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Molecular analysis of mucopolysaccharidosis type VI in Poland, Belarus, Lithuania and Estonia

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ABSTRACT

Mucopolysaccharidosis VI (MPS VI) is a rare autosomal recessive disorder caused by a deficiency of N-acetylgalactosamine-4-sulfatase (ARSB). Over 130 *ARSB* gene mutations have been identified thus far and most mutations are unique to individual families. We aimed to analyze the spectrum of mutations in the *ARSB* gene responsible for the disorder in Poland, Belarus and Baltic States. Twenty one families with MPS VI patients, in whom diagnosis was confirmed biochemically and enzymatically, were studied. Direct sequencing of patient genomic DNA was used to identify *ARSB* mutations. In total, fourteen different disease-causing mutations were found. Three novel mutations included insertion c.375_376insT, a missense mutation c.499G>A (p.G167R) and deletion/insertion c.750_754delinsCCTGAAGTCAAG. We also report 11 previously described mutations (p.A33V, p.W57C, p.Q88X, p.T92K, p.Q97X, p.R152W, p.R160Q, p.R160X, p.Y210C, p.Y266S, p.G302R). The mutation p.R152W was present at a high prevalence of 50% (21/42) the mutated alleles in this group of patients. High prevalence of p.R152W mutation in Poland, Belarus and Baltic States indicates a possible founder effect and suggests that screening for this mutation may be appropriate in MPS VI patients from this region. Our study has also provided evidence to support genotype–phenotype correlation.

1. Introduction

Maroteaux–Lamy syndrome (mucopolysaccharidosis type VI, MPS VI, OMIM 253200) is a rare lysosomal storage disorder with an autosomal recessive mode of inheritance. MPS VI is caused by deficient activity of the lysosomal acid hydrolase N-acetylgalactosamine-4-sulfatase (4-sulfatase, arylsulfatase B, ARSB, EC 3.1.6.12), which is required in the sequential degradation of some glycosaminoglycans (GAGs), namely dermatan sulfate (DS) and chondroitin sulfate (CS) [1]. Failure to degrade these GAGs results in the accumulation of the undegraded or partially degraded substrates in the lysosomes of cells and a characteristic clinical phenotype.

Clinical features include short stature, coarse facial features, stiff joints, skeletal malformations, *dysostosis multiplex*, respiratory problems, heart involvement, corneal clouding, and hepatosplenomegaly [1–3]. The rate of clinical progression of MPS VI patients varies considerably, generating a wide, continuous clinical spectrum from severe to relatively attenuated [1,2].

With the cloning of the *ARSB* gene and advances in sequencing technology, the identification of *ARSB* mutations was made possible [4,5]. The *ARSB* gene has been assigned to chromosome 5q11-q13, comprising 8 exons spanning less than 440 kb. This gene encodes a polypeptide of 533 amino acids with a signal peptide of 37 amino acids [6–8].

According to the records provided by the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk) and recent publications, over 130 *ARSB* gene mutations have been identified thus far. Allele frequencies of the different mutations are very low, and most mutations are unique to individual families. This allelic heterogeneity is thought to contribute to the wide spectrum of clinical presentation observed in MPS VI patients. Mutations have been reported in several populations from various countries, including Russia [9–12], Australia [9,11,13,14], USA [10,15–19], France [10,16,18,20], Germany [10,16], Portugal [18], Italy [21,22], Asia [23–26], Brazil and Chile [18,27,28].

Although no specific ethnic group has been associated with an increased risk of MPS VI, some populations have been found to have

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increased frequencies of specific mutations. The 1533del23 mutation is common among Brazilian MPS VI patients, occurring in 23% of alleles. This mutation also occurs in Portuguese MPS VI patients, although the frequency is unknown [27,28].

This is the first report describing *ARSB* mutations in 24 patients from Poland, Belarus, Lithuania, and Estonia.

2. Materials and methods

2.1. Patients

The present series comprises a total of 21 unrelated families affected by MPS VI. All patients were recruited with the help of their attending clinicians as a part of an international collaboration between Poland, Belarus and Baltic States (Lithuania, Latvia and Estonia). Four MPS VI families of Polish origin were diagnosed at The Children's Memorial Health Institute (Warsaw, Poland). Six Lithuanian families were diagnosed at the Center for Medical Genetics, Vilnius University Hospital, Santariskiu Klinikos (Vilnius, Lithuania) and two of the families claimed Polish origin, one family with 3 siblings claimed Lithuanian/Russian origin and three families claimed Lithuanian origin. Nine families from Belarus were diagnosed at the National Research and Applied Medicine Center "Mother and Child", Clinical Diagnostic Genetic Laboratory (Minsk, Belarus): eight claimed Belarusian origin and one claimed Russian/Tatarian origin. Two Estonian families claiming Estonian origin were diagnosed at the Department of Genetics, Tartu University Hospital (Tartu, Estonia). So far no patient has been diagnosed in Latvia (Dr. Zita Krumina, Medical Genetics Clinic, Children's University Hospital, Riga, Latvia, personal communication). Diagnosis of MPS VI was confirmed biochemically by demonstrating abnormal excretion of dermatan sulfate in urine and deficient activity of ARSB in leucocytes and/or fibroblast cell lines.

2.2. Molecular analysis

DNA was isolated from venous blood according to Miller et al. or extracted from blood spots on Whatman cards according to REDExtract-N-Amp™ Blood PCR Kit (Sigma-Aldrich) protocol [29].

The *ARSB* gene was analyzed by direct sequencing. PCR and sequencing primers were designed with Primer3 or Mutation Discovery softwares (primers and PCR conditions available upon request). Fluorochromatograms were analyzed using Mutation Surveyor™ software (SoftGenetics). Sequences NM_000046.3 and NP_000037.2 were used as a reference.

Protein multiple alignments were performed using Alamut version 1.53 software. Databases used in the study: NCBI (http://www.ncbi.nlm.nih.gov), Ensembl (http://www.ensembl.org/index.html), HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), and HGVS (http://www.hgvs.org/). In order to determine the prevalence of the p.A33V variant in the Caucasian population, 101 anonymous randomly selected DNA samples from the same ethnic background from the DNA bank of the Health Care Center Genomed were used as a control group.

2.2.1. Ethical consideration

The protocol was approved by the human-subjects institutional review board at The Children's Memorial Health Institute. Written informed consent had to be provided by the parents or legal guardians. The study was designed and conducted in compliance with the principles of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines for Good Clinical Practice.

3. Result

3.1. Clinical evaluation

Clinical characteristics of some patients have been reported previously elsewhere [30,31]. Clinical data, the genotypes, clinical phenotypes and ethnic origins of the 24 patients studied here are listed in Table 1. The clinical phenotype, defined as previously reported [18,32], was available for all patients and ranged from relatively attenuated to severe: 30% (n = 7) of patients presented with the severe form of the disease, 33% (n = 8) with the intermediate form, and 37.5% (n = 9) with the attenuated form.

3.2. Analysis of the ARSB mutations

The entire open reading frame of the *ARSB* gene and all the exon-intron boundaries were investigated in the 24 MPS VI patients representing 21 unrelated families (42 mutated alleles) by direct sequencing of genomic PCR products. The molecular examination of the patients with MPS VI allowed identification of 41 out of 42 (97.6%) of the mutant alleles.

Characteristics of the 14 distinct *ARSB* mutations identified are reported in Table 2. The mutational spectrum comprised 9 missense mutations, 3 nonsense mutations and 2 frameshift mutations (one insertion and one deletion/insertion). Three of these mutant alleles have not been previously reported. The novel lesions resulted from insertion c.375_376insT, a single nucleotide substitution (c.499G>A) causing missense mutation (p.G167R) and deletion/insertion c.750_754delinsCCTGAAGTCAAG. Two novel mutations (c.375_376insT and p.G167R) were found in heterozygous state in association with p.R152W. Mutation c.750_754delinsCCTGAAGTCAAG was found in a homozygous state (biparental inheritance was confirmed).

In addition, we found several previously described mutations in this series of patients. Fig. 1 depicts the location of the various mutations and polymorphisms detected in relation to the structure of the *ARSB* gene.

3.3. Missense and nonsense mutations

A total of 12 *ARSB* mutations were either missense or nonsense. Eleven of them (p.A33V, p.W57C, p.Q88X, p.T92K, p.Q97X, p.R152W, p.R160Q, p.R160X, p.Y210C, p.Y266S, p.G302R) have already been described. The G to A transition at nucleotide 499 resulting in substitution of glycine for arginine 167 (p.G167R) is a novel mutation.

3.4. Frameshift mutations

We found one insertion (c.375_376insT) and one deletion/insertion (c.750_754delinsCCTGAAGTCAAG) that have not been reported so far (Fig. 1). They are both frameshift mutations causing premature termination of protein synthesis. An insertion of a thymine base between nucleotides 375 and 376 results in a change of the 126th glutamic acid codon (GAA) into opal termination codon (TGA). A deletion of 5 nucleotides with a concurrent insertion of 12 nucleotides (c.750_754delinsCCTGAAGTCAAG) causes a frameshift and premature stop codon 8 codons downstream of this mutation, reducing the encoded mutant ARSB to 257 amino acids compared to 533 in a wild type protein.

3.5. Polymorphisms

In the studied group frequent polymorphisms were identified (Fig. 1). The p.A33V occurred in the examined group with a frequency of 19% (4 homozygous cases, 8/42 alleles). The frequency in the control group was 5% (5 heterozygous cases, 5/202 alleles).

Table 1
Clinical phenotypes and genotypes encountered in 24 MPS VI patients included in the study.

F	Pt	Ethnic origin[a]	Age at onset/ diagnosis	Clinical signs[b]	Clinical classification[c]	Allele 1[d]	Allele 2[d]
1	1	Polish	1 y/3 y	CC, CCO, CFF, GR, H (mild), IH, MC, JS, RR, SK, UH	Severe	**c.750_754delinsCCTGAAGTCAAG**[f] p.A33V[e]	**c.750_754delinsCCTGAAGTCAAG** p.A33V[e]
2	2	Polish	6 mo/5 y	CCO, CFF, GR, JS, SK	Intermediate	p.Y210C	?
3	3	Polish	6 y/6 y	CC, CCO, CFF, GR, IH, MC, JS, SK	Intermediate	p.Q88X	p.R152W
4	4	Polish	21 y/37 y	CC (mild), CCO (severe), CF, CFF (mild), GR (mild), JS (mild), SK	Attenuated	p.R152W	p.R152W
5	5	Belarusian	nd/10y	CC, CCO, CFF, CTS, GR, H, MC, JS, SCC, SK, UH	Severe	p.Y266S p.A33V[e]	p.Y266S p.A33V[e]
6	6	Belarusian	8 y/9 y	CC, CCO, CFF, CTS, GR, H, MC, JS, SK, UH	Intermediate	p.Q97X	p.R152W
7	7	Russian (Belarus)	8 y/16 y	CC, CCO, CFF, CTS, MC, JS, SK	Attenuated	p.Y210C	p.T92K
8	8	Belarusian	4 y/14 y	CC, CCO, CFF, CTS, GR, H, MC, JS, SK	Intermediate	p.W57C	p.R152W
9	9	Belarusian	8 mo/5 y	CC, CCO, CFF, GR, IH, MC, JS, SK, UH	Severe	p.Y266S p.A33V[e]	p.Y266S p.A33V[e]
10	10	Belarusian	1 y/2 y	CCO,CFF, GR, H, MC, JS, SK	Severe	p.Y266S p.A33V[e]	p.Y266S p.A33V[e]
11	11	Belarusian	15 y/22 y	CC, CCO, CFF, CTS, GR (mild), H, JS, SK	Attenuated	p.R152W	p.R152W
	12	Belarusian	15 y/22 y	CC, CCO, CFF, CTS, GR (mild), H, JS, SK	Attenuated	p.R152W	p.R152W
12	13	Belarusian	5y/13y	CCO, CFF, CTS, GR (mild), JS, SK	Attenuated	p.R152W	p.R152W
13	14	Belarusian	9.5 mo/5y	CC, CCO, CF, CFF, CTS, GR (severe), H, MC, JS, SK	Severe	p.R160X	p.R160Q
14	15	Lithuanian	2 y/23 y	CCO (mild), CF (mild), CFF (mild), GR (mild), IH (mild), MC, JS (mild), SK (mild)	Attenuated	p.R152W	p.R152W
15	16	Polish (Lithuania)	7 y/30 y	CCO (moderate), CFF (moderate), GR (mild), HC, IH (mild), MC, JS, SCC, SK	Attenuated	p.R152W	p.R152W
16	17	Lithuanian	9 mo/11 y	CCO (mild), CFF (severe), GR (severe), HC, MC, JS (severe), SK (severe)	Intermediate	p.R152W	**c.375_376insT**
17	18	Lithuanian/Russian (Lithuania)	14 mo/10 y	CCO (severe), CFF, GR (severe), H (mild), JS (severe), SK (severe), SCC	Intermediate	p.R152W	**p.G167R**
	19	Lithuanian/Russian (Lithuania)	16 mo/6 y	CCO (severe), CFF, GR (severe), H (mild), MC, JS (severe), SK (severe)	Intermediate	p.R152W	**p.G167R**
	20	Lithuanian/Russian (Lithuania)	15 mo/12 y	CC, CCO (mild), CFF, GR (severe), H (mild), MC, JS (severe), SK (severe)	Intermediate	p.R152W	**p.G167R**
18	21	Lithuanian	4 y/11 y	CC, CFF, GR, JS, SK	Intermediate	p.R152W	**p.G167R**
19	22	Polish (Lithuanian)	6 y/15 y	CFF (mild), CCO (mild), GR (mild), HC, JS (moderate), MC, SK (moderate)	Attenuated	p.R152W	p.R152W
20	23	Estonian	1 mo/11 y	CC, CCO, CFF, GR, H, MC, JS, SK	Intermediate	p.R152W	p.G302R
21	24	Estonian	12 y/19 y	CC, CCO (mild), CFF, GR (mild), HC, JS, SCC, SK	Attenuated	p.R152W	p.R152W

F = family; pt = patient; mo = months; y = years; ? = unknown; Novel mutations are marked in bold.
[a] Ethnic origin as declared by patients, in brackets a country of citizenship if different than the country of origin.
[b] CC, corneal clouding; CCO, cardiac complications; CF, cardiac failure; CFF, coarse facial features; CTS, carpal tunnel syndrome; GR, growth retardation; H, hepatomegaly; HC, hydrocephalus; HS, hepatosplenomegaly; IH, impaired hearing; MC, macrocephaly; JS, joint stiffness; RR; recurrent rhinitis; SCC, spinal cord compression; SK, skeletal abnormalities; UH, umbilical hernia.
[c] Clinical classification defined as previously reported [18,32].
[d] NCBI Reference Sequence: NM_000046.3 (c.DNA considering A of the ATG translation initiation codon as nucleotide +1); NP_000037.2.
[e] p.A33V was previously described as a mutation [18] discussed here as a possible polymorphic change.
[f] The mutation carrier test was performed in order to confirm the biparental inheritance of identified mutations and to exclude large deletions.

3.6. Genotype–phenotype correlation

Genotypes, phenotypes and geographical origins of the 24 patients studied here are listed in Table 1. Fifty two percent of the unrelated patients were found to be homozygous for either known *ARSB* mutations (10 patients) or novel lesions (1 patient), while 48% of patients were compound heterozygotes (including one in whom a second defect in the *ARSB* allele has not been identified).

Overall, we found 12 distinct genotypes including one that was partially characterized.

Among the analyzed group, 19 (90.5%) unrelated patients were either homozygous or compound heterozygotes for the four most common alleles (p.A33V, p.R152W, p.Y266S, p.Y210C).

Mutations p.R152W and p.Y210C were associated with a relatively attenuated MPS VI phenotype. Homozygosity for p.R152W mutation yielded very mild phenotypes in patients, while heterozygous patients generally presented with an intermediate phenotype (Table 1). Nonsense mutations (p.Q88X, p.Q97X, p.R160X) as well as a missense mutation p.Y266S were associated with a severe MPS VI phenotype.

3.7. Frequency and geographical distribution of ARSB mutations in the Central and Eastern European population

As reported in Table 2, in the whole series comprising patients from Poland, Belarus and Baltic States, the frequency of the most common mutations was: 50% for p.R152W, 14.3% for [p.A33V; p.Y266S] and 4.8% for p.G167R, p.Y210C and p.E250DfsX8. Altogether, these mutations were represented on 69% of *ARSB* alleles. Rare or private mutations comprised 31% of *ARSB* lesions identified, and 40% of these were novel. When we analyzed the geographical distribution and relative frequencies of the most frequent mutation, we observed a cluster of families with the p.R152W mutation in Poland, Belarus and Baltic States with the center located in Lithuania suggesting the existence of the founder effect (Fig. 2).

4. Discussion

At present, over 130 mutations in the *ARSB* gene have been reported in the literature suggesting a high molecular heterogeneity for MPS VI in many populations. These mutations have been identified primarily in

Table 2
Characteristics of the changes in the *ARSB* gene identified in 24 MPS type VI patients.

Loc	Change at DNA level[a]	Predicted effect on protein structure	Type of mutation	No. of alleles (%)[b]	References
Ex. 1	c.98 C>T	p.A33V	Missense (polymorphism)[c]	19	[18]
	c.171 G>C	p.W57C	Missense	2.4	[18]
	c.262 C>T	p.Q88X	Nonsense	2.4	[30]
	c.275 C>A	p.T92K	Missense	2.4	[18]
	c.289 C>T	p.Q97X	Nonsense	2.4	[19]
Ex. 2	**c.375_376insT**	**p.E126X**	**Frameshift**	2.4	Present study
	c.454 C>T	p.R152W	Missense	50	[11]
	c.478 C>T	p.R160X	Nonsense	2.4	[11]
	c.479 G>A	p.R160Q	Missense	2.4	[11]
	c.499 G>A	**p.G167R**	**Missense**	4.8	Present study
Ex. 3	c.629A>G	p.Y210C	Missense	4.8	[13]
Ex. 4	**c.750_754delinsCTGAAGTCAAG**	**p.E250DfsX8**	**Frameshift**	4.8	Present study
	c.797A>C	p.Y266S	Missense	14.3	[18]
Ex. 5	c.904 G>A	p.G302R	Missense	2.4	[22]

Loc = location; Ex = exon; ins = insertion; del = deletion; aa = amino acid; Novel mutations are marked in bold.
[a] NCBI Reference Sequence: NM_000046.3 (c.DNA considering A of the ATG translation initiation codon as nucleotide +1).
[b] For the calculation of allele frequencies in this study the total number of alleles was regarded as 42 because the number of analyzed families was 21.
[c] The change p.A33V was previously described as a disease causing mutation [18] discussed here as a possible polymorphic change.

the patients from North and South America, Western Europe, Australia and Asia. Apart from two reports by Voskoboeva describing thirteen mutations, there are no other reports from Eastern and Central Europe [11,12]. Thus, this report is the first exhaustive mutational analysis performed in a large group of MPS VI patients from Poland, Belarus, Lithuania and Estonia. In this study, the *ARSB* gene profile of 21 families was examined and a total of 14 different mutations, scattered along the *ARSB* gene, were detected. Such high molecular heterogeneity is in agreement with the wide mutational spectrum previously reported for the disease in many other populations.

An uncommon finding of this study was the frequency of homozygosity in our sample: 52% (11/21) of the families bore homozygous mutations. It is unusual that a patient with a rare disease, such as MPS VI, would be homoallelic for a mutation unless a mutant allele was common or the patient was a product of a consanguineous mating. The latter explanation is presumed to be likely for some of some patients (1, 5, 9 and 10) since they had more than one homozygous change in DNA sequence. However, that was not the case in patients homozygous for the p.R152W mutation.

Mutation p.R152W was present in 14 families (7 in homozygosity and 7 in heterozygosity) and therefore accounted for 50% of the total number of mutant alleles (Table 2). Until now, mutations described in MPS VI worldwide have been unique and have rarely been found in unrelated patients. In 2003, Petry et al. [27] reported a deletion (1533del23) among Brazilian MPS VI patients, which was found in 23% of alleles. The p.R152W mutation was first reported by Voskoboeva et al. in an Ukrainian patient [11]. All of the patients with this mutation in our study were from Central and Eastern Europe (Poland, Lithuania, Belarus, and Estonia, Fig. 2). The presence of several MPS VI families residing in a small geographic area with frequent appearance of the mutant allele in homozygous form would be considered *a priori* a classic case of founder effect. The geographical regions of Lithuania, Belarus and Ukraine had close historical relations with Poland. Because no known consanguinity was declared among the patients studied here, p.R152W may represent a founder mutation in the context of the Central and Eastern European population. If confirmed, this should render the mutation a primary target in molecular studies of MPS VI in this geographical region. Additionally, screening the

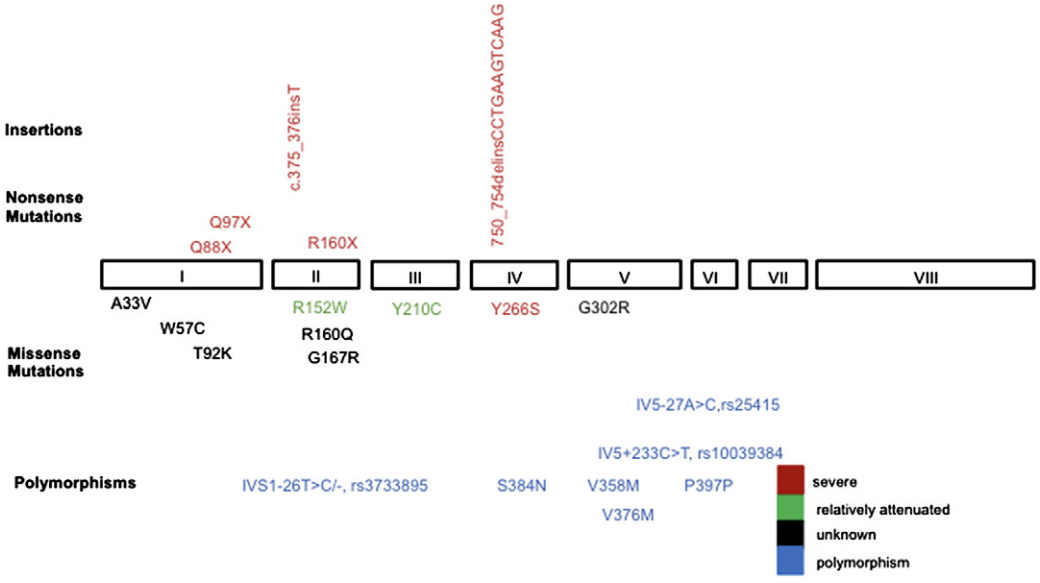

Fig. 1. Location of all the MPS VI mutations in the human *ARSB* gene identified in this study. The exons are represented by boxes and numbered by roman numerals. The predicted severity of the mutations is color-coded as shown by legend. Disease phenotype was categorized as relatively attenuated, intermediate and severe on the basis of clinical criteria.

Fig. 2. Geographical distribution of mutations in the present series. Black dots refer to families with patients homozygous for p.R152W mutation; black and white dots refer to families with patients heterozygous for p.R152W mutation; white dots refer to families with patients with other mutations. The p.R152W mutation was first reported by Voskoboeva et al. in an Ukrainian patient [11].

extended families or neighborhoods as well as haplotype analysis of these patients should be considered.

Genotype–phenotype correlation for most MPS VI patients has been difficult due to a large number of mutations, which are often private or novel. In general, mutations resulting in gross alternations in the *ARSB* sequence such as nonsense mutations and mutations resulting in a truncated protein due to sequence deletions or insertions are generally found in patients with the severe form of the disease. On the other hand, missense mutations, which comprise the majority of mutations in MPS VI, have been found in patients with both severe and attenuated phenotypes [18]. A number of mutations in our study appeared to result in disease that progresses relatively slowly, while others appeared to result in disease that progresses rapidly with early onset of a severe phenotype. The p.R152W mutation results from nucleotide substitution CGG>TGG in exon 2 of the *ARSB* gene which leads to a missense change of arginine to tryptophan residue. In our study, eight patients (4, 11–13, 15, 16, 22, 24) from seven families were homozygous for the p.R152W mutation. All of these patients presented with slower progression of the disease. MPS VI features developed later in the course of the disease and most often included mild or very mild coarse features, restricted joint range of motion, and skeletal malformations. At the time of the study, two of the patients (4 and 24) were deceased (at the age of 38 and 24 years respectively), while the two eldest patients were 34 years of age confirming that this mutation is associated with greater longevity. Despite classification as relatively attenuated or slowly progressing, these patients may develop serious complications including cardiac and neurological problems as seen in patients 4, 16, and 24 [31]. Summarizing, individuals homozygous for p.R152W may remain undetected because of the mild resulting phenotype [31].

Mutation p.R152W was also detected in a heterozygous state in patients 3, 6, 8, 17–21, and 23 (Table 1) resulting in all patients in intermediate phenotypes suggesting that the severity of alleles such as p.W57C, p.Q88X, p.Q97X, and p.G302R, seemed to be alleviated by the p.R152W allele.

Mutations c.375_376insT, p.G167R and c.750_754delinsCCTGAAGTCAAG are reported here for the first time, representing a contribution to the knowledge of the mutational spectrum of MPS VI. The insertion (c.375_376insT) and del/ins (c.750_754delinsCCTGAAGTCAAG) mutation both lead to premature interruption of the open reading frame and consequently a shortening of ARSB polypeptide of almost 80% and over 50% respectively. With so significant a reduction in size, truncated protein products are nonfunctional and in homozygotic state are likely to result in a severe phenotype.

The third novel mutation c.750_754delinsCCTGAAGTCAAG was found in patient 1 in a homozygous state. The same patient also carried previously described change p.A33V and polymorphism p.S384N, both in a homozygous state. Mutation p.A33V is located in exon 1, while changes c.750_754delinsCCTGAAGTCAAG and p.S384N are in exon 4. The polymorphism p.S384N seems, however, not to influence the polypeptide structure as the reading-frame shift resulting from mutation c.750_754delinsCCTGAAGTCAAG leads to premature termination of translation after the 257th amino acid. The patient presented with early symptoms and a rapidly advancing phenotype.

In the case of patient 1 effect of p.A33V mutation seems less striking than that of the co-existing novel mutation c.750_754delinsCCTGAAGTCAAG (also homozygous here) leading to the loss of 50% of the ARSB polypeptide. Interestingly the only prior mention of the p.A33V change was made regarding a patient from North America who also had two other mutations: p.W57C and p.Y266S. However, it was not stated which of these three changes were present on one allele [18]. Our results indicate that p.A33V was likely present on the same allele as p.Y266S because, in addition to patient 1, p.A33V was detected in three other patients (5, 9 and 10) in a double mutant [p.A33V; p.Y266S] allele in a homozygotic state with a frequency of 14.3% in the studied alleles. There is no indication of any relation among families of these patients so one may assume that an allele of the patient described by Karageorgos et al. [18] may reveal an Eastern European origin. The association of p.A33V with other mutations (Table 1) renders its effect on disease development questionable; p.A33V may only represent a polymorphic change. Patients 5, 9, and 10, who had the [p.A33V; p.Y266S] double mutant allele, presented with a severe MPS VI phenotype. If p.A33V is indeed a polymorphic change, the severe phenotype seen in these patients could be attributed to the p.Y266S mutation. Unfortunately, as p.A33V did not occur in isolation, we are unable to determine conclusively whether it is a disease-causing mutation or a polymorphism. Analysis using Alamut software for p.Ala33Val (c.98C>T) showed that either cysteine nucleotide or Ala residue is weakly conserved. Analysis predicted also small physicochemical difference between Ala and Val (64 scores according to Grantham matrix; possible range between 0 and 215). Perhaps in silico analysis to predict its effect in the protein would be helpful to resolve this matter.

The p.Y210C mutation, detected in several different populations, has been reported to result in an intermediate to mild phenotype [12–14,18,19] as the enzyme with the p.Y210C mutation is predicted to be relatively stable and to retain moderate catalytic activity [33]. Until recently the p.Y210C mutation has only been detected as a heterozygous mutation and it was speculated that homozygous individuals may, because of their late presentation with clinical problems not obviously suggestive of MPS VI, never be diagnosed and remain undetected in the general population [14]. Gottwald et al. have recently reported the first patient homozygous for p.Y210C mutation with essentially musculoskeletal phenotype [34]. In a study performed by Karageorgos et al. this mutation was reported as the most frequent (18% of MPS VI patients) [18], while in our study it represented 4.8% (2/42) of the mutant alleles and was present in two families (9.5%). In patient 7, who had a relatively attenuated form of the disease, this mutation was identified together with the previously reported p.T92K mutation [18]. In patient 2 p.Y210C was the only mutation found. The unidentified mutation in this patient could reside in the promoter region, which was not analyzed in this study, or involve partial gene deletion in the unidentified allele, which would not have been detected by the PCR and sequencing approaches used in this study. Neither would a recombination event that is also a possibility in this case. The patient presented with an intermediate phenotype. As mutation p.Y210C has been reported to result in an intermediate to mild phenotype, we can speculate that the other unrecognized mutation may be responsible for the more severe phenotype of this patient.

Consideration should be given to the clinical implications of these findings for the investigation, counseling and treatment of MPS VI patients. Genetic testing for p.R152W mutation in Eastern European population may permit early identification of attenuated and difficult to recognize patients either at diagnosis or through newborn screening. Recognition of the clinical consequences of different genotypes may have therapeutic implications and could permit more accurate patient stratification for entry into therapeutic programme. Patients with the attenuated forms may benefit more because devastating and irreversible changes may not have yet happened despite the diagnostic odyssey that many patients suffer through.

Finally, some limitations of this study should be discussed. Although MPS VI is a disease caused by mutations in the *ARSB* gene, genotype–phenotype correlation suggested in most studies was imperfect. It has been proposed that the absence of absolute correlation between genotype and phenotype for “single gene” diseases is a consequence of three nondiscrete influences: functional activity thresholds, modifier genes, and systems dynamics [35–37]. In case of MPS VI there might be two thresholds of protein function that could explain the clinical phenotype. In the two threshold nondiscrete model, specific alleles might be associated at all times with either the mild or the severe phenotype, and other mutant alleles would be indeterminate [35]. In this indeterminate range the clinical phenotype would depend on other factors inherited independently, such as modifier genes and metabolic flux through related pathways [36]. In other words, it has be kept in mind that there is no obvious clear distinction between simple Mendelian and complex traits: genetic diseases represent a continuum with diminishing influence from a single primary gene influenced by modifier genes, to increasingly shared influence by multiple genes [36].

5. Conclusions

In addition to augmenting the MPS VI molecular spectrum and clinical phenotypes knowledge base, the information obtained in this study resulted in the following conclusions:

1. Mutations p.R152W and p.Y210C were associated with a relatively attenuated MPS VI phenotype. Homozygosity for p.R152W mutation yielded very mild phenotypes, while in heterozygous form, the p.R152W mutation resulted in intermediate phenotypes.
2. Nonsense mutations (p.Q88X, p.Q97X, p.R160X) as well as a missense mutation p.Y266S were associated with a severe MPS VI phenotype.
3. The p.R152W mutation accounted for 50% of the mutant alleles in our patient series which may be of great epidemiological relevance. As there is no known consanguinity among the patients studied here, p.R152W may represent a founder mutation in the context of the Central and Eastern European population, which, if confirmed, should render the mutation a primary target in molecular studies of MPS VI in this geographical region. This possibility should be further evaluated.

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