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Genetic Heterogeneity of 72 Patients With Mucopolysaccharidosis In Tunisia

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***Abstract*— The mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases, presenting with a progressive multisystem disorder, with extreme clinical heterogeneity. The purpose of this study was to investigate six genes involved in different types of mucopolysaccharidosis (MPS), a group of lysosomal storage diseases. Mutation screening was performed for a total of 72 MPS Tunisian patients with MPS I (n=43), MPS II (n=5), MPS IIIA (n=7), MPS IIIB (n=3), MPS IIIC (n=2), or MPS IVA (n=12). The methodological approaches included genomic PCR sequencing, RT-PCR for MPS IVA and tetra-primer ARMS PCR assay for MPSI. The present study revealed one novel splice site mutation in one MPSI patient, in addition to the 11 previously Tunisian mutations reported for the first time by our research team: three in the *IDUA* gene (MPS I) including two missense, one nonsense; four in the *SGSH* gene (MPS IIIA) including a mutation involving the start codon, one small duplication, one small deletion and a large deletion of exons 1 to 5; two in the *NAGLU* gene (MPS IIIB) including one missense mutation and one nonsense mutation; two in the *HGSNAT* gene (MPS IIIC) including one missense and one nonsense mutation, two in the *GALNS* gene including one missense and one splicing mutation. These data demonstrate the remarkable mutational heterogeneity characterizing all types of MPS tested, although high consanguinity rate in the Tunisian population. We report biochemical and molecular aspects of these patients to prevent (e.g. genetic counseling, prenatal diagnosis) the recurrence of affected child since these diseases are still not rare in our area and as specific therapy is not available in our country.**

***Index Terms*— Genetic counseling, glycosaminoglycan, Lysosomal storage diseases, mucopolysaccharidoses, mutations.**

I. Introduction

Lysosomal storage diseases (LSDs) are a heterogeneous group of rare inherited disorders caused by the deficiency of a specific lysosomal enzyme leading to the accumulation of undigested or partially digested macromolecules such as mucopolysaccharides, glycoproteins and sphingolipids.

Mucopolysaccharidoses (MPS) are a group of LSDs caused by the deficiency of a lysosomal enzyme involved in the catabolism of glycosaminoglycans (GAG). The accumulation of GAG in various tissues and organs leads to cellular dysfunction. Patients with MPS disorders usually appear normal at birth but develop progressively symptoms over the first few months of life or even after many years. Clinical signs include short stature, coarse facial features, organomegaly, skeletal and joint affection, airway and cardiac involvement, hearing and vision impairment. Mental retardation is present in the severe forms of MPS I, MPS II (and MPS VII) and all subtypes of MPS III. Nowadays, eleven enzyme defects that cause seven different types of MPS have been identified [1].

There are six different MPS disorders: MPS I (Hurler yndrome/Hurler-Scheie syndrome/Scheie syndrome; OMIM #607014, 607015, 607016), MPS II (Hunter syndrome; OMIM #309900), MPS III (Sanfilippo syndrome) including 4 subtype MPS IIIA (OMIM #252900), MPS IIIB (OMIM #252920), MPS IIIC (OMIM #252930), MPS IIID (OMIM #252940), MPS IV (Morquio syndrome) including 2 subtypes MPS IVA (OMIM #253000), and MPS IVB (OMIM #253010), MPS VI (Maroteaux-Lamy syndrome; OMIM #253200) and MPS VII (Sly syndrome; OMIM #253220). MPS III and MPS IV have four and two subtypes respectively, each one caused by the deficiency of a different enzyme. These inherited diseases may constitute a relatively more important social and economic concern in Tunisia because of the prevalence of first-cousin marriages.

The diagnosis of MPS patients is usually made by the combination of urine screening methods, enzyme analysis and DNA molecular analysis.

We report the molecular characterization of 72 patients with a clinical and/or biochemical diagnosis and in some cases, a family history of one of four different MPS types (MPS I, MPS II, MPS III (A, B or C) and MPS IVA).
Overall, most mutations are private with only p.P533R mutation (*IDUA* gene, MPS I) being common and specific in our population. We describe tetra-Primer ARMS PCR method to identify this common mutation. This method offers extremely fast, economical, and simple detection.

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II. Patients and Methods

A. Patients

Peripheral blood samples and urine from 72 patients with a clinical suspicion of MPS disorder were analyzed in the Laboratory of Biochemistry, Farhat Hached Hospital (Sousse, Tunisia) for biochemical (quantification and electrophoresis of urinary glycosaminoglycans) and molecular investigation for the following disorders: MPS I (n=43), MPS II (n=5), MPS IIIA (n=7), MPS IIIB (n=3), MPS IIIC (n=2) and MPS IVA (n=12). All studied patients are from a consanguineous marriage, and there were no known relationships among the families. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and approved by the Ethics Committees of the respective Tunisian hospitals. Informed consent was obtained from all patients and their families for being included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

B. Biochemical diagnosis

The diagnosis of these diseases was based on the following approach after a clinical and paraclinical suspicion.

1) Quantitative analysis of total urinary glycosaminoglycans

Study of urinary glycosaminoglycans was performed first. Urinary GAGs were quantified using a DMB (dimethylmethylene blue) test [2]. The quantity of DMB bound to sulfated glycosaminoglycans was measured via spectrophotometry at wavelength of 656 nm.

Electrophoresis on cellulose acetate plate was performed to identify which type of GAG is present in excess (e.g., dermatan sulfate, heparan sulfate, keratan sulfate). Discontinuous electrophoresis on cellulose acetate plates separated the different GAGs based on their charge and differential solubility in ethanol, and the mucopolysaccharides were visualized by staining with alcian blue [2].

2) Enzyme analysis

Alpha-L-iduronidase (MPSI; EC 3.2.1.76) activity was determined in leucocytes using the phenyl-alpha-L-iduronide artificial substrate, in the Biochemistry Laboratory of Sousse-Tunisia. Enzyme analysis for iduronate-2-sulfatase (MPS II; EC 3.1.6.13), heparan-N-sulfatase (MPS IIIA; EC 10.1.1), N-acetyl-α-glucosaminidase (MPSIIIB; EC 3.2.1.50), α-glucosaminide-N-acetyltransferase (MPS IIIC, EC 2.3.1.78) and N-acetylgalactosamine-6-sulfatase (MPS IVA; EC 3.1.6.4) were performed in leucocytes using a fluorimetric substrate, in the Laboratory of Inherited Metabolic Diseases in Lyon-France.

C. Molecular analysis

1) DNA sequencing analysis

Genomic DNA was isolated from venous blood by the phenol/chloroform procedure, according to standard protocols as described previously [3]. All the exons and flanking intron/exon junctions of the *IDUA*, *IDS*, *SGSH*, *NAGLU*, *HGSNAT* and *GALNS* genes were amplified and sequenced. For patients with a family history of known or suspected pathogenic mutations or for the index cases' parents, targeted PCR and sequencing were performed in the Laboratory of Inherited Metabolic Diseases in Lyon-France (*IDUA*, *IDS* and *GALNS*) and in the Laboratory of Medical Biochemistry in Rouen, France (*SGSH*, *NAGLU* and *HGSNAT*).

2) Tetra primer-ARMS-PCR of p.P533R mutation in the IDUA gene

The tetra primer-ARMS-PCR procedure was used to identify the p.P533R mutation.

Allele specific primers were designed using computer programs. Primer sequences and annealing temperature are provided in Table 1. PCR reaction consisted of 50 ng of DNA, 1 X HotStarTaq buffer, 2 mM MgCl2, 200 µM of each dNTP, 10 pmol of each primer, 2.5 U of HotStarTaq (Qiagen) and 1 X Q solution. The final reaction volume was 18 µl. Thermal PCR profile consisted of an initial denaturation at 95°C for 15 min, 35 cycles of denaturation at 94°C for 30s, annealing at 68°C for 30s and extension at 72°C for 30s followed by a final extension step at 72°C for 10 min. PCR products were resolved in 2% agarose gel and were visualized under UV light. The PCR amplification of normal and mutant alleles was carried out in the same reaction. Both wild and mutant homozygotes and heterozygote samples were used as positive controls.

The mutations p.P533R (*IDUA* gene) and c.120+1G>A (*GALNS* gene) frequencies were determined by direct counting of the alleles in the tested population.

III. Results

A. Molecular analysis

1) IDUA gene

A total of 8 different suspected pathogenic sequence alterations were detected in 43 MPS I patients (Table 2). Among them, one novel splicing mutation c.1650+1G>A in addition to the three previously Tunisian mutations reported for the first time by our research team but still unreported in HGMD (www.hgmd.org) (c.530T>C (p.F177S), c.1734T>C (p.L578Q) and c.1587_1588insGC (p.L530AfsX31) [4], [5]. These mutations were associated with a severe phenotype except for the p.L578Q mutation.

The novel alteration was a splice site mutation at the donor site of intron 11 (c.1650+1G>A) and was identified in the *IDUA* gene at the homozygous state in three patients who developed a severe form of MPS I. A splice site mutation can cause activation of an alternative cryptic splice site in preference to using the legitimate splice site. The g>a mutation at the 5'-donor splice site of intron 11 presumably caused exon skipping, the loss of exon 12, and aberrant polypeptide that was misfolded (http://rulai.cshl.edu/tools/ESE3).

The missense mutation (p.F177S) was predicted to be pathogenic by both prediction tools PolyPhen (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) in one homozygous patient with a severe phenotype [4].

Two patients who were compound heterozygotes (p.P533R/p.L578Q) manifested clinically milder symptoms

than homozygous p.P533R patients with the severe and/or intermediate phenotype. The novel missense mutation p.L578Q was also predicted pathogenic by both PolyPhen and SIFT [5].

Another third mutation, a small insertion of 2 nucleotides, has been identified in one homozygous patient presenting a severe phenotype: c.1587_1588insGC (p.L530AfsX31) leads to a frameshift and consequently the occurrence of a premature stop codon 30 residues downstream [4].

We also identified Eleven SNPs (dbSNP; http://www.ncbi.nlm.nih.gov/), in the *IDUA* gene (Table 3). Of these polymorphisms, seven were within *IDUA* exons, and have been previously described and used for linkage analysis and haplotypes determination in MPSI patients [5]. For the p.P533R mutation, the same associated polymorphism was shared by all affected family members. For the p.F602X and p.L530AfsX31 mutations, the same previously described haplotype [5] was identical for all the affected patients of this study.

For the detection of p.P533R mutation, we have developed a simple and reliable tetra-primer ARMS PCR assay. The method was performed and tested for a group of p.P533R heterozygote or homozygote patients.

Primers 11-12F2IDUA and 11-12R1IDUA were used as internal control primers, producing a control band of 542 bp, whereas primers 11-12F1WIDUA and 11-12R1IDUA produced a 421 bp wild band in a healthy subject, reflecting the absence of a C>G transversion in codon 533 in exon 11. Primers11-12F2IDUA and 11-12R2MIDUA produced a 164 bp band in a positive homozygous control. Heterozygous p.P533R was shown by the presence of 164 bp with 421 bp and the 542 bp control band (Fig. 1). Parental studies confirmed the segregation of the p.P533R allele.

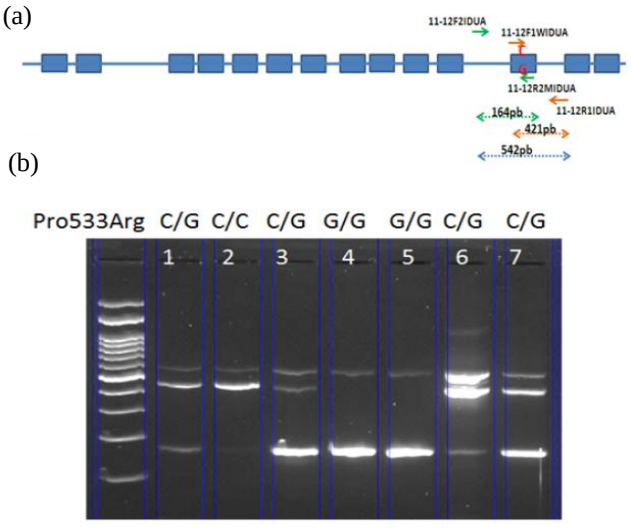

Fig. 1 Schematic illustration showing primers in the proposed tetra-primer ARMS PCR assay method to detect p.P533R mutation in *IDUA* gene. (a) Exons are represented as blue boxes while introns are represented by single line. (b) Agarose gel electrophoretogram. PCR products using DNA from heterozygous p.P533R patient as template (lanes 1, 3, 6 and 7): three bands were detected. Using DNA from a healthy subject (lane 2), two bands were detected. Using DNA from a patient homozygote for p.P533R (lanes 4 and 5), two bands were detected.

2) IDS gene

Molecular analysis of the *IDS* gene was performed for five unrelated patients suspected of Hunter syndrome (MPS II). Two different mutations located within exon 3 were identified: c.262C>T (p.R88C) and c.281G>A (p.G94D) (Table 2). Both mutations have been previously described by others [6] and in Tunisia [7]. Carrier testing has not been performed in the mothers of affected boys. In addition, one unreported intronic polymorphisms [IVS7+38 (c.1006+38T>C)] and five previously reported polymorphisms (dbSNP; http://www.ncbi.nlm.nih.gov/), including two located within IDS exons, were identified in the studied MPS II patients (Table 3).

3) SGSH gene

Molecular analysis of the *SGSH* gene was performed for 7 patients suspected for Sanfilippo syndrome type A (MPS IIIA). A total of 7 different mutations were identified by our research team [8] (Table 2): a mutation involving the start codon c.2T>C (p.Met1?), one small duplication c.29dup (p.L11AfsX22), [8] c.1080del C (p.V361SfsX52) and a large deletion of exons 1 to exon 5, (g.75802301_75809393del7093 pb) which removed the first five exons of the *SGSH* gene and part of the *SLC26A11* adjacent gene [8].

Of the 7 MPS IIIA patients, one was a compound heterozygote for a previously reported mutation c.1129C>T (p.R377C) and the large deletion, g.75802301_75809393del7093 pb.

4) NAGLU gene

Molecular analysis of the *NAGLU* gene was performed for 3 patients suspected for Sanfilippo syndrome type B (MPS IIIB). All of these patients were found homozygotes.

Two mutations were identified (previously reported by Ouesleti et al 2011) and were novel (Table 2).

One of the alterations was a nonsense mutation c.1674C>G (p.Y558X). The other one was a missense mutation c.1811C>T (p.P604L) that was found homozygote in a pair of affected siblings. Both PolyPhen and SIFT predicted this missense change to be pathogenic.

5) HGSNAT gene

Molecular analysis of *HGSNAT* gene was performed for 2 patients suspected for Sanfilippo syndrome type C (MPSIIIC). These patients were compound heterozygote.

Two mutations identified in the *HGSNAT* gene were novel and previously reported by Ouesleti et al 2011 (Table 2). One of the alterations was a nonsense mutation c.1209G>A (p.W403X). The other novel change c.1879G>T (p.W627C) was due to a modification of a hydrophobic amino acid by another that contains sulfur in the side chain altering subsequently the structure of protein. Both PolyPhen and SIFT predicted this missense change to be pathogenic.

6) GALNS gene

Molecular analysis of the *GALNS* gene was performed for 12 patients suspected for Morquio A syndrome (MPS IVA). All of these patients were homozygotes. A total of six different suspected pathogenic sequence alterations were detected. The most common mutation observed in the

Table. 1 Primers for tetra-primer ARMS-PCR method for detection of p.P533R mutation

Primer	Sequence[a]	Tm[b] (°C)	C[c] (µM)	Expected products
11-12F1**W**IDUA	5'CCCCGCGCTGCGGCTGC***C***3'	69.6	0.2	
11-12R1IDUA	5'CCCTAGGAGAACCCACACCCCACTGG3'	71.1	0.3	421 pb
11-12F2IDUA	5'CCTGAGGTCGGGCCGAGCGTC3'	69.6	0.2	
11-12R2**M**IDUA	5'CACGTGCACCAGCAAAAGCGACCC***C***3'	66.0	0.2	164 pb
11-12F2IDUA	5'CCTGAGGTCGGGCCGAGCGTC3'	69.6	0.2	
11-12R1IDUA	5'CCCTAGGAGAACCCACACCCCACTGG3'	71.1	0.3	542 pb

[a] specific-enhancing mismatches are shown in bold italics

[b] The melting temperature (Tm)

[c] Final reaction concentration

Table. 2 Genotypes identified in 72 MPS patients

Type of MPS	Gene	Number of patients	Allele 1	Allele 2	Effect of alleles 1 and 2 on protein	References
MPS IH (n=39 patients)	IDUA	4	c.1805delT; p.F602X	c.1805delT; p.F602X	Phenylalanine to stop	[4]
		21	c.1598C>G ; p.P533R	c.1598C>G ; p.P533R	Proline to Arginine	[4],[5]
		6	1882C>T ; p.R628X	1882C>T ; p.R628X	Arginine to stop	[5]
		1	1882C>T ; p.R628X	non identified	Arginine to stop	[4]
		2	c.1743C>G ; p.Y581X	p.Y581X	Tyrosine to stop	[5]
		1	c.1587_1588insgGC; p.L530AfsX31	c.1587_1588insgGC; p.L530AfsX31	Frame- shift, premature stop	[4]
		1	c.530T>C; p.F177S	c.530T>C; p.F177S	Phenylalanine to serine	[4]
		3*	c.1650+1G>A; IVS11+1G>A	c.1650+1G>A; IVS11+1G>A	aberrant polypeptide	This report
MPS IH/S (n=2)		2	c.1598C>G ; p.P533R	c.1598C>G ; p.P533R	Proline to Arginine	[5]
MPS IS (n=2)		2	c.1598C>G ; p.P533R	c.1734T>C ; p.L578Q	Proline to Arginine Leucine to Glutamine	[5]
MPS II (n=5 patients)	IDS	4	c.262C>T; p.R88P	c.262C>T; p.R88P	Arginine to Proline	[7]
		1*	c.281G>A ; p.G94D	c.281G>A ; p.G94D	Glycine to Asparagine	[23]
MPS IIIA (n=7 patients)	*SGSH*	1	p.M1?	p.M1?	Occurs in the ATG initiation codon	[8]
		1	c.1129C>T ; p. R377C	g.75802301_75809393del7093	Arginine to Cystein deletion exons 1 to 5	[8]
		1	c.1093C>T; p.Q365X	c.1093C>T; p.Q365X	Glutamine to stop	[8]
		1	c.29dup; p.L11AfsX22	c.29dup; p.L11AfsX22	Frame-shift, premature stop	[8]
		1	c.197C>G; p.S66W	c.197C>G; p.S66W	Serine to Tryptophane	[25]
		1	c.1080del ; p.V361S fsX52	c.1080del ; p.V361S fsX52	Frame-shift, premature stop	[8]
		1	g.75802301_75809393del7093	g.75802301_75809393del7093	deletion exons 1 to 5	[8]
MPSIIIB (n=3 patients)	*NAGLU*	1	c.1674C>G; p.Y558X	c.1674C>G; p.Y558X	Tyrosine to stop	[8]
		2	c.1811C>T ; p.P604L	c.1811C>T ; p.P604L	Proline to Leucine	[8]
MPSIIIC (n=2 patients)	*HGSNAT*	2	c.1209G>A ; p.W403X	c.1879G>T; p.W627C	Tryptophane to Stop tryptophane to Cystein	[8]
MPSIVA (n=12 patients)	*GALNS*	1	c.287G>C; p.G66R	c.287G>C; p.G66R	Glycine to Argenine	[12], [10]
		7	c.120+1G>A; IVS1+1G>A	c.120+1G>A; IVS1+1G>A	loss of exon 2, aberrant polypeptide	[11]
		1	c.341C>T; p.A85T	c.341C>T; p.A85T	Alanine to Threonine	[11]
		1	c.1156C>T; p.R386C	c.1156C>T; p.R386C	Arginine to Cystein	[19]
		1	c.421T>A ; p.W141R	c.421T>A ; p.W141R	tryptophane to Arginine	[19]
		1	c.1168 delc ; p.L390X	c.1168 delc ; p.L390X	Leucine to stop	[19]

*Studied patients in this study

Table. 3 Single nucleotide variations (SNV) identified in MPS Tunisian patients

Gene	Intronic single nucleotide variations	Exonic single nucleotide variations
IDUA	c.159-33C>T (rs373759069) c.299+161C>A (rs184055797) c.299+173G>C (rs369516569) c.590-8C>T (rs6848974) c.1525-38T>C (rs1131853)	c.24C>A (rs11248061) c.60G>A (rs10902762) c.314G>A (rs3755955) c.924G>C (rs6830825) c.543T>C (rs6815946) c.1230C>G (rs115790973) c.1467C>T (rs115929690) V554IG>A
IDS	c.168C>T (rs1141608) c.1006+38T>C*	c.508-816C>T (rs4844027)
GALNS	c.567+134A>G c.403+684C>A c.1569+70T>A c.244+86G>A* c.567+105T>C* c.633+125A>G* c.633+138C>A*	c.1431G>A (rs2303271) c.1266T>G (P420P) c.1177G>C (rs2303269) c.708C>T (rs1064315) c.199C>A (rs11862754)

*Mutation found in this report

GALNS gene was c.1156C>T (p.R386C) which has been already found in a patient of Turkish origin [9].

Two of the mutations were previously reported [10, 11] in the Tunisian population. One of the alterations was a G to A transition in the conserved 5′ donor splice site of intron 1 (GACgt >GACat: designated c.120+1G>A (IVS1+1G>A) and a G to C transversion in codon 66 of exon 2, resulting in a substitution of a glycine 66 with an arginine (p.G66R) (Table 2).

RT-PCR was used to amplify the region from exon 1 to exon 4 of the *GALNS* mRNA and showed that the splice site mutation c.120+1G>A removes exon 2 of the *GALNS* gene [11].

Twelve SNPs (dbSNP; http://www.ncbi.nlm.nih.gov/) were identified in the *GALNS* gene (Table 3). Of these polymorphisms, five were exonic. Six polymorphisms have been previously described and used for linkage analysis in MPSIVA patients and haplotype determination [12]. Four previously unreported intragenic polymorphisms [IVS2+86 (c.244+86G>A)], [IVS6+125 (c.633+125A>G)] and [IVS6+138 (c.633+138C>A)] (Table 3) were associated with the p.L390X mutation in a homozygous patient.

Haplotyping of known *GALNS* polymorphisms revealed that the splice site mutation was on a common background which suggests that these mutant alleles were "identical by descent" and were derived from a common ancestor [13].

IV. Discussion

We report on the molecular characteristics of 72 MPS patients belonging to unrelated families.

In Tunisian the incidence for all types of mucopolysaccharidoses is estimated to 2.3 cases per 100,000 live births [14]. The prevalence of MPS type I, III and IVA, these most frequently identified in the collected data was estimated to 0.63, 0.7 and 0.45 per 100,000 live births, respectively [15].

Molecular analysis revealed 27 different mutations, including 8 in the *IDUA* gene, 2 in the *IDS* gene, 7 in the *SGSH* gene, 2 in the *NAGLU* gene, 2 in the *HGSNAT* gene and 6 in the *GALNS* gene. Our results are in accordance with the high degree of allelic heterogeneity among these genes. It is worth noting that p.P533R mutation at the homozygous state was associated with a wide spectrum of clinical phenotypes i.e. mild, intermediate or severe. In the *IDS* gene only two missense mutations located within exon 3 were identified in five unrelated patients. Examination of the distribution of mutations located within the coding region of the *IDS* gene revealed that exon 3 presents significantly more mutations than the other exons [16].

A total of 7 mutations located within exon 1 and 8 were identified in the *SGSH* gene. All the mutations described in this gene were distributed unequally among the exons of this gene.

Our analyses identified one novel splicing mutation in one MPSI patient, in addition to the 11 mutations previously identified in Tunisia reported for the first time by our research team: three in the *IDUA* gene; four in the *SGSH* gene; two in the *NAGLU* gene; two in the *HGSNAT* gene, two in the *GALNS* gene. Homozygous mutations were found in 68 patients and compound heterozygous mutations were found only in 4 patients. This may be explained by the high percentage of consanguinity in our study related. In previously study, consanguinity was found in 83% of the Tunisian families [15]. In Tunisia, a high inbreeding resulting from consanguinity rate marriage between first second or third degree cousins has been identified in all investigated Tunisian MPS patients [7], [17]. Thus, unrelated families originating from the same region presented common MPS mutations, suggesting an eventual founder effect.

The most frequent disease-causing mutations in the studied patients were the mutations, p.P533R (*IDUA*) and c.120+1G>A (*GALNS*), which accounted for approximately 55.3% (47/85) and 58.3% (14/24) of the alleles, respectively.

The c.1598C>G (p.P533R) mutation in the *IDUA* gene was the most frequent disease-causing mutation in our patients and was previously described as a common mutation in the Northern African population which accounted for approximately 99% of the total mutant alleles [18]. This finding suggested that these mutant alleles were "identical by descent" and were derived from a common ancestor.

For economic and technical reasons, we have selected and developed the tetra primer-ARMS PCR assay as a method for characterizing the common mutation p.P533R. The technical analysis is simple, cost-effective, fast and feasible in our laboratory. This method will be implemented in screening purpose and also in a context of MPS I family study. The molecular results complete the biochemical exploration (urinary GAG measurement, electrophoresis profile and enzymatic activity) and will be helpful in case of prenatal diagnosis which is requested due to the high inbreeding rate encountered in Tunisia. The same tetra-primer ARMS PCR method is in progress to be used for the common c.120+1G>A (MPS IVA) investigation.

V. Conclusion

This study allowed determining the most frequent mutations of MPS in Tunisia. The identification of these mutations in unrelated families' permits accurate carrier detection and genetic counseling of at-risk relatives, and should facilitate molecular prenatal diagnosis.

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