Molecular Genetics and Metabolism Reports 42 (2025) 101186

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Mucopolysaccharidoses types I and IIIA: Diagnosis and identification of novel polymorphisms associated with common mutations in Moroccan patients

Houda El Fissi [a,*], Fadoua Bouzid [a,b], Mohammed Said Sebbar [c], Mohammed Amine Serghini [a], Fouad Msanda [a], Najat Alif [a]

[a] Laboratory of Biotechnologies and Valorization of Natural Resources, Department of Biology- Faculty of Sciences, Ibn Zohr University, Agadir, Morocco
[b] Department of Environment and Life Sciences, Faculty of Applied Sciences, Ibn Zohr University, Ait Melloul, Morocco
[c] Private pediatrician

ARTICLE INFO

Keywords:
Mucopolysaccharidosis type I
Mucopolysaccharidosis type IIIA
P.P533R
P.Arg377Cys
Polymorphisms
RFLP

ABSTRACT

Background: Mucopolysaccharidoses types I and IIIA are lysosomal storage diseases caused by mutations in the *IDUA* and *SGSH* genes, leading to deficiencies in α-L-iduronidase and heparan sulfamidase, respectively. These progressive, autosomal recessive disorders require early diagnosis.
Purpose: The study targeted and investigated the p.Pro533Arg mutation, known to cause mucopolysaccharidosis type I, and the p.Arg377Cys mutation, associated with mucopolysaccharidosis IIIA, in newly recruited Moroccan families. In parallel, variants/polymorphisms associated with these mutations were searched for.
Methods: Researchers employed RFLP assays for the p.Pro533Arg and p.Arg377Cys mutations and genomic PCR sequencing for variant detection. PolyPhen-2, MutPred2, SIFT, and MutationTaster were used to assess the pathogenicity of these variants, helping to evaluate their potential impact on disease.
Results: The p.Pro533Arg mutation was found in newly recruited families with Hurler syndrome, consistent with previous findings. Similarly, the p.Arg377Cys mutation was present in a newly recruited family with Sanfilippo A syndrome. DNA sequencing revealed five SNPs four in the *IDUA* gene and one in the *SGSH* gene with three *IDUA* SNPs and one *SGSH* SNP being novel.
Conclusion: The p.Pro533Arg and p.Arg377Cys mutations are common among Moroccan patients with MPS I and MPS IIIA, respectively. The ability to detect these mutations using restriction endonucleases allows for molecular diagnosis in affected families. Five polymorphisms were identified among them four are novel.

1. Background

Mucopolysaccharidoses (MPS) are a heterogeneous group of rare inherited diseases of glycosaminoglycans catabolism, caused by the deficiency of lysosomal enzymes [1]. Eleven known deficient enzymes give rise to seven different types of mucopolysaccharidoses. Recently, several papers have mentioned three additional types: MPS-plus [2,3], MPS X [4,5], and MPS IIIE, with the latter belonging to type III [6].

MPS Type I is an autosomal recessive class of mucopolysaccharidosis that is due to a deficiency in alpha-L-iduronidase (IDUA; EC 3.2.1.76; OMIM: 252800). This deficit leads to the generalized intracellular accumulation of heparan sulfate and dermatan sulfate. The disease has a wide range of clinical phenotypes that have historically been classified into three forms: Hurler (OMIM 607014) has a life expectancy of less than 10 years, Hurler-Scheie (OMIM: 607015) is an intermediate form that has a lifespan of less than 25 years, and Scheie (OMIM: 607016) can survive until the mature age [7,8]. More than 295 mutations have been identified to date [9]. The disease has a worldwide prevalence of about 1:100,000 [10].

MPS IIIA (Sanfilippo type A) is a subtype of MPS type III. The disease,

Abbreviations: MPS, Mucopolysaccharidosis; OMIM, Online Mendelian Inheritance in Man; *IDUA*, alpha-L-iduronidase gene; *SGSH*, *N*-sulfoglucosamine sulfohydrolase gene; RFLP, Restriction Fragment Length Polymorphism; pb, peer of bases; SNP, Single Nucleotide Polymorphism; LD, Linkage Disequilibrium.

* Corresponding author.
E-mail addresses: houdaelfissi@gmail.com (H. El Fissi), fadoua.bouzid@edu.uiz.ac.ma (F. Bouzid), sebbar.m.said@hotmail.com (M.S. Sebbar), m.serghini@uiz.ac.ma (M.A. Serghini), f.msanda@uiz.ac.ma (F. Msanda), n.alif@uiz.ac.ma (N. Alif).

https://doi.org/10.1016/j.ymgmr.2025.101186
Received 7 June 2024; Received in revised form 3 January 2025; Accepted 3 January 2025

inherited in an autosomal recessive pattern, is caused by a deficiency of *N*-sulfoglucosamine sulfohydrolase (Sulfamidase; SGSH; EC 3.10.1.1; OMIM: 252900). Sanfilippo A is considered the most severe subtype with early onset symptoms and a shorter life expectancy [11,12]. To date, 152 mutations have been identified in the *SGSH* gene [9]. There is no worldwide prevalence for subtype A, but MPS III is considered the most frequent type of mucopolysaccharidoses with a birth prevalence of 0.28–4.04 per 100,000 [13].

These syndromes are progressive in nature and are characterized by multisystem involvement [14–16]. The definitive diagnosis is always based on the enzyme activity test, which is considered the gold standard. However, it is always recommended to carry out a molecular analysis to establish genotype-phenotype correlation [17], tough, this can be particularly challenging, especially with novel variants, where correlations may not always be possible.

Our previous studies have shown that the p.Pro533Arg (NM_000203.5:c.1598C>G p.(Pro533Arg*); rs121965021) mutation is responsible for MPSI in Moroccan patients [18,19]. We have identified the p.Arg377Cys (NM_000199.5:c.1129C > T p(Arg377Cys*); rs772311757) mutation in a limited number of two patients with Sanfilippo A [20]. Our suspicion of these mutations in newly recruited families prompted us to seek them out using the RFLP method. We consider this method efficient and useful for screening these two syndromes. In parallel, we have identified the polymorphisms associated with these syndromes in patients, their relatives, and in controls.

We specifically recruited patients affected by MPS I and MPS IIIA, enabling us to directly investigate the genetic and clinical characteristics associated with these types within our patient population. Additionally, we continue to investigate the other types of MPS.

2. Materials and methods

2.1. Ethical approvals

The ethics committee of the MOHAMED VI university hospital center in Marrakech has approved all procedures. Written and signed informed consents were obtained from the parents or the legal guardians of the patients for participation in this study.

2.2. Study cohort

The RFLP technique was carried out on four new families: three with MPS I and one with MPS IIIA. Research of SNPs was conducted on 16 families: 14 with MPS I, including patients ($N = 14$), unrelated parents (father or mother of each patient, $N = 8$), and relatives ($N = 2$), and two with MPS IIIA, including two patients and one parent. Fifty unrelated healthy individuals were examined as healthy controls. When a nuclear family had multiple affected individuals, only one individual was considered. Similarly, in cases where patients belonged to related families, only one family was included in the analysis.

After clinical suspicion, the biochemical investigation identifies the type of accumulated GAGs. The determination of alpha-L-iduronidase and heparane sulfamidase activities, in patients and healthy controls, in parallel to enzyme controls, β-D-glucuronidase and α-D-*N*-acetyglycosaminidase, respectively, confirmed the diagnosis.

2.3. RFLP- genotyping

Five milliliters of venous blood were collected from patients, their parents and relatives, and healthy controls. DNA was extracted on the same day of collection whenever possible, following the DNA extraction method detailed in Sambrook and Russell (2001), Chapter 7 [21]. The quality and integrity of the DNA samples were assessed using a Nano-Drop spectrophotometer to ensure their suitability for downstream analyses."

Primers and PCR conditions used are listed in Tables 1 and 2, respectively. Then, we proceeded to the RFLP technique on Exons XI and XII of the *IDUA* gene and exon VIII of the *SGSH* gene using the restriction endonucleases *Bsi*EI and *Hha*I, respectively. Digestion with each endonuclease was carried out for an hour at 37 °C in a final volume of 20 µl, containing 10 µl of PCR products, 10 units of enzymes, and 1× Buffer Tango as recommended by the manufacturer. Digested products were run on a 2.5 % agarose special (Low EEO) gel, stained with ethidium bromide, and visualized under UV.

light.

Table 1
Primers and conditions for polymerase chain reaction amplification and sequencing.

Genes	Exons	Primers sequences	Product size in pb	Tm (C°)
IDUA	III	S: 5′TCCCACATGCTCCGTTGT3′ R: 5′GAATGGACATCCAAGGACTCAGA3′	274	50
	VII	S: 5′TGCGGCTGGACTACATCTC3′ R: 5′ AGGTTCTGATGCTGCGC3′	447	52
	XI and XII	S: 5′GTGTGGGTGGGAGGTGGA3′ R: 5′CATGGGTGAAGGGGTCG3′	459	65
SGSH	VIII	S: 5′TTGGATTGGAGAAGGGAGC3′ R: 5′CCGGTAGTAGTAATGACGGAGG3′	457	63

Table 2
: PCR conditions.

Mixture	PCR	Temperature °C	Duration (min)
1× PCR buffer:	Initial denaturation	*95*	10
200 ng genomic DNA	Denaturation	95	1
0.2 mM dNTP	Annealing	Tm	1
1.5 mM $MgCl_2$	Extension	72	1
5 units Taq DNA polymerase	Final extension	72	10
Total reaction volume: 25 µl	Number of cycles: 35	-	-

2.4. Sequencing

In order to study the mutational and polymorphism spectrum, we performed sequencing of three amplified genomic fragments: exon III, and VII for the *IDUA* gene and exon VIII for the *SGSH* gene. Bidirectional sequencing analysis for PCR was carried out using a big-dye terminator for the amplified and purified products. DNA sequencing analysis was carried out in both forward and reverse sequences. Primers and PCR conditions are listed in Tables 1 and 2. The amplified products were purified using the ExoSAP-IT kit, then directly sequenced using a sequencing kit (BigDye v3.1) and an automatic sequencer (ABI 3130xl Genetic Analyzer, 16 capillary sequencer, Applied Biosystems).

2.5. Data analysis

The alignment and comparison of sequences were performed by using the BLAST program. To test the pathogenicity of variants, four different softwares: Polyphen-2, Mutpred2, Sift and MutationTaster where scores and sensitivity are displayed were used. Allele and genotype frequencies of SNPs were determined using PopGene32 software [22]. Deviation from Hardy-Weinberg expectations and investigation of the differences in allele frequencies between the studied samples were tested using the Chi-square (X^2) test with StatCalc software Epi Info™ 7.2.2.2. Pairwise linkage disequilibrium (LD) among SNPs was analyzed using Lewontin's D' statistic and the squared correlation statistic r2 through SNPStats online analysis program.

3. Results

3.1. RFLP of DNA from the family with Hurler disease

As for the *IDUA* gene, the *Bsi*EI restriction enzyme has one restriction site on the amplified DNA from the wild-type samples, cleaving the PCR amplicons into two products with lengths of 311 and 148 bp. We presented the genotypic pattern of one family among the three newly recruited MPS I families. The restriction digest from a ten-year-old girl patient produces three fragments with lengths of 277, 148, and 34 pb as the enzyme cuts the DNA at two sites, confirming that she is homozygous for the p.Pro533Arg mutant allele. Samples from his parents and his brother exhibit four different fragments with lengths of 277, 148, 311, and 34 bp, confirming that they are heterozygous for the mutant allele. Meanwhile, his paternal grandmother shows bands of 311 and 148 bp, indicating that she is homozygous for the wild allele (Fig. 1).

3.2. RFLP of DNA from the family with Sanfilippo A disease

Digestion of the 457 bp fragment from the exon VIII of *SGSH* by *Hha*I resulted in three fragments of 211, 77, and 169 pb in the presence of two cut sites in wild-type samples. The p.Arg377Cys mutation abolishes a recognition site. The digested amplicon from a seven-year-old girl patient produces two fragments with sizes 211 and 246 pb as the enzyme cuts only once, confirming that she is homozygous for the p.Arg377Cys mutant allele. Samples from his father exhibited four fragments 211, 77, 169, and 246 pb, confirming that he is obligatory heterozygous (Fig. 2).

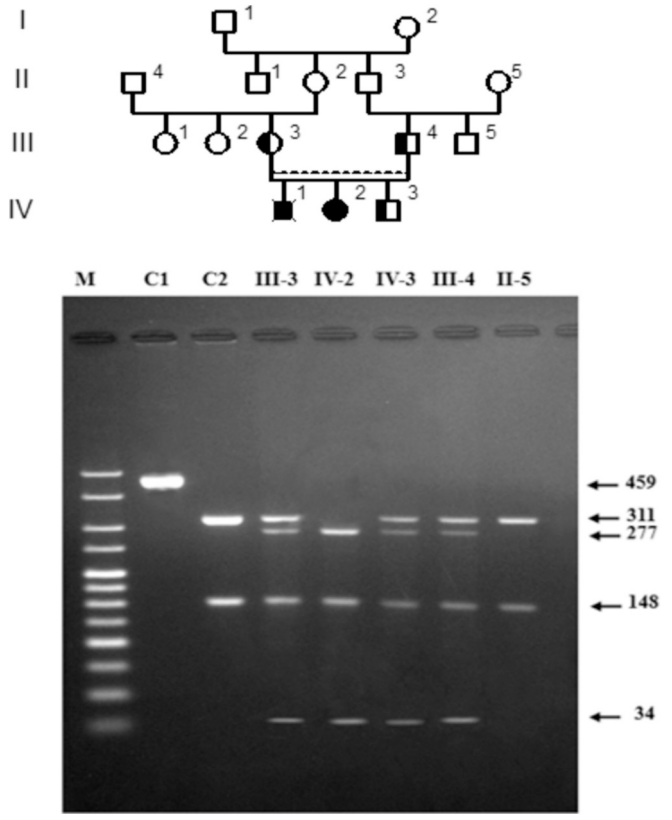

Fig. 1. Detection of the p.Pro533Arg mutation by *Bsi*EI enzyme. Electrophoresis profiles of digested DNAs onto 2.5 % agarose gel with ethidium bromide. RFLP of the exon XI and XII of the *IDUA* gene. Lane M, 25 pb DNA ladder. Lanes C1, C2, undigested (459 pb) and digested (311 and 148 pb) from control individuals, respectively. Lane IV-2, the proband shows 277 and 34 pb which indicates his homozygosity for the mutant allele. Lanes III-3 (her mother), III-4 (her father), and IV-3 (her brother) show a wild band of 311 pb, and mutated bands of 277 and 34 pb, which means that they are heterozygous. Lane II-5 (her paternal grandmother) shows wild bands. She is homozygous for the wild allele. Roman and Arab chiffers indicating generations and individuals, respectively, are equivalent to those of the family tree.

3.3. SNPs in IDUA and SGSH genes

During the exploration of the *IDUA* and *SGSH* genes, we specifically sequenced 3 exons from the *IDUA* gene and 1 exon from the *SGSH* gene to search for any SNPs. The nucleotide sequence from MPS I and IIIA patients, their respective parents and relatives, and controls were aligned.

The nucleotide sequence alignment aimed to detect SNPs within the *IDUA* and the *SGSH* genes in MPS I and IIIA patients, their respective parents and relatives, and controls.

The Fig. 3 displays the five SNPs detected in the present study: four in the *IDUA* gene and one in the *SGSH* gene. In the *IDUA* gene the p.Val325, was previously reported, while three are novel. The first one is the G106 variant, which was detected with a novel nucleotide change indicated in bold of GGC to GGA. The second one is the p.Ala266Glu, where GCG was changed to GAG, and the third one is the IVS3–17 t > a. In the *SGSH* gene, the detected variant is novel. It is the p.Arg346Lys, where CGG is changed to CTG. All of these SNPs were detected in a heterozygous state.

The p.Gly106 was found in one patient and two unrelated parents. The A266E was observed in three patients and two unrelated parents. p. Val325 (rs2153022311) in exon VII was detected in all homozygous patients for the p.Pro533Arg mutation and their relatives. The IVS3–17 t > a variant was present in three patients and three unrelated parents. The mutant allele frequencies of p.Gly106, p.Val325, and IVS3–17 t > a are higher in patients (50 %, 6.25 %, and 18.75 %, respectively) than in controls (1 %, 4 %, and 5 %, respectively). Regarding p.Ala266Glu, the

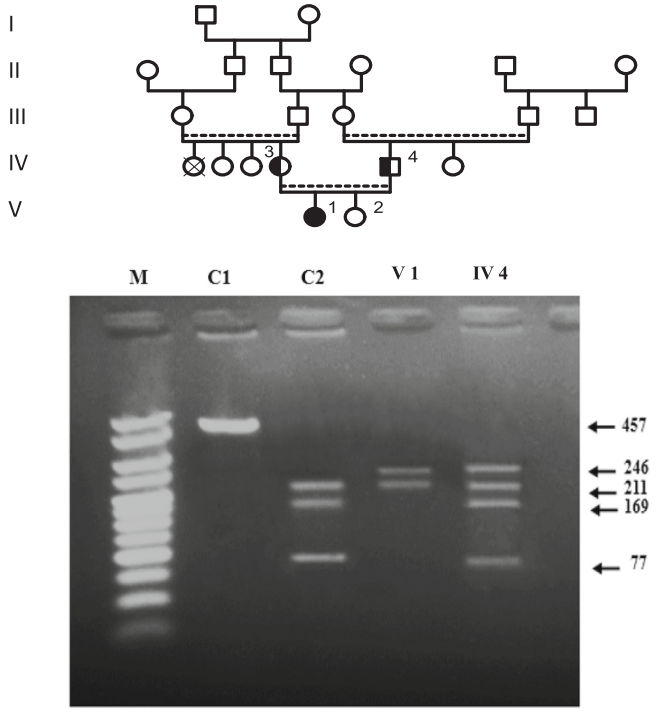

Fig. 2. Detection of the p.Arg377Cys mutation. Electrophoresis profiles of digested DNAs onto 2.5 % agarose gel with ethidium bromide. RFLP of the exon VIII of the *SGSH* gene with *Hha*I. Lane M, 25 pb DNA ladder. Lanes C1, C2, undigested (457 pb) and digested (211, 169, and 77pb), respectively, from healthy control. Lane V1 (the proband), shows a band of 246 pb, which indicates his homozygosity. Lane IV4 (her father) shows both cut (169 and 77pb) and uncut (246 pb) fragments. Numbers are equivalent to those of the family tree.

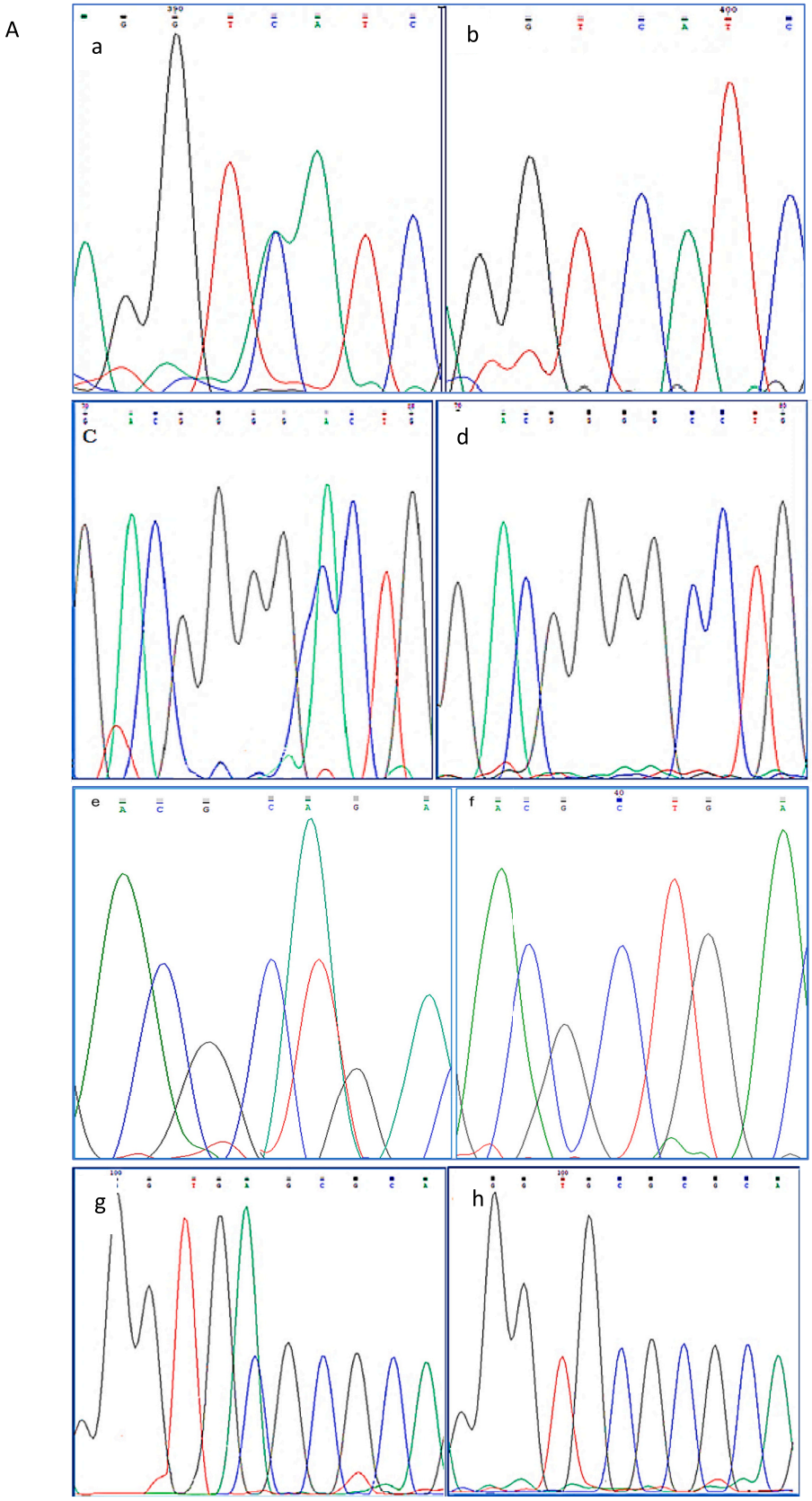

Fig. 3. (A) SNPs in *IDUA* gene; a and b: Heterozygous and wild allele of p.Val325, respectively; c and d: Heterozygous and wild allele of p.Gly106, respectively; e and f: Heterozygous and wild allele of IVS3–17 t > a, respectively; g and h: Heterozygous and wild allele of p.Ala266Glu, respectively. (B) SNP in *SGSH* gene: (a) and (b) wild allele and Heterozygous of p.Arg346Lys, respectively.

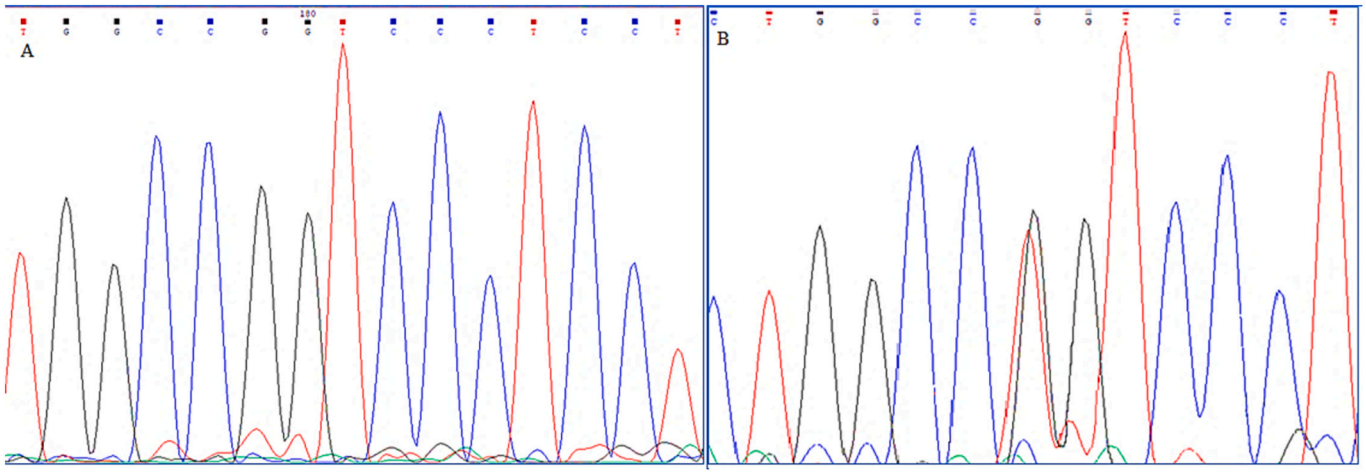

Fig. 3. (*continued*).

mutant allele frequency is 18 % in patients compared to the zero frequency observed in the control subjects (Table 3).

The pathogenicity of the p.Ala266Glu variant was evaluated using MutationTaster, which classified it as a polymorphism. This prediction was consistent with MutPred2, which indicated the variant as non-pathogenic with a score < 0.5. However, SIFT predicted the variant to be intolerant with a score < 0.05. Additionally, PolyPhen-2 classified the variant as possibly damaging under both the HumDiv and HumVar models, with a score < 0.990 (Table 4).

The χ^2 test identified that the genotypic and allelic frequencies of p.Gly106 and IVS3–17 t > a did not significantly differ between controls and patients, where the *P*-value for the p.Gly106 and the IVS3–17 t > a are 0.41 and 0.094, respectively. However, a significant difference occurs between controls and patients regarding allelic and genotypic frequencies of p.Val325 and p.Ala266Glu ($p < 0.05$) (Table 3).

We also investigated interactions between pairs of SNPs in the *IDUA* gene. The linkage disequilibrium (LD) analysis revealed a strong LD, on one hand, between the pairs p.Val325- p.Gly106, and p.Val325-IVS3–17 t > a, and, on the other hand, between p.Gly106 -IVS3–17 t > a, and p.Gly106 - p.Ala266Glu ($D' > 0.8$) (Table 5).

Regarding the *SGSH* gene, we found the variant p.ArgR346Lys in exon VIII (chr17:80210924) (Fig. 3). NCBI reported p.Arg346Gln with the rs number rs367883023. In this study, the nucleotide change is different: G > T instead of G > A. G > T leads to an amino acid change of arginine to leucine while G > A leads change of arginine to glutamine. p.ArgR346Lys was observed in a heterozygous state in one patient and her father, and two controls. None of the patients or controls were homozygous for this variant. Analysis of its pathogenicity by Polyphen2,

Table 3
Genotype and allele frequencies of the SNPs of *IDUA* and *SGSH* genes.

Gene	SNP; Tribucleotide change in NCBI /* Tribucleotide change in our study	HGVS nomenclature	rs number/ Nucleotide change in NCBI; nucleotide change in our study	Genotype and allele		Cases N = 14; Cases/ Genotype and allele frequency (%)	Parents and relatives *N* = 10 Subjects/ Genotype and allele frequency (%)	Controls *N* = 50 Subjects/ Genotype and allele frequency (%)	χ^2	*P*-value	OR
IDUA	p.Val325: (GTC > GTA)	NM_000203.5: c.975C>A p. (Val325*)	rs2153022311/C > A	Genotype	CC	0/0	0/0	46/92	20.62	0.00001	NA
					CA	8 /100	8/100	4/8			0
					AA	0/0	0/0	0/0			NA
						6 (?)	2 (?)	0 (?)			
				Allele	C	8 /50	8 /50	96/96			
					A	8 /50	8 /50	4/4			
	p.Gly106: (GGC > GGT)	NM_000203.3: c.318C>T p. (Gly106*)	rs1174027022/C > T; *C > A	Genotype frequency	CC	7/87.5	6/75	49/98	0.65	0.41	1
					CA	1/12.5	2/25	1/2			2.619
					AA	0/0	0/0	0/0			NA
						6 (?)	2 (?)	0 (?)			
				Allele frequency	C	15/93.75	14/87.5	99/99			
					A	1/6.25	2/12.5	1/1			
	IVS3–17 t > a		**/ t > a	Genotype frequency	TT	5/62.5	5/62.5	45/90	2.8	0.094	1
					TA	3/37.5	3/37.5	5/10			3.75
					AA	0/0	0/0	0/0			NA
						6 (?)	0 (?)	0 (?)			
				Allele frequency	T	13/81.25	13/81.25	95/95			
					A	3/18.75	3/18.75	5/5			
	p.Ala266Glu:/* GCG > GAG		**/C > A	Genotype frequency	CC	5/62.5	6/75	50/100	11.46	0.00071	1
					CA	3/37.5	2/25	0/0			16.8
					AA	0/0	0/0	0/0			NA
						6 (?)	0 (?)	0 (?)			
				Allele frequency	C	13/81.25	14/87.5	100/100			
					A	3/18.75	2/12.5	0/0			

(*) Change in our study; (**) Unreported rs; (?) Not known; (NA) Not applicable; (OR) Odds Ratio.

Table 4
Analysis of pathogenicity of variants by prediction tools.

	Mutpred2		Sift		Polyphen2				Mutationtaster
					HumDiv		HumVar		
	Score	Prediction	Score	Prediction	Score (Sensitivity)	prediction	Score (Sensitivity)	prediction	Prediction
IDUA/p.A266E	0.391	Non-pathogenic	0.02	Intolerant/ Deleterious	0.939 (0.8)	Possibly damaging	0.673 (0.79)	Possibly damaging	polymorphism
SGSH/p.R346L	0.563	Pathogenic	0.17	Tolerated	0.993 (0.7)	Probably damaging	0.873 (0.71)	Possibly damaging	Disease-causing

Table 5
Pairwise Linkage Disequilibrium Extent and Correlation Coefficient among *IDUA* SNPs.

SNPs	D′	r
p.Val325- p.Gly106	0.9963	0.3596
p.Val325- IVS3–17 t > a	0.8072	0.452
p.Val325- p.Ala266Glu	0.4854	0.3796
p.Gly106 - IVS3–17 t > a	0.9967	0.6423
p.Gly106 - p.Ala266Glu	0.9966	0.4598
IVS3–17 t > a- p.Ala266Glu	0.483	0.3458

Mutpred, and MutationTaster shows that p.ArgR346Lys is considered disease-causing, while SIFT indicated that it is tolerated (>0.05) (Table 4).

4. Discussion

Here, we report a DNA analysis including pathogenic and SNPs variants of the *IDUA* and *SGSH* genes in families affected by MPS I and IIIA, respectively. Among these families, four are newly recruited.

The cumulative number of MPS I and MPS IIIA cases across our previous study (14 MPS I and two MPS IIIA cases) and the current study (three MPS I and one MPS IIIA case) is 17 and three, respectively. It is worth noting, that MPS III is the most frequent type in several European countries [23–25], and also in Tunisia [26], with whom the Moroccan population shares a certain genetic and ethnic similarity, so this number of cases cannot reflect the real frequency of this disease in Morocco. However, data compiled from 33 countries, as reported by Celik et al. in 2021 [27], has led to the conclusion that the prevalence and incidence of all MPS are underestimated due to insufficient methods for identifying all cases of MPS. Therefore, a combination of biochemical and molecular diagnosis will improve the detection and identification of new cases with MPS IIIA.

4.1. Pathogenic mutations

In the newly recruited families, we searched for the p.Pro533Arg mutation known to be responsible for MPS I in Moroccan patients, and the p.Arg377Cys mutation thought to be responsible for MPS IIIA. Based on the literature [28,29] and bioinformatics research with Gene Runner software [30], the targeted p.Pro533Arg and p.Arg377Cys mutations can be highlighted by restriction endonucleases *BsiEI* and *HhaI*, respectively. In the MPS I studied family, since the paternal grandmother is homozygous for the wild allele, it is likely that the mutant allele was inherited from the paternal grandfather. Unfortunately, his DNA was not available. In our global cohort, including previous families, the three newly recruited families with Hurler syndrome, like the twelve out of fourteen previously studied have the p.Pro533Arg mutation. This maintains a high frequency of over 93 % (26 out of 28) and (32 out of 34), in both previous [18] and current studies, respectively. This is the highest frequency recorded so far of this mutation, followed by that reported in Algeria and Tunisia with frequencies of 81, and 62.5 % respectively [31,32].

The female patient with Sanfilippo A investigated in this study is the third, in our series to possess the p.Arg377Cys mutant allele (6/6). Even though the number of cases is limited to three (Two in the previous [20] and one in the present studies, respectively), the result suggests that this p.Arg377Cys mutant allele could potentially be a common variant, providing a basis for studying a larger number of cases. This mutation is rare since it is reported only once in the homozygous state in an Italian patient [28] and in the heterozygote state in a Tunisian patient [33] and one Chinese [34].

The observed high frequencies of both the p.Pro533Arg and p.Arg377Cys mutations suggest a founder effect in Morocco, emphasizing the importance of conducting rapid molecular analysis for their detection [19,20]. It is worth mentioning that investigated families with MPS I and MPS IIIA, are first-degree consanguineous. The high rate of inbreeding in Morocco is strongly associated with autosomal recessive disorders [35,36]. This also promotes the spread of the deleterious mutations in the population.

4.2. SNPs variants

During the exploration of the *IDUA* and *SGSH* genes, we have noticed the presence of four SNPs in the *IDUA* gene: p.Gly106, p.Ala266Glu, p.Val325, and IVS3–17 t > a, and one in the *SGSH* gene. To our knowledge, four of them are novel: p.Gly106, p.Ala266Glu, and IVS3–17 t > a in the *IDUA* gene and p.Arg346Lys in the *SGSH* gene. The p.Gly106 variant in exon III (chr4:1000630) was reported in NCBI with the rs number of rs1174027022, but in this study, the nucleotide change is different: **C > A** instead of **C > T**. Nevertheless, this novel change maintains the amino acid as synonymous. The p.Ala266Glu variant in exon VII (chr4:1001988) and the IVS3–17 t > a variant in intron 3 (chr4:1000595) are novel, as they are not reported anywhere. The p.Arg346Lys variant in *SGSH* gene is novel, as the substituted amino acid is leucine (C**G**G > C**T**G) in our study instead of glutamine (C**G**G > C**A**G) in NCBI, where the R346Q is reported with the rs number of rs367883023. The novel changed nucleotides observed in this study, in both cases of the *IDUA* and *SGSH* genes, are transversions. When present, we have found these polymorphisms in only a heterozygous state.

The genotype frequencies of p.Val325 and p.Ala266Glu, in our patient group, are significantly different from those in the controls. In both polymorphisms, the nucleotide change is C to A. For Ala266Glu, the CC genotype decreases from 100 % in controls to 62.5 % in patients, and 75 % in parents and relatives. The absolute absence of the wild-type CC genotype for p.Val325 in all patients and their parents and relatives, and its presence at 100 % in controls could potentially suggest a protective effect against the onset of the disease. Instead, the CA genotype in both p.Val325 and p.Ala266Glu variants may interact with other genetic factors, contributing to the expression of the disease.

The p.Gly106 and p.Val325 variants are considered synonymous. In the p.Ala266Glu variant, the amino acid change has led us to analyze its pathogenicity. Two of the four software programs used have considered this variant as tolerant. We can confirm its non-pathogenicity from the point of view that the MPS I causing mutation is identified in the homozygous state in all patients. On the other hand, the zero frequency of the variant in controls could suggest that this polymorphism can

increase the risk of the disease since the major phenotype observed in our cohort study is Hurler. Furthermore, it has been hypothesized that some polymorphisms may alter the clinical phenotype of the patient due to structural effects on the *IDUA* gene, especially for those with missense mutations. Besides, other polymorphisms were reported to be associated with the Hurler / Scheie and Scheie phenotypes [37–39].

4.3. Haplotype analysis

The study of interactions between these SNPs showed a strong LD between p.Val325, on the one hand, and p.Gly106 on the other hand, with the other SNPs. These results suggest a probable association between these polymorphisms and the p.Pro533Arg/MPSI disease.

The presence of these novel polymorphisms in the Moroccan population can be explained by the high rate of consanguinity in this country. Indeed, rare genotypes inherited through consanguineous marriages are maintained within this population [32]. Thus, these variants may serve as biomarkers of MPS I disease in this population. Additionally, compiling these polymorphisms with other haplotypes (as reported in our latest study [40]) can establish a more detailed genotype background and also contribute to determining the origin of mutations within Moroccan MPS I patients.

Regarding the *SGSH* gene, the p.Arg346Lys polymorphism was found in a Sanfilippo A patient and her father. The pathogenicity of this variant was evaluated with four different prediction tools, all of which confirmed its potential pathogenicity except for the Sift software. However, we lack evidence to confirm whether this missense mutation indeed causes the disease. Our classification of it as non-pathogenic is based on the fact that we detected this variant in two individuals from our control group. However, the Lod Score disequilibrium and Correlation Coefficient were not determined for the *SGSH* gene. This is because, on the one hand, we have only a limited number of patients, and on the other hand, we did not conduct the screening on DNA samples from controls.

5. Conclusion

Genetic testing for all hereditary diseases, not just mucopolysaccharidoses, should be recommended because specific treatment for those of which treatment exists can, if implemented early, prevent deterioration and improve the quality of life. We describe a RFLP test for the MPS I/p.Pro533Argand MPS IIIA/p.Arg377Cys mutations that is a more affordable alternative compared to next-generation sequencing (NGS) and Sanger sequencing methods. These mutations should be initially sought in patients of Moroccan descent. In our study, we identified five polymorphisms, four of which are novel. Understanding the association between these polymorphisms and pathogenic mutations is crucial for improving diagnosis.

Funding

No funding was received for this article.

Consent

Written informed consent was obtained from the parents or legal guardians of the patients for their participation and publication of this work. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Ethics approval and consent to participate

The families gave informed consent before withdrawal of blood samples and written informed consent was obtained and signed by all MPSI, MPS IIIA and controls families, in addition the verbal consent was also obtained during consultation. The study was approved by the ethic committee of the MOHAMED VI university hospital center in Marrakech, with reference number of 9/2021.

Consent to publication

Written informed consent was obtained from the parents or legal guardians of the patients for their participation and publication of this work. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

CRediT authorship contribution statement

Houda El Fissi: Writing – original draft, Methodology, Investigation, Conceptualization. **Fadoua Bouzid:** Methodology, Investigation. **Mohammed Said Sebbar:** Investigation. **Mohammed Amine Serghini:** Writing – review & editing. **Fouad Msanda:** Writing – review & editing. **Najat Alif:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgments

The authors are grateful to the families and healthy blood donors for their participation in this work. We are grateful to Mahfoud-Filali Samir and the staff of his laboratory for collecting venous blood.

Availability of data and materials

The datasets analyzed during the current study are available in the ensemble database: https://www.ensembl.org/index.html and NCBI.

References

[1] M. Stapleton, N. Arunkumar, F. Kubaski, et al., Clinical presentation and diagnosis of mucopolysaccharidoses, Mol. Genet. Metab. 125 (2018) 4–17, https://doi.org/10.1016/j.ymgme.2018.01.003.
[2] S. Novgorodova, N. Maksimova, Comparative clinical characterization of the new recessive mucopolysaccharidosis-plus disease in the Yakut and Turkish populations, Med. Genet. 19 (2020) 90–92.
[3] Z. Cyske, L. Gaffke, K. Pierzynowska, et al., Mucopolysaccharidosis-plus syndrome: is this a Type of Mucopolysaccharidosis or a separate kind of metabolic disease? Int. J. Mol. Sci. 25 (2024) 9570.
[4] M. Sun, C.K. Kaminsky, P. Deppe, et al., A novel homozygous missense variant in ARSK causes MPS X, a new subtype of mucopolysaccharidosis, Genes Dis. (2024) 11.
[5] S. Verheyen, J. Blatterer, M.R. Speicher, et al., Novel subtype of mucopolysaccharidosis caused by arylsulfatase K (ARSK) deficiency, J. Med. Genet. 59 (2022) 957–964.
[6] K. Wiśniewska, J. Wolski, M. Żabińska, et al., Mucopolysaccharidosis Type IIIE: a real human disease or a diagnostic pitfall? Diagnostics 14 (2024) 1734.
[7] L.A. Clarke, R. Giugliani, N. Guffon, et al., Genotype-phenotype relationships in mucopolysaccharidosis type I (MPS I): insights from the international MPS I registry, Clin. Genet. 96 (2019) 281–289.
[8] Mucopolysaccharidosis Type I, in: L.A. Clarke, M.P. Adam, et al. (Eds.), GeneReviews(®), University of Washington, Seattle Copyright © 1993–2024, University of Washington, Seattle. Seattle (WA), 1993.
[9] HGMD, Human Gene Mutation Database, Available from, https://www.hgmd.cf.ac.uk/ac/index.php, 2024.
[10] C. Cantú-Reyna, D.L. Vazquez-Cantu, H. Cruz-Camino, et al., Mucopolysaccharidosis Type I in Mexico: case-based review, Children 10 (2023) 642, https://doi.org/10.3390/children10040642.
[11] N. Benetó, L. Vilageliu, D. Grinberg, et al., Sanfilippo syndrome: molecular basis, disease models and therapeutic approaches, Int. J. Mol. Sci. 21 (2020) 7819.
[12] A. Tebani, L. Abily-Donval, I. Schmitz-Afonso, et al., Unveiling metabolic remodeling in mucopolysaccharidosis type III through integrative metabolomics and pathway analysis, J. Transl. Med. 16 (2018) 1–14.
[13] Y. Pearse, M. Iacovino, A cure for Sanfilippo syndrome? A summary of current therapeutic approaches and their promise, Med. Res. Arch. (2020) 8.
[14] S.J. Knottnerus, S.C. Nijmeijer, L. IJlst, et al., Prediction of phenotypic severity in mucopolysaccharidosis type IIIA, Ann. Neurol. 82 (2017) 686–696.

[15] S.D. Kingma, E.J. Langereis, C.M. De Klerk, et al., An algorithm to predict phenotypic severity in mucopolysaccharidosis type I in the first month of life, Orphanet J. Rare Dis. 8 (2013) 1–11.
[16] G. De Ponti, S. Donsante, M. Frigeni, et al., MPSI manifestations and treatment outcome: skeletal focus, Int. J. Mol. Sci. 23 (2022) 11168.
[17] F. Kubaski, F. de Oliveira Poswar, K. Michelin-Tirelli, et al., Diagnosis of mucopolysaccharidoses, Diagnostics 10 (2020) 172, https://doi.org/10.3390/diagnostics10030172.
[18] N. Alif, K. Hess, J. Straczek, et al., Mucopolysaccharidosis type I: characterization of a common mutation that causes hurler syndrome in Moroccan subjects, Ann. Hum. Genet. 63 (1999) 9–16, https://doi.org/10.1046/j.1469-1809.1999.6310009.x.
[19] F. Bouzid, M.S. Sebbar, A. El Mousadik, et al., IDUA gene polymorphisms among mucopolysaccharidosis and healthy Moroccan subjects, Gene Rep. 21 (2020) 100798, https://doi.org/10.1016/j.genrep.2020.100798.
[20] F. Bouzid, Z. Brakez, I. Benyamna, et al., Sanfilippo a disease: biological and molecular study of Moroccan patients, British J. BioMed. Res. 5 (2019) 1226–1233, https://doi.org/10.24942/bjbmr.2019.578.
[21] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular cloning: a laboratory manual, Cold spring harbor laboratory press, 1989.
[22] F.C. Yeh, R.C. Yang, T. Boyle, et al., POPGENE 32, Microsoft Windows-Based Freeware for Population Genetic Analysis, Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, 2000.
[23] A.S. Alyazidi, O.Y. Muthaffar, L.S. Baaishrah, et al., Current concepts in the Management of Sanfilippo Syndrome (MPS III): a narrative review, Cureus 16 (2024).
[24] G. Malm, J.E. Månsson, Mucopolysaccharidosis type III (Sanfilippo disease) in Sweden: clinical presentation of 22 children diagnosed during a 30-year period, Acta Paediatr. 99 (2010) 1253–1257.
[25] V. Delgadillo, M.D.M. O'Callaghan, L. Gort, et al., Natural history of Sanfilippo syndrome in Spain, Orphanet J. Rare Dis. 8 (2013) 1–11.
[26] S.A. Khan, H. Peracha, D. Ballhausen, et al., Epidemiology of mucopolysaccharidoses, Mol. Genet. Metab. 121 (2017) 227–240.
[27] B. Celik, S.C. Tomatsu, S. Tomatsu, et al., Epidemiology of mucopolysaccharidoses update, Diagnostics 11 (2021) 273.
[28] P. Di Natale, N. Balzano, S. Esposito, et al., Identification of molecular defects in Italian Sanfilippo a patients including 13 novel mutations, Hum. Mutat. 11 (1998) 313–320, https://doi.org/10.1002/(SICI)1098-1004(1998)11:4%3C313::AID-HUMU9%3E3.0.CO;2-P.
[29] L. Gort, A Chabás and M J Coll, analysis of five mutations in 20 mucopolysaccharidosis type I patients: high prevalence of the W402X mutation, Hum. Mutat. 11 (1998) 332.
[30] G. Runner, 6.5. 52 Beta, Frank Buquicchio and Michael Spruyt 2019, 2019. http://www.generunner.net/.
[31] L. Chkioua, S. Khedhiri, Z. Jaidane, et al., Mucopolysaccharidosis type I: identification of alpha-L-iduronidase mutations in Tunisian families, Archives de pediatrie: organe officiel de la Societe francaise de pediatrie. 14 (2007) 1183–1189, https://doi.org/10.1016/j.arcped.2007.06.018.
[32] A. Tebani, L. Zanoutene-Cheriet, Z. Adjtoutah, et al., Clinical and molecular characterization of patients with mucopolysaccharidosis type I in an Algerian series, Int. J. Mol. Sci. 17 (2016) 743, https://doi.org/10.3390/ijms17050743.
[33] S. Ouesleti, V. Brunel, H.B. Turkia, et al., Molecular characterization of MPS IIIA, MPS IIIB and MPS IIIC in Tunisian patients, Clin. Chim. Acta 412 (2011) 2326–2331.
[34] G. Lee-Chen, S. Lin, M. Ko, et al., Identification and characterization of mutations underlying Sanfilippo syndrome type a (mucopolysaccharidosis type IIIA), Clin. Genet. 61 (2002) 192–197, https://doi.org/10.1034/j.1399-0004.2002.610304.x.
[35] N. Dahbi, A. El Khair, K. Cheffi, et al., Consanguinity, complex diseases and congenital disabilities in the Souss population (southern Morocco): a cross-sectional survey, Egypt. J. Med. Hum. Genet. 25 (2024) 27.
[36] L. Romdhane, N. Mezzi, Y. Hamdi, et al., Consanguinity and inbreeding in health and disease in north African populations, Annu. Rev. Genomics Hum. Genet. 20 (2019) 155–179.
[37] A. Ghosh, J. Mercer, S. Mackinnon, et al., IDUA mutational profile and genotype–phenotype relationships in UK patients with Mucopolysaccharidosis Type I, Hum. Mutat. 38 (2017) 1555–1568.
[38] N. Venturi, A. Rovelli, R. Parini, et al., Molecular analysis of 30 mucopolysaccharidosis type I patients: evaluation of the mutational spectrum in Italian population and identification of 13 novel mutations, Hum. Mutat. 20 (2002) 231–239.
[39] N. Atceken, R.K. Özgül, D.Y. Yilmaz, et al., Evaluation and identification of IDUA gene mutations in Turkishpatients with mucopolysaccharidosis type I, Turkish J. Med. Sci. 46 (2016) 404–408.
[40] L. Chkioua, H. El Fissi, Y. Amri, et al., Mucopolysaccharidosis type I: founder effect of the p. P533R mutation in North Africa, BMC Genomics 25 (2024) 948.