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**Short Report**

# Identification of a Novel Arylsulfatase B Gene Mutation in Three Unrelated Iranian Mucopolysaccharidosis Type-VI Patients with Different Phenotype Severity

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## ABSTRACT

**Background:** Mucopolysaccharidosis type-VI (MPS-VI), which is inherited as an autosomal recessive trait, results from the deficiency of N-acetylgalactosamine 4-sulfatase (arylsulfatase B) activity and the lysosomal accumulation of dermatan sulfate. In this study, *ARSB* mutation analysis was performed on three unrelated patients who were originally from the West Azerbaijan province of Iran. **Methods:** After PCR and direct DNA sequencing, DNA extraction was performed. **Results:** Sequencing analysis revealed a novel homozygous missense mutation in the *ARSB* gene at c.1457A>G [p. D486V] in three unrelated Iranian MPS-VI patients with different phenotype severity. **Conclusion:** The mutation type in three patients was the same; probably, because of a foundation effect on their population. *Iran. Biomed. J. 16 (3): 169-171, 2012*

*Keywords:* Mucopolysaccharidosis type-VI (MPS-VI), Mutation, Iran

## INTRODUCTION

Maroteaux-Lamy syndrome (mucopolysaccharidosis type-VI [MPS-VI], MIM# 253200), is a rare, autosomal recessive lysosomal storage disorder caused by a deficiency of arylsulfatase B (ARSB) enzyme that is involved in the degradation of glycosaminoglycans (GAG) dermatan and chondroitin sulfate. The *ARSB* gene, located on chromosome 5q13-q14, contains 8 exons [1], which produce the arylsulfatase B polypeptide with 533 amino acids [2]. Therefore, *ARSB* gene mutations lead to lysosomal storage and urinary excretion of these partially degraded substrates. Patients with MPS-VI usually have normal intellectual development, but show many physical symptoms found in Hurler syndrome. A wide variation in the clinical severity is observed: the infantile (severe), juvenile (intermediate) and adult (mild) forms [3]. Although over 130 *ARSB* mutations have been reported in different countries and different ethnic populations, no *ARSB* mutation analysis has been reported in the Iranian population. Experimentally, it seems that Maroteaux-Lamy syndrome is the most common type of mucopolysaccharidosis in Iran. In this study, three unrelated Iranian patients with MPS-VI and severe phenotypes were described and homozygous missense mutation in their *ARSB* gene was also characterized.

## MATERIALS AND METHODS

***Clinical features of the patients.*** The three patients were born in first-cousin consanguineous marriages and were originally from the West Azerbaijan province of Iran, but they were completely unrelated. The clinical features of the three patients have been listed in Table 1. The patients were informed of the aim of the study and gave their informed consent to the genetic analysis. The three patients were diagnosed as most likely having the severe form (early onset) of MPS-VI based on the medical interview, physical exam,

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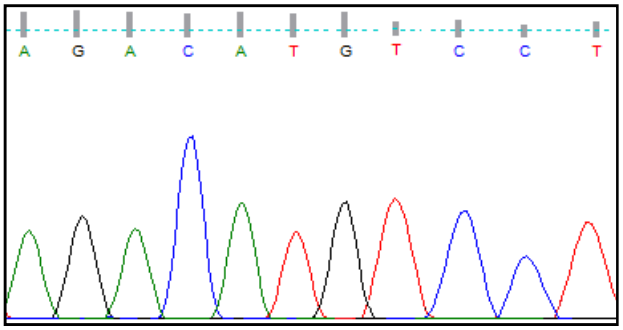


**Fig. 1.** Comparison of missense mutation at exon 8 of *ARSB* gene at c.1457A>T [p.D486V] in patients with normal sequence of cDNA and protein.

electrocardiogram, echocardiogram, ophthalmologic evaluations, GAG quantification in urine, measurement of the activity of N-acetylgalactosamine-4-sulfatase (ARSB) in leukocytes and cultured fibroblasts. However, to confirm the diagnosis, molecular analysis of the *ARSB* gene was performed on genomic DNA from the three patients and their samples were amplified by PCR. PCR products were analyzed by direct sequencing of DNA fragments.

***PCR and sequencing.*** Genomic DNA was isolated from peripheral EDTA-treated blood cells by Qiagen DNA Mini kit (cat No: 51304). Genomic DNA (250 ng) of each patient was subjected to PCR amplification of the exons 1-8, each of which contained one exon (exons 2-7) or the coding region of one exon (exons 1 and 8). The primers used to amplify exons 1, 7 and 8 were described by Isbrandt and colleagues [4], and those used to amplify exons 2, 3, 4, 5 and 6 were described by Petry and colleagues [5]. PCR reactions (50 µL) were carried out using 100 ng genomic DNA, 2 mmol/L dNTP, 1× PCR buffer (10 mmol/L Tris HCl, pH 8.3; 50 mmol/L KCl), 20 pmol each primer (forward and reverse), 1.5 mmol/L $MgCl_2$ and 1U SmartTag DNA polymerase (Roche Diagnostics, Mannheim, Germany). Exon 1 was amplified using 1.5 mmol/L $MgSO_4$ and 1U Taq Platinum Pfx DNA polymerase (Invitrogen Life Technologies, Brazil) The reaction mixture was cycled at 94°C for 15 min, followed by 40 cycles at 94°C for 45 s, at 48-65°C for 45 s, at 72°C for 1 min, and a final extension at 72°C for 5 min [4, 5]. PCR products were separated on 2% agarose gels, run in 0.5 × Tris/Borate/EDTA Buffer at 110 V for 50 min, stained in 0.002 mg/mL ethidium bromide and visualized by means of a UV light PCR. PCR products were analyzed by direct sequencing of DNA fragments, amplified by the ABI 3700 capillary sequencer (Macrogen, Korea). Sequence results were compared with the published sequence (GenBank).

## RESULTS

ARSB activity in leukocytes was 50, 50 and 65 nmole/h/mg protein (reference values: 72-174) in patients 1, 2 and 3, respectively, and urinary excretion of GAG was 53, 45 and 28 g/mol Cre.

Interestingly, direct sequencing of all exons of the *ARSB* gene revealed the same novel homozygous missense mutation in all three patients. However, their parents were heterozygous for the missense mutation. The missense mutation was located at exon 8 of the *ARSB* gene at c.1457A>G [p.D486V] (Fig. 1). This mutation produces deficient protein products leading to the early onset of Maroteaux-Lamy syndrome (nucleotide changes were numbered starting from the A of the ATG initiation codon of the GenBank cDNA clone).

**Table 1.** Clinical features of the three Iranian patients with mucopolysaccharidosis type-VI.

| Clinical features | Patient 1 | Patient 2 | Patient 3 |
|---|---|---|---|
| Age of onset (years) | 2.5 | 2.5 | 3 |
| Coarse facial features | + | + | - |
| Hepatosplenomegaly | + | - | - |
| Dysostosis multiplex | + | + | - |
| Growth retardation | + | + | + |
| ENT disease | + | + | + |
| Cardiac disease | + | - | - |
| Ophthalmological disease | + | - | - |
| CNS disease | - | - | - |
| Mental retardation | - | - | - |
| Inguinal hernia | + | - | - |

CNS, central nervous system




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## DISCUSSION

This is the first report of a novel missense mutation in the *ARSB* gene in three unrelated Iranian patients. Based on the patients' clinical symptoms and their GAG and ASB activities, MPS-IV diagnosis was probable and was therefore necessary to be confirmed by molecular analysis.

Our patients had a missense mutation at exon 8 of the *ARSB* gene at c.1457A>G [p.D486V]. However, another study indicated that the missense mutation near this location, which was previously reported by Karageorgos *et al.* [6] at c.1450A>G [p.R484G], causes severe type of MPS-IV. Our novel missense mutation was found to be very near to the mutation in Karageorgos's study and had the same amino acid change. Although all three patients had the same mutation and all were in the same age group, the severity of the disease was different among them. Patient 1 had a more severe form of MPS-VI in comparison with the other two patients, and patient 3 was only presented with growth retardation and ear, nose and throat disorders. However, none of them had central nervous system disorders and mental retardations.

Experimentally, it would seem that Maroteaux-Lamy syndrome is the most common type of mucopoly-saccharidosis in Iran; however, there have been no documented reports yet. Our data show that the mutation type in all three unrelated patients was the same. This may be because they were originally from the same region and existence of a foundation effect in their population. In conclusion, more cases from this Iranian province and other locations of the country are needed to identify the most common mutations in the various ethnic groups of the Iranian population.

## ACKNOWLEDGMENTS

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