GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 00, Number 00, 2016

Pp. 1–5
DOI: 10.1089/gtmb.2016.0221

ORIGINAL ARTICLE

Novel Mutations, Including a Large Deletion in the *ARSB* Gene, Causing Mucopolysaccharidosis Type VI

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Objective: Mucopolysaccharidosis type VI (MPS VI; Maroteaux-Lamy syndrome), a rare autosomal recessive lysosomal storage disease, is caused by mutations in the *N*-acetylgalactosamine-4-sulfatase (*arylsulfatase B*, or *ARSB*) gene, resulting in a deficiency of ARSB activity. This study aimed to characterize the clinical and molecular features of four unrelated Thai patients with MPS VI. Two were products of consanguineous marriages. *Materials and Methods:* The diagnosis was confirmed by biochemical and genetic tests. We performed mutation analysis by polymerase chain reaction-sequencing on the entire coding region of the *ARSB* gene. Array-based comparative genomic hybridization (aCGH) analysis combined with direct sequencing was also used to search for the deletion boundary. *Results:* The causative mutations were detected in all cases. Of four different mutations identified, three have never been previously described, which included two missense mutations (p.C155Y and p.R388T) and a deletion encompassing exons 2 and 3. Both missense mutations were absent in 110 unaffected ethnic-matched control chromosomes and an in-house database of 180 Thai exomes. The p.C155Y and p.R388T mutations were located in the highly conserved residues. aCGH analysis combined with direct sequencing identified the breakpoints of a large 13,788 base pair deletion. It is the largest deletion of *ARSB* described to date in patients with MPS VI. *Conclusion:* This study expanded the known mutational spectrum of ARSB; we identified three novel mutations; two of which are missense mutations and one that represents the largest deletion mutation identified to date in this gene.

Keywords: mucopolysaccharidosis type VI, *ARSB*, novel mutations, Thai

Introduction

Mucopolysaccharidosis type VI (MPS VI; MIM 253200) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the *N*-acetylgalactosamine-4-sulfatase (*arylsulfatase B*, or *ARSB*) gene, resulting in deficiency of ARSB enzyme activity. The inability to efficiently degrade glycosaminoglycans, dermatan sulfate, and chondroitin 4-sulfate leads to accumulation predominantly of dermatan sulfate within the lysosomes of cells causing progressive damage in several tissues and organs. Patients with MPS VI present with a great variability of clinical phenotypes, including disease onset and rate of progression. The clinical severity ranges from a severe or infantile form to a mild or adult form. Patients with the rapidly progressing form usually present before 2 years of age with skeletal abnormalities, coarse facial features, corneal clouding, cardiac abnormalities, hepatosplenomegaly, and growth retardation. They generally have relatively normal intelligence. For patients with the mild form, they have later onset of symptoms usually after the second decade and less severe phenotypes (Litjens *et al.*, 1989; Isbrandt *et al.*, 1994; Valayannopoulos *et al.*, 2010). Diagnosis may be difficult in some cases due to the wide spectrum of clinical manifestations and unawareness of the disease.

The *ARSB* gene, which encodes arylsulfatase B, is located on chromosome 5q11-q13 and contains eight exons. At least 160 different disease-causing mutations scattered throughout the *ARSB* gene have been identified, with the most common being missense mutations (Human Gene Mutation Database, www.hgmd.cf.ac.uk, accessed May 2016). The nonsense splice-junction alterations, nucleotide deletions or insertions, and exon deletions have also been reported (Karageorgos *et al.*, 2007; Costa-Motta *et al.*, 2011; Saito *et al.*, 2012; Brands *et al.*, 2013).

In this study, we described four unrelated Thai patients with MPS VI. Three novel alterations in the *ARSB* gene,

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Table 1. Clinical and Molecular Characteristics of Thai Patients with Mucopolysaccharidosis Type VI

Case No.	*Age of onset*	*Age at diagnosis*	*First manifestation*	*Consanguinity*	ARSB *activity*[a]	*Mutation*	*Age at last follow-up (years)*
1	2 years	14 years	Coarse face, skeletal deformity	N	2238	c464G>A (pC155Y), c1163G>C (pR388T) (compound heterozygous)	21
2	3 years 5 months	9 years 10 months	Skeletal deformity and joint stiffness	Y	N/A	138-kb deletion (homozygous)	15
3	5 years	6 years 9 months	Skeletal deformity and joint stiffness	N	472	c479G>A (pR160Q) (homozygous)	7
4	2 years	4 years 7 months	Coarse face, inguinal hernia	Y	955	c464G>A (pC155Y) (homozygous)	5

[a]nmol/mg Prot/h, reference range = 923 ± 496.
N, no; N/A, not available; Y, yes.

including two missense mutations and a 13.8-kb deletion encompassing exons 2 and 3 of the *ARSB* gene, were identified. This is the first study to describe the deletion covering the large genomic region associated with MPS VI.

Materials and Methods

Subjects

Four unrelated patients were included in this study. Two were products of consanguineous marriages. Diagnosis of MPS VI was based on leukocyte ARSB activity and mutation analysis. Clinical features and laboratory findings are shown in Table 1.

The study was approved by the institutional review board of the Faculty of Medicine of Chulalongkorn University and written informed consent was obtained from each family.

Mutation analysis of the ARSB *gene*

After informed consent, genomic DNA was extracted from peripheral blood leukocytes obtained from four patients and their available parents according to standard protocols. The entire coding regions of the *ARSB* gene were assessed by polymerase chain reaction (PCR) and direct sequencing. PCR primers are shown in Table 2.

The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH), according to the manufacturer's recommendations, and sent for direct sequencing in both directions at Macrogen, Inc. (Seoul, Korea). The sequence was analyzed using Sequencher (version 4.2; Gene Codes Corporation, Ann Arbor, MI). For each novel missense mutation, restriction fragment length polymorphism (RFLP) analysis was used to confirm its presence in the patient as well as to screen in 110 control chromosomes from unaffected ethnic-matched individuals. The p.C155Y (c.464G>A) mutation eliminates the *Bsm*I site, while the p.R388T (c.1163G>C) mutation creates the *Mfe*I site. The *Bsm*I and the *Mfe*I restriction enzymes were therefore used in PCR-RFLP to screen for the presence of each mutation. Sorting Intolerant from Tolerant (SIFT; http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html) and PolyPhen (http://genetics.bwh.harvard.edu/pph2) were used for prediction of the possible impact of amino acid substitutions on the stability and function of mutant proteins.

Table 2. Primer Sequences and Polymerase Chain Reaction Conditions for *ARSB* Mutation Analysis

Exon	*Primers' name*	*Sequence (5′–3′)*	*Annealing temp (°C)*	*PCR cycle*	*Size (bp)*
1	ARSBE1F	CAGCTGAGTTTCCAAGAAGC	58	35	699
	ARSBE1R	AGCCGCCGGGACCCATAACT			
2	ARSBE2F	ATCAGTAAGGTAGAAGGCCA	50	35	353
	ARSBE2R	CATTTGATTGCACTTGGGTG			
3	ARSBE3F	TAGCCTCGTCACGGGTAATC	54	35	450
	ARSBE3R	AGGCTGATTCTGGGATACTG			
4	ARSBE4F	GCATTCTGTAGGTTGTCTTG	50	35	456
	ARSBE4R	GTCTCCACTATTGCAGTTTG			
5	ARSBE5F	GGTGGGAAAAGGCAAGGAAT	54	35	444
	ARSBE5R	CTCAATGGAGTCAGGCTGCT			
6	ARSBE6F	GTGATTGTGGAGACCTCCAA	52	35	299
	ARSBE6R	GCTAGGCTAGAGACACACTA			
7	ARSBE7F	GCTACTGTTCTGCAAGGGTA	52	35	299
	ARSBE7R	GGCAGATAGACTGGAGATAC			
8	ARSBE8F	TATGTTTCCACACCCACAAC	50	35	451
	ARSBE8R	AATGAGACAAGAGTCGTGAG			

PCR, polymerase chain reaction.

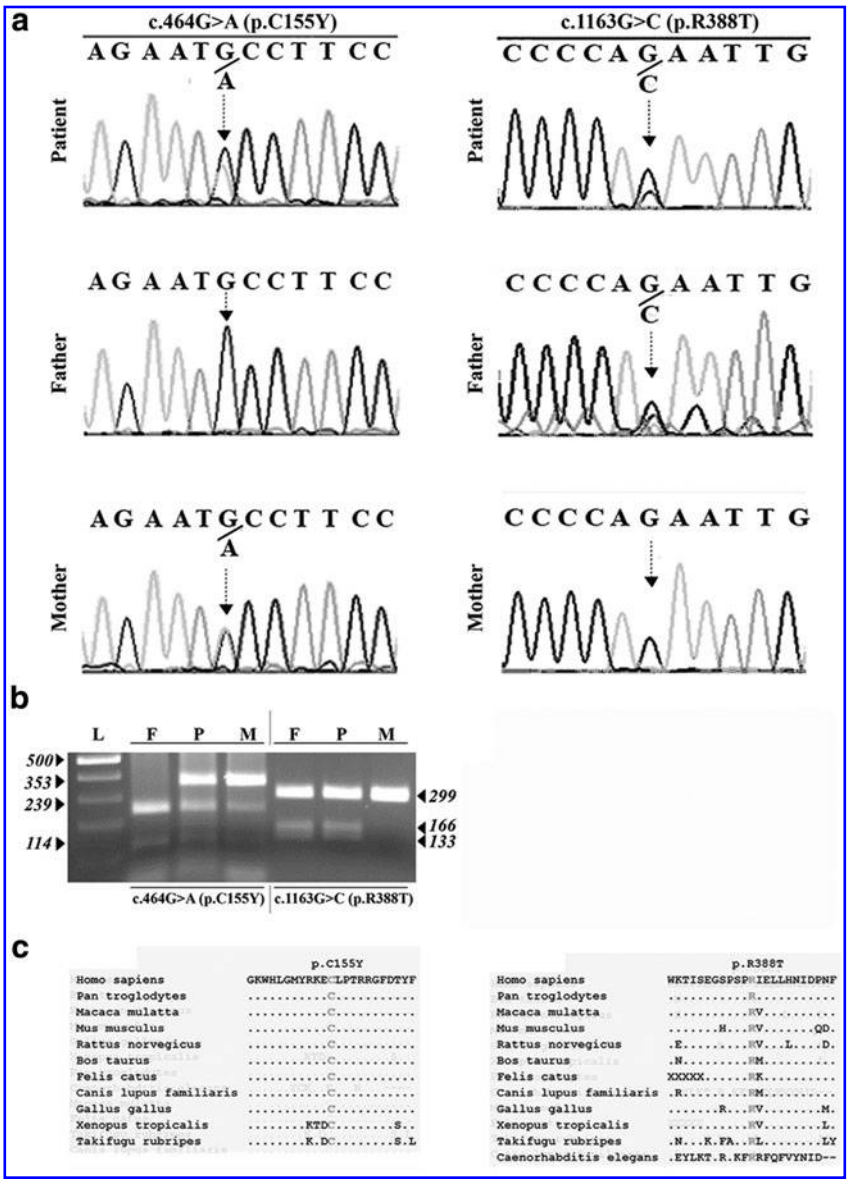

FIG. 1. Mutation analysis of the novel missense *ARSB* mutations. **(a)** Electropherogram showing that the c.464G>A (p.C155Y) was inherited from the mother, while the c.1163G>C (p.R388T) was inherited from the father. **(b)** PCR-RFLP analysis. The c.464G>A mutation eliminates the *Bsm*I site, while the c.1163G>C mutation creates the *Mfe*I site. *Bsm*I digested the wild-type allele into 239 and 114-bp products (*an arrow head*). The mutant allele does not have the recognition site, leaving the 353-bp PCR product intact. *Mfe*I digested the mutant allele into 166 and 133-bp products (*an arrow head*). The wild-type allele does not harbor the recognition site, leaving the 299-bp PCR product intact. F, father; L, 100-bp marker; P, patient; M, mother. The 500-bp band is indicated by *an arrow head*. **(c)** Sequence alignment of ARSB from various species. The site of each mutation is indicated by *an arrow*. PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

Array-based comparative genomic hybridization analysis

Genomic DNA of the proband from Family 2 was sent to Macrogen, Inc., for array-based comparative genomic hybridization (aCGH) analysis on chromosome 5 using NimbleGen 385K, according to the company's instructions. Briefly, genomic DNA was labeled with fluorescent dyes, cohybridized to a NimbleGen Human CGH 385K Chromosome 5 Tiling Array, and scanned using a 2-μm scanner. Log2-ratio values of the probe signal intensities (Cy3/Cy5) were calculated and plotted versus genomic position using Roche NimbleGen NimbleScan 2.5 software. Data were displayed in Roche NimbleGen SignalMap software. The deletion breakpoints were further analyzed by PCR. A primer pair, MPSVI_int1-3del_F1: 5′-GGC TAT TAT ACA CTA AAG AAC ATT TCG-3′ and MPSVI_int1-3del_R1: 5′-CAG GAG TTG TAT AAC ATT TGA TAG TGT-3′ covering the chr5: 78,258,570–78,272,591 region, was designed to detect the deleted allele.

Results

PCR-direct sequencing revealed novel missense *ARSB* mutations in the proband of Family 1. He was found to be compound heterozygous for two different mutations. The c.464G>A mutation in exon 2 was transmitted from the mother and expected to result in a cysteine to tyrosine substitution at codon 155 (p.C155Y) (Fig. 1a). The c.1163G>C mutation in exon 6 was transmitted from the father (Fig. 1a) and expected to result in an arginine to threonine substitution at codon 388 (p.R388T). PCR-RFLP using *Bsm*I and *Mfe*I restriction enzymes was performed to confirm the presence of the c.464G>A and c.1163G>C mutations, respectively (Fig. 1b). It was also used to screen both mutations in 110 ethnic-matched unaffected controls and none were observed. Both mutations were also absent in our in-house database of 180 exomes. In addition, they were located at highly conserved amino acid residues (Fig. 1c).

A homozygous deletion encompassing exons 2 and 3 of the *ARSB* gene was identified in the proband of Family 2. He was

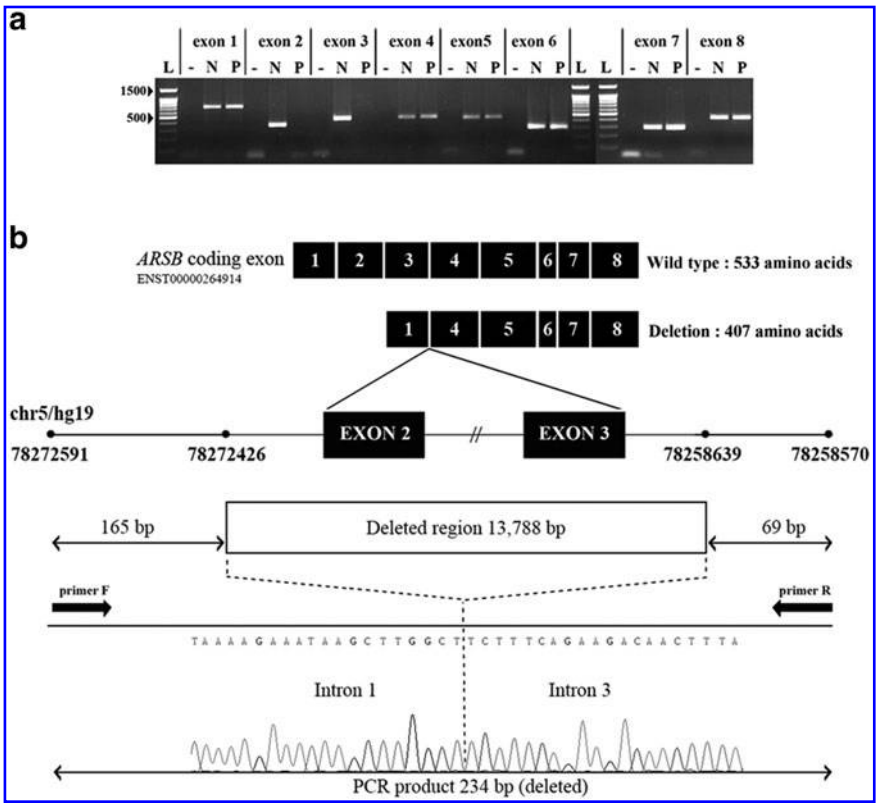

FIG. 2. Mutation analysis of the large deletion. **(a)** PCR analysis showing the absence of exons 2 and 3. L, 100-bp marker; –, no DNA; N, normal control; P, patient. **(b)** Electropherogram of the patient showing the precise breakpoints of the 13,788-bp deletion. Schematic representation of the deleted region identified in the patient showing the location of the primers and the expected size. The sequences were arranged in the minus (–) strand direction (GRCh37/hg19).

found to be homozygous for the deletion as revealed by an absence of the PCR products of exons 2 and 3 (Fig. 2a). To search for the deletion boundary, the proband's DNA sample was subjected to aCGH analysis covering chromosome 5. The presence of the deleted allele was further verified in the patient and the parents by PCR analysis across the predicted deletion points. Direct sequencing confirmed the deletion size of 13,788 base pairs expanding from position chr5: 78,258,639–78,272,426 (Fig. 2b). Both parents were carriers for this deletion.

A homozygous c.479G>A (p.R160Q) mutation was found in a female patient from Family 3. Her mother was heterozygous for the mutation. Her father's DNA sample was unavailable. Another female patient of Family 4 was found to be homozygous for the c.464G>A (p.C155Y) mutation, which was also identified in Family 1. Both parents were found to be heterozygous for this mutation.

Discussion

In this study, we described four unrelated patients with MPS VI. Two were products of consanguineous marriages. Three novel alterations were identified, including two missense mutations and a deletion. Patient 1 was compound heterozygous for the c.464G>A (p.C155Y) and c.1163G>C (p.R388T) mutations. A 13.8-kb deletion encompassing exons 2 and 3 of the *ARSB* gene was detected in patient 2. A previously known mutation, c.479G>A (p.R160Q), was identified in patient 3 (Voskoboeva *et al.*, 1994). Interestingly, patient 4 was homozygous for the novel c.464G>A (p.C155Y) mutation, which was found in patient 1.

The newly identified c.464G>A and c.1163G>C mutations were inherited from the patient's mother and father, respectively. Several lines of evidence support both variants as causative mutations. They were not identified in 110 ethnic-matched unaffected control chromosomes by RFLP and 180 in-house exomes. ClustalX revealed that the cytosine residue at codon 155 and the arginine at codon 388 were highly conserved (Fig. 1c). PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) predicted the c.464G>A (p.C155Y) to be probably damaging with a score of 0.978 and the c.1163G>C (p.R388T) to be probably damaging with a score of 1.000. In addition, SIFT (http://sift.jcvi.org) predicted the c.464G>A to be tolerated with a score of 0.211 and the c.1163G>C to be deleterious with a score of 0.001. Another missense mutation, the c.479G>A (p.R160Q) identified in our patient 3 has been previously reported in a patient with the intermediate form of MPS VI (Voskoboeva *et al.*, 1994). Only the c.479G>A was detected and inherited from her mother. Her father's DNA was unavailable.

A novel deletion was also found in our study. The deletion encompassing exons 2 and 3 of the *ARSB* gene was initially revealed by an absence of the PCR products of exons 2 and 3, which was further confirmed by aCGH analysis covering chromosome 5. Using aCGH combined with PCR-direct sequencing, we were able to identify a 13,788-bp deletion within the *ARSB* gene as a disease-causing allele inherited from patient 2's parents (Fig. 2b). To our knowledge, this deletion is the largest identified to date in patients with MPS VI. There have been three studies reporting large deletions in the *ARSB* gene with two encompassing exon 5 (Arlt *et al.*, 1994; Villani *et al.*, 2010) and one involved exon 4 (Lin *et al.*, 2015). Our newly identified 13.8-kb deletion encompassing exons 2 and 3 resulted in a deletion of 126 amino acids covering from residue p.105I to p.230K at the sulfatase domain of ARSB (www.rcsb.org/pdb/explore/remediatedSequence.

do?structureId=1FSU). This deletion was predicted to result in a shortened ARSB protein from 533 to 407 amino acids long. Our patient with this homozygous deletion had onset of disease at around 3 years and 5 months (Table 1). The previous study reported a patient with a homozygous exon 4 deletion who had early onset MPS VI. The patient received recombinant human ARSB at the age of 2 years with an improvement in skeletal deformities and developmental milestones (Lin *et al.*, 2015).

Genotype–phenotype correlations in MPS VI have been widely discussed with some studies involved in sufficient number of cases demonstrating possible correlations (Garrido *et al.*, 2007; Karageorgos *et al.*, 2007; Valayannopoulos *et al.*, 2010). Due to the limited number of our cases, it is difficult to conclude any genotype–phenotype correlation.

In conclusion, we successfully characterized four unrelated Thai patients with MPS VI and identified causative mutations in all cases. Three novel mutations in *ARSB* were detected, expanding the mutational spectrum of *ARSB* causing MPS VI. This study is also the first to characterize the largest deletion encompassing exons 2 and 3 of the *ARSB* gene.

Acknowledgments

The authors would like to thank the patients and their families for participating in this study. This work was supported by the Grant for Development of New Faculty Staff, Chulalongkorn University (GDNS 57-032-30-003), Thailand Research Fund (BRG5980001), and the Chulalongkorn Academic Advancement into its 2nd Century Project.

Author Disclosure Statement

No competing financial interests exist.

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