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Mutational Analysis of Mucopolysaccharidosis in Iranian Patients

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Abstract

Mucopolysaccharidosis (MPS) is a rare and heterogeneous metabolic disorder with wide phenotypic distributions throughout the world. This study aimed to determine the genetic polymorphisms, contributing to the most common types of MPS in 19 unrelated Iranian patients. The sequence of the coding region and exon-intron boundaries of the MPS genes were analyzed by Sanger sequencing method. We used the biochemical and clinical characteristics of MPS subjects for genetic analysis. A novel IDUA variant (c.99T>C, p.H33H), a novel nonsense change (c.514C>T, p.R172*) in exon 5 of IDS gene, c.74G > A (p. p.R24H) in SGSH gene, and three variants including (c.607C>T (p.R203*), c.259G>C (p.A87P), and c.683G>A (p.R228Q)) in NAGLU (n-acetyl-alpha-glucosaminidase) were predicted as novel pathogenic mutations. In conclusion, this study broadened genotypic spectrum of Iranian MPS patients, facilitating the definition of disease-associated mutations, which help to provide a more effective approach in MPS carrier detection.

Keywords: Mucopolysaccharidosis, Mutation Screening, Iran, Genetic Counseling, Sanger Sequencing

1. Background

Mucopolysaccharidosis (MPS) is a set of inborn metabolic errors caused by an absence of specific lysosomal enzymes involved in glycosaminoglycan (GAG) catabolism (1). This metabolic block resulted in the GAGs accumulation in various organs, leading to progressive and multisystemic features (2). Based on the impairment of 11 involved enzymes, nine various clinical types and numerous subtypes of MPS have been identified (3). The subdivisions of MPS include Hurler Disease (MPS I), Hunter Syndrome (MPS II), Sanfilippo Syndrome (MPS III), Morquio Syndrome (MPS IV), Maroteaux Lamy Syndrome (MPS VI), Sly Syndrome (MPS VII), and hyaluronidase deficiency (MPS IX) (4).

It is very important to develop the genotypic catalog of specific disorders for a population. In this respect, there is inadequate research based on a limited number of cases in Iran. Therefore, the present study aimed to measure the rate of mutation and compare the data to the studies conducted in neighboring countries so as to identify the status of MPS disease in the Iranian population. Despite important information provided by genetic testing, there are limitations in this regard. Identification of common mutations prevents the need for next generation sequencing (NGS) based tests in MPS candidate patients. This results in lower diagnostic costs for patients and can be identified and categorized at a very low cost, and appropriate therapies can be considered.

2. Objectives

In this study, we examined the clinical and biochemical features and genetic analysis of 19 Iranian patients with MPS to identify novel MPS-associated mutations.

3. Methods

3.1. Ethical Statement

The sample included a total of 19MPS patients referred to the Taban Health Care and Diabetes Clinic (THCDC) from 2009 to 2015. The present study was approved by the Ethical Committee of National Institute of Genetic Engineering and Biotechnology (NIGEB) (IR.NIGEB.EC.1397.8.23.C),

Tehran, Iran. Informed consent was obtained from patients. The clinical characteristics of MPS patients were summarized in Table 1.

3.2. DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted using PrimePrep Genomic DNA extraction kit (GeNet Bio) from peripheral blood samples of MPS patients, collected in tubes containing ethylenediaminetetraacetic acid (EDTA) in a final volume of 2 mL. Briefly, the PCR reaction was carried out in a total volume of 25 μL containing 50 ng of DNA, 2.5 μL of 10 $\times$ PCR buffer, 0.1 mM of each dNTP, 1 mM of $MgCl_2$, 0.1 μM of each primer, and 0.3 units of Taq polymerase enzyme (CinnaGen, Iran) using a thermocycler (Eppendorf, Hamburg). The DNA was denatured at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min. The annealing temperature was based on the temperature (°C) value of each primer set (Table 2). The extension temperature was at 72°C for 1 min, with final extension at 72°C for 10 min.

3.3. Sequencing Analysis

The double-stranded DNA of PCR products from MPS patients was examined using an automated ABI sequencing machine (Applied Biosystems 3100, Kavosh Fanavaran Kawsar Company, Tehran, Iran). The DNA sequences were confirmed for any nucleotide variation and then analyzed using Finch TV software (http://www.geospiza.com/finchtv/).

3.4. In Silico Analysis of the Variants

The impact of novel mutations was verified through Human Gene Mutation Database (HGMD). In order to predict the functional effects of novel variations, the sequence changes were evaluated using in silico prediction algorithms SIFT (5), polyphen (6), and I-Mutant 2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0.html).

4. Results

4.1. Epidemiological and Clinical Data

In this study, 19 unrelated Iranian MPS patients were affected by MPS I (31%), MPS II (10%), MPS IIIA (31%), and MPS IIIB (26%) (Table 1).

4.2. Sequence Analysis

All the coding exons and intronic boundary regions of the IDUA, IDS, SGSH, and NAGLU genes were assessed by polymerase chain reaction (PCR) in all patients. The list of primers used in this study was presented in Table 2. Direct sequencing of the PCR products showed 16 variants in these genes, among which two nonsense and three missense alterations had been reported in the disease databases previously and two variants were present in the local population database (http://www.iranome.ir) (Table 3).

Except the mentioned variants, remaining variants were not found in the disease databases (Clin Var, OMIM, HGMD, and literature in PubMed till to Jul 29, 2019). Six variants were predicted as disease-causing by Mutation Taster.

4.3. Amino Acid Substitutions

Among the cases, six patients had single base-pair substitutions in IDUA gene, five of these variants were detected in exon 1, and a single base pair substitution (c.1205G>A) was detected in exon 9 of one sample. Of the five mutations identified in the IDUA gene, three mutations were the missense type leading to a change in the amino acid sequence, one mutation was the nonsens type that leads to creation of stop codon, and one mutation was the silent type.

We found a single base pair substitution (c.514C>T) in exon 5 of IDS gene in one sample. This mutation is of the nonsense type and leads to the formation stop codons. In addition, four single base-pair substitutions in NAGLU gene were detected in five samples, including a single base-pair substitution (c.2209C>A) in exon 6 of two samples, two single base-pair substitution (c.607c>T and c.683G>A) in exon 3 of two samples, and a single base-pair substitution (c.259G>C) in exon 1 of one sample. Of these four mutations, three were missense mutations, and one was nonsense mutation. Moreover, our data showed five single base-pair substitutions in SGSH gene in 6 samples, including a single base pair-substitution (c.74G>A) in exon 1 of one sample, a single base-pair substitution (c.456G>A) in exon 3 of one sample, and two single base-pair substitution (c.364G>A and c.456G>A) were found in two and one of our samples, respectively.

Of these five mutations, four were missense mutations, and one was silent mutation that does not alter the amino acid sequence (type of mutations was summarized in Table 3).

5. Discussion

MPS is a group of hereditary, rare, and incurable 'lysosomal storage diseases' (7). It is estimated that 1 in 25,000 newborn children will have some type of the MPS in the United States in 2013 (8). MPS demonstrates remarkable genotypic heterogeneity, explaining the association of genotype-phenotype variability (9, 10). The present study indicated clinical and molecular features of 19 patients

Table 1. Epidemiologic and Clinico-Biochemical Features of Patients

Patients	MSP Type	Sex	PCM	Clinical Feature	Main Symptom	Face Abnormality	Biochemical Urine GAG Accumulation Detected	MR	Cardiac Manifestations	Deafness
1	MSP I	M	+	Hepatospelnomegaly, spinal cord curvature	Sever NDD, cloudy cornea, hearing loss	Coarse face, irregular's dental, gum hyperplasia, corneal opacity	DS>HS	+	MI AI	BT
2	MSP I	F	+	CVS	#	#	#	#	#	#
3	MSP I	M	+	Skeletal deformity, stiff joint, respiratory infection, claw hand	Deafness, cloudy cornea, dolichocephalic, CHD, moderate NDD	gum hyperplasia, cloudy cornea Coarse face,	DS>HS	+	MI AS	Mixed
4	MSP I	F	+	Umbilical hernia, gargoyles, sever Hepatomegaly, mild splenomegaly	cloudy cornea 1 year, deafness 2years	Mild coarse face, irregular's dental	DS, HS	+	MS	BT
5	MSP I	M	+	Short stature, claw hand, dysostosis multiplex	Macrocephaly, viceromegaly,	cloudy cornea, Coarse face,	DS>HS	+	MI	Mixed
6	MSP I	F	+	Seizure, hepatomegaly, short stature	Macrocephal, ombelical hernia	Facial dysmorphic features, mild Coarse face	DS, HS	N	MI AI	P
7	MSP II	M	_	dysostosis multiplex, Skeletal deformity; Hepatospelnomegaly, joint; stiffness; dolichocephaly, claw, hand	Carpal tunnel, large tongue, Hydrocephaly, repeated diarrhea, large; tongue, J-shape sella; turcica	Coarse face, clear cornea, hypertelorism	DS, HS	+	Cardiomyopathy, Valvulopathy	BT
8	MSP II	M	_	AF	#	#	#	#	#	#
9	MSP IIIB	M	+	Mild speech difficulty, insomnia, mild Skeletal deformity,	Psychotic, ADHD, NDR, hairsotysm, mild speech difficulty	Moderate Coarse face	HD CS	+	N	N
10	MSP IIIB	F	+	mild Skeletal deformity,	Difficult mood	Coarse face	ND	+	Valvulopathy	N
11	MSP IIIA	F	+	Mild speech difficulty	Aggressive behavior	Mild coarse face	HS CS	+	N	MILD BT
12	MSP IIIB	M	+	insomnia, mild Skeletal deformity,	ADHD, hairsotysm	Hair sot face	HS	+	N	N
13	MSP IIIB	F	+	moderate speech difficulty, mild short stature, Seizure,	Hairsotysm, NDR, otitis	Mild coarse face	HS	Mild +	N	N
14	MSP IIIA	M	+	Seizure, mild short stature	Claw hand, NDD, recurrent media otitis	MPS like phenotype	HS DS CS	+	Cardiomyopathy	Mixed
15	MSP IIIB	M	+	Mild speech difficulty, insomnia	ADHD, NDR,	Mild coarse face	HS, CS	+	N	N
16	MSP IIIA	M	+	Otitis media, speech difficulty,	NDD, NDR, Claw, hand, Psychotic	coarse face	Hs cs	+	N	Mixed
17	MSP IIIA	M	+	Seizure, Speech delay	Claw hand, Hairsotysm	Mild coarse face	ND	+	Valvulopathy	MILD BT
18	MSP IIIA	F	+	mild Skeletal deformity	NDR, Aggressive behavior	MPS like phenotype	HS CS	+	N	N
19	MSP IIIA	F	+	Insomnia, Skeletal deformity,	ADHD, Psychotic	coarse face	ND	Mild+	CHD	Mixed

from unrelated Iranian families, manifesting various biochemical and clinical characteristics of MPS disease. Additionally, MPS is promptly diagnosed through urine and blood biochemical analysis (8, 11). However, a definite diagnosis of different types of MPS requires a wide range of comprehensive biochemical and molecular genetic techniques (12). In this study, to evaluate mutations of IDUA, IDS, SGSH, and NAGLU genes, we sequenced 19 blood samples acquired from MPS patients using a Sanger sequencing method. In our study, 15 variants in these genes were reported in MPS patients, of which eight variants were novel. Out of 15 variants, 9 variants were disease-causing mutations. Of the changes in IDUA identified in this study, the novel change c.99T>C (p.H33H) was found in one patient, which was a disease causing one, and it had not been published previously. However, the other variant c.1205G>A (p.W402*) was disease causing, and it had been reported by Zanetti et al. in Italian population (13) and Atceken et al. in Turkish population (14). The IDUA gene contains 14 exons (15). It should be noted that in our study, 80% of IDUA mutations were found in exon 1, and only 10% were in exon 9. Chkioua et al. showed novel splice site mutation in intron 11 of IDUA gene in four MPS I patients from four families from northern Tunisia (16). Sánchez reported that 14% of the IDUA gene mutations in his 7-member population were in exons 5 and 9 (17). Chkioua et al. reported that exon 1 mutations were detected in 75% of the patients, and exon 9 mutations were detected in 25% of patients in eight families with MPS I (18). Therefore, our results are consistent with those of Chkioua et al. The novel nonsense change c.514C>T (p.R172*) in exon 5 of IDS gene was found in more than one patient (in 10% our sample size). This variant was predicted as disease causing. Chistiakov et al. analyzed 17 children with hunter syndrome and found exon 5 of IDS gene mutations in 27% of mutations (19).

Exon 5 of IDS gene mutation accounted for only 5.5% of

Table 3. Exon Variants in the MPS Genes in Patients

Patients	MPS Type	Gene	Exon/(Intron)	Variant	Protein Change	Point Mutation Type	Zygosity	Clinical Significance
1	I	IDUA	1	c.32T>G	p.L11R	Missense	Hom	Polymorphism
2, 3			1	c.T99G	P.H33Q	Missense		Polymorphism
4			1	c.99T>C	p.H33H	Missense		Disease Causing
5			9	c.1205G>A	p.W402*	nonsense		Disease Causing
6			1	c.33G>T	normal	silent		Disease Causing
7, 8	II	IDS	5	c.514C>T	p.R172*	nonsense	Hemi	Disease Causing
11	IIIA	SGSH	2	c.150G>T	normal	Silent	Hom	
14			3	c.256A>G	P.N86D	Missense		Disease Causing
16, 18			4	c.364G>A	p.G122R	Missense		Disease Causing
17			4	c.456G>A	p.I152I	Missense		Disease Causing
19			1	c.74G>A	p.R24H	Missense		Likely Pathogenic
9	IIIB	NAGLU	3	c.607c>T	p.R203*	nonsense	Hom	Disease Causing
12			1	c.259G>C	p.A87P	Missense		Polymorphism
10			3	c.683G>A	p.R228Q	Missense		Disease Causing
15, 13			6	c.2209C>A	p.R737S	Missense		Polymorphism

the total mutations observed in the study by Zhang (20). SGSH gene contains 8 exons, and interestingly, all the novel mutations in this study were found in exons 1 to 4. Mutational analysis on 23 patients from the UK with Sanfilippo syndrome type A showed that 30% of total mutations were located in exons 1 to 4 (21).

Yassaee et al. showed that SGSH mutations were in exons 2 and 7 in 11 families (22). Of the 5 variants found in the SGSH gene, three variants (c.364G>A (p.G122R), c.456G>A (p. p.I152I), and c.74G>A (p. p.R24H)) were predicted as disease causing, and the last two variants were not reported in any population; however, c.364G>A had been published in 2000 by Beesley et al. (21). In the study of NAGLU gene variants, three variants including (c.607c>T (p.R203*), c.259G>C (p.A87P), and c.683G>A (p.R228Q)) were predicted as novel pathogenic variants. In addition, Yassaee et al. revealed that NAGLU mutations were located in exons 2, 5, and 6 (22); but our data demonstrated that NAGLU mutations were in exons 1, 3, and 6. Similar studies have been performed in different countries. For example, Bekri's study described the clinical and molecular features of 13 Algerian MPS 1 patients by molecular study of the IDUA gene (23).

Moreover, in a study conducted in 2014, molecular studies were performed in seven Mexican MPS I patients, and p.W402X was listed as a common mutation (17). Vafiadaki et al. identified the primary genetic lesion in 57 unrelated Korean MPS II patients, and they found various types of mutation in 42 patients (24). In the study by Beesley et al., mutational analysis was performed on the sulphamidase gene from 23 patients in the UK. In the study by Beesley et al., mutational analysis was performed on the sulphamidase gene from 23 patients in the UK, and 13 different new mutations were found (21). Our results showed positive mutation spectrum of IDUA, IDS, SGSH, and NAGLU genes in the Iranian cohort. The high fraction of detected novel variants highlights the mentioned genes mutation heterogeneity. However, this heterogeneity generates challenges in the interpretation of genotype and phenotype correlation. Meanwhile, if these mutations are studied and proven in a larger sample population, they could help to identify and categorize the patients with MPS symptoms. Due to the MPS heterogeneity and the clinical picture complexity, whole exome sequencing (WES) is usually recommended to all clients as a first tire test in Iran, which is a time consuming and costly test. If we have Iranian common mutations in the mentioned exons of target genes, we can screen the patients with MPS symptoms and diagnose them earlier at a lower cost.

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Footnotes

Authors' Contribution: Study concept and design: M. Houshmand & S. Farshidi & S. Saberi; Analysis and interpretation of data: S. Saberi & R. Jazayeri & B. Kamalidehghan & M. Houshmand; Drafting of the manuscript: R. Jazayeri; Critical revision of the manuscript for important intellectual content: R. Jazayeri & M. Houshmand.

Conflict of Interests: There was no conflict of interest.

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Table 2. Primer Sequences for IDUA, IDS, SGSH, and NAGLU genes

	Primers	Sequences
IDUA	Exon 1	F-GAGTCATCGGTCCTCAGAGC
		R-AGGACCCACCCACAAACAC
	Exon 2	F-CGCTGCCAGCCATGCTGAGGCTCG
		R-CCTCCCATCTGTGCCTCTGTAAGG
	Exons 3-4	F-GGGTTATTTTCCAAGGGGAAG
		R-CCAACCTATCCCTTGTCACC
	Exons 5-6	F-ATGCAGACGCCCTTCATC
		R-CCTGCTCCAGGATGGAGA
	Exons 7-8	F-CCACGACGGTACCAACTTCT
		R-TCCCCTTGGTGAAGGAGTC
	Exon 9	F-CTGGGGACTCCTTCACCAAG
		R-CAGGTAGCGCGTGACGTA
	Exon10	F-GTAAGCCGGGGTTCCAGG
		R-CGGTCCTCAGGGTTCTCC
	Exon 11	F-TGTGGGTGGGAGGTGGAG
		R-GAGGGAAGGGCTGTGATGG
	Exon 12	F-ACAGTGTGTGGGGTGAAGG
		R-TTGCTGGTGCACGTGTGT
	Exons 13-14	F-CCTAGGGGACATGAGATGGA
		R-CTCCAGCTGGGTCCTCATC
IDS	Exon 1	F- CTGTGTTGCGCAGTCTTCAT
		R- ATGCAGGAAAGGACAGATGG
	Exon 2	F- CCATCTGTCCTTTCCTGCAT
		R- TAACAAGATGTCCCGCACAA
	Exon 3	F- GCTGTGGCGATGCTTACCTCTG
		R- AAGAGAACCCAGACTCTGGACA
	Exon 4	F- GGCTTAGGGACCAGGAAGTC
		R- AACAAGTAGCACCCACCAGC
	Exon 5	F- CCTGCCTGGAAAACAAGAAA
		R- GGCCTTGACCTCTAAATCCC
	Exon 6	F- ACGTGGGGAATGCTAGTGAG
		R- GTTGGGAGAGTCCTGATCCA
	Exon 7	F- GCTGTGACTCTGTGGGTGAA
		R- CCAGGTTAAAAATGGGGGTT
	Exon 8	F- CAGCCTGTCAAGAATGAGCA
		R- ACCCCCAAAGCCTATGATTC
	Exon 9	F- CATATGGAGCCCAGACAGGT
		R- GGAAGGGAGCACATCACATT
	Exon 1	F- GAGACCAGAGAGCCGGAG

		R- ATTGACCACGGGTGGGG
	Exon 2	F- CTCACTCCCAGTGCTGTTTC
		R- GGGAGACGTGGCAGAGG
	Exon 3	F- GAGAACAGGTGCGGCAG
		R- ACCTCCTGGGCTCTGGC
	Exon 4	F- GAAGGGAGCAGAAAGGGTTG
		R- ATCCCGCCGGAAGACTC
	Exon 5	F- CCGAGGGGCTTCCTGTG
		R- CAAGCTCGTAGGAGGCCAG
	Exon 6	F- GTTCTGGGCTAACCCATTTG
		R- GACCCTCACCCACATTATGC
	Exon 7	F- GTCTACACACACCACCCGC
		R- CCCGTCCCAGATCCACTC
	Exon 8	F- GAGGGCAGCTCCTGTGTG
		R- CATCTCCAGAAGCTGAGCAA
	Exon 9	F- CTGGTACAAGGACCTCCGTC
		R- AAGGACAACTGTGTCCCCTG
NAGLU	Exon 1a	F- CCCAAGGGAGTATCCTGGTA
		R- TGGCAGCCACAGAAGTCG
	Exon 1b	F- CTTGGACACCTACAGCCTGG
		R- AGGCTCTGAAAGGCAGAGTG
	Exon 2	F- GGTACCTGGTCTCAGCTCCA
		R- GAAAACACCTACGGTGGCTC
	Exon 3	F- CCAGCACAAAGAAGCAATGA
		R- ATCTATCACCGATTCTGCCC
	Exon 4	F- CTGCGTGTATCCTGGGAGAT
		R- GGAATAAAATTCCCTCTCTGAGC
	Exon 5	F- GTGAACACTATGGCGGCTTC
		R- GTCCCTCTGCCTACCCCTAC
	Exon 6a	F- GCCCTCTGTTTCATCACTCC
		R- GGTGGGAGACCCCATACC
	Exon 6b	F- TGGTCTATTCCCTCATGGCT
		R- GTCACTAGCCAGCACCTCGT
	Exon 6c	F- CTATGAGGAGGCAAGAAGCG
		R- AGCGGGGGTAATATTTGAGG
	Exon 6d	F- CGTTCTCAGCAAGCAGAGGT
		R- TATAGCCCTGAGTCCTCCCA