A Novel Pathological *ARSB* Mutation (c.870G>A; p.Trp290stop) in Mucopolysaccharidosis Type VI Patients

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Keywords

ARSB · c.870G>A · Mucopolysaccharidosis type VI · Mutation

Abstract

Mucopolysaccharidosis (MPS) type VI, also known as Maroteaux-Lamy syndrome, is a lysosomal storage disorder, characterized by the deficiency of the arylsulfatase B enzyme. The clinical phenotype and severity of the illness varies according to the residual enzyme activity. Typical features are a short stature, shortened trunk, protuberant abdomen, flexed-knee stance, arched back, corneal clouding, joint stiffness and contractures as well as a waddling gait. Patients typically have Hurler-like dysmorphic facial features: microcephaly, prominent forehead and eyes, a broad nose, low nasal bridge, thick lips, and hyperplastic gums with widely spaced teeth. Complications of the illness include obstructive airway, cardiac valvular problems, splenomegaly, hernias, and pneumonia. Unlike other MPS diseases, MPS VI is characterized by normal intellectual development. Since the disease is due to deficient glycosaminoglycan (mucopolysaccharide) metabolism, elevated urinary glycosaminoglycan levels are a main indicator of MPS. Diagnosis is confirmed by enzyme assays, specifically low arylsulfatase B activity in conjunction with the normal activity of other lysosomal enzymes. Enzyme replacement therapy and hematopoietic

stem cell therapy are showing positive results in the management of the condition. The more severely affected patients, with a rapidly advancing form of the disease, have a short life span and succumb, most commonly to heart failure, by early adulthood. The frequency of *ARSB* variants in patients with MPS VI are as follows: 59.5% missense, 13.5% small deletions, 12% nonsense, 5% splice site or intronic variants, 3% small duplications, 3% large deletions, and 1% stop-loss. We report an Albanian family with siblings diagnosed with MPS VI after clinical examination, biochemical tests, and molecular analysis. Hereby, a novel c.870G>A nonsense homozygous mutation was found responsible for the loss of the enzyme activity.

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Mucopolysaccharidosis type VI (MPS VI; MIM 253200), or Maroteaux-Lamy syndrome, is an autosomal recessive lysosomal storage disorder caused by a deficiency of N-acetylgalactosamine-4-sulfatase (arylsulfatase B, *ASB*) which is required for the degradation of dermatan sulfate. This deficiency in *ASB* results from mutations in the *ARSB* gene and causes intralysosomal storage and urinary excretion of the glycosaminoglycan, dermatan sulfate [Kantapu-

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Fig. 1. a Patient II.1. Fifteen-year-old Albanian boy. Note coarse face, short neck, claw hands, and low nasal bridge. **b** Patient II.2. Twelve-year-old Albanian boy with the same clinical findings and phenotype as in patient II.1.

tra et al., 2014; Ittiwut et al., 2017]. Clinical features of MPS VI vary and can include growth retardation, coarse facial features, stiff joints, skeletal malformations, respiratory difficulties, corneal clouding, hepatosplenomegaly, and cardiac abnormalities. Unlike many other lysosomal storage disorders, mental development is generally normal, although other symptoms (such as hearing loss) may partially cause developmental delay in some individuals [Kara-georgos et al., 2004, 2007a, b]. The prevalence of MPS VI varies among populations, with estimates ranging from 0.0132/100,000 live births in Poland, to 8.0/100,000 live births in Saudi Arabia, and 20.0/100,000 live births in Brazil. While several variables likely influence prevalence rates across populations, it is expected that populations with higher degrees of consanguinity display a higher prevalence of the disease. Only 18% of the published 201 *ARSB* variants have been recorded in public databases (ClinVar, Emv-Class, and ClinVita) [Tomanin et al., 2018]. Pathogenic variants in *ARSB* do not appear to be concentrated in any particular region or domain of the ASB protein. Although pathogenic variants in or near the active site pocket were identified, many are located away from the active site. c.629A>G; p.Tyr210Cys was reported as the most common pathogenic variant in patients with MPS VI; c.962T>C p. Leu321Pro was the second most common allele (6.6%). p. Leu321Pro was found primarily in Turkish originated patients, where it is believed to be a founder mutation [Kantaputra et al., 2014]. Homozygote individuals are particularly represented in the analyzed population of MPS VI patients, 55% of all reported cases [Tomanin et al., 2018].

Clinical Description

Clinical features of the first patient (II.1), a 15-year-old male, were a coarse face with large nose and thick lips, low nasal bridge, large nostrils, epicanthus, cloudy cornea, macrocephaly with a 56.6 cm head circumference (54.3 cm in healthy controls) [Kara et al., 2016], teeth abnormalities and dental caries, claw hands, joint limitation, hirsutism, umbilical hernia, kyphoscoliosis, short neck, and hepatomegaly. Patient II.2, the 12-year-old brother, presents with the same phenotypic features (Fig. 1) and a head circumference of 54 cm (52.8 cm in healthy controls) [Kara et al., 2016]. Additionally, the boy is afflicted with cloudy cornea and a small membrane in the retina.

Materials and Methods

Genomic DNA was isolated from peripheral blood samples. All exons of the *ARSB* gene were amplified by PCR from gDNA. Purified PCR products were sequenced using Big Dye Termination cycle sequencing. The pathogenicity of the identified variant allele was confirmed by the absence of this mutation in 20 unrelated Albanian and in 30 Turkish healthy controls. Analysis of ASB activity was determined by a fluorometric assay [Valayannopoulos et al., 2010; Yu et al., 2013], following a standard protocol, using 20 μ L of cell homogenate, 80 μ L of assay buffer (0.05 M Na-acetate buffer, pH 5.6), and 100 μ L of substrate [5 mM 4-methylumbelliferyl sulphate, Sigma M7133] in assay buffer) combined in a microwell plate. The activity (expressed as nmol/mg protein/h) was derived from a standard curve prepared with known quantities of 4-methylumbelliferyl at pH 5.6. Although enzyme activity varies among individuals (e.g., due to the specific *ARSB* variant, assay method, etc.), most individuals with MPS VI demonstrate ASB activity <10% of the lower limit of the normal level across the disease spectrum [Yu et al., 2013].

Fig. 2. Electropherograms of the family. **a** Carrier father (I.1). **b** Carrier mother (I.2). **c** c.870G>A homozygous patient (II.1). **d** c.870G>A homozygous patient (II.2). **e** Carrier son (II.4) **f** c.870G>A homozygous (reverse direction) patient (II.1).

Results

Laboratory Findings

The index patient (II.1) had increased urinary creatinine: 71 mg/mmol of creatinine (normal range: 0–12 years, 2–12 mg/mmol of creatinine) together with increased urinary excretion of chondroitin/dermatan sulphate. The enzyme levels in the blood showed α -L-iduronidase: 23 nmol/mg of protein/h (6–50 nmol/mg of protein/h), iduronate-sulfatase: 154 nmol/mg of protein/h (115–154 nmol/mg of protein/h), and ASB: 1.2 nmol/mg of protein/h (10–63 nmol/mg of protein/h). Patient II.2. had increased urinary creatinine: 57 mg/mmol of creatinine (0–12 years; 2–12 mg/mmol of creatinine) and increased urinary excretion of chondroitin/dermatan sulphate was observed. Enzyme levels in the blood α -L-iduronidase: 23 nmol/mg of protein/h, iduronate-sulfatase: 135 nmol/mg of protein/h, and ASB: 1.2 nmol/mg of protein/h. ASB activity was 15.1 nmol/mg/h for the father and 15.3 nmol/mg/h for the last child (II.4). The index patient and his brother had a novel c.870G>A (p.Trp-

290stop) homozygous mutation in the *ARSB* gene (Fig. 2). Both parents were found to be carriers of this mutation as shown in Figure 3. Two months later, the index patient died at the age of 15. The third pregnancy (II.3) was a stillbirth. Mutation analysis was performed from amniotic fluid for II.4. The fetus (II.4) and the father also had a c.1072G>A polymorphism besides the c.870G>A (p.Trp-290stop) variation. The baby (II.4) was born 5 months after the mutation analysis.

Discussion and Conclusion

Due to the fact that there is no study evaluating the *ARSB* mutation in Albanian patients with MPS VI, we aimed to share our findings in this report. The term “mutation” is used in this study to describe a genetic variation with less than 1% frequency. A “novel” mutation or variant of unknown significance refers to a variation which has not been reported previously. The literature and databases (HGMD, ClinVar, Ensembl and dbSNP) were

Fig. 3. Pedigree of the family.

checked for previously reported mutations or variants of unknown significance to determine whether a mutation is novel or not. In addition, the values of enzyme activity of the father and the last born son (II.4.) with the same genotype were found to be normal. The c.870G>A (p.Trp290Ter, p.W290*) variation was determined to be disease-causing using in silico prediction tools

(MutationTaster, PolyPhen, and SIFT). Consequently, c.870G>A nonsense variation was found responsible for the loss of the enzyme activity. Preimplantation genetic testing was recommended to the parents for future pregnancies. Molecular diagnostic testing of *ARSB* variants is essential for timely diagnosis of MPS VI.

Statement of Ethics

The study was approved by the local ethics committee and was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

V.S.H. and M.B. designed the study, performed the experiments and analysis of the data, and wrote the manuscript. A.B.-L. designed, drafted, and revised the study.

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