ORIGINAL ARTICLE

MOLECULAR ANALYSIS OF MUCOPOLYSACCHARIDOSIS TYPES I IN MOROCCO: IDENTIFICATION OF NOVEL MUTATION AND FIVE POLYMORPHISMS

| Fadoua Bouzid [1*] | Ilham Benyamna [1] | Abdelhamid Elmousadik [1] | Abdellah Moustaine [2] | and | Najat Alif [1] |

[1]Laboratory of Biotechnologies and Valorization of Natural Resources | School of Sciences | IBN Zohr University | Agadir | Morocco |
[2.] Pediatric practice | Agadir | Morocco |

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Abstract

Bachground: Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disease caused by the deficiency of the α-L-iduronidase (IDUA) enzyme. This deficiency leads to the accumulation of dermatan sulfate and heparan sulfate. **Objective**: The aim of this study is to detect IDUA mutational spectrum in 14 Moroccan patients with MPS I. **Methods**: Diagnosis of MPS I was confirmed by a biochemical study. The molecular analysis was performed by direct sequencing of *IDUA* gene from DNA patients' sample, their parents and control subjects. **Results**: In this series, we have detected the previously reported mutation p.P533R at homozygous state in 92.85% of studied patients and an unreported deletion mutation p.P515fs (c.1545delC) at heterozygous state in one patient, showing the Hurler phenotype. In addition, five polymorphisms in the homozygous or heterozygous states including two new variants were detected in investigated patients. These non-pathogenic variants indicate a high degree of allelic heterogeneity explaining different observed phenotypes.

Keywords: *Mucopolysaccharidosis type I; IDUA gene; p.R533P; p.P515fs; polymorphisms*

1. INTRODUCTION

Mucopolysaccharidosis the type I (MPS I) is an autosomal recessive metabolic disease caused by the deficiency of α-L-iduronidase (IDUA). This enzyme is involved in the catabolism of heparan sulfate (HS) and dermatan sulfate (DS). The deficiency of IDUA leads to accumulation of these two GAGs in lysosome, resulting in damaging various tissues [1].

The *IDUA* gene located on chromosome 4p16.3, contains 14 exons, is transcribed into a 2.3 kb mRNA and translated into a protein of 653 amino acids [2]. Up to now, more than 200 different mutations have been reported in *IDUA* gene [3]. Of these, missense/nonsense mutations have the highest rates of 58 %, splice site mutations (15.9 %), deletions (14 %) and insertions (7.3 %). The most common mutations in the world are p.W402X, p.Q70X, p.P533R and p.G51D [4].

MPS type I is a progressive multi-systemic disorder with a wide range of clinical manifestations. It presents three clinical subtypes: Hurler (MPS I-H), Hurler-Scheie (MPS I-HS), and Scheie (MPS I-S) syndromes [5]. MPS I-H is the most common, while MPS I-S is a rare form. Patients with the severe type (MPS I-H) are characterized by umbilical hernias, facial dysmorphia, skeletal deformities, hepatosplenomegaly, corneal clouding, mental delay, chronic upper respiratory and cardiac disease. In Hurler phenotype, the first symptoms are generally appearing before 12 months of life. Patients with the intermediate phenotype (MPS I-HS) have relatively physical symptoms, including hepatosplenomegaly, slight facial dysmorphia and a mental delay. The attenuated phenotype (MPS I-S) involves normal intelligence and a near-normal life span [6, 7].

Hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT) can effectively treat MPS I. Enzyme replacement therapy has proven to be safe and effective for the following subtypes: Hurler-Scheie and Scheie [8], while the application of HSCT with ERT is recommended for the successful treatment of Hurler syndrome [9, 10].

In this work, we identify the remaining mutation in a patient who was, in a previous study, reported to be compound heterozygous for the p.P533R and describe correlations between the clinical phenotypes and the *IDUA* gene variants in a total of fourteen MPS I patients. This study documented database on haplotype in ethnic populations and provide informations on the genetic mechanisms underlying the frequent mutation.

2. METHOD

2.1 Patients

This study was carried out, in parallel, on 14 unrelated MPS I Moroccan patients and 50 control subjects. Patients were recruited from different regions in Morocco and there were no known relationship between the families. When a family has more than one patient, only one of them is presented in this study. Clinical features of each patient are reported in Table 2.

*Corresponding author Author

All procedures were in accordance with the ethical standards of the Ethics Committees of the Medical Moroccan society. The consent was obtained from the patients and their families for being included in this study as well as for their identifying information.

2.2 Biochemical assay

The diagnosis of MPS in all studied patients was suspected by phenotypic analysis. The biochemical screening based on quantitative and qualitative urinary GAGs analysis [11, 12] oriented the diagnosis to MPS I for females and MPS I or II for males. The measurement of leukocyte α-L-iduronidase activity confirmed the diagnosis of MPS I [13]. This measurement was not done for two females because their heparinized blood was not obtained.

2.3 Molecular analysis

2.3.1 DNA extraction and PCR amplification: Genomic DNA was extracted from the peripheral blood (5 ml) of the affected child and their parents, and controls using the phenol/chloroform method. The quantity and quality of DNA were measured using a spectrophotometer.

PCR analysis was performed in a total volume of 25 µl containing 150 ng genomic DNA; 5X of Taq buffer; 1.5 mM of $MgCl_2$; 200 µM of dNTP; 0.4 µM of each primer and 0.2 µl of 5U/µl Taq DNA polymerase (Bioline). The running conditions were: pre-denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing temperature at 50°C to 62°C for 20 s with an elongation period of 15 s at 72°C. A final cycle of elongation was performed at 72°C for 3 min. PCR products were resolved in agarose gel at 2 % and visualized under UV light.
In order to study the *IDUA* mutational spectrum, we performed sequencing of six amplified genomic fragments, exons 1, 2, 3, 7 and both 11 and 12. Primer sequences used for amplification of the investigated exons are presented in Table 1.

Table 1: Pair primers sequences of the *IDUA* exons 1, 2, 3, 7 and both 11 and 12.

Exons	Sequences
Exon 1	5'ACCCAACCCCTCCCAC3' 5'GCTCCGGTCTCTGAAGCT3'
Exon 2	5'GAACGTGTGTGTCAGCCG3' 5'GCTCGGAAGACCCCTTGT3'
Exon 3	5'TCCCACATGCTCCGTTGT3' 5'GAATGGACATCCAAGGACTCAGA3'
Exon 7	5'TGCGGCTGGACTACATCTC3' 5'AGTAGCAGGTTCTGATGCTGC3'
Exon 11 and Exon 12	5'GTGTGGGTGGGAGGTGGA3' 5'CATGGGTGAAGGGGTCG3'

2.3.2 Direct sequencing: The amplified products obtained in the presence of the pairs primer 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).

2.3.3 Bio-informatic analysis: The sequences were aligned to the IDUA gene reference sequence (NG_008103.1) using logical BLAST (https://blast.ncbi.nlm.nih.gov) [14] to identify DNA variants. All variants identified were analyzed against using the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk) [15], Ensembl (https://www.ensembl.org) [16]. Sequence variants of mutant alleles were compared with the wild alleles sequence from 50 genomes of healthy unrelated Moroccan individuals.

3. RESULTS

3.1 Clinical features

In our series, we have observed 10 patients who have Hurler phenotype, 3 cases with Hurler/Scheie phenotype and one patient was presented with mild form of Scheie. One case, patient 7, was treated with Laronidase replacement therapy. The clinical manifestations of the 14 MPS I patients (7 females, 7 males) showed, at variable degrees, the presence of a short stature, a coarse face, an hepatosplenomegaly, a cardiac abnormality, a mental retardation and skeletal deformities. Eleven patients showed the corneal clouding. There is umbilical hernia in thirteen patients. All these patients displayed the obstructive airway disease. The clinical characteristics of each patient are presented in Table 2 bellow.

Table 2: Clinical characteristics of the 14 MPS I patients.

Patient	Sex	Age at diagnosis	Consanguinity of the parents/degree	Short stature	Coarse face	Hernia	Cardiac disease	Mental delay	Corneal clouding	Age at death
1	Female	7	1st cousins	+	+	+	+	-	+	-
2	Female	20	-	++	+	+	-	+	-	?
3	Male	3	1st cousins	+	+	-	+	+	+	-
4	Male	4	-	++	++	+	+	+	-	21
5	Female	9	-	++	+	+	+	+	+	?
6	Male	2	-	+	+	+	-	?	+	-
7	Male	10	1st cousins	++	+	+	+	+	+	-
8	Female	17	1st cousins	+	+	+	+	-	+	-
9	Female	10.5	1st cousins	++	+	+	+	+	+	11
10	Female	10	2nd cousins	++	+	++	+	+	+	?
11	Male	11	1st cousins	+	+	++	-	+	+	-
12	Male	9	1st cousins	+	+	+	-	-	-	-
13	Female	3	2nd cousins	+	+	+	+	+	+	-
14	Male	17	1st cousins	+	+	+	+	+	+	-

Note: **(+)** = Presence; **(-)** = Absence**; (?)**= Not Known.

3.2 Urinary GAGs and IDUA enzyme analysis

All studied patients have elevated urinary GAGs levels. The chromatography patterns of these accumulated GAGs showed the presence of both heparan and dermatan sulfate abnormal bands compared to their healthy peers. Patients for whom determination was done (7 males and 5 females) do not have IDUA activity. Therefore, the biochemical analysis confirmed the diagnosis of MPS I in all the fourteen patients.

3.3 Mutation Detection

3.3.1 IDUA mutation analysis: For the Fourteen patients, electrophoresis on agarose gel at 2% of PCR products from exons 1, 2, 3, 7 and both 11 and 12 showed no large rearrangement (data not shown). So, we have screened PCR products from these six exons by direct sequencing. Of the fourteen patients with the previously reported p.P533R mutation, one, patient 3 in table 2, is heterozygous for this pathogenic mutation (Figure 1A). His father is also heterozygous (Figure 1B). Conversely to that, his mother doesn't carried the p.P533R allele (Figure 1C), instead of that she beers the c.1545delC (Figure 1D). We can assume that this affected child has inherited the p.P533R allele from his father and the p.P515fs mutation from his mother. This novel mutation would be due to the deletion of the single base C of the third nucleotide proline codon 515, at cDNA nucleotide position 1545 in exon 11 of *IDUA* gene. The deletion c.1545delC (p.P515fs) might leads to a premature stop codon after 8th amino acid of the affected residue. The impact of this variant would be deleterious to the protein function. We have not detected the p.P515fs nor the p.P533R mutations in 100 studied control chromosomes (Figure 1E, 1F). On the other hand, the p.P533R mutation was confirmed to be heterozygous among the remaining parents of the patients.

Table 3: MPS I mutations in Moroccan patients.

Mutation	Position ADNc	Position ADNg	Nucleotide change	Exon	Codon protein	Predicted protein change	Reference
p.P533R	1598	3233	C>G	Exon 11	533	Proline to Arginine	[17]
p.P515fs	1545	3180	C>-	Exon11	515	deletion, premature stop codon	a

a*: Novel sequence change

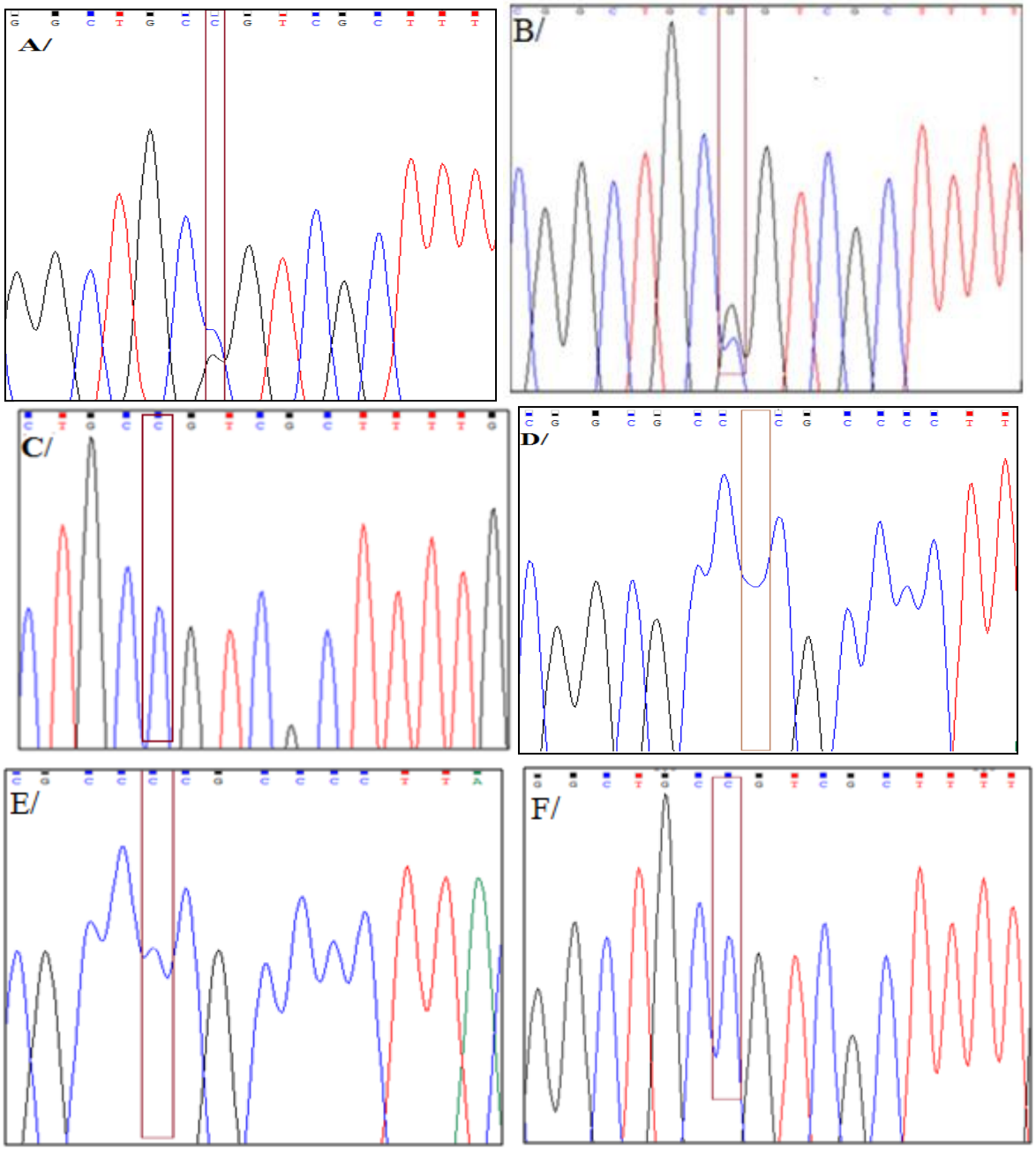

Figure 1: (A, B) Heterozygous state of p.P533R allele in the patient 3 and his father, respectively. (C, D) Normal allele of the p.P533R mutation and c.1545delC mutation in the patient 3's mother, respectively. (E, F) Normal alleles of the c.1545delC and p.P533R mutations, respectively.

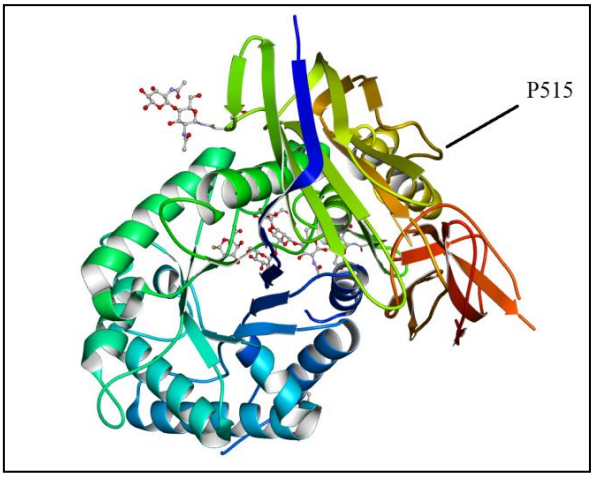

Figure 2: The predicted three-dimensional structures of human IDUA enzymes; the wild type amino acid at position 515.

3.3.2 Polymorphisms analysis

In addition to the pathogenic mutations, five sequence variants were identified, including two previously frequent polymorphisms: A8A and A20A in exon 1. The IVS2-44C >T polymorphism, in intron 2, was identified in homoallelic state in only patient 2. The R105Q polymorphism, in exon 3, and the IVS12+75G>T polymorphism, in intron 12, in heteroallelic state (Figure 3A, 3C) were identified in some patient's parent. The polymorphisms analysis revealed the novel intronic polymorphisms which are IVS10-13delC (Figure 3B) and IVS10-5delC, deletions mutations both based in intron 10.

On the other hand, we have observed that the IVS12+72T>G polymorphism was found, at heterozygous form, in some patient's parents, who are obligatory heterozygous, their healthy relatives, with or without p.P533R allele, and also in unrelated studied subjects (Figure 4A). However, none of investigated patients who are homoallelic for the p.P533R mutation have this polymorphism (Figure 4B). Therefore, all polymorphisms detected are presented in Table 4. The characteristics and the nucleotide positions of these polymorphisms are shown in Table 5.

Figure 3: Sequences of some variants detected of the *IDUA* gene.

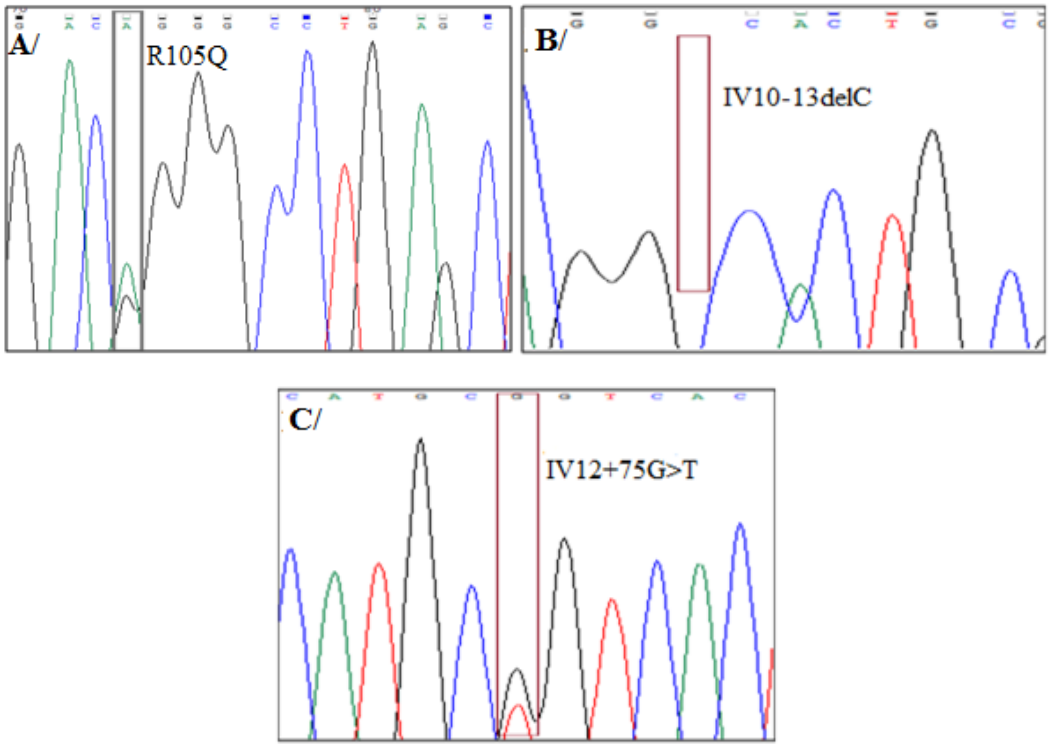

Figure 4: A/ Sequences of the IV12+72T <G polymorphism at heterozygous state in all studies: parents, patient's relative and unrelated studied subjects B/ wild allele in all studied patients homoallelic for p.P533R

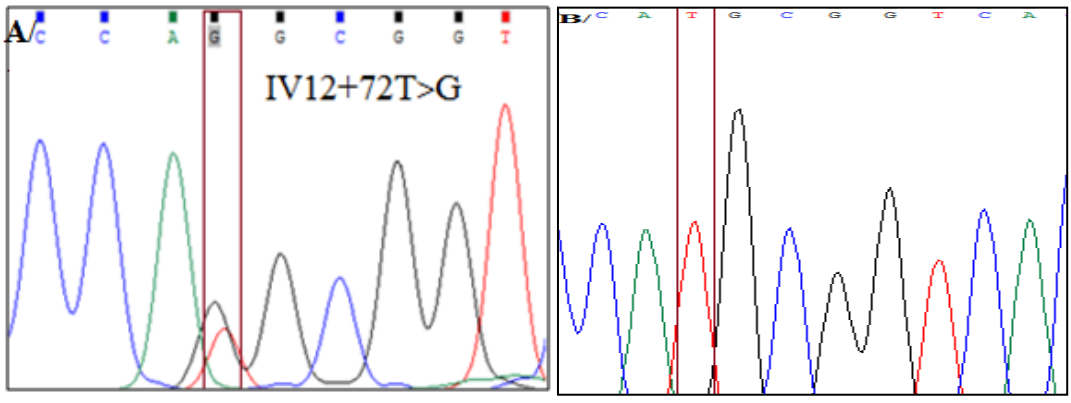

Table 4: Phenotype-genotype correlation for the Moroccan MPS I patients.

Patients	Phenotype	Mutations		Polymorphisms						
		Allele 1	Allele 2	A8A	A20A	R105Q	IVS2-44C/T	IVS10-13delC	IV10-5delC	IVS12+75 G>T
1	Hurler	P533R	P533R	+/+	+/+	-/-	-/-	-/-	-/-	-/-
2	Hurler/Scheie	P533R	P533R	+/+	+/+	-/-	+/+	+/+	+/+	-/-
3	Hurler	P533R	P515fs	-/-	-/-	-/-	-/-	+/+	-/-	-/-
4	Hurler	P533R	P533R	+/+	+/+	**-/-**	-/-	+/+	+/+	-/-
5	Hurler	P533R	P533R	-/-	-/-	**-/-**	-/-	+/+	-/-	-/-
6	Hurler	P533R	P533R	-/-	-/-	-/-	-/-	+/+	+/+	-/-
7	Hurler	P533R	P533R	-/-	-/-	-/-	-/-	+/+	+/+	-/-
8	Scheie	P533R	P533R	-/-	-/-	**-/-**	-/-	+/+	-/-	-/-
9	Hurler	P533R	P533R	+/+	+/+	-/-	-/-	?	?	-/-
10	Hurler	P533R	P533R					ND		
11	Hurler	P533R	P533R					ND		
12	Hurler/Scheie	P533R	P533R					ND		
13	Hurler	P533R	P533R	-/-	-/-	-/-	-/-	?	?	-/-
14	Hurler/Scheie	P533R	P533R					ND		

Note: **(+)** = Presence; **(-)** = Absence; **(?)** = Not Known; (ND) = Not determined.

Table 5: Characteristics and position of polymorphisms in Moroccan MPS I families.

Nucleotide change	DNAc	DNAg	Exon/intron	Codon	References
C/A	24	230	Exon1	A8A	[2]
G/A	60	642	Exon1	A20A	[2]
C/T	300-44	586	Intron 2	IVS2-44C >T	[18]
G/A	314	626	Exon3	R105Q	[2]
C/-	1525-13	3143	Intron10	IVS10-13delC	a
C/-	1525-5	3155	Intron10	IVS10-5delC	a
G/T	1727+75	3515	Intron 12	IVS12+75G>T	e
T/G	1727+72	3512	Intron 12	IVS12+72T >G	e

a: novel polymorphisms; **e**: the gnomAD Genomes.

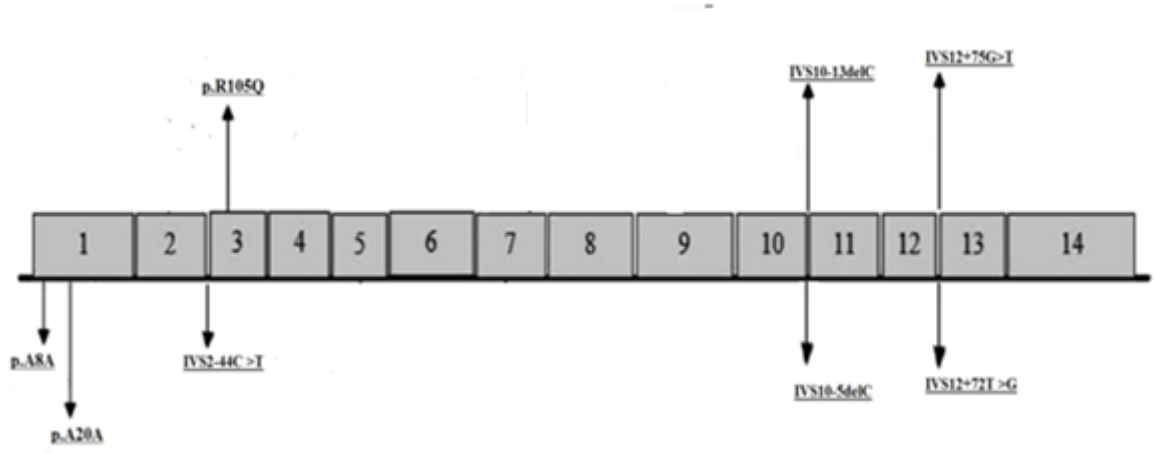

Figure 4: Spectrum of polymorphisms in Moroccan MPS I families

4. DISCUSSION

Because we are interested in MPS I, we conducted this study on a cohort of 14 families from different regions of Morocco. The proportion of consanguineous marriages in the referred families was 71.42% with first cousin unions being the most common. This result was similar to previous studies, showing that consanguinity of first cousins is the most represented in our population [17, 19].

In the present paper, MPS I patients show a broad clinical spectrum ranging from the severe "Hurler" phenotype to the attenuated "Scheie" phenotype. All Hurler (MPS I-H) patients presented the severe small size, a coarse face, severe organomegaly, hernia, cardiac abnormality, corneal clouding, and mental retardation. Patients with the intermediate phenotype (MPS I-HS), were characterized by the slight skeletal deformities, joint stiffness, organomegaly, mental retardation and long life. In one patient with the attenuated phenotype (MPS I-S) intelligence was mild to normal. The clinical manifestations observed in the present study were similar to those reported in other studies [2, 20].

4.1 Mutation diagnosis

The aim of this study was to measure the mutational profile, compare it to studies from other regions and provide insights about the current Moroccan population. In this report, the molecular analysis of the 28 MPS I chromosomes identified two pathogenic mutations. The spectrum of mutations in the selected cohort is homogeneous. Indeed, the P533R mutation was found at 96.42% (27/28) of the studied alleles. This mutation was already indicated as the most frequent in the Moroccan population (92% of mutant alleles) [17]. This been reported in 10 Tunisian MPS I patients at 62.5% [21] and in a group of 13 Algerian patients at 84% [22]. Therefore, this mutation would be native in North Africa.

In our previous work [17], by the Fluorescence Assisted Mismatch Analysis (FAMA) [23], both the patient 3 and his father were described to be heterozygous for the p.P533R mutation while his mother was homozygous for the wild allele. These results were confirmed at that time by sequencing. In this study, we show that the second MPS I allele for this compound heterozygous patient would be the novel mutation c.1545delC which is transmitted by his mother.

This mutation as well as small deletions or insertions could not be detected by FAMA. We are surprized to find compound heterozygous the patient 3, although he is born to first cousins parents. Generally, in autosomal recessive disorders, the consanguinity increase apparition of the same mutant allele.

The p.P515fs mutation predicted to result in a frameshift of the protein sequence. The IDUA mRNA transcript of the mutated allele might lead to an earlier premature translation termination codon, resulting in truncated IDUA protein at residue 523 of the total 653 amino acids. Combined with clinical features of the patient and according to predicted results this deletion would be very likely pathogenic. In addition to that, DNA samples from 50 Moroccan controls were negative for both p.P533R and c.1545delC mutations.

4.2 Common Moroccan mutation

The niece of a woman whose two children are affected with MPS I, was informed about her heterozygosity for the P533R allele. Thanks to her university level, she was well aware that she had to avoid wedding to a relative, so that her children would not be affected. She was shocked when her first son, with the classmate she wedded, is affected. The couple, then, discovered that the husband is also heterozygous for this mutant allele. On the other hand, screening for the common P533R allele in 150 randomly healthy individuals revealed one mutant allele among 300 [24]. These observations suggest that P533R allele is widely spread among Moroccan population.

4.3 Polymorphism diagnosis

Screening for genetic variations in different alleles facilitates the evaluation of the genetic profile of each population and the establishment of the phenotype/genotype correlation of genetic disorders to prevent severe symptoms. Currently, the results obtained from numerous studies provide insights into the mutational spectrum of the *IDUA* gene [25].

Many of the polymorphisms variants of the sequence have been described in the *IDUA* gene [26, 27]. The effect of these sequence variants on the functioning of *IDUA* has not been yet well defined. However, it has been shown that the presence of polymorphisms can alter the patient's clinical phenotype [28]. In this work, an analysis of polymorphisms was conducted on 10 of 14 patients to establish a phenotype/genotype correlation. Patients for whom polymorphism analysis was done are presented in table 4. The A8A and A20A polymorphisms are present in 33.3% of the subjects. This result is in agreement with the literature, which describe that these two variants are very frequent. Indeed, it was reported in almost 70% and 80% of the cases described in Tunisia and Algeria [21, 22], respectively. In this study, we have found the A8A and A20A polymorphisms are associated with the phenotypes: Hurler and Hurler/ Scheie. We have detected the IVS2-44C >T polymorphism at homozygous state in one patient (P2), showing the intermediate phenotype. The novel intronic polymorphisms (IVS10-13delC and IVS10-5delC) were observed in 90% of patients presenting the severe phenotype.

The R105Q polymorphism was found, at heteroallelic state, in patient 2's mother. Furthermore, we have searched for A314A, N297N that are described in literature [2, 29], in ten among fourteen patients but none of them have these polymorphisms.

Differences in environment and backgrounds of patients with the same genotype may explain, in part, differences observed in their clinical phenotype [30]. For instance, the patient 2 who has beneficiated, since her childhood, from several chirurgical interventions was aged 20 years while patient 9 who did not, has died at early age. However, there is a significant relationship between the *IDUA* genotypes and various phenotypes that are observed in MPS I.

Some genetic studies have succeeded to investigate the origin of the frequent mutation from haplotypes analysis. In, our studied patients, the p.P533R missense mutation was associated with specific *IDUA* haplotype (A8A, A20A). This haplotype was not observed in 100 control alleles. Since this mutation is common between unrelated MPS I Moroccan families and, also, between those of Tunisian and Algerian [21, 22], we can conclude that this mutation would be a foundational mutation in Morocco and in the populations of North Africa.

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