Another Novel Missense Mutation in *ARSB* Gene in Iran

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Abstract- Mucopolysaccharidosis VI (MPS-VI) is an infrequent autosomal recessive disorder caused by mutations in *ARSB* gene and deficiency in lysosomal enzyyme *ARSB* activities subsequently. This enzyme is essential for the breaking of glycosaminoglycans (GAGs) such as dermatan sulfate and chondroitin sulfate. *ARSB* dysfunction results in imperfect breakdown of GAGs and their accumulation in urine. Mutations in *ARSB* gene are the main players in MPS-VI disease and its clinical consequences. Most reported mutations are point mutations but there are some other examples in literature. Here we report a novel missense mutation in *ARSB* gene that is inherited as an autosomal recessive mode and probably explain the clinical status of the proband. This mutation replaces the threonine 92 by proline and alters *ARSB* structure. This is the most feasible scenario for clinical condition we described here. This novel mutation should be remarked for PND and PGD to improve the health and management of such families.

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Introduction

Mucopolysaccharidosis type VI (OMIM#253200), a lysosomal storage disorder with autosomal recessive pattern of inheritance (1), was first described as a novel dysostosis associated with increased urinary excretion of chondroitin sulfate (2). The incidence rate for MPS-VI was reported in approximately 1 in 320,000 live births in three decades of registration (3).

The disease results from an insufficiency of arylsulfatase B enzyme. Clinical traits and harshness exhibits a wide spectrum of symptoms from slowly to rapidly progressing forms (4,5), but short stature, hepatosplenomegaly, dysostosis multiplex, stiff joints, corneal clouding, cardiac abnormalities, and facial dysmorphism are familiar symptoms (6) while mental retardation is a highly unusual finding (7). Severe forms may have onset from birth, increased urinary glycosaminoglycans (generally>100 µ/mg creatinine), severe dysostosis multiplex, short stature, and death before the 2nd or 3rd decade of life (8). One milder phenotype with more gently progression has later onset, mildly elevated urinary glycosaminoglycans (generally<100 µ/mg creatinine) and mild dysostosis multiplex and death in the 4th or 5th decades of life (8). The disease is caused by mutations in the *ARSB* gene.

The *ARSB* gene also known as ASB, G4S, or MPS6 is discoverable at 5q14.1 (9) and spans around 2.6Kb (10). Arylsulfatase B belongs to the sulfatase family and is encoded by eight constructing exons which are interrupted by seven introns. Homozygous (11) or compound heterozygous mutations in *ARSB* gene are responsible for MPS-VI (12-14). Separate reports indicated different mutations of the *ARSB* gene in MPS-VI patients. Some of them were substitutions (15), and the others were splice site mutations (15) while all of them results in irrecoverable deficiency in *ARSB* activity leading to the accumulation of dermatan sulfate (8). In this report, we describe an affected person with a novel missense mutation in *ARSB* gene responsible for this clinical condition.

Case Report

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The patient is an eight years old boy of a consanguineous marriage (Figure 1A). He first showed respiratory distress and heart defects at age four. His paternal aunt has mental retardation and the proband has MPS symptoms. The proband and his family were referred to Sarem Medical Genetics Laboratory.

Clinical findings

An 8-year-old boy was referred to Sarem Medical Genetics Laboratory for genetic counselling and mutation detection. In the clinical examination he showed corneal clouding, funnel shaped chest, cardiomyopathy, valve lesions, hepatosplenomegaly, umbilical and inguinal hernia, large head, bulging frontal bone and restricted joint movement. He displayed hearing impairment, respiratory distress, but without coarse facial features and no history of seizures. Proband's height and weight at age four were 103 cm (upper than 95th percentile) and 19600 gr (in normal range), respectively. His parents were healthy persons in a consanguineous ethnic Iranian marriage (pedigree is shown in Figure 1A). The proband was born from a full term pregnancy and urinary analysis showed his urinary dermatan sulfate was strongly positive. Other evaluation showed deficient arylsulfatase B enzyme, decreased WBC and fibroblast cells. The patient's heart was slightly enlarged and lungs were normal in size. In addition, mild hepatomegaly and splenomegaly were the other findings. Owing to biochemical assays, clinical findings and pedigree information, we hypothesized that the proband was affected by *Maroteaux-Lamy syndrome*.

Figure 1

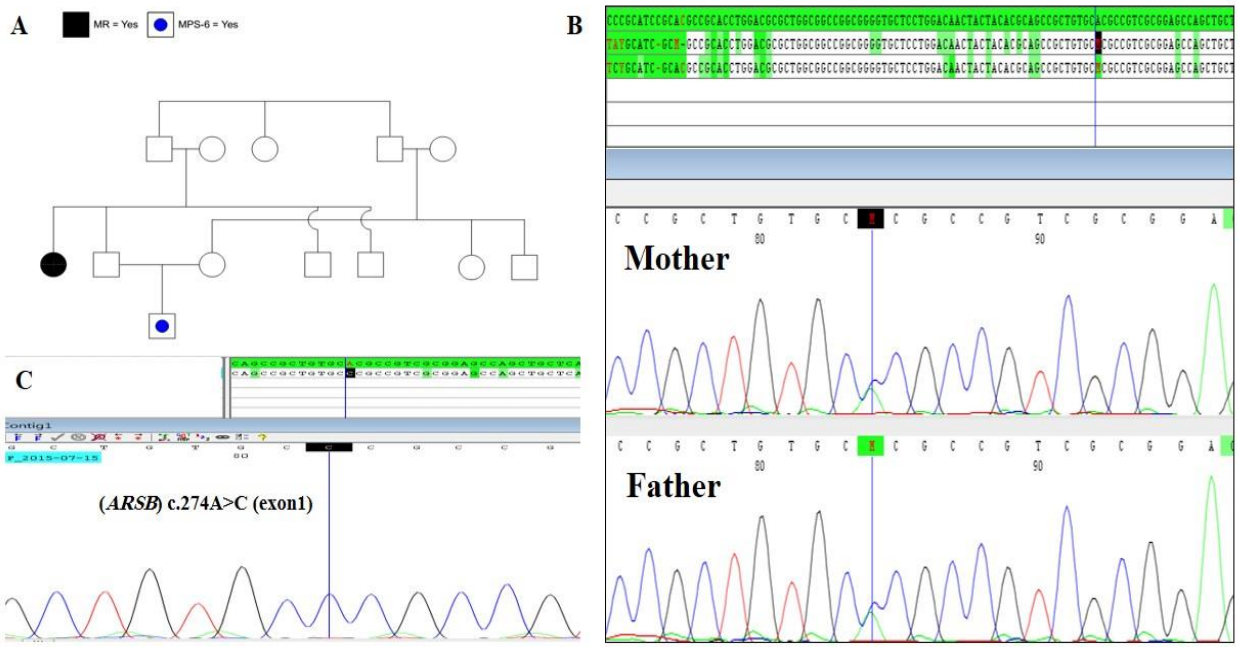

Figure 1. A) Family pedigree, the proband pointed by arrow. **B)** The novel pathogenic c.274A>C (p.T92P) variant at exon 1 (ENST00000264914 transcript) of *ARSB* gene which is presented in heterozygote state in both parents. **C)** The novel pathogenic c.274A>C (p.T92P) variant at exon 1 (ENST00000264914 transcript) of *ARSB* gene in homozygote state in the patient

Molecular genetic characterization

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 for being included in the study and in addition Informed consent was obtained from the parents and peripheral blood was drained from the antecubital vein. Using DNA purification kit (Roche, Switzerland), genomic

DNA was extracted from the peripheral blood leukocytes for all family members. PCR primers were designed using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) and evaluated by oligo analyzer tool (https://eu.idtdna.com/calc/analyzer) (Table 1). Isolated DNAs were engaged in multiple PCR reaction to amplify *ARSB* gene coding regions. Our PCR products were sequenced on ABI 3130 Genetic Analyzer (Applied Biosystems, USA). Sequences were aligned to the GRCh37/hg19 human reference genome using BLAST program and analyzed by Codon code aligner software (CodonCode Corporation, USA). We applied mutation taster (http://www.mutationtaster.org/), SIFT (http://sift.jcvi.org/) and Polyphen (http://genetics.bwh.harvard.edu/pph2/) online tools for the prediction of clinical outcomes of variants as well as ExAC (http://exac.broadinstitute.org/), HGMD (http://www.hgmd.cf.ac.uk/ac/all.php) and ClinVar(http://www.ncbi.nlm.nih.gov/clinvar/) databases for studying previous reported mutations of the gene. We portrayed a 3D structure of mutated protein using SWISS-MODEL (http://swissmodel.expasy.org/interactive) and also considered its evolutionary conservation.

Molecular genetic findings

Over PCR-sequencing method, two variants in *ARSB* gene were identified in the patient. One of these variants revealed in homozygous state (Figure 2A), c.1072G>A (P.V358M) ENST00000264914, analyzed by mutation taster and SIFT utilities. This variant could not explain any clinical condition and was recognized as single nucleotide polymorphism (Figure 2B). We also discovered another variant in *ARSB* gene in homozygous state, c.274A>C (p.T92P) ENST00000264914. Analysis of the variant was conducted with mutation taster, SIFT and Polyphen, from which it was discovered as a disease causing variant existing in homozygous state at the first exon of *ARSB* gene (Figure 1C). Furthermore, the latest variant in parents was tracked where it unveiled in heterozygote state in the father and mother (Figure 1B). This variant is non-existent in ExAC, HGMD and ClinVar databases and was thus considered a novel mutation. This variant was classified as a missense mutation thus *ARSB* protein 3D structure using the SWISS-MODEL (Figure 3A, B and C) was provided and additionally, it was realized that the altered amino acid (Threonine) is conserved through evolution (Figure 3D).

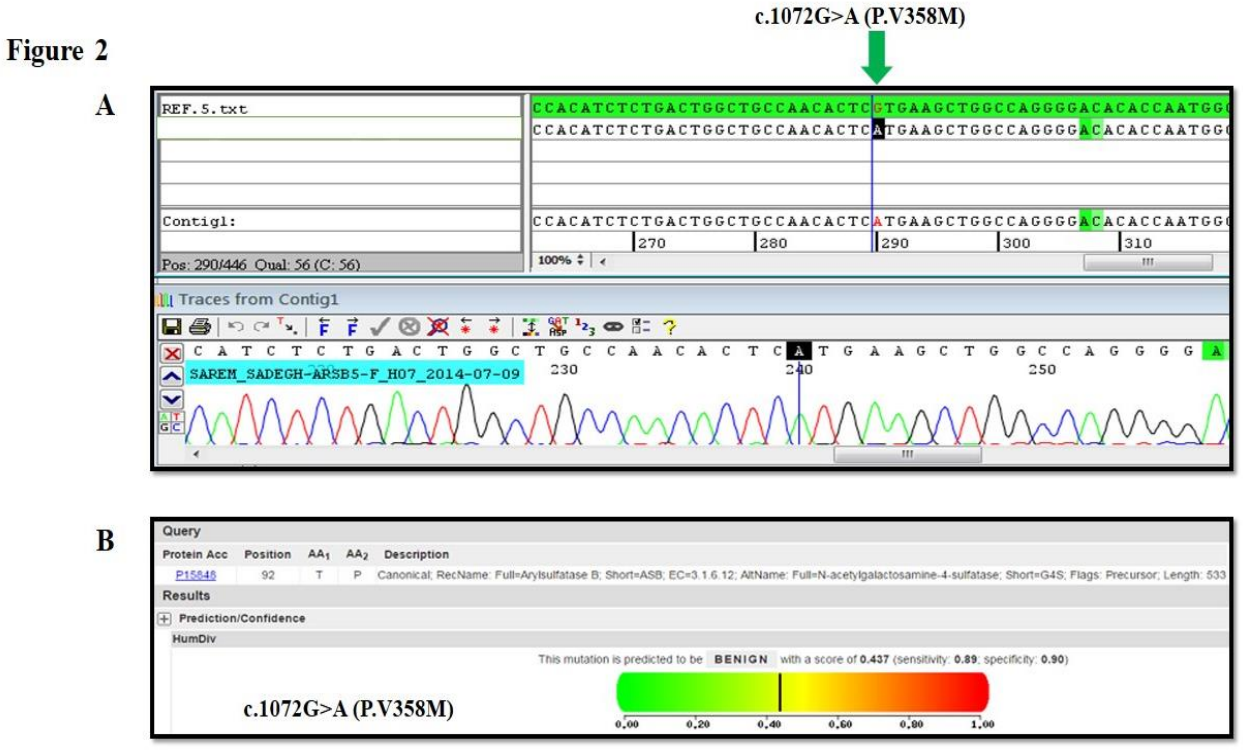

Figure 2. A) The c.1072G>A (p.V358M) variant at exon 5 (ENST00000264914 transcript) of *ARSB* gene. This variant occurred in homozygous state and **B)** it has no pathogenic effect according to our *in silico* analysis through PolyPhen2

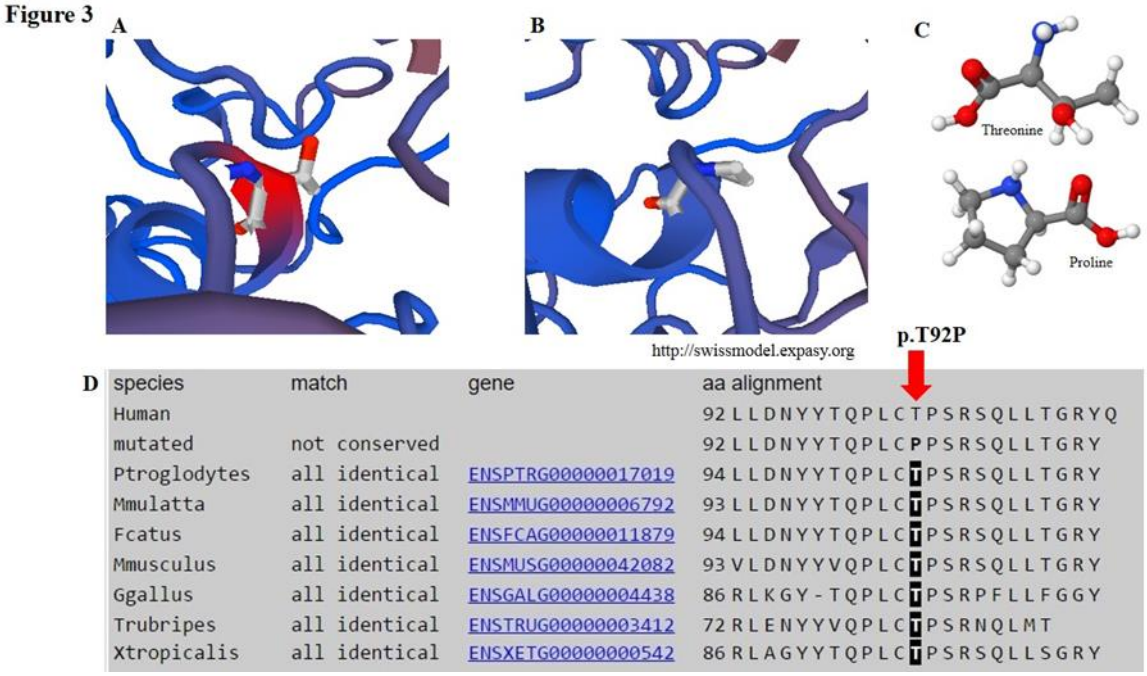

Figure 3. A) The 3D structure of normal *ARSB* protein and, **B)** Mutated *ARSB* protein harboring p.T92P alteration. **C)** Spatial structure of threonine and proline amino acids. **D)** *ARSB* protein amino acid conservation regarding to mutation position. Position of mutation (p.T92P) pointed by arrow and threonine amino acids were highlighted. As it seems threonine 92 is a conserved amino acid in ARSB protein.

Table 1. Properties of used primers

Primer ID	Sequence	Exon	Product size(bp)
***ARSB*1F**	5´- ACCTAGGCTGGAACGACGT-3´	1	509
***ARSB*1R**	5´- ATAACTGGGTCGGCGGTC-3´		
***ARSB*2F**	5´- CAAGTCAACAGATATGATCAG -3´	2	371
***ARSB*2R**	5´-TTCATTTGATTGCACTTGG-3´		
***ARSB*3F**	5´-GTCACGGGTAATCAATTGC-3´	3	410
***ARSB*3R**	5´- GGACTTCCCTAGAATTTATTAG-3´		
***ARSB*4F**	5´- GCATAAATCTGAACTGTCTTATCC-3´	4	426
***ARSB*4R**	5´- TTACCATGTCTCCACTATTGCA-3´		
***ARSB*5F**	5´- GCACCATTTAGTAACAATGTAC-3´	5	423
***ARSB*5F**	5´- ACACAAAAGCTATCATTCTTG-3´		
***ARSB*6F**	5´- GACCTCCAAATTCATGACAG-3´	6	281
***ARSB*6R**	5´- TAGAGACACACTAGGTAATC-3´		
***ARSB*7F**	5´- AATGCTGATTTATAACAACACG-3´	7	267
***ARSB*7R**	5´- ACTGGAGATACTGCCCTG-3´		
***ARSB*8F**	5´-CTTGGCCTCAGACTCCTT-3´	8	432
***ARSB*8R**	5´-TTGGGATAACAAATGAGACAAG-3´		

Discussion

Mucopolysaccharidosis type VI is a member of LSDs (Lysosomal Storage Diseases) caused by arylsulfatase B insufficiency. Its homodimer hydrolyzes sulfate groups of N-Acetyl-D-galactosamine, chondriotin sulfate, and dermatan sulfate (16). Clinical features are variable (6) and the patient exhibited this paradigm with no further symptoms.

Normally, *ARSB* enzyme must be present in normal persons and it could be unidentifiable for enzyme activity in severe MPS-VI patients (17). There is some redundancy in enzyme activity of patients with increasing slope starting from the most severe patients to more attenuated forms (18). Our records indicated a clear deficiency of arylsulfatase B enzyme which diverts our attribution to the Maroteaux-Lamy syndrome (Table 2).

Table 2. Biochemical test results for studied patient

	Enzyme	Normal(nmol MU/mg protein)	Result(nmol MU/mg protein)	Disease
Patient	Arylsulphatase B	100-330	25	Marateuax-Lamy (MPS VI)

MPS-VI follows the autosomal recessive mode of inheritance (11) in the pedigree (Figure 1).

Although it is a rare event, but spontaneous gastrointestinal bleeding in a patient with pancreatitis and a demonstrable or suspected pseudocyst demands both a high degree of clinical awareness about the possibility of a false aneurysm, and a defined diagnostic and therapeutic stratagem (10).

In this study, *ARSB* gene was sequenced in order to find the genetic problem, upon which a nonpathogenic variant (c.1072G>A (P.V358M) ENST00000264914) (Figure 2A) and a novel missense mutation (c.274A>C) was discovered. Based on this study's in silico predictions, substitution of G nucleotide by an in position 1072 of *ARSB* cDNA has no pathogenic effect (Figure 2B) and it cannot explain paband's clinical representations. The other variant (c.274A>C) is not documented in 1000 genomes, ExAC and ClinVar databases and is thus a novel mutation in exon 1 causing an amino acid replacement (p.T92P). This might alter the protein structure and possibly its function (Figure 3A, B). Based on our in silico studies, this novel mutation are able to explain clinical manifestations of the proband. This case study confirms the mode of inheritance in the family. Indeed, threonine 92 is conserved through evolution (Figure 3D) thus highlighting its significance in protein function. Threonine is slightly heavier than proline and their 3D structures are diverse (Figure 3C). This alteration is just upstream of a helix structure starting at amino acid 93, thus this alteration might affect protein structure and function and cause clinical manifestations of the case persisting on this report. This substitution occurred in N-acetylgalactosamine-4-sulfatase domain which is responsible for hydrolysis of the 4-sulfate groups of the N-acetyl-D-galactosamine 4-sulfate units of chondroitin sulfate and dermatan sulfate (19). This domain is very critical for *ARSB* function and any modification might change the role.

In this report we discussed a pathogenic alteration in *ARSB* gene, converting threonine to proline residue and malfunctioning the protein. According to our study, postulation of this mutation as a reason for MPS-VI disease is not unrealistic. Nowadays, the lists of pathogenic mutations are growing because of the use of modern technologies in genetic diagnosis (20). Therefore, pathogenic variants-for example this novel missense mutation-should be considered to improve PND, PGD and PGS services. Iranian populations were not included in 1000 genomes project. Therefore, there are few data about the Iranian population and their mutations in the literature and this study contributes to the improvement of genetic services.

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