

Identification of Missense Mutation Pro77Arg As A Founder Mutation in Morquio-A Syndrome in Indian Gujarati Patel Ethnicity

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Research article

Keywords: Mucopolysaccharidosis type IVA (MPS IVA), Morquio A syndrome, GALNS, GAG, founder mutation, skeletal dysplasia, p.P77R.

Posted Date: September 21st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-65487/v1>

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Abstract

Background: Morquio A syndrome (MPS IVA) is a mucopolysaccharide group storage disorder caused due to the deficient activity of the lysosomal enzyme N-acetylgalactosamine-6-sulfatase encoded by *GALNS*. The present study represents the mutation spectrum of *GALNS* in 25 Gujarati Patel patients of India clinically and biochemically confirmed with Morquio-A disorder.

Methods: Urinary GAG quantitation and leucocyte enzyme assay was carried out in all 25 patients. This was followed by molecular characterization by amplification and sequencing of the exons and adjacent intronic regions of *GALNS* gene. Haplotype analysis was performed in patients showing p.P77R variant, using microsatellite markers D16S3121, D16S3026 and D16S3023 and SNPs.

Results: We identified 11 mutations that include eight missense mutations: (p.L36R, p.D39G, p.P77R, p.C79R, p.P125L, p.P151L, p.G255A and p.L350P), one splice site mutation: (c.121-7C>G), one small insertion: (c.1241_1242insA, p.I416HfsTer2) and one small deletion: (c.839_41delACA). Of these, three missense mutations (p.D39G, p.G255A and p.L350P), one splice site and the two indels mentioned above are novel. In the present study, we found maximum number of mutant alleles in exon 2, and of note, the variant p.P77R was seen in fourteen patients.

Conclusion: p.P77R variant was predominantly found in Gujarati Patel community and the results of haplotype analysis indicated it to be the founder mutation in this community. Further, a study of 200 unrelated healthy control participants from Gujarat has identified this mutation in the heterozygous status in two individuals. Overall, our study suggests that p.P77R is likely to be a founder mutation for Morquio-A syndrome in Gujarati Patel ethnicity.

Background

Morquio- A syndrome, (MIM# 253000) is an autosomal recessive glycosaminoglycan storage disorder with progressive skeletal dysplasia, among other symptoms. It is caused due to deficient activity of the lysosomal enzyme N-acetylgalactosamine-6-sulfatase (GALNS, E.C.3.1.6.4) resulting from mutations in *GALNS* gene (OMIM* 612222) (1, 2). The deficiency of GALNS in Morquio-A syndrome patients results in the accumulation of glycosaminoglycans (GAG) such as keratan sulfate (KS) and chondroitin-6-sulfate (C6S) (3). The clinical features are characterized by a noticeable short stature, protrusion of the chest, kyphoscoliosis, genu valgum, laxity of joints and abnormal gait (4–6). Spine is predominantly involved in these patients (7), without affecting neuronal function.

This is a rare lysosomal storage disorder, with an estimated incidence ranging from one in 76,000 in Northern Ireland to one in 640,000 births in Western Australia (4, 8, 9) and one in 450,000 in the Netherlands, and Portugal (8, 10). Though study in Asian population is scarce, the available estimates of birth prevalence include 1 in 500,000 live births in Japan, 1 in 304,000 in Taiwan (11), and 1 in 701,000 live births in Malaysia (12).

As of July 2020, 327 mutations in the *GALNS* have been reported with 242 as missense/nonsense mutations, 37 indels, 32 splicing site mutations, and 3 complex rearrangements (13). The ten most frequently reported *GALNS* alterations from patients with Morquio-A across the world are- c.1156C > T, c.337A > T, c.901G > T, c.120 + 1G > A, c.1171A > G, c.935C > G, c.871G > A, c.860C > T, c.953T > G and c.757C > T (14). Among these, the mutations that have been commonly observed in particular ethnicities are: c.120 + 1G > A (Tunisian- 91%), c.757C > T (Pakistani- 89%), c.953T > G (Chinese-58%) c.1171A > G (64%) and c.337A > T (52%) in the Irish population. Thus, the prevalence of certain alleles in particular ethnicities is likely to be due to founder effects.

Bidchol et al. (15) were the first to report the mutation spectrum of the *GALNS* gene in Indian Morquio-A syndrome patients. In that study, the patients studied were primarily from the Northern and Southern regions of the country. Nonetheless, considering the diverse population of India with different ethnicities, the scope for better diagnosis and management of Morquio-A syndrome would be enhanced if the mutation spectrum is known specifically for the individual ethnicities.

In present study we aimed to identify the mutation spectrum in Gujarati Indian patients with Morquio-A syndrome.

Materials And Methods

Study subjects and ethics statement

The study was carried out in 25 children from Gujarat-India, affected with dysostosis multiplex as per the clinical diagnosis. The age range of the subjects was 11 months to 21 years. Clinical details were noted in a case record form and an informed consent was obtained from each family. The study protocol was approved by Institutional Ethics committee of FRIGE, Ahmedabad (Reg No- E/13237) as per the Helsinki declaration. 5ml peripheral blood was collected from the patients for leucocyte enzyme assay and isolation of DNA.

Urinary GAG Testing and Leucocyte Enzyme Assay

Urinary GAG quantitative study was performed by dimethyl methylene blue (DMB) dye method (16). The concentration of urine creatinine of individual patients was measured by a biochemistry analyzer using a commercial kit and expressed in mg/mmol of creatinine. The pre-treatment of urine prior to qualitative analysis of GAG by electrophoresis, was performed by a method described by Hopwood and Harrison, 1982 (17). Lysosomal enzyme β -galactose-6-sulfate-sulfatase activity was carried out from leucocytes using fluorogenic synthetic substrate 4-methylumbelliferyl- β -galactose-6-sulfate-triethyl ammonium (18). The fluorescence of free 4-methylumbelliferone (4 MU) was measured by a spectrofluorometer (LS55-Perkin Elmer) to determine the β -galactose-6-sulfate-sulfatase activity. The enzyme activity was expressed as the amount of substrate in nmol cleaved per hour per mg of protein in the cell lysates.

Molecular characterization

For all enzymatically confirmed cases of Morquio-A syndrome, genomic DNA was isolated from peripheral blood by the standard salting out method (19). The exonic regions and the flanking intronic sequences of the *GALNS* gene were amplified by PCR in 14 fragments using prescribed primers (See Additional file 1). A 10 μ l reaction mixture for the DNA amplification of each fragment was made up of 100ng genomic DNA, 1mM dNTPs, and 10XPCR buffer. 30 cycles of amplification were performed, each consisting denaturation at 94°C for 1 minute, annealing at 60-65°C suitable for each exon for 45 seconds and extension at 72°C for 45 seconds in a thermal cycler. The final extension time was of 10 minutes. PCR products along with the appropriate negative controls and a 100 base-pair DNA ladder were then subjected to electrophoresis in 2% agarose gel and purified using Exo-SAP-IT™ (USB Corporation, OH, USA). The purified products were sequenced using BigDye Terminator v3.1 and capillary electrophoresis was performed using an automated sequencer ABI-3500 (Applied Biosystems, CA, USA) for mutation analysis of *GALNS*. The sequences through the bidirectional sequencing was analyzed by comparing with the reference sequence of *GALNS* (RefSeq cDNA NM_000512.5). The mutations identified were annotated with Human Gene Mutation Database, dbSNP and McGill University database

Bioinformatic analysis

The functional effects of non-synonymous single nucleotide substitution (nsSNPs) were predicted using software SIFT (Sorting Intolerant From Tolerant) (<http://sift.jcvi.org>), Polyphen2 (Polymorphism Phenotyping v2) (<http://genetics.bwh.harvard.edu/pph2/>), MutPred (<http://mutpred.mutdb.org/>) and PROVEAN (<http://provean.jcvi.org>). The pathogenicity of indels were confirmed by MutationTaster (<http://www.mutationtaster.org/>). Evolutionary conservation of amino acid residues of *GALNS* was analyzed using ClustalW program (<http://www.uniprot.org/help/sequence-alignments>).

Haplotype analysis was performed in all Morquio-A syndrome patients using microsatellite markers D16S3121, D16S3026 and D16S3023 (Figure 1) and SNPs. To confirm the results obtained from haplotype analysis, 200 normal unrelated individuals were screened using ARMS-PCR for P77R variant (See Additional file 1). A reaction mixture of 13 μ l was prepared by adding the components in their required quantity that included Emerald master mix from Takara (10 μ l/ tube); distilled water (2 μ l/ tube) and 5pmol common reverse primer (1 μ l/ tube). Normal forward and mutant forward primers (1 μ l/ tube) and 100ng of DNA was added along with the reaction mixture to the respective PCR tubes. The PCR was conducted as per the required condition such as, initial denaturation at 94°C for 5 minutes of 1 cycle followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing (normal primer - 50°C, mutant primer - 61°C) for 45 seconds, elongation at 72°C for 1 minute and the final elongation was at 72°C for 5 minutes of 1 cycle. Amplified products were separated on 2% agarose gel at 100V for 30 minutes and viewed on UV image analyzer.

Results

The present study included 25 Gujarati Indian patients clinically and enzymatically diagnosed as Morquio-A. Seven of the 25 patients had consanguineous parents and remaining 18 patients were endogamous. The age of the patients at diagnosis ranged from 11 months to 21 years with a mean age of 4.32 years. The detailed information about the clinical features of all the patients is provided in Table 1. The clinical features present in all the patients included short stature, short fingers and short neck-trunk, and frontal bossing. Kyphosis was seen in 10/25 patients and platyspondyly in 5/25 patients. The other prominent symptoms associated with Morquio-A were observed with a low frequency in the present study such as corneal clouding was seen only in 1/20 (5.00%), knock knee in 6/25 (24.00%) patients. On radiological assessment most of them showed anterior beaking of vertebral bodies, acetabular dysplasia, platyspondyly and multiple dysplastic epiphyses.

Table 1
Clinical, biochemical and molecular details of the Gujarati Patel patients with Morquio-A syndrome

Sr. No	Case ID	Age at diagnosis	Sex	Religion / caste	Consanguinity	Clinical features	Enzyme activity (nmol/hr/mg)	Variant	Zygoty
1	P1	3 years	F	Hindu Patel	No	Kyphosis, Anterior beaking of bones, Short fingers	6.0	p.P77R c.230C > G	Homozygous
2	P2	1.9 years	M	Hindu Patel	No	Anterior offset of D1-14 relation to Da vertebral body, exaggerated lumbar lordosis with mild kyphotic curvature at dorsa-lumbar junction, anterior inferior beaking of lower dorsal and lumbar vertebral body	3.7	p.P77R c.230C > G	Homozygous
3	P3	11 months	F	Muslim	Yes	Kyphotic deformity in lower thoracic and upper lumbar spine with gibbous formation at D11-D12, minimal scoliosis with convexity towards lumbar side	0.75	p.P77R c.230C > G	Homozygous
4	P4	1.5 years	M	Muslim	Yes	Skeletal dysplasia	11.1	p.L36R c.107T > G	Homozygous
5	P5	8 years	F	Hindu Patel	No	Skeletal deformity noted at age of 8-9 months, keeping neck extended, knock knee, kyphosis of back, delayed motor development, frontal bossing, corneal clouding, short neck and trunk, widening of wrist and elbow, mild hypotonia	4.8	p.P77R c.230C > G	Homozygous
6	P6	1 year	F	Hindu Soni	No	Skeletal abnormality, D-L junction prominence with scoliosis, large skull, frontal bossing, kyphosis, mild platyspondyly, bullet shaped phalanges, wide ribs	4.15	c.1241_1242insA (p.I416HfsX2)	Homozygous
7	P7	2 years	M	Hindu Ahir	No	Short stature, D/L kyphosis, hydrocephalus	4.3	p.D39G c.116A > G	Homozygous
8	P8	6 years	M	Darbar	Yes	Skeletal dysplasia, hypo scoliosis, pigeon chest, knock knee, rickets	5.6	p.P151L c.452C > T	Homozygous

*Another variant remains unidentified, ** Checked only for p.P77R which was absent

Sr. No	Case ID	Age at diagnosis	Sex	Religion / caste	Consanguinity	Clinical features	Enzyme activity (nmol/hr/mg)	Variant	Zygosity
9	P9	2 years	M	Hindu Patel	No	Bullet shaped metacarpals, platyspondyly, D-L kyphosis, bilateral hip dysplasia	7.7	p.P77R c.230C > G	Homozygous
10	P10	1 year	M	Hindu Patel	No	DL Kyphosis, platyspondyly, abnormal CFE B/L femur	3.0	p.P77R c.230C > G	Homozygous
11	P11	6 years	M	Hindu Patel	No	Short stature, short neck, short fingers, skeletal dysplasia	1.1	P77R c.230C > G and p.G255A c.764G > C	Compound heterozygous
12	P12	1.7 years	F	Hindu Patel	No	Mild skeletal abnormality, sometimes gait disturbance, mild coarse face	0.198	p.P77R c.230C > G	Homozygous
13	P13	2.5 years	M	Hindu Patel	No	Bullet shaped phalanges bones, platyspondyly, anterior beaking of vertebral bodies, distorted acetabulum, genu valgum	0.05	p.L350P c.1049T > C	Homozygous
14	P14	3.7 years	F	Hindu Patel	No	Short stature, genu valgum, spondyloepiphyseal dysplasia, acetabulum dysplasia, epiphyseal dysplasia	0.37	p.P77R c.230C > G	Homozygous
15	P15	4 years	F	Hindu	No	Mild anterior beaking of vertebral bodies, short and wide metacarpals noted with pointed base, Madelung deformity in wrist	0.08	c.839_41delACA and p.P125L c.374C > T	Compound Heterozygous
16	P16	12 years	F	Hindu	No	Dysostosis multiplex, deep acetabula, Varus deformity of upper lip	3.4	p.C79R c.235T > C	Homozygous
17	P17	21 years	M	Hindu Patel	No	Skeletal dysplasia	0.03	p.P77R c.230C > G	Homozygous

*Another variant remains unidentified, ** Checked only for p.P77R which was absent

Sr. No	Case ID	Age at diagnosis	Sex	Religion / caste	Consanguinity	Clinical features	Enzyme activity (nmol/hr/mg)	Variant	Zygoty
18	P18	5 years	M	Javia	No	Skeletal dysplasia, bilateral genu valgum, ankle deformity, bilateral Plano valgus feet, spondylo epiphyseal dysplasia	0.25	p.P77R c.230C > G	Homozygous
19	P19	3.5 years	M	Muslim	Yes	Dysmorphic face, dysostosis multiplex, short stature	0.04	p.L36R c.107T > G	Homozygous
20	P20	4 years	F	Muslim	Yes	Short stature, difficulty in standing from sitting, spine lumbar lordosis, wrist broadening	0.8	c.121-7C > G	Homozygous
21	P21	1.6 years	M	Hindu Patel	No	Mild scoliosis, mild prominence of cerebral sulci & both lateral ventricles, inferior beaking of lower dorsal & lumbar vertebra, kyphotic deformity, relatively short metacarpals with pointed proximal ends	0.02	p.P77R c.230C > G	Homozygous
22	P22	4.6 years	M	Hindu Patel	No	Short stature, frontal bossing, pectus carinatum, genu valgum, kyphoscoliosis	0.04	Not determined	**
23	P23	4 years	F	Hindu Patel	No	Short stature, wrist deformity, mild pes carinatum, platyspondyly, anterior beaking of all vertebrae, bilateral acetabular dysplasia, knocked knees	0.09	p.P77R c.230C > G	Heterozygous*
24	P24	6 years	F	Muslim	Yes	Chest deformity, bending of knees, coarse face, increased wrist angle, knock knee, flat foot, pectus carinatum	0.025	p.P77R c.230C > G	Homozygous
25	P25	2 years	F	Muslim	Yes	Skeletal dysplasia	0.025	p.P77R c.230C > G	Homozygous

*Another variant remains unidentified, ** Checked only for p.P77R which was absent

The urinary GAG analysis and the GALNS enzyme activity were determined for all the patients. Elevated levels of urinary GAG were observed in all the patients with excess of KS and C6S by 1D electrophoresis. The enzyme activity of N-acetylgalactosamine-6sulfate sulfatase in all the patients was in the range of 0.02–11.1 nmol/hour/mg of protein and the mean enzyme activity was 2.30 nmol/hour/mg of protein which was less than 10% of the mean normal enzyme activity (Table 1).

The molecular testing of the *GALNS* gene has identified 11 mutations in the *GALNS* gene from the 24 patients (Fig. 2, Table 1). No mutation could be identified in one patient (P22). The mutations found include eight missense mutations (84%): c.107T > G (p.L36R), c.116A > G (p.D39G), c.230C > G (p.P77R), c.235T > C (p.C79R), c.374C > T (p.P125L), c.452C > T (p.P151L), c.764G > C (p.G255A) and c.1049T > C (p.L350P), one splice

site mutation (4%): c.121-7C > G, one small insertion (4%): c.1241_1242insA (p.I416HfsTer2) and one small deletion (4%): c.839_41delACA. Three missense mutations, one splice site mutation and two indels were novel (p.D39G, p.G255A, p.L350P, c.121-7C > G, c.839_41delACA and c.1241_1242insA/p.I416HfsTer2) (Fig. 2). All the mutations were present in homozygous state except two mutations in compound heterozygous state (p.P77R/p.G255A and c.839_41delACA /p.P125L) and one in heterozygous (p.P77R) state with another variant remaining unidentified.

All the novel mutations were tested for their pathogenicity using pathogenicity prediction tools MutationTaster, MutPred, PolyPhen2, SIFT and PROVEAN. All the three missense mutations were predicted as pathogenic by aforementioned bioinformatics predictive tools. The respective scores and likely basis of effect on the protein for the non-synonymous single nucleotide substitutions found are presented in Table 2. The pathogenicity of the novel splice site mutation and small indels was confirmed by MutationTaster. The sequence alignment and ortholog of *GALNS* are highly conserved among various species for the residues D39, P77, C79, P125, P151, G255, L350, in which missense mutations were found in this study. Additional file 2 gives the details of conservation of *GALNS* in orthologs (Additional file 2).

Table 2
Output of in silico analysis of GALNS missense variants detected in Morquio-A patients

Sr. No.	Exon/ Intron	Nucleotide Change	Change in Protein	Type of variant	MutPred analysis for missense variants	PolyPhen-2 analysis	SIFT result	PROVEAN (cutoff=-2.5)
1.	Exon 1	c.107T >G	p.L36R	Missense	0.859 Loss of stability, Gain of methylation at L36, Loss of loop, Gain of disorder, Loss of sheet	1 Probably damaging	0 Damaging	-3.555 Deleterious
2.		c.116A >G	p.D39G	Missense	0.823 Loss of stability, Gain of catalytic residue at D39, Loss of sheet, Gain of disorder, Loss of helix	0.997 Probably damaging	0 Damaging	-5.863 Deleterious
3.	Exon 2	c.230C >G	p.P77R	Missense	0.948 Gain of methylation at P77, Gain of MoRF binding, Gain of phosphorylation, Gain of catalytic residue at S82, Loss of disorder	1 Probably damaging	0.01 Damaging	-4.973 Deleterious
4.		c.235T >C	p.P79R	Missense	0.965 Altered Metal binding; Gain of Catalytic site at C79; Altered Ordered interface; Gain of Intrinsic disorder; Loss of Allosteric site at R83	1 Probably damaging	0 Damaging	-10.060 Deleterious
5.	Exon 4	c.374C >T	p.P125L	Missense	0.907 Altered Ordered interface; Altered Metal binding; Gain of Helix; Loss of Relative solvent accessibility; Loss of Acetylation at; Altered DNA binding; Loss of Allosteric site at L128; Loss of Methylation at K130	1 Probably damaging	0.06 Damaging	-4.654 Deleterious
6.	Exon 5	c.452C >T	p.P151L	Missense	0.920 Loss of disorder, Loss of ubiquitination at K153, Gain of loop, Loss of methylation at K153, Gain of MoRF binding	1 Probably damaging	0 Damaging	-8.970 Deleterious
7.	Exon 8	c.764G >C	p.G255A	Missense	0.857 Loss of Relative solvent accessibility; Altered Transmembrane protein; Altered Coiled coil; Loss of Allosteric site at E260	1 Probably damaging	0.11 Damaging	-5.881 Deleterious
8.	Exon 10	c.1049T > C	p.L350P	Missense	0.958 Gain of Relative solvent accessibility; Altered Metal binding; Altered Transmembrane protein; Gain of Allosteric site at T348; Gain of Catalytic site at T348	1 Probably damaging	0.30 Damaging	-4.100 Deleterious
<p>Effects of the missense variants on the structure and function of <i>GALNS</i>, as predicted by use of bioinformatics tools. Novel variants are in red. All the variants are likely to be pathogenic, based on predictions by all the tools used. In MutPred, probability > 0.75 is considered a "very confident hypothesis" for a variant to be causative of disease. In PolyPhen-2, the scores for neutral to damaging range from 0–1, with 1 being "probably damaging". According to SIFT, a variant is "damaging" if the score is ≤ 0.05 and tolerated if the score is > 0.05. In PROVEAN, an amino acid substitution with a score < -2.5 is deleterious.</p>								

Table 3A: Haplotype analysis of founder mutation p.P77R in Gujarati Patel patients with Morquio-A syndrome using microsatellite markers

Markers	Genomic position	Position wrt <i>GALNS</i>	Length of the marker (bp)	Range in our population (bp)	Range in other populations (bp)
D16S3121	88,455,110-88,455,199	575 kb Upstream	83	69-85	79-85
D16S3026	89,426,440-89,426,641	569 kb Upstream	202	202-217	204-210
D16S3023	89,432,214-89,432,296	358 kb Downstream	90	83-105	87-97

Table 3B: Haplotype analysis of founder mutation p.P77R in Gujarati Patel patients with Morquio-A syndrome using SNPs.

Variation (db SNP ID)	Exon/ Intron	Genomic position	Alleles
rs8062286	Intron 1	10873	C>T
rs35588597	Intron 1	10902	delG
Novel	Intron 1	11441	C>A
rs12709105	Intron 1	11524	C>T
rs12598981	Intron 1	12339	C>A
rs12444242	Intron 1	17332	G>A
rs12447646	Intron 1	17551	C>T
CM950524	Exon 2	19247	C>G
rs71395332	Intron 2	19347	G>A
rs377453859	Intron 2	19941	G>C
rs8054994	Intron 4	21092	C>A
rs12444543	Intron 5	25595	T>C
rs2269333	Intron 6	25857	T>C
rs2269334	Intron 6	25891	A>G
rs2269335	Intron 6	25904	C>A
rs12934499	Intron 6	26099	G>A
rs1064315	Exon 7	26192	C>T
rs11076721	Intron 7	26525	A>T
rs3743546	Intron 9	34959	T>C
rs3859024	Intron 10	35362	G>C
rs747232	Intron 11	37349	G>A
rs2303271	Exon 13	43909	G>A
rs11076716	Intron 13	47410	G>C
rs11076715	3' UTR	47564	G>A
rs1141390	3' UTR	47895	T>C
rs1135364	3' UTR	48139	A>G
rs1135366	3' UTR	48180	A>G
rs77936719	3' UTR	48229	C>G

We found five previously known missense mutations (p.L36R, p.P77R, p.C79R, p.P125L, p.P151L) (14, 15, 20–22). Out of these, the two missense variants p.P77R and p.C79R have been reported in Indian patients (15), whereas the remaining variants were reported from Mexican, Japanese and Italian population. The proportion of p.L36R in our cohort was 8% and that of p.P77R was 60%. This indicates the higher prevalence of p.P77R in our study population, specifically in the Patel community. This high frequency of the variant in the population is likely to be due to the founder effect. In order to confirm this, we carried out haplotype analysis in patients using dinucleotide microsatellite markers. The results for the same is depicted in Table 3A. Also, all the patients with p.P77R showed a unique identical genotype for the 28 SNP alleles investigated in the present study (Table 3B). A further study comprising 200 unrelated normal Gujarati individuals was carried out to check for the presence of this variant in the population using ARMS-PCR. None of the individuals showed the presence of the variant except for two individuals who were found to be in carrier state. This data also provides evidence that the p.P77R variant is a founder mutation for Morquio-A syndrome especially in Patel community of Gujarati Indians.

Discussion

The present study is the first to report the clinical and genetic profiles of patients with Morquio-A syndrome in Indian Gujarati patients. Our study cohort consisted of 25 patients with consanguinity in 28% and endogamy in the remaining. A previous study by Bidchol et al (15) was also carried out in Indian patients but mainly comprising northern and southern region of the country and none from Gujarat.

The mean age at the first symptom was 4.32 years. The mean age reported in various studies differ across populations with Bidchol et al (15) reporting a higher mean age of 6.7 years at presentation, whereas, Montano et al (23) and Lin et al (24) have reported a lower mean age of 2.1 years and 2.0 years respectively. This depends on awareness among clinicians, patients and availability of a diagnostic facility. In the present study, majority of the referrals were from pediatric orthopedic specialists. Use of urine GAG electrophoresis with high excretion of KS with CS seems to be the reason for early diagnosis.

The clinical phenotype of the patients consisted of marked features like short stature, short fingers to kyphosis, scoliosis, genu valgum and skeletal dysplasia. Leong et al (12) reported such severe phenotypes in all the Malaysian patients with Morquio A syndrome. A similar phenotypic spectrum was reported in Indian Morquio-A syndrome patients (15) where short stature, genu valgum and pectus carinatum were the most commonly observed. The International Morquio-A syndrome registry has given the 5 most common initial symptoms in Morquio-A syndrome to be short stature (49.9%), genu valgum (45.1%), kyphosis (44.4%), pectus carinatum (43.6%), and abnormal gait (37.8%). In this study we observed short stature (100%), genu valgum (32%) and kyphosis (40%). We found, mild kyphosis and scoliosis were common at an early age, as is expected. However, serious skeletal abnormality and dysostosis multiplex was observed in patients at an advanced age. These findings support the fact that the phenotypic severity increases with advancement of age as reviewed (25).

All the patients had persistent increased urine excretion of GAGs predominantly KS with or without HS (heparan sulphate). The similar observation has been reported in Morquio-A syndrome patients (3). This clearly indicates the use of urinary GAG electrophoresis as the first line screening test and as a differential diagnosis of Morquio-A syndrome. The GALNS enzyme activity in the KS positive patients ranged from undetectable to less than 10% of the total enzyme activity. Our results are consistent with that observed by Leong et al (12) in the Malaysian population and other reported studies (15, 26, 27).

On mutational analysis of the *GALNS* gene in the biochemically diagnosed Morquio-A syndrome patients, we found that out of the 14 exons, exons 1, 2, 5 and 11 harbor most mutations. The study by Bidchol et al (15) showed exon 8 to harbor most of the mutations followed by exon 1 and 7. This is in contrast to our observation of exon 2 (p.P77R) being the hot spot site for mutation. This is likely to be due to absence of Gujarati patients in the earlier study from India.

Our study has identified a total of eleven mutations, out of which eight are missense mutations. This finding is similar to that reported by Bidchol et al (15) as well as matches with the trend observed in HGMD, where for Morquio A, maximum missense mutations have been reported followed by other types. Five out of eight missense variants in our study have been previously reported. None of the ten most common mutations as per the earlier report (3) were found in our study. This suggests that there is heterogeneity at the molecular level. Thus, we can infer that mutation spectrum is influenced by ethnicity. One of the missense variants (p.P77R) more frequently seen in our study has been reported earlier to be present in Indian patient (20) with unknown ethnicity. This mutation was also reported being linked to severe phenotype (28), indicating the association of the variant with severe phenotype in Indian patients in the present study. Bidchol et al (15) has also reported a mutation at the same position (p.P77S), suggesting that the proline at position 77 is important for the enzyme activity and any change can lead to clinical manifestations of Morquio-A syndrome. As reported by Sukegawa (29), proline at 77 has a key role in preventing the internalization in hydrophobic patch of the protein. The positively charged side chain of arginine instead of proline is expected to have a destabilizing effect in this process. The missense variant p.L36R was also found in two patients. This variant has been previously reported by Tomatsu et al (30) and Morrone et al (14) indicating its association with attenuated phenotype. Another missense variant p.P151L reported in this study was also reported previously by Tomatsu et al (20). Proline is conserved at 151 position, thereby any substitution at this site will lead to alteration in

packing of protein (29). The missense variant p.C79R was first reported by Bidchol et al (15) in an Indian patient. Our study also reports this variant in one patient in homozygous condition. The missense variant p.P125L was found in a patient in compound heterozygous condition. This variant has been reported earlier by Tomatsu et al (21) in Japanese population showing severe phenotype for Morquio-A.

Our study has also identified 6 novel mutations that include 3 missense mutations, 1 splice site, 1 small insertion and 1 small deletion. The novel missense mutation p.D39G is an active site mutation which was found in one patient (P7). The noticeable phenotype was short stature, kyphosis and hydrocephalus. Morrone et al (14) and Tuyuz et al (28) have reported p.D40N to be associated with severe phenotype in Morquio-A syndrome patient. The other two novel missense variants p.G255A and p.L350P were found in compound heterozygous and homozygous conditions respectively. The splice site variant c.121-7 C > G identified in the present study has not been reported till date. The patient with this mutation presented with X-ray showing spine lumbar lordosis and wrist broadening. Generally, mutations in the splice site region leads to the formation of abnormal protein due to splicing defect. In our study, we have also identified a small insertion p.I416HfsTer2 in one patient which is a novel mutation. The prominent clinical features of skeletal abnormality, kyphoscoliosis, frontal bossing and platyspondyly were observed in this patient. A small deletion in exon 8 was found in a patient in compound heterozygous condition. As per the data by Tomatsu et al (30), 20 small deletions have been reported in the *GALNS* gene. The deletion in the present study was not previously reported in Indian population.

A set of bioinformatics tools have predicted the pathogenicity of the novel mutations by in silico analysis as described earlier. And all of them were at the conserved regions and mutations at these positions are highly likely to be pathogenic and causative of Morquio-A syndrome.

Interestingly, p.P77R was predominantly found in the Patel community of Gujarat. In order to confirm the founder effect, haplotype study was carried out in unrelated individuals of the same ethnicity using dinucleotide microsatellite markers. All the tested individuals were negative for this mutation except two individuals showing a carrier status. This confirms that p.P77R is a founder mutation in the Gujarati Patel population. Our study further indicates the reason of significant burden of rare diseases in India as has been observed recently (31). A greater attention needs to be paid to the different subpopulations and the effect of rare disease on community health needs to be elucidated.

Conclusion

The mutation spectrum for Morquio-A syndrome patients of Gujarati Indians is unique, wherein P77R is likely to be the founder mutation in Gujarati Patel ethnicity. This can be further utilized as a molecular marker and for carrier screening of the aforementioned population.

List Of Abbreviations

GAG: glycosaminoglycans, KS: keratan sulfate, C6S: chondroitin-6-sulfate, ARMS-PCR: amplification refractory mutation system PCR, HS: heparan sulphate

Declarations

Ethics approval and consent to participate

- Present study under submission has been approved by the institutional ethics committee [FRIGE's Institute of Human Genetics] wide approval number FRIGE/IEC/5/2010 dated 7th March, 2010. This process is in accordance with the Helsinki declaration.
- An informed consent was obtained from the parents before enrolling the patients for the investigations [This was in accordance with the requirement of the institutional ethics committee].
- An informed consent for publication was also obtained from the individuals included in the submission [This was in accordance with the requirement of the institutional ethics committee].

Consent for publication

Informed written consent was obtained from all the participants for publication of their clinical details and/or clinical images. A copy of the written consent is available for review by the editor of this journal.

Availability of data and materials

The dataset generated and/or analyzed during the current study is submitted in the ClinVar repository. Additional File 3 gives information on the ClinVar accession IDs of all the variants of *GALNS* gene generated or analyzed in the present study.

Competing interests

All authors declare that they have no conflict of interests (financial or non-financial).

Funding

This work is partially supported by Indian Council of Medical Research (ICMR) Project No.: BMS:54/2010. The funding agency was not directly or indirectly involved in the study design, specimen collection, analysis, interpretation and preparation of the manuscript.

Details of the contributions of individual authors

Designing the study, carrying out the protocol for enzyme assays and molecular study - JS, RB, CP, FJS, SVN, SS. Preparation of manuscript - JS, AN, FJS, SS. Clinical analysis - PN, MS, MC. Data analysis - JS, RB, SS. Haplotype and *in silico* studies- NP, PD, VT, RB and SS. JS and SS will act as guarantors of the study.

Acknowledgements

We thank Dr. Harsh Sheth and Dr. Sunil Trivedi for critical reading and evaluating the manuscript and also, Ms. Satahki Panda and Ms. Niyati Kapasi for their partial help in preparing the manuscript. We are grateful to all the patients and their families for the support, as without their consent this study would not have been possible. We acknowledge Indian Council of Medical Research (ICMR) for partial support to the work.

References

1. Matalon R, Arbogast B, Justice P, Brandt IK, Dorfman A. Morquio's syndrome: Deficiency of a chondroitin sulfate N-acetylhexosamine sulfate sulfatase. *Biochem Biophys Res Commun.* 1974;61(2):759–65.
2. Nakashima Y, Tomatsu S, Hori T, Fukuda S, Sukegawa K, Kondo N, et al. Mucopolysaccharidosis iv a: Molecular cloning of the human N-acetylgalactosamine-6-sulfatase gene (GALNS) and analysis of the 5'-flanking region. *Genomics.* 1994;20(1):99–104.
3. Peracha H, Sawamoto K, Averill L, Kecskemethy H, Theroux M, Thacker M, et al. Molecular genetics and metabolism, special edition: Diagnosis, diagnosis and prognosis of Mucopolysaccharidosis IVA. Vol. 125, *Molecular Genetics and Metabolism.* 2018. p. 18–37.
4. Hendriksz CJ, Harmatz P, Beck M, Jones S, Wood T, Lachman R, et al. Review of clinical presentation and diagnosis of mucopolysaccharidosis IVA. *Mol Genet Metab.* 2013;110(1–2):54–64.
5. Theroux MC, Nerker T, Ditro C, MacKenzie WG. Anesthetic care and perioperative complications of children with Morquio syndrome. *Paediatr Anaesth.* 2012;22(9):901–7.
6. Rodriguez ME, Mackenzie WG, Ditro C, Miller TL, Chidekel A, Shaffer TH. Skeletal dysplasias: Evaluation with impulse oscillometry and thoracoabdominal motion analysis. *Pediatr Pulmonol.* 2010;45(7):679–86.
7. Solanki GA, Martin KW, Theroux MC, Lampe C, White KK, Shediak R, et al. Spinal involvement in mucopolysaccharidosis IVA (Morquio-Brailsford or Morquio A syndrome): Presentation, diagnosis and management. *J Inher Metab Dis.* 2013;36(2):339–55.
8. Nelson J. Incidence of the mucopolysaccharidoses in Northern Ireland. *Hum Genet.* 1997;101(3):355–8.
9. Nelson J, Crowhurst J, Carey B, Greed L. Incidence of the mucopolysaccharidoses in Western Australia. *Am J Med Genet.* 2003;123A(3):310–3.
10. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *J Am Med Assoc.* 1999;281(3):249–54.
11. Leadley RM, Lang S, Misso K, Bekkering T, Ross J, Akiyama T, et al. A systematic review of the prevalence of Morquio A syndrome: challenges for study reporting in rare diseases. *Orphanet J Rare Dis.* 2014;9:173.
12. Leong HY, Abdul Azize NA, Chew HB, Keng WT, Thong MK, Mohd Khalid MKN, et al. Clinical, biochemical and genetic profiles of patients with mucopolysaccharidosis type IVA (Morquio A syndrome) in Malaysia: The first national natural history cohort study. *Orphanet J Rare Dis.* 2019;14(1):1–10.
13. The Human Gene Mutation Database. [accessed on 2020 Jul 9]. Available from: <http://www.hgmd.org>
14. Morrone A, Tylee KL, Al-Sayed M, Brusius-Facchin AC, Caciotti A, Church HJ, et al. Molecular testing of 163 patients with Morquio A (Mucopolysaccharidosis IVA) identifies 39 novel GALNS mutations. *Mol Genet Metab.* 2014;112(2):160–70.
15. Bidchol AM, Dalal A, Shah H, S S, Nampoothiri S, Kabra M, et al. GALNS mutations in Indian patients with mucopolysaccharidosis IVA. *Am J Med Genet A.* 2014/09/22. 2014 Nov;164A(11):2793–801. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/25252036>
16. De Jong JGN, Wevers RA, Laarakkers C, Poorthuis BJHM. Dimethylmethylene blue-based spectrophotometry of glycosaminoglycans in untreated urine: A rapid screening procedure for mucopolysaccharidoses. *Clin Chem.* 1989;35(7):1472–7.
17. Hopwood JJ, Harrison JR. High-resolution electrophoresis of urinary glycosaminoglycans: an improved screening test for the mucopolysaccharidoses. *Anal Biochem.* 1982 Jan 1;119(1):120–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/6803608>
18. van Diggelen OP, Zhao H, Kleijer WJ, Janse HC, Poorthuis BJHM, van Pelt J, et al. A fluorimetric enzyme assay for the diagnosis of Morquio disease type A (MPS IV A). *Clin Chim Acta.* 1990;187(2):131–9.

19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16(3):1215.
20. Tomatsu S, Fukuda S, Cooper A, Wraith JE, Rezvi GMM, Yamagishi A, et al. Mucopolysaccharidosis type IVA: Identification of six novel mutations among non-Japanese patients. Vol. 4, *Human Molecular Genetics*. 1995. p. 741–3.
21. Tomatsu S, Fukuda S, Cooper A, Wraith JE, Ferreira P, Natale P Di, et al. Fourteen novel mucopolysaccharidosis IVA producing mutations in GALNS gene. *Hum Mutat.* 1997;10(5):368–75.
22. Tomatsu S, Orii KO, Bi Y, Gutierrez MA, Nishioka T, Yamaguchi S, et al. General implications for CpG hot spot mutations: Methylation patterns of the human iduronate-2, sulfatase gene locus. *Hum Mutat.* 2004;23(6):590–8.
23. Montañó AM, Tomatsu S, Gottesman GS, Smith M, Orii T. International Morquio A Registry: Clinical manifestation and natural course of Morquio A disease. *J Inherit Metab Dis.* 2007;30(2):165–74.
24. Lin HY, Chuang CK, Chen MR, Chiu PC, Ke YY, Niu DM, et al. Natural history and clinical assessment of Taiwanese patients with mucopolysaccharidosis IVA. *Orphanet J Rare Dis.* 2014;9(1).
25. Khan S, Alméciga-Díaz CJ, Sawamoto K, Mackenzie WG, Theroux MC, Pizarro C, et al. Mucopolysaccharidosis IVA and glycosaminoglycans. Vol. 120, *Molecular Genetics and Metabolism*. 2017. p. 78–95.
26. Moreno Giraldo LJ, Escudero Rodríguez ÁM, Sánchez Gómez A, Satizabal Soto JM. Clinical and molecular characteristics of colombian patients with mucopolysaccharidosis IVA, and description of a new galns gene mutation. *Mol Genet Metab reports.* 2018 Jul 20;16:53–6. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30094185>
27. Xie J, Pan J, Guo D, Pan W, Li R, Guo C, et al. Mutation analysis and pathogenicity identification of Mucopolysaccharidosis type IVA in 8 south China families. *Gene.* 2019;686:261–9. Available from: <https://doi.org/10.1016/j.gene.2018.11.051>
28. Tüysüz B, Alkaya DU, Toksoy G, Güneş N, Yıldırım T, Bayhan İA, et al. Mutation spectrum and pivotal features for differential diagnosis of Mucopolysaccharidosis IVA patients with severe and attenuated phenotype. *Gene.* 2019/04/11. 2019 Jul 1;704:59–67. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/30980944>
29. Sukegawa K. Biochemical and structural analysis of missense mutations in N-acetylgalactosamine-6-sulfate sulfatase causing mucopolysaccharidosis IVA phenotypes. *Hum Mol Genet.* 2000;9(9):1283–90.
30. Tomatsu S, Montañó AM, Nishioka T, Gutierrez MA, Peña OM, Trandafirescu GG, et al. Mutation and polymorphism spectrum of the GALNS gene in mucopolysaccharidosis IVA (Morquio A). Vol. 26, *Human Mutation*. 2005. p. 500–12.
31. Consortium Gu, Sivasubbu S, Scaria V. Genomics of rare genetic diseases-experiences from India. *Hum Genomics.* 2019 Sep 25;14(1):52. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/31554517>

Figures

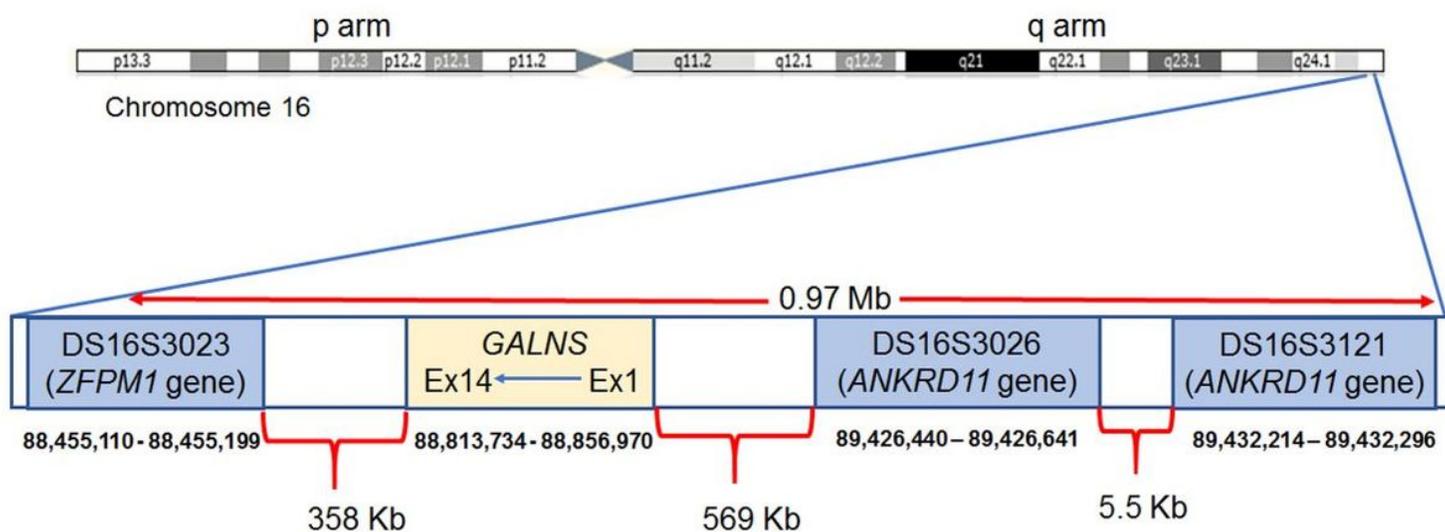


Figure 1

Schematic representation of dinucleotide microsatellite markers used for Haplotype analysis.

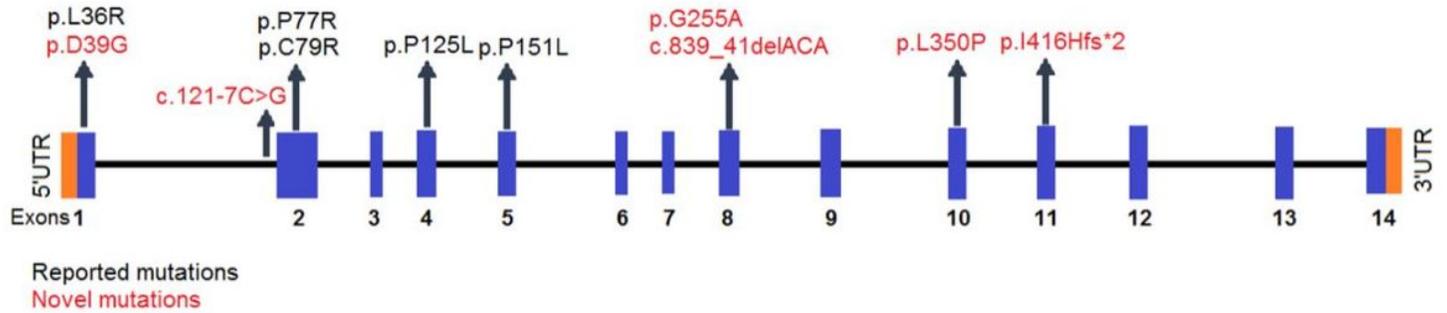


Figure 2

Schematic representation of variants found in GALNS in the current study.

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