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Genetic Variants in Mucopolysaccharidosis Type I Patients from Pakistan: Identification of a Novel 14-Nucleotide Deletion and A Highly Prevalent Missense Variant in a Cohort of 10 Families

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Key words
DNA sequencing, *IDUA* gene, MPS-I, Mucopolysaccharidosis-I, Consanguinity

ABSTRACT

Autosomal recessive mucopolysaccharidosis-I (MPS-I), an inborn error of metabolism, in which heparan sulfate and dermatan sulfate accumulate in the cells due to deficiency of enzyme alpha-iduronidase (IDUA) is more common in consanguineous populations. Previously, variants in alpha-iduronidase (*IDUA*) gene are reported to cause MPS-1 phenotypes. The aim of the present study was to identify genetic variants in the *IDUA* gene in ten unrelated MPS-1 affected consanguineous local families reporting to the children Hospital, Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan. Blood samples from affected and unaffected family members were collected and sequencing of *IDUA* gene was performed. *In silico* analysis of all identified disease-causing variants was done to check their affect on protein structure and function. Clinical examination of all MPS-1 patients showed coarse facial features, skeletal malformation, hernia, corneal clouding, abdominal distention and hepatosplenomegaly. Sequencing of *IDUA* gene revealed ten missense variations and eight synonymous variations. *In silico* tools including mutation taster, SIFT, PolyPhen and Provean suggested three variants as disease-causing. Among disease-causing variants, a previously reported missense variant i.e., c.1469T>C causing p.Leu490Pro was identified in 80% of our analyzed families. Furthermore, a novel 14 nucleotide deletion i.e., c.568_581del AACGTCTCCATGAC causing p.Asn190Hisfs*204 and a single nucleotide deletion i.e., c.784delC causing p.His262Thrfs*55 were segregating with MPS-I phenotype. This study reports a previously reported missense variant in 80% of screened families, a novel (c.568_581del AACGTCTCCATGAC) and a previously reported disease-causing deletion each in affected family.

INTRODUCTION

Mucopolysaccharidosis-I (MPS-I) (OMIM# 252800) is a lysosomal storage disorder LSD, that results from accumulation of glycosaminoglycans (GAGs) i.e., dermatan sulfate and heparin sulfate in various tissues of the body and leads to failure of multiple organs. MPS-I is inherited as an autosomal recessive disorder affecting 1 in 100,000 live births and is considered as pan-ethnic (Muenzer *et al*., 2009; Ngiwsara *et al*., 2018). It is a progressive disorder characterized by coarse facial features, defective hearing, stunted growth, hernias, corneal clouding, hepatosplenomegaly, dysostosis multiplex, sleep apnea, progressive brain abnormalities, cardiac abnormalities and joint contractures (Beck *et al*., 2014; Ngiwsara *et al*., 2018; Gul *et al*., 2019). The disease pathophysiology is not yet understood. Previously MPS-I mice models have shown that the buildup of GAGs causes the deactivation of osteoclastic protease cathepsin K, which further leads to the reduced degradation of cartilage and pathology of bone which is observed in MPS-1 phenotypes (Wilson and Bromme, 2010). The progression in arthropathy is intervened by the storage of glycosaminoglycans (GAGs), which causes elevation in recruitment of cytokine and chemokine (IL-1-beta, TNF-α etc.) which further increases the chondrocyte apoptosis, synovial hyperplasia and joint

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destruction along with inflammation (Simonaro *et al*., 2008, 2010).

Variants in *IDUA* gene (OMIM# 252800) encoding a lysosomal enzyme called alpha-L-iduronidase (EC 3.2.1.76) are reported to cause MPS-1 phenotypes (Hopwood and Morris, 1990; Muenzer, 2011; Beck *et al*., 2014). *IDUA* gene is located on chromosome 4p16.3 with 14 exons encoding a 653 residues polypeptide (Scott *et al*., 1993). Three subtypes of MPS-1 based on disease severity include Hurler (MPS I-H) (OMIM 607014) the most severe neuropathic form with life expectancy of less than 10 years, Hurler-Scheie (MPS I-H/S) (OMIM 607015) intermediate form with mild or no neurological involvement and patients may survive till third decade of life (Ngiwsara *et al*., 2018), Scheie (MPS I-S) (OMIM 607016) being milder of all with late onset and normal life span with available therapies (Muenzer *et al*., 2009; Bertola *et al*., 2011). Diagnosis of MPS-1 is confirmed by assay of enzyme activity and variants analysis of *IDUA* gene (Ngiwsara *et al*., 2018). Most common treatment method is enzyme replacement therapy (ERT) (Braulke and Bonifacino, 2009; Muenzer *et al*., 2009) and hematopoietic stem cell transplantation (HSCT). ERT is also not effective for neuronal conditions due to the inability to cross the blood brain barrier (Scarpa *et al*., 2017). For severe form of disease i.e., MPS1-H, HSCT should be done before age of two years, for this purpose molecular genetics diagnosis to identify causative variants for each patient is essential but not available in Pakistan (Terlato and Cox, 2003; Afroze and Brown, 2017). However, alternative therapeutic measures i.e., use of pharmacological chaperones and gene therapy are still under investigations (Penati *et al*., 2017; Pereira *et al*., 2018). Therefore, genetic diagnosis of MPS-1 cases especially from consanguineous families with expected high prevalence (Afroze and Brown, 2017; Afzal *et al*., 2018; Gul *et al*., 2019) will lead to identification of novel pathogenic variants to aid in better understanding of genotype-phenotype correlations and therapeutic interventions.

In this study we aimed to study the genetic basis of the mucopolysaccharidosis type I patients and to reveal their molecular findings and variants for causing the phenotype and to provide the families with genetic counselling.

MATERIALS AND METHODS

Patients recruitment

A total of ten MPS-1 affected unrelated consanguineous families were recruited from The Children Hospital, Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan during the time period from November 2017 to December 2018. Detailed pedigrees were drawn by interviewing family members. All patients were diagnosed initially through phenotypic and radiological findings (X-Ray). Among probands, there are 7 female patients and 3 male patients. Among ten families, seven have more than one affected alive/deceased patients while 3 families have single affected individual (Table I). The blood samples were collected from MPS-1 patients and parents of each patient to confirm the heterozygosity. Blood samples were stored in 10 ml vacutainer tubes (BD vacutainer K2 EDTA 18 mg) prior to DNA extraction. Dry blood spot (DBS) was performed only for the selected patients (MPS-03 and MPS-06) with severe phenotypes through tandem mass spectrometry (TMS), which measured the levels of enzyme in peripheral blood leukocytes.

Amplification of IDUA *gene and its nucleotide sequencing*

Genomic DNA was extracted by a modified method as described by Kaul *et al*. (2010). Quantity and purity of extracted DNA was accessed using a µDrop Plate reader (Multiskan™, Thermo Fisher Scientific, and Waltham, MA, USA). Primers for amplification of all exons of *IDUA* gene (NM_000203.5) and their flanking splice sites were same as described previously by Gul *et al*. (2019). DNA of proband of each MPS-1 family was amplified in a 25µl reaction volume using T100 thermal cycler (Bio-Rad, CA, USA) and same reaction composition and cycling profile as described previously (Gul *et al*. 2019). The amplified PCR products were loaded on the 1.5% agarose gel along with 1kb size ladder to evaluate product size and purified by using DNA purification Kit (*Wiz Bio* Solutions, Seongnam, Korea). Sequencing was performed using Big Dye Terminator Ready reaction mix (Applied Biosystems, Foster City, CA, USA) following manufacturer instructions on an automated ABI 3100 genetic analyzer. Results were analyzed by BioEdit (version 7.0.5.3). After identification of disease-causing variant/s in each proband, these variants were screened using DNA of available unaffected family members to check segregation with disease phenotype.

In silico *analysis*

All identified variants listed in Tables I and II were checked for disease-causing potential through mutation taster (http://www.mutationtaster.org/) and their affect on protein structure and function was predicted through different bioinformatics tools like Chimera (version 1.13.1). Sorting intolerance from tolerance (SIFT) (https://sift.bii.a-star.edu.sg/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), Provean (http://provean.jcvi.org/), Hope (https://www3.cmbi.umcn.nl/hope/), Mutpred LOF (http://mutpredlof.cs.indiana.edu/), I-Mutant (https://folding.biofold.org/i-mutant/i-mutant2.0.html), Panther (http://www.pantherdb.org/), SNPs and GO (http://snps.

biofold.org/snps-and-go/snps-and-go.html), and PHD-SNP (http://snps.biofold.org/phd-snp/phd-snp.html), Mutation assessor (http://mutationassessor.org/r3/). I-Tasser was used to model protein structures affected by frameshift variants identified in this study. 3D structure models for the frameshift variants of IDUA protein were built using I-Tasser (http://zhanglab.ccmb.med.umich.edu/I-TASSER/). I-Tasser employs an integrated approach including comparative modeling, threading, and *ab-initio* modelling (Roy *et al*., 2010).

RESULTS

All ten families reported here belonged to Punjab province of Pakistan. Family and clinical details of each proband are listed in Table I. At the time of enrollment, three families (MPS-01, MPS-02 and MPS-05) had single affected individual, four families (MPS-03, MPS-04, MPS-08 and MPS-10) had two patients, whereas family MPS-07, MPS-09 and MPS-06 had three, four and five patients, respectively. Among five cases of MPS-06, four patients died before the age of five years and the only alive patient was six months old. MPS-1 patients of all enrolled families showed disease phenotype i.e., bone deformities, respiratory problems, hepatosplenomegaly, coarse facial features, growth problems, hernia, corneal clouding, joint contractures, and sleeping disorders (Table I). However, neurological problems were detected in MPS-03 patient, cardiac problems were present in MPS-01 and MPS-06 patients, kyphosis/gibbus formation were found in seven cases (MPS-01, 02, 03, 06, 08, 09 and 10) and dysostosis multiplex was present in MPS-04 patient. The age of patients ranged from 6 months to 14 years at the time of enrollment (Table I).

Upon sequencing of all *IDUA* (NM_000203) gene coding exons and the intron-exon boundaries using DNA samples of probands of all enrolled families, a total of 20 variants were identified (Tables I, II) including thirteen previously reported and seven novel variants. Among these variants, there are two deletions and 18 single nucleotide substitutions. Ten nucleotide substitutions lead to missense variations whereas eight were synonymous variants (Tables I, II). According to mutation taster results, both deletions were disease-causing however, except p. L490P, all missense and synonymous variants were benign variants. We identified a homozygous disease-causing variant in each family (Table I).

NM_000203.5 (IDUA): *c.568_581delAACGTCTCCATGAC; p. (Asn190Hisfs*204)*

DBS performed indicated the complete absence of enzyme in patient i.e., 0.00 (cut-off values >0.7). Sequence analysis of *IDUA* gene in family MPS-03, discovered a fourteen-nucleotide deletion segregating with disease phenotype in a consanguineous family (Fig. 1B). This novel deletion i.e., NM_000203.5 (*IDUA*): c.568_581delAACGTCTCCATGAC; p. (Asn190His*fs**204) was predicted to cause substitution of asparagine to histidine at 190 position followed by a frameshift with an in frame stop codon leading to a truncated protein of 392 amino acids i.e., Asn190His*fs**204 as predicted by mutation taster. The longer the deletion is,and more highly conserved region is (Fig. 3A), higher are the chances for the variant to be pathogenic and de novo variant with paternity and maternity confirmed (PS1) is also strong candidate for pathogenicity according to ACMG standards and guidelines. The heterozygosity of the variant was confirmed in parents (Fig. 1B)

Table I. Characteristics of MPS I patients and their identified pathogenic variants in *IDUA* gene.

Family ID	Gender	Parental consanguinity	Age at enrollment	Nucleotide change	Protein change	Status/exon
MPS-01	Male	Yes	10 years	c.1469T>C	p.Leu490Pro	Homozygous/10
MPS-02	Female	Yes	14 months	c.1469T>C	p.Leu490Pro	Homozygous/10
MPS-03	Male	Yes	1.5 years	c.568_581del AACGTCTCCATGAC	p.Asn190His *fs**204	Homozygous/5
MPS-04	Female	Yes	6 years	c.1469T>C	p.Leu490Pro	Homozygous/10
MPS-05	Female	Yes	19 months	c.1469T>C	p.Leu490Pro	Homozygous/10
MPS-06	Female	Yes	6 months	c.784delC	p.His262Thr*fs**55	Homozygous/6
MPS-07	Female	Yes	8 years	c.1469T>C	p.Leu490Pro	Homozygous/10
MPS-08	Female	Yes	14 years	c.1469T>C	p.Leu490Pro	Homozygous/10
MPS-09	Male	Yes	3 years	c.1469T>C	p.Leu490Pro	Homozygous/10
MPS-10	Female	Yes	8.5 years	c.1469T>C	p.Leu490Pro	Homozygous/10

Table II. List of identified benign variants, ACMG classification, frequencies and exonic positions in *IDUA* gene of MPS I patients.

S. No	Nucleic acid change	Amino acid change	ACMG interpretation	Global minor allele frequency	Exon/ Intron	Accession numbers
	Exonic variants					
1	c.24C>A	No change	BS1	0.39257	Exon 1	rs11248061
2	c.60G>A	No change	BS1	0.39437	Exon 1	rs10902762
3	c.99T>G	p.His33Gln	BS1	0.10803	Exon 1	rs10794537
4	c.314G>A	p.Arg105Gln	BS1	0.16873	Exon 3	rs3755955
5	c.543T>C	No change	BS1	0.21985	Exon 5	rs6815946
6	c.942G>C	No change	BS1	0.21865	Exon 7	rs6830825
7	c.1164G>C	No change	BS1	0.21865	Exon 8	rs6836258
8	c.1081G>A	p.Ala361Thr	BS1	0.21765	Exon 8	rs6831280
9	c.1230C>G	No change	BS1	0.21785	Exon 9	rs115790973
10	c.1232T>C	p.Val411Ala	N/A	N/A	Exon 9	This report
11	c.1292C>T	p.Ala431Val	N/A	N/A	Exon 9	This report
12	c.1360G>A	p.Val454Ile	BS1	0.21865	Exon 9	rs73066479
13	c.1364C>G	p.Ala455Gly	N/A	N/A	Exon 9	This report
14	c.1654A>T	p.Thr552Ser	N/A	N/A	Exon 12	This report
15	c.1786A>C	p.Ser596Arg	N/A	N/A	Exon 13	This report
	Intronic variants					
16	g.15984T>C	No change	BS1	0.21885	Intron 9/10	rs115134980
17	g.15989C>G	No change	N/A	N/A	Intron 9/10	This report

ACMG, American college of medical genetics and genomics; BS, strongly benign, N/A: not available.

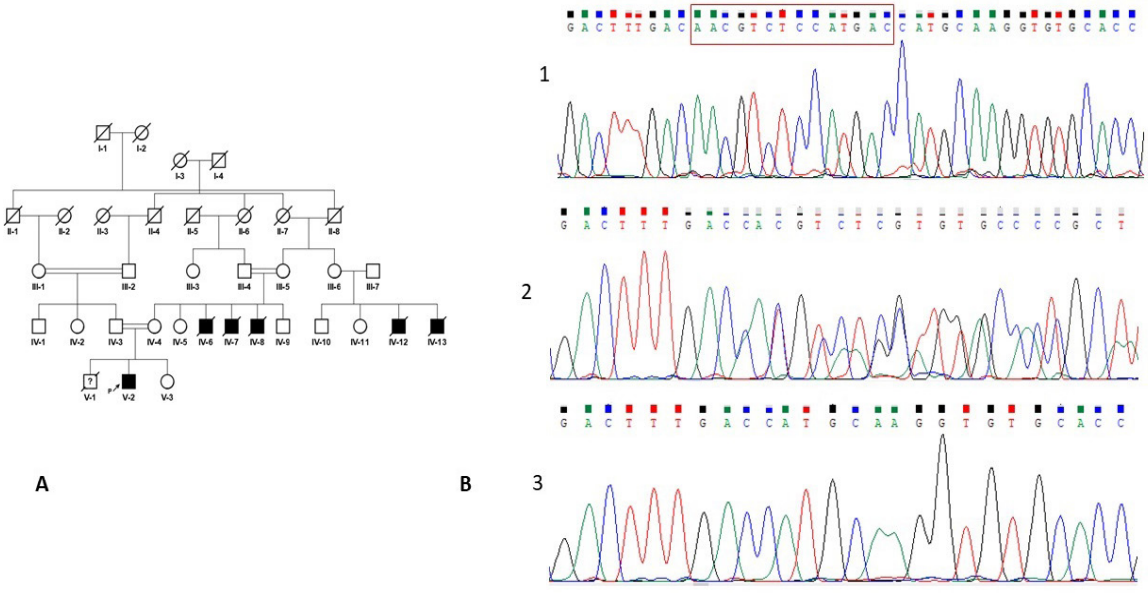

Fig. 1. A, Pedigree of Family MPS-03. Squares and circles symbolize males and females, respectively. Clear symbols indicate unaffected family members while filled symbols indicate MPS-1 patients. Consanguineous marriages are indicated by double lines. B, Chromatograms showing the novel deletion of fourteen nucleotides i.e., NM_000203.5 (*IDUA*): c.568_581delAACGTCTCCATGAC; p. (Asn190Hisfs*204) (i) homozygous wild type (ii) heterozygous carrier (iii) homozygous mutant.

(Richard *et al*., 2015). Polyphen-2 *in silico* tool predicted p.Asn190His variant as probably damaging with score of 1. According to PROVEAN p. Asn190His*fs**204 variation is deleterious with a probability score of -1197.876 (Cut-off value= -2.5). The effect of p.Asn190His substitution on the structure and function of protein is detected through HOPE and it highlighted that mutant amino acid i.e., asparagine (N) is larger in size than the wild type amino acid histidine (H). Asn is located in a preferred secondary structure i.e., turn while histidine prefers to be in another secondary structure thus destabilized the local conformation. Histidine is highly conserved in the region so its substitution will be probably damaging for the protein. Mutpred-LOF gave a score of 0.56678 (>0.5) confirms the pathogenicity. I-Mutant showed the greater decrease in stability of protein with a score of -2.52 Kcal/mol.

*NM_000203.5 (*IDUA*): c.784delC; p.(His262Thrfs*55)*

In family MPS-06, the DBS indicated 0.00 (cut-off values >0.7) values for enzyme measurement in peripheral blood leukocytes. Also, molecular identification revealed a previously reported homozygous deletion of cytosine nucleotide i.e., c.784delC causing a substitution of histidine with threonine at 262 position followed by a frameshift with an in frame stop codon thus causing a shorter protein of 315 amino acids i.e., p. His262Thr*fs**55 was segregating with MPS-1 phenotype (Fig. 2). According to Richard *et al*. (2015) if the small in-frame deletion is in the highly conserved region (Fig. 3B) and is de novo with paternity and maternity confirmed, the variant is strongly pathogenic (PS2) (Richard *et al*., 2015). *In silico* analysis through SIFT predicted histidine to threonine substitution at 262 position to affect protein function (probability score of 0.00). Polyphen-2 score for p.His262Thr was 1 and PROVEAN score for p. His262Thr*fs**55 was -917.011, respectively predicting this frameshift and in frame stop codon as deleterious to protein function. When the effect of p.His262Thr variant on the structure and function of protein was analyzed through HOPE, it revealed the size of mutant residue is small and hydrophobicity of the mutant amino acid is greater than the wild type. Histidine at position 262 form hydrogen bonds with Glutamic acid at 182 position and this change in amino acid leads to loss of hydrogen bonds in the core and thus disturb the correct folding of protein. Histidine at 262 position is highly conserved so alteration in this residue is probably damaging for protein. Mutpred-LOF score was 0.56907 which confirms the pathogenicity (>0.5 = pathogenic). I-Mutant showed the greater decrease in the stability of protein with a score of -0.81. Both PHD-SNP and SNPandGO confirms the variant p.His262Thr as disease-causing.

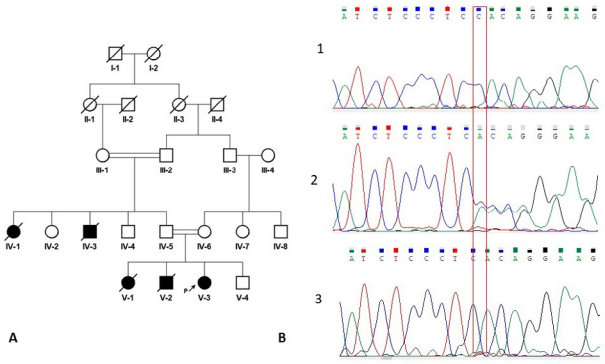

Fig. 2. A, Pedigree of Family MPS-06. Squares and circles symbolize males and females, respectively. Clear symbols indicate unaffected family members while filled symbols indicate MPS-1 patients. Consanguineous marriages are indicated by double lines. B, Chromatograms showing the novel deletion of cytosine nucleotide i.e., NM_000203.5 (*IDUA*): c.784delC; p.(His262Thrfs*55). (i) homozygous wild type (ii) heterozygous carrier (iii) homozygous mutant.

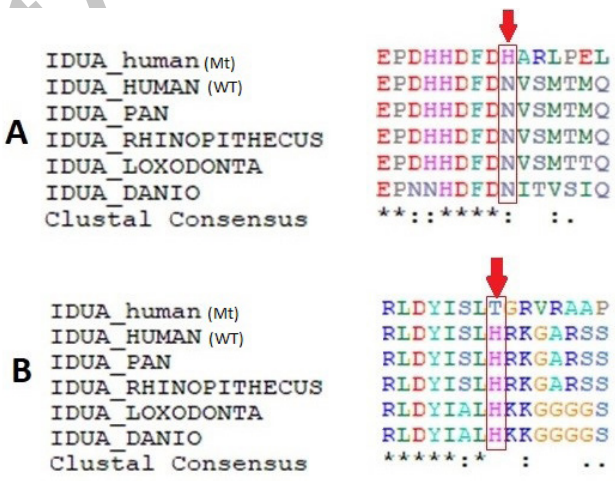

Fig. 3. Conservation of amino acid residues altered by A, novel fourteen nucleotide deletion i.e., NM_000203.5 (*IDUA*): c.568_581delAACGTCTCCATGAC; p.(Asn190His*fs**204) B, deletion of cytosine i.e., NM_000203.5 (*IDUA*): c.784delC; p.(His262Thrfs*55) among other IDUA orthologs. Mt stands for mutant and WT for wild type protein.

There are three domains of IDUA protein including a TIM barrel domain (residues 42–396), a β-sandwich domain (397–545) and an immunoglobulin-like domain (residues 546–642) (McKusick *et al*., 1972; Poletto *et al*., 2018). We checked conservation of amino acid residues for both frameshift variants as shown in fig. 3 which revealed that wild type sequence was conserved

among different orthologs of IDUA (Fig. 3). Furthermore, modeling of wild type and both frameshift mutant variants of the IDUA protein identified in MPS-03 and MPS-06 families respectively was performed using I-Tasser (Fig. 4). As both frameshift variants i.e., p.Asn190His*fs**204 and p.His262Thr*fs**55 lead to truncated proteins of 392 and 315 amino acids thus the structural modeling showed loss of two of the three domains of IDUA protein i.e. in the mutant proteins. The enzyme active site predicted for the wild type as well as for both truncated proteins is same at 175 residue. But, the ligand binding sites for the wild type protein were predicted to be twelve in number at residues 53, 91, 181, 182, 264, 299, 304, 305, 306, 349, 352 and 363 but these were predicted to be eleven at 91, 143, 181, 185, 188, 219, 246, 294, 299, 322, 334 residues for p.Asn190His*fs**204 mutant and only seven at residue number 53, 91, 181, 182, 264, 299 and 304 for the p.His262Thr*fs**55 mutant. These predictions showed that both mutant proteins were truncated causing protein structure and function defects.

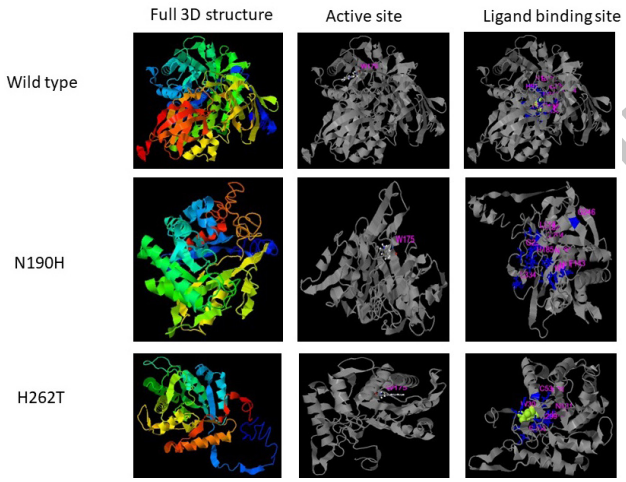

Fig. 4. Protein structure prediction using I-TASSER software. A, wild type protein. B, the truncated IDUA protein caused by 14 nucleotide deletions leading to p.Asn190His*fs**204 and C, the truncated IDUA protein caused by cytosine nucleotide deletion leading to p.His262Thr*fs**55 as compared to wild type protein. The tertiary structure of wild type and both mutants, the predicted active site of enzyme and the predicted ligand binding sites of the WT and mutant proteins are shown as indicated.

*NM_000203.5 (*IDUA*): c.1469T>C; p. (Leu490Pro)*

We identified a high frequency of a previously reported NM_000203.5 (*IDUA*): c.1469T>C; p. (Leu490Pro) variant in our study on Pakistani MPS-1 families (Table I). Among ten analyzed families, the percentage of p.L490P was 80% (8/10) (MPS-01,02,04,05,07,08,09,10), suggesting that most collected families of MPS-1 have this variant (Gul *et al*., 2019, 2023). For this variant according to Richard *et al* (2015), when prevalence increases statistically in affected individuals than controls that variant is also considered as strong candidate for pathogenicity (PS4). P.Leu490Pro is one of the high prevalent variant in patients with MPS-I throughout the world with confirmation of paternity and maternity heterozygosity state. Mutpred score for p.Leu490Pro was 0.564. I-Mutant results showed the greater decrease in the stability of protein with reliability index score of 5 and thermal value DDG is -0.91 Kcal/mol. According to PHD-SNP and SNP and GO analysis this variant is disease-causing with scores of 2 and 0.709, respectively.

DISCUSSION

Inbreeding is customary in Pakistan, which predisposes to increased burden of autosomal recessive diseases in society including LSDs. Among mucopolysaccharidosis, MPS-I with different phenotypic variation is found to be more prevalent worldwide including Pakistani population (Chkioua *et al*., 2011; Cheema *et al*., 2017). The ratios of different phenotypes of MPS-1 were reported as Hurler 60.9%, Hurler/Scheie 23%, Sheie 12.9% and 3.2% undetermined by Beck *et al*. (2014).

Variants in a single gene are responsible for three MPS-1 phenotypes; thus, identification of causative variants for each patient is important to develop genotype-phenotype correlation (De Ru *et al*., 2012). Consequently, molecular genetics diagnosis leads to therapeutic management as hematopoietic stem cell transplantation is used for patients presenting with severe phenotype i.e., Hurler and show better results with earlier age at treatment (Ghosh *et al*., 2017). This data necessitates molecular genetic screening of MPS-1 cases especially from consanguineous populations that will not only identify prevalent variants to establish molecular genetics diagnosis testing but will have clinical utility for disease management. For this purpose, the present study was performed to screen *IDUA* gene in ten MPS-1 segregating unrelated consanguineous Pakistani families (Table I).

Variant's analysis of *IDUA* gene confirmed one homozygous disease-causing variant in each of our screened families. Among identified variants, a previously reported missense variant i.e., p.L490P was detected in eight out of ten analyzed families, and it also provide the possibility of this variant to be a founder variant in Pakistani cohort but still more studies are required to confirm its status (Table I) (Tieu *et al*., 1995). The disease phenotype of all of NM_000203.5 (*IDUA*): c.1469T>C; p.

(Leu490Pro) carrying patients was Hurler-Scheie similar to previously reported Pakistani patients carrying same variant (Arora *et al*., 2007; Gul *et al*., 2019, 2023; Zahoor *et al*., 2019). The substitution of leucine at position 490 with proline may affect enzymatic activity of protein causing an intermediate phenotype (Ghosh *et al*., 2017). The nature and position of substituted amino acid and also newly inserted amino acid describe the impact of that very substitution on structure of protein (Firasat *et al*., 2018). According to HOPE, leucine at position 490 is located in helix-loops-helix region (482-508 amino acids residues) of β-sandwich domain (482-508) which is important for interaction with other two domains of enzyme, and its substitution with proline may result in loss of this interaction (Maita *et al*., 2013; Gul *et al*., 2019).

Sequencing of *IDUA* coding sequence in remaining two families revealed, a novel deletion of 14 nucleotides (c. 568_581delAACGTCTCCATGAC) in exon 5 and a previously reported single nucleotide deletion i.e., c.784delC, in exon 6 in family MPS-03 and MPS-06, respectively. According to mutation taster both deletions lead to the frameshift of the coding sequence thus creating a pre-mature stop codon i.e., c. 568_581delAACGTCTCCATGAC leading to p.Asn190His*fs**204 and c.784delC causing p.His262Thr*fs**55 with truncated proteins of 392 and 315 amino acids, respectively. Previously, c.784delC causing p. His262Thr*fs**55 was reported by Al-Jasmi *et al*. (2012) in an MPS-1 patient of Pakistani origin with central nervous system involvement indicating Hurler Phenotype (Al-Jasmi *et al*., 2012). Here in our study MPS-06 patient carrying same variant also had by birth onset of disease. According to Maquat (2005), all those transcripts which generate pre-mature termination codon at 50-55 base pairs upstream of exon-exon junction complex undergo the process of non-sense mediated decay (NMD) in mammals (Maquat, 2005). Furthermore, *IDUA* variants which cause premature protein truncations lead to absence of enzyme and severe phenotype (Keeling *et al*., 2013; Huang *et al*., 2018). Absence of enzyme was further confirmed by dried blood spot (DBS) tandem mass spectrometry (TMS) for MPS-03 and MPS-06 patients, which gave a value of 0.00 (cut-off values >0.7) for both cases. Thus, both of these variants lead to truncating proteins predicted to be degraded by NMD leading to absence of α-L-iduronidase enzyme and excessive accumulation of GAGs hence showing severe phenotype with by birth onset of disease and neuropathic involvement and in our study patients. Furthermore, asparagines amino acid at 190 position of IDUA protein is among one of the six potential sites i.e., N110, N190, N336, N372, N415 and N451 which are subject to post-translational glycosylation (Scott *et al*., 1991), thus substitution with histidine at this position may impact protein function as described previously by Maita *et al*. (2013).

The remaining 17 variants including 9 missense and 8 synonymous were benign variants according to mutation taster (Table II). Previously it is reported that benign variants may also contribute to variable enzyme activity in healthy subjects (Scott *et al*., 1993) thus may contribute to the phenotypic variability among patients, along with different pathogenic variants. Consequently, including one novel deletions, we described three variants and their genotypic-phenotypic correlation in the Pakistani MPS-1 patients.

CONCLUSION

These results indicate the heterogeneous *IDUA* variant spectrum in this population. Mutational screening of the *IDUA* gene facilitates an accurate diagnosis of MPS-1 clinical subtypes, which are necessary for selection of treatment options (Poe *et al*., 2014), provides carrier status, and will contribute to the development of novel therapeutic approaches.

DECLARATIONS

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IRB approval and Ethical statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional/ethical review boards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethical Committee Quaid-i-Azam University, Islamabad (BEC-FBS-QAU2019-198).

Data transparency

All data and materials in this study comply with the field standards.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Patients signed informed consent regarding publishing their data and photographs (if required).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Submission declaration and verification

This description has not been published previously or under consideration anywhere else. This work is approved by all authors and responsible authorities. If accepted, this work will not be published anywhere else in any form without written consent of the copy right holder.

Statement of conflict of interest

The authors have declared no conflict of interest.

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