



## Case Report

# A case of mucopolysaccharidosis type VI in a polish family. Importance of genetic testing and genotype-phenotype relationship in the diagnosis of mucopolysaccharidosis



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## ABSTRACT

**Background and objectives:** Mucopolysaccharidosis type VI (MPS VI) is a rare, autosomal recessive lysosomal storage disorder caused by deficient enzymatic activity of N-acetyl galactosamine-4-sulphatase, which is caused by mutations in the arylsulphatase B (ARSB) gene. To date, 163 different types of mutations in the ARSB have been reported. However, the full mutation spectrum in the MPS VI phenotype is still not known. The aim of this study was to perform molecular testing of the ARSB gene in the patient and his family members to confirm MPS VI.

**Methods:** Molecular characterisation of the ARSB gene was performed using Sanger sequencing. We studied a child suspected of having MPS VI and 16 other relatives.

**Results:** We identified a C-to-T transition resulting in an exchange of the Arg codon 160 for a premature stop codon (R160\*, in exon 2). The transition was in CpG dinucleotides.

**Interpretation and conclusions:** The study provided some insights into the genotype-phenotype relationship in MPS VI and the importance of genetic testing when diagnosing MPS, which is not a mandatory test for the diagnosis and only very occasionally performed. Additionally, we present here the history of a family with confirmed MPS VI, which is extremely rare especially in south-eastern Poland. What is more, the position where the mutation is located is very interesting because it is the region of CpG, which is the site of the methylation process. Thus, this opens the possibility of a new approach indicating the involvement of an epigenetic mechanism that should be examined in the context of the pathomechanism of MPS.

## 1. Introduction

Mucopolysaccharidosis type VI (MPS VI, OMIM: 253200) also known as Maroteaux-Lamy syndrome is a rare autosomal recessive lysosomal storage disorder caused by deficiencies in the activity of N-acetyl galactosamine-4-sulfatase (4-sulfatase, arylsulphatase B, ARSB, EC 3.1.6.12). ARSB is an enzyme critical for the degradation of glycosaminoglycans (GAGs), dermatan sulphate and chondroitin sulphate. Molecular defects that cause MPS VI lead to an accumulation of GAGs in connective tissue, lysosomes and urinary excretion of partially degraded dermatan sulphate (DS) [1,2].

In general, for MPS, a prevalence of approximately 0.22 cases per 100,000 births was found for MPS I, while for MPS II, the prevalence

was estimated at 0.45 cases per 100,000 births; for MPS IV A and B, at 0.14 cases in 100,000 births; and for MPS VI, at 0.03 cases per 100,000 births. Fig. 1 demonstrates the prevalence rates for MPS [6]. The incidence of MPS VI varies greatly between populations, ranging from 0 in Northern Ireland to 20 per 100,000 live births in Monte Santo county in Northeast Brazil. In Central and Eastern Europe, the incidence rate ranges from 0.0363 to 0.64 per 100,000 live births in Poland and Lithuania, respectively. It is the rarest form of mucopolysaccharidosis in Poland [3–6].

It is believed that the relatively high prevalence in Lithuania and other Eastern Europe countries is caused by the founder effect with a high carrier frequency of the p.(Arg152Trp) mutation in the ARSB gene.

MPS VI has a wide variety of clinical phenotypes depending on the

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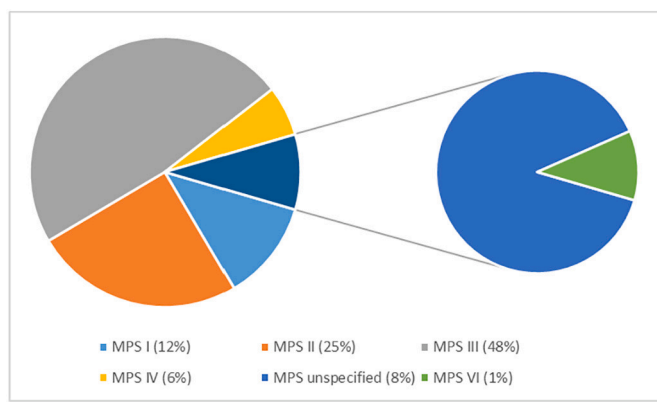


Fig. 1. Relative rates of mucopolysaccharidoses in Poland (including 392 patients). Data according to Jurecka et al. [6].

patient. These include growth retardation, coarse facial features, skeletal deformities, hepatosplenomegaly, corneal opacity, dysostosis multiplex, joint stiffness, and respiratory and cardiac abnormalities. In contrast to other types of mucopolysaccharidosis, patients with MPV VI exhibit normal mental development. Three common clinical forms of MPS VI are observed: severe or infantile, intermediate or juvenile, and mild or adult. The majority of patients with the infantile form die in childhood due to cardiopulmonary complications. In contrast, patients with the adult form of MPS VI have less severe clinical symptoms and their life-span often reaches the seventh or eighth decade [1,2].

MPS VI is determined by mutations in the arylsulfatase B (*ARSB*) gene. The *ARSB* gene is located on chromosome 5q11-q13, spanning less than 440 kb. It contains 8 exons which encode a polypeptide consisting of 533 amino acids with a signal peptide of 37 amino acids. According to the records provided by the Human Gene Mutation Database (HGMD, [www.hgmd.cf.ac.uk](http://www.hgmd.cf.ac.uk)) 163 mutations have been identified, most of them being single base, missense or nonsense variations. Most of them are unique to individual families while a few have been attributed to the founder effect. This heterogeneity is believed to contribute to the wide spectrum of symptoms in MPS VI patients [7–11].

## 2. Clinical case study

A 14 month-old male patient was admitted to our hospital suffering from hand contracture, thought to be the result of arthritis.

The boy was from the first pregnancy, first delivery, born by caesarean section (pelvic fetal position, IUGR) in the 37th week, Apgar score 9/10, birth weight 2570 g, with features of intrauterine dystrophy. Perinatal tests revealed GBS infection in the mother, intrauterine infection was excluded in the newborn. In the 4th week of life, hip dysplasia was diagnosed, and the boy remained under the care of the orthopedic clinic (initially diapers, then a Pavlik harness) and was undergoing rehabilitation. In the 2nd month of life, left-sided inguinal hernia developed. In approx. The 6–7th month, the mother noticed a “hump” of the spine (thoracic lumbar vertebrae, Th-L, borderline). From about 7th month, there were contractures in the knee joints. In addition, delayed motor development, and increased muscle tone were observed during infancy. The parents were not related to one another. The parents were young (mother 21 years old and father 24 years old) and without any clinical symptoms. There was no family history of any illnesses.

The boy was in a good general condition. Physical examination revealed thick facial features, a prominent forehead, narrow palate, contractures in the knee and hip joints and in the interphalangeal joints of hands, umbilical hernia, left-sided inguinal hernia, funnel chest, increased muscular tone, and dysmorphic features.

The patient was referred to the Rheumatology Outpatient Clinic (which has extensive experience as it also functions as the Center of Rare Diseases) due to contractures in the joints of the hands, knees and abnormal gait. The boy was suspected to be suffering from MPS I or VI. The most common type of MPS II among the Polish population was excluded, based on our clinical experience and the observed phenotype as well as family history. In the family history, there were no incidences of MPS II, which is an X-linked recessive disease. The observed symptom of the disease was a mild stiffness of the finger joints. The results of basic laboratory tests were within the reference intervals for his age. Electrophoresis of excreted GAG revealed dermatan sulfate in the serum and urine. Ultrasound of the abdomen showed the liver size at the upper norm limit with a homogeneous parenchyma and moderately enlarged spleen with a uniform echogram of the parenchyma. Ultrasound of the hip joints revealed no exudate, synovial hyperplasia, and no increased vascular flow. The joint capsule was adjacent. A clear thickening up to 7.7 mm of the joint capsule on both sides and significantly increased echogenicity of the joint capsules were found. Both joints were symmetric, the femoral head round, without visible pathology, and the periarticular space was without pathology [12,13]. We did not observe any other clinical manifestations such as cardiac involvement, corneal clouding or upper airway obstruction. Growth retardation was observed as shown in Fig. 2. The patient was treated with enzyme replacement therapy, ERT (Naglazyme).

Since the patient presented with characteristic clinical symptoms of MPS VI, abnormal development of the spine in the thorax-lumbar part and normal intellectual function, we decided to perform a molecular study of the *ARSB* gene as there was a suspicion that it might be MPS VI. Blood samples were collected from the patient, his parents, grandparents and the parents' siblings.

## 3. Molecular genetic studies

Total genomic DNA was obtained from peripheral blood collected in EDTA. Isolation was performed using a NucleoSpin Blood Kit (Macherey Nagel). Sequences of primers specific to the *ARSB* gene were used according to Petry et al. as previously described [Petry et al., 2005]. Polymerase chain reaction (PCR) was used to amplify exons 1–8 and the adjacent intronic regions of the *ARSB* gene. The amplification was performed using a FastStart PCR Master Kit (ROCHE Diagnostics). The PCR products were purified with a NucleoSpin Gel and PCR Clean-up (Macherey Nagel) and sequenced with a BigDye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies, Foster City, CA, USA). The products of cycle sequencing were purified of unbound fluorescent dyes with a BigDye XTerminator Purification Kit (Life Technologies, Foster City, CA, USA) and separated on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were analysed using the AB DNA Sequencing Analysis Software v.5.2. (Applied Biosystems) and then were compared to the *ARSB* gene reference (NCBI GeneBank Reference Sequence Accession Numbers: NM\_000046.5 and ENSEMBL database Accession Numbers: ENST00000264914.9).

## 4. Ethics statement

Informed consent was obtained from the patient's parents and all family members.

## 5. Results

### 5.1. Mutation analysis

At first, we identified a C-to-T transition, resulting in an exchange of the Arg codon 160 for a premature stop codon [p.(Arg160\*)], in exon 2 in the patient (Fig. 3). The variant was presented in the homozygous state in the patient. This variant has been previously reported and is recorded in The Human Gene Mutation Database (HGMD) as causing

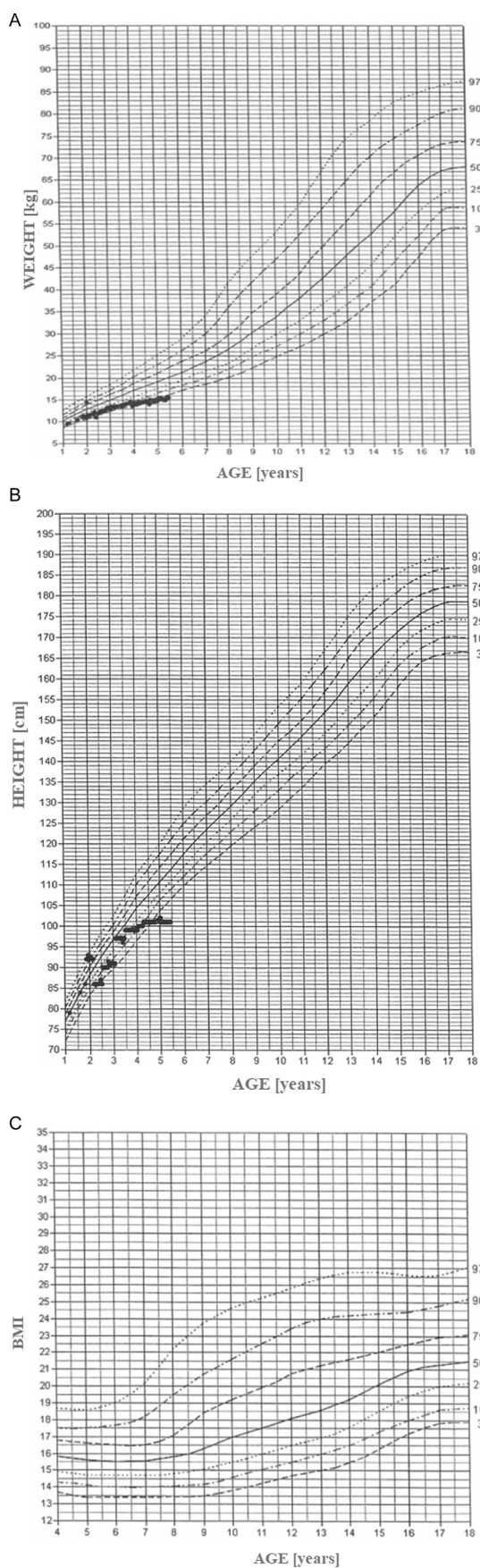


Fig. 2. Associations of age and z scores of weight (A), height (B), and body mass index (BMI) (C) of the patient.

the MPS VI phenotype. We next performed genetic testing in the patient's parents. We also identified the presence of the same variant in the heterozygous state in both parents, which confirmed the diagnosis of MPS VI which is inherited autosomally recessively.

## 5.2. Other family members

Among the relatives on the father's side of the family, the same variant was identified in the heterozygous state. On the mother's side, only her father did not have the variant. The family history with frequency of Arg160\* is presented in Fig. 4.

## 6. Discussion

In this report, we present the history of MPS VI diagnosed in a Polish family. Confirmation of this type of MPS was unexpected as Poland has the lowest prevalence of MPS VI.

The patient was suspected to be suffering from either MPS I or VI. Based on our clinical experience and knowing the clinical symptoms and diagnostic results, we excluded MPS II (the most common in the Polish population). The boy presented several characteristic clinical symptoms of MPS VI, mostly related to skeletal deformities without mental retardation. The first observed symptom in the boy was a mild stiffness of the finger joints and then growth retardation.

This is the first case of MPS VI diagnosed in the south-eastern region of Poland. The highest prevalence of MPS VI was reported in Russia, Kazakhstan, Central and Eastern Europe and Belarus [3,6]. The patient was the first child of young and healthy parents and was born to a family without a history of MPS or any past illnesses. However, following genetic testing, interviews with the parents revealed that many ancestors on both sides of the family were born in territories occupied by Russia during the World War II. Another aspect of this case is that the mutation that we identified may be the founder variant for MPS VI in the Russian population.

No specific ethnic group has been associated with an increased risk of MPS VI. However, it may be the case that some populations have increased frequencies of specific mutations causing the disease. Petry et al., described the deletion of 23 nucleotides at position 1533 of the *ARSB* gene, which is very characteristic for the Brazilian population [14,15]. The same variant is very common (with a frequency 23% of alleles) in Portuguese MPS VI patients [15]. We mention this here because, based on the literature, we can speculate that there are founder mutations linked to various ethnic populations. This is not a new fact, but grouping and exploring these founder mutations could help to characterise the genetics of different types of MPS. There are unique common mutations in different genes that are responsible for different types of MPS. Some common mutations in a particular MPS may directly correspond to the high prevalence of that particular MPS in a certain region or ethnic group. It is known that different types of MPS are distributed with different frequencies in different regions on the world. The exact characterisation of mutations that are associated with the birth prevalence of certain types of MPS and the countries or even the regions over the world could be very helpful in diagnosing the disease and especially in confirming the phenotype. Moreover, it may help clinicians in assessing the relative risks and benefits of the currently available therapies. The low prevalence of MPS among countries, especially Poland, and the large number of mutations as well as inconsistent phenotype testing limit the ability to predict phenotype from genotype.

The variant that we identified in the Polish family was [p.(Arg160\*)]. This variant was identified for the first time in 1994 in Germany, where it was encountered 7 times. This variant has already been found in diverse populations and ethnic groups such as Belarusian, Indian, Italian, Russian, Spanish and Taiwanese patients [16–21]. This substitution results from a transition in a CpG dinucleotide region. This is the second very interesting point in our study. Until now, the most



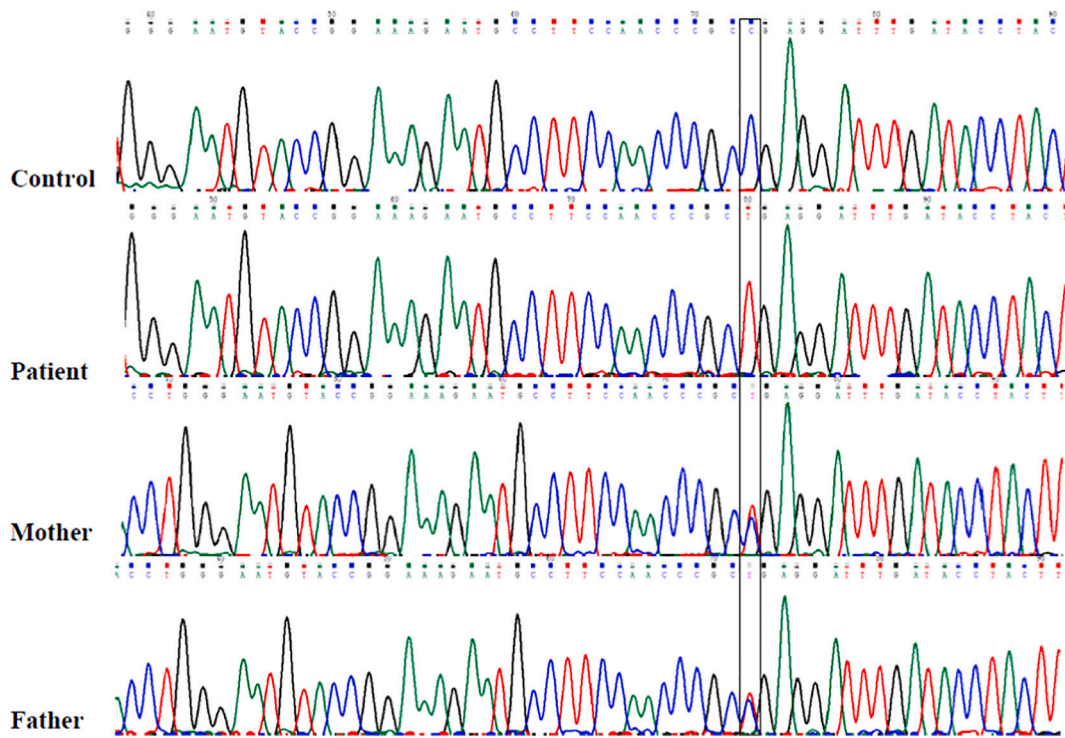


Fig. 3. ARSB sequencing electropherograms showing [p.(Arg160\*)] variant (indicated by the frame) in the patient and his parents in comparison to a healthy control as a reference.

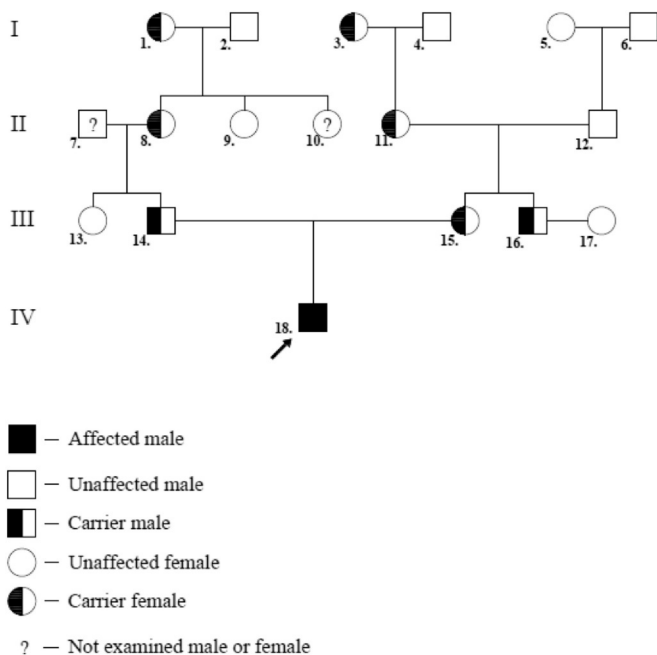


Fig. 4. History of the family. The patient is indicated by the black arrow. A symbol with a question mark “?” indicates an individual who did not undergo clinical evaluation and genetic testing.

unique variants identified in the *ARSB* gene were missense (~59%); followed by small deletions (~13%), nonsense (~12%), splice site or intronic variants (~5%), small duplications (~3%), large deletions (~3.0%) and stop-loss mutations (~1.0%). In our study, we identified a C-to-T transition at the 478 nucleotide position within a CpG dinucleotide. It is also known that the mutation rate varies throughout the genome in a contextual fashion [22]. It was estimated that ~24% of missense or nonsense variants in the *ARSB* gene are transitions in a CpG

dinucleotide [23]. The nonsense variant that we confirmed in the patient is described as one of a total of seven nonsense mutations reported in the gene. Among them, p.(Arg160\*) is one of two (with p.(Arg315\*)) of the transitions located in CpG nucleotides consisting of ~28% which are predicted to change the gene's activity and are strongly related to the phenotype's severity [22]. \*\*\*.

Knowing that 10–60% of SNPs that cause human diseases result from transitions at CpG dinucleotides, we can speculate that CpG methylation may have important implications for the etiology of not only MPS VI, but also of all types of MPS. It may provide new insight into the etiology of the disease which has a wide heterogeneity on both phenotype and genotype levels. It could be worth evaluating the relationship between methylation status and CpG hotspot mutations in the whole genome in MPS VI patients, which has not previously been studied. In patients diagnosed with MPS type II and IV, it was demonstrated that the methylation pattern in iduronate-2-sulfatase (*IDS*) and lysosomal *N*-acetylgalactos-amine-6-sulfatase (*GALNS*) genes causing the disease correlated with the likelihood of mutation. Methylation regulates gene expression and can play a crucial role in the occurrence of MPS VI-causing mutations [24]. The variant that we confirmed in our patient has been suggested to be a mutational hotspot in the *ARSB* gene [18,20].

The third and the final aspect that we want to discuss here is the importance of the genotype-phenotype relationship that should be included at the beginning of the diagnostic process of MPS VI. Nowadays, the diagnosis of mucopolysaccharidosis is generally established based on a combination of clinical symptoms and laboratory tests. The patients may show growth retardation, coarse facial features, skeletal deformities, frequent upper-airway infections, enlarged liver and spleen, hearing loss, joint stiffness, or coarse hair. All of these symptoms and signs suggests a mucopolysaccharidosis but do not provide a specific diagnosis. The first step in MPS VI diagnosis is the assessment of urinary GAGs excretion with dermatan sulphate and chondroitin sulphate being compounds excreted in high amount. Both quantitative and qualitative analysis of urinary GAGs is performed. A positive result is usually followed by measurement of the *ARSB* enzyme activity in

leucocytes and fibroblasts being considered optimal samples [25]. Although the biochemical tests are sufficient to diagnose MPS VI, they do not allow for correlation between enzyme activity and clinical phenotype. Due to the high frequency of broad compound heterogeneity and incomplete identification of polymorphisms, the correlation between genotype and phenotype, and prediction of severity is very limited. This lack of reliability means that it is not possible to appropriately manage the disease based on genotype at this time. Here, we would like to emphasise the high importance of molecular testing, which contributes to the establishment of the genotype-phenotype relationship in this disease. As mentioned above, our patient did not have all of the specific symptoms of the disease, his parents were young and healthy, and there was no family history of the disease.

Thus, in this paper, we present not only the case study, but especially the high value of molecular testing in the context of diagnosing mucopolysaccharidosis and the genotype-phenotype relationship. In MPS patients, early intervention with ERT, which can preserve or restore function by removing excess material accumulated in the lysosome, is very important. The therapeutic potential of ERT is possible only when the diagnosis is performed accurately. The genetic diagnosis allows for rapid treatment initiation and also for genetic counselling. The physical condition of our patient on the day of hospitalisation was very critical. After molecular diagnosis, and ERT treatment, within two years, he was able to function normally with healthy children of his age. In reporting this case, we would like to emphasise the importance of molecular diagnosis related to a proper treatment decision and to a delayed disease progression (in this case, even reversing clinical manifestation of the disease), and also to publish this specific clinical description of the patient with MPS VI from the Polish population, which may help in better understanding the genotype-phenotype interactions. MPS VI is characterised by its severity, broad clinical spectrum, and high level of genetic diversity displayed in individuals. For most variants in the *ARSB* gene (about 45%) there is not enough evidence in the databases and literature for their classification and pathogenicity. The systematic reporting and classification of alleles pathogenic for the disease may be very helpful in reducing the time consumed during diagnosis, and also in reducing the ethical complications, based on our example, in which MPS VI has the lowest frequency in the Polish population.

#### Declaration of Competing Interest

The authors report no financial or other conflict of interest relevant to the subject of this article.

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