in our patient. We also thank Laura Yeh, medical technologist, for the technical assistance.

## DISCLOSURE

Conflict of interest: None

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