Case Series

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Genotypes and phenotypes of Sri Lankan Patients with Mucopolysaccharidosis type IVA

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Abstract

Mucopolysaccharidosis type IVA is a rare autosomal recessive lysosomal storage disorder occurring worldwide in all ethnic groups. It is caused by bi-allelic variants in the GALNS gene (OMIM 612222). We report five cases of mucopolysaccharidosis type IVA with short stature and severe skeletal dysplasia. An optimized diagnostic strategy that combined enzymatic testing and genetic screening was applied. All the tested urine samples showed increased urinary glycosaminoglycan / creatinine ratios. In all five cases, the enzyme activity of galactosamine-6-sulfate sulfatase was pathologically decreased. Gene-targeted sequencing revealed a previously unreported homozygous c.139-12T>C variant of the GALNS gene in one patient and three previously reported missense variants in four patients; c.253T>C (p.Cys85Arg), c.626C>T (p.Ala209Val) and c.878C>T (p.Ser293Leu). Genetic studies not only confirm the diagnosis of mucopolysaccharidosis IVA, but also enable predicting the prognosis and facilitate genetic counseling. Enzyme replacement therapy is not available in Sri Lanka to date. However, the quality of life in these patients can be improved by a multidisciplinary approach.

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Introduction

Mucopolysaccharidosis type IV (MPS IV), also known as Morquio syndrome, is a rare autosomal recessive lysosomal storage disorder (LSD) caused by deficiency of two enzymes catalyzing degradation of glycosaminoglycans; N-acetylgalactosamine-6-sulfate-sulfatase (GALNS) deficiency in Morquio A (MPS IVA) and beta-galactosidase (GLB1) deficiency in Morquio B (MPS IVB). Accumulation of keratan sulfate (KS) and chondroitin-6-sulfate in tissues is responsible for the characteristic clinical manifestations of MPS IV that include, short-trunk dwarfism, skeletal dysplasia, fine corneal deposits, hearing impairment (conductive or sensorineural), dental abnormalities and cardiac valve abnormalities.[1]

Case Presentation

The clinical characteristics of the probands are listed below and are in accordance with those of patients previously diagnosed with MPS IVA, as described in the literature.

Case 1

This female child had, kyphoscoliosis, genu valgum, ulnar deviation of the wrist and joint hypermobility. She also had Mongolian blue spots over the posterior aspect of the trunk. She was clinically diagnosed to have MPS IV at the age of two years.

Case 2

Among the clinical features of this female child were short neck, lumbar lordosis, pectus carinatum, genu valgum, abnormal gait, joint hypermobility and dental abnormalities. The diagnosis of MPS IV was made at the age of 10 years.

Case 3

Lumbar lordosis, pectus carinatum, genu valgum, platyspondyly, hip dysplasia, and dental abnormalities were the presenting clinical features of this two-and-a-half-year-old female child. On slit lamp examination there were stromal opacities in the cornea. Ultrasound scan of abdomen revealed a liver with coarse echotexture. The 2D-echocardiogram was normal.

Case 4

This two-year-old male child was born to consanguineous parents who are first cousins. He presented with short neck, pectus carinatum, joint hypermobility and umbilical hernia. The 2D-echocardiogram of the child showed mildly myxomatous mitral valve and trivial mitral regurgitation.

Case 5

This three-and-a-half-year-old male child was born to consanguineous parents. He had pectus carinatum, genu valgum, platyspondyly, dental abnormalities, corneal opacity and inguinal hernia. Dysostosis multiplex and short stature were common to all the cases.

Quantification of total glycosaminoglycans (GAGs) in urine: Urine total GAGs were analyzed at Institute of Human Genetics, Foundation for Research in Genetics and Endocrinology (FRIGE) House, Gujarat, India. GAGs were determined by dimethylmethylene blue (DMMB) dye-binding assay with slight modifications to the method reported by De Jong et al.[2] Absorbance of the GAG-DMMB complex was measured at 520 nm immediately after mixing 20 µl of standard / sample and 100 µl of distilled water with 100 µl of DMMB, in Cobas Bio centrifugal analyzer. The urine creatinine levels were measured and results were expressed in terms of mg GAG / mmol creatinine. All four patients whose total GAG levels were quantified showed increased excretion of total GAGs (Table 1).

Quantification of enzyme activities: The enzyme studies were carried out again at CENTOGENE GmbH, Rostock, Germany. GALNS activity was measured in dried blood spots by LC / MRM-MS (liquid-chromatography / multiple-reaction-monitoring mass-spectrometry) based method.[3] In all five cases enzyme activity of GALNS was pathologically decreased (table 1).

Table 1. Urine glycosaminoglycan levels and blood GALNS activity in patients with MPS IVA.

Case	Age as on the date of urine sample collection	Urine GAG / creatinine ratio (mg GAG / mmol creatinine)	GALNS activity (µmol / L / h)
Case 1	2.7 years	9.2 (4.4 – 8.0)	< 0.3 (≥ 2.0)
Case 2	10.5 years	53.5 (1.9 – 4.3)	< 0.1 (≥ 2.0)
Case 3	2.5 years	10.9 (4.4 – 8.0)	< 0.3 (≥ 2.0)
Case 4	2.2 years	14.75 (4.4 – 8.0)	< 0.1 (≥ 2.0)
Case 5	3.2 years	Not documented	< 0.1 (≥ 2.0)

N.B: Lower limit of detection [LOD] and lower limit of quantification [LOQ] of the GALNS activity at CENTOGENE are 0.1 µmol/L/h and 0.3 µmol/L/h respectively. Reference intervals are given in parenthesis.

Genetic diagnostic testing: The GALNS and GLB1 genes were analyzed by sequencing PCR products that cover the entire coding region and the highly conserved exon-intron splice junctions; GALNS by bi-directional Sanger Sequence Analysis while GLB1 by NGS-Illumina as the laboratory started updating the platform for sequencing the genes to NGS-Illumina from 2017. Our patients were investigated during the transition period. The reference sequence of the GALNS gene is NM_001323544.1. The reference sequence of the GLB1 gene was NM_001317040.1. Deletion/duplication analysis for case 5 was carried out by quantitative PCR assay (qPCR) by using gene-specific amplicons targeting every coding exon of the GALNS gene. No pathogenic variants were detected in GLB1 gene. Pathogenic variants in GALNS gene were detected in all five cases resulting in a diagnosis of MPS IVA. The genotypes of the cases are summarized in table 2.

Table 2. Genotypes observed in patients with MPS IVA.

A	B	C	D	E	F
1	M	No	Compound heterozygous	c.253T > C p.(Cys85Arg) c.626C > T p.(Ala209Val)	NM_001323544.1
2	F	Yes	Homozygous	c.878C > T p.(Ser293Leu)	NM_001323544.1
3	F	No	Homozygous	c.253T > C p.(Cys85Arg)	NM_001323544.1
4	M	Yes	Homozygous	c.253T > C p. (Cys85Arg)	NM_001323544.1
5	M	Yes	Homozygous	c.139-12T > C p. (?)	NM_001323544.1

Note: A = Case, B = Gender, C = Parental Consanguinity,

D = Zygosity, E = Genotype, F = Reference sequence

Discussion

Quantification of urinary KS by LC-MS / MS (Liquid-chromatography-tandem mass-spectrometry) is a specific and quantitative method for analysis of KS as opposed to spectrophotometric estimation of total GAG excretion or qualitative analysis using thin layer chromatography or electrophoresis. Measuring enzyme activity from a DBS is only a screening test and enzyme activity analysis in fibroblasts or leukocytes is recommended if screening is negative. Variant analysis not only confirms the results of enzyme activity but also facilitates genetic counseling of the family.[4] In the present study, an optimized diagnostic strategy that combined enzymatic testing and genetic screening was applied. Fig. 1 illustrates how the diagnostic strategy in the present case study is related to the pathogenesis of MPS IVA.

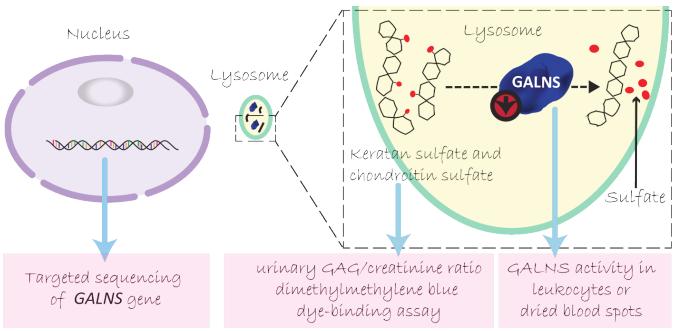

Figure 1. Relating pathogenesis of Mucopolysaccharidosis type IVA to its diagnostic approach.

N-acetylgalactosamine-6-sulfate-sulfatase (GALNS) enzyme encoded by GALNS gene removes 6-sulfate groups of the N-acetyl-D-galactosamine 6-sulfate units of chondroitin sulfate and of the D-galactose 6-sulfate units of keratan sulfate. Deficiency of GALNS enzyme results in tissue accumulation and urinary excretion of the glycosaminoglycans. GALNS variants, reduced GALNS activity and increased urinary glycosaminoglycan excretion can be detected by targeted gene sequencing, enzyme assays and dimethylmethylene blue assay respectively.

The c.253T > C (p.Cys85Arg) variant has been previously reported in Indian patients.[5] The variant is reported as c.253T > C (p.Cys85Arg) or c.235T>C (p.Cys79Arg) depending on the reference sequence used; NM_001323544.1 and NM_000512.4 respectively. c.626C > T (p.Ala209Val) has been reported earlier (as c.608C > T p.Ala203Val due to the use of a different reference sequence) from Indian and Brazilian patients with severe disease phenotype of MPS IVA.[5,6] Case 2 in the present study had significantly high urine GAG levels. The variant detected in this patient, c.878C > T (p.Ser293Leu) has been reported before using a different reference transcript as c.860C>T (p.Ser287Leu) in American, Australian, and Polish patients with severe phenotype.[6] In case 5, we detected a previously unreported homozygous variant in intron 1 of the GALNS gene, c.139-12T > C. To date, this variant is not described in the Exome Aggregation Consortium, Exome Sequencing Project or the 1000 Genomes Browser. Software analyses (Alamut v.2.7.1) predicted an effect on splicing. All the patients reported in the present study showed significantly low GALNS activity.

Enzyme replacement therapy with elosulfase alfa is not available in Sri Lanka to date, but can be brought down on special request. Nevertheless, quality of life in these patients can be improved also by a multidisciplinary approach, once the diagnosis is properly done. Caring paediatrician or physician may provide the patient with palliative care with nonsteroidal anti-inflammatory drugs (NSAIDs) for joint pain, antibiotics for pulmonary infections. Oxygen supplementation should be arranged for pulmonary compromise and obstructive sleep apnoea. Moreover, the coordinating physician should interact with other health care professionals such as orthopedic surgeons, otorhinolaryngologists, geneticists, dentists and physiotherapists to form an effective multidisciplinary team.[7-9] Finally, we hope that our study will raise awareness for MPS IVA in Sri Lanka, eventually resulting in improved diagnostic and therapeutic options.

Conclusions

The optimized diagnostic strategy that combined enzymatic testing, urinary glycosaminoglycan excretion, and genetic screening yielded clinically useful laboratory information for genotype-phenotype correlations. All the patients showed significantly increased urinary glycosaminoglycan / creatinine ratios and significantly low GALNS activity that could be correlated with deleterious mutations detected in our patients.

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