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Case Report

Characterization of a novel exonic deletion in the *GALNS* gene causing Morquio A syndrome

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ABSTRACT

Mucopolysaccharidosis IVA or Morquio A syndrome is a rare lysosomal storage disorder caused by *N*-acetylgalactosamine-6-sulfatase deficiency. A diagnosis can be provided by the identification of reduced *N*-acetylgalactosamine-6-sulfatase activity as well as detection of compound heterozygous or homozygous pathogenic variants in *GALNS*. We present a case of two sisters of healthy non-consanguineous parents with a severe classical phenotype of Morquio A syndrome. Both patients were found to carry a novel homozygous deletion of exon 9, which was initially suspected by next generation sequencing (NGS) due to lack of coverage, but could not be confirmed by this methodology. Therefore, an allele specific polymerase chain reaction assay was designed to confirm the exon 9 deletion and determine the precise deletion breakpoints (c.899-397_1003-1862del) for our patients. Recognizing limitations of molecular testing is important to ensure proper diagnosis and subsequent treatment for individuals with Morquio A syndrome.

1. Introduction

Mucopolysaccharidosis IVA (MPS IVA) or Morquio A syndrome (OMIM #253000); is an autosomal recessive lysosomal storage disease caused by the deficiency of *N*-acetylgalactosamine-6-sulfatase (GALNS) (E.C. 3.1.6.4), which is encoded by the *GALNS* gene (OMIM *612222) [1,2]. The condition is characterized by intracellular accumulation of the glycosaminoglycans (GAGs) keratan sulfate and chondroitin-6-sulfate, which classically leads to progressive development of clinical features, including skeletal and joint abnormalities, short stature, cardiorespiratory compromise, impaired vision, hearing loss, and hepatomegaly [3]. The extreme clinical variability of Morquio A syndrome, which can vary from minimally symptomatic with normal stature to the classic phenotype can be attributed at least in part to heterogeneity in *GALNS* mutations [2]. The diagnosis of Morquio A syndrome is established by analysis of GALNS enzyme activity or by the identification of biallelic pathogenic variants in *GALNS* on molecular genetic testing. Missense, nonsense, and splicing variants, as well as small deletions, small insertions, gross insertions/duplications, and gross deletions have been found in *GALNS*. Sequence analysis of *GALNS* identifies approximately 94% of variants whereas deletion/duplication analysis identifies another 2–3% [3]. We present a case of two sisters with Morquio A syndrome with a unique homozygous deletion of exon 9 of the *GALNS* gene.

2. Case report

Two sisters, seen by our center beginning at ages 10 and 8 years respectively, were born to non-consanguineous parents of East Indian background with no family history of Morquio A syndrome or other mucopolysaccharidoses or skeletal dysplasia.

Patient 1 was delivered by emergency Cesarean section at 8 months gestation due to fetal distress weighing 2.6 kg. She first presented with trouble walking and abnormal gait at age 1.5 years and skeletal survey was suggestive of dysostosis multiplex. She was diagnosed at age 2 years with Morquio A syndrome based on reduced *N*-acetylgalactosamine-6-sulfatase activity in leukocytes. On examination at age 7 years, she had normal cognition, truncal shortening, scoliosis, small umbilical hernia, distal joint laxity and waddling gait. MRI of the brain and cervical spine, performed at age 6 years, noted cervical arthropathy and gibbus

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deformity at the cervicothoracic junction. Corneal clouding was noted at age 5 years. She underwent bilateral genu valgum surgery at age 6 years. Echocardiogram performed at age 5 years in India showed mild tricuspid regurgitation with normal biventricular function. At age 10 years on presentation to our center she primarily used a wheelchair for long distances and fatigues easily. At age 12 years her height of 97.4 cm was well below the 1st centile on the CDC girls growth chart for age but approximately 50th centile on the Morquio A growth chart (Fig. 1) [4]. During a 6-min walk test, patient 1 was only able to ambulate for 3 min and 113.4 m, which is less than the average distance of approximately 650 m for a 10 year-old in the healthy general population [5]. It is also less than the average distance of 763 ft for 5–11-year-old patients with Morquio A syndrome in the Morquio A Clinical Assessment Program (MorCAP) study [5]. Echocardiogram showed thickened mitral leaflets with trivial stenosis as well as mildly dilated aortic sinotubular junction and ascending aorta. Sleep study showed severe obstructive sleep apnea, which led to tonsillectomy and adenoidectomy, but due to obstructive sleep apnea utilizes nighttime CPAP at home.

Patient 2 was delivered by repeat Cesarean section at full term weighing 3.2 kg. No pregnancy or fetal complications were reported as an indication for cesarean delivery. She had an umbilical hernia that was surgically repaired at age18 months. Parents noted frequent falls around age 3 years. She was subsequently identified to have genu valgum and skeletal survey indicated early signs of dysostosis. Given the family history, she was tested for Morquio A syndrome and *N*-acetylgalactosamine-6-sulfatase activity was deficient. She underwent occipitocervical decompression and fusion surgery at age 5 years for cervical C1-C2 instability, occipitocervical stenosis and early myelomalacia. Corneal cloudiness was identified at age 7 years. Echocardiograms at age 7 and 8 years showed mildly thickened mitral valve with mild tricuspid regurgitation and normal biventricular function. On presentation to our center at age 8 years, she had severe bilateral genu valgum, hip dysplasia, leg length discrepancy, pectus carinatum and used a wheelchair for ambulation. At age 11 years her height was 103 cm, which falls well below the 1st centile on the CDC girls growth chart for age but approximately the 75th centile on the Morquio A growth chart (Fig. 1) [4]. During a 6-min walk test she was able to ambulate 75.6 m but only completed 2 min, which is less than the average distance of approximately 600 m for a 9-year-old in the healthy general population [5]. She underwent bilateral distal femur and proximal tibia lateral hemiepiphysiodesis for genu valgum. Sleep study performed at age 8 years showed snoring without obstructive sleep apnea.

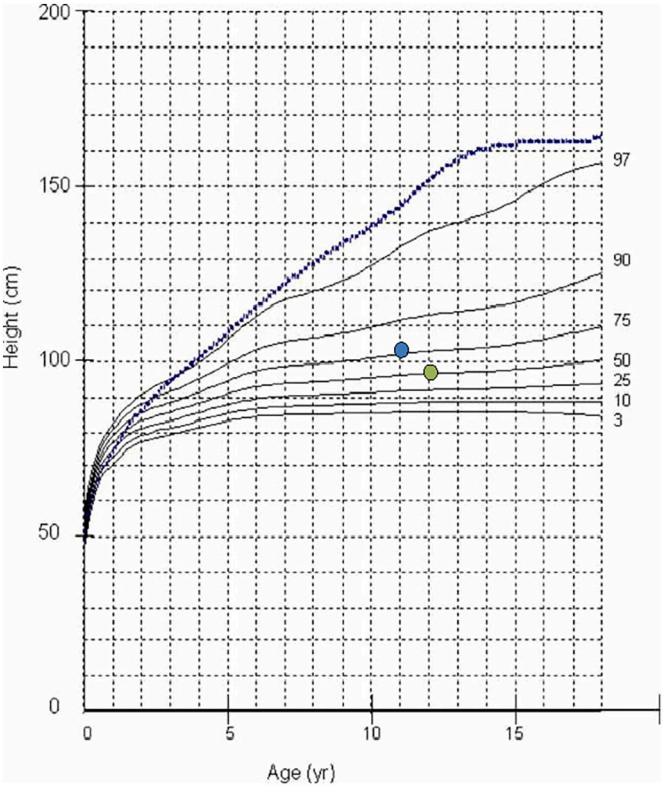

Fig. 1. Morquio A growth charts adapted from Montaño et al. 2008 [4]. Patient 1 (green) was 97.4 cm at age 12 years, which correlates to <1st centile on CDC girls growth chart for age but approximately 50th centile on the Morquio A growth chart. Patient 2 (blue) was 103 cm at age 11 years, which correlates to <1st centile on the CDC girls growth chart for age but approximately the 75th centile on the Morquio A growth chart.

Both patients have had elevated keratan sulfate (KS) values in urine since establishing care with our center. Prior KS values are unavailable. Patient 1 had a KS value of 1.14 mg/mmol/creat (reference <0.5 mg/mmol/creat for all values) at age 10 years, 0.69 and 1.3 mg/mmol/creat at age 11 years and 0.88 mg/mmol/creat at age 12 years. Patient 2 had KS values of 1.2 and 0.7 mg/mmol/creat at age 9 years, 1.5 mg/mmol/creat at age 10 years, and 0.96 mg/mmol/creat at age 11 years.

Both patients received intravenous enzyme replacement therapy (ERT) with Elosulfase alfa 2 mg/kg/week for 9 months beginning at ages 7 and 6 years respectively before transferring to our center. Parents noted improvement on ERT including improved energy and exercise tolerance especially for the first 4–5 days post-infusion. After an 18-month gap in therapy, both patients were restarted on weekly ERT infusions at 2 mg/kg/week. Both patients had reactions to ERT and had to undergo desensitization protocol and enhanced premedication regimens.

Prior NGS genetic testing for Morquio A syndrome did not identify sequence variants associated with Morquio A syndrome, however it was noted that a large region of the *GALNS* gene corresponding to exon 9 was not covered in the sequencing data. This was suggestive of a homozygous deletion in *GALNS*. After establishing care with our center, repeat enzyme testing was sent to Greenwood Genetic Center, which identified absent *N*-acetylgalactosamine-6 sulfatase activity for patient 1 and significantly reduced activity of 0.18 nmol/17 h/mg for patient 2 (reference range 49–255 nmol/17 h/mg; affected range < 7 nmol/17 h/mg). Repeat molecular testing at Greenwood Genetic Center identified a homozygous deletion encompassing only all of exon 9 of the *GALNS* gene. Further analysis identified the exact breakpoints as c.899-397_1003-1862del. Genomic microarray on blood cells of patient 1 did not show any large runs of homozygosity.

3. Materials and methods

3.1. Functional and biochemical assays

N-acetylgalactosamine-6-sulfatase (GALNS) enzyme analysis was performed as previously described by van Diggelen et al. [6] The activities of nine other lysosomal enzymes were within normal limits.

3.2. Molecular analysis

PCR amplification of the coding exons for *GALNS* produced an appropriate product for all exons with the exception of exon 9. Several repeated attempts failed. Sanger sequencing analysis of the amplified products did not identify any potentially pathogenic variants, which supported the previously obtained molecular results by NGS. Therefore, we hypothesized that a deletion encompassing exon 9 was present in these sisters. Long range PCR (+-3kB) was attempted from exon 8 to exon 10 and a smaller than expected band indicated a deletion was in fact present (data not shown). The PCR product was sequenced to identify the specific breakpoints (Fig. 2). A PCR primer was designed within intron 9 (Fig. 3) to develop an allele specific PCR for this deletion. A 731 bp fragment was expected from the normal allele while a 489 bp fragment was expected from the allele containing the deletion (Fig. 4).

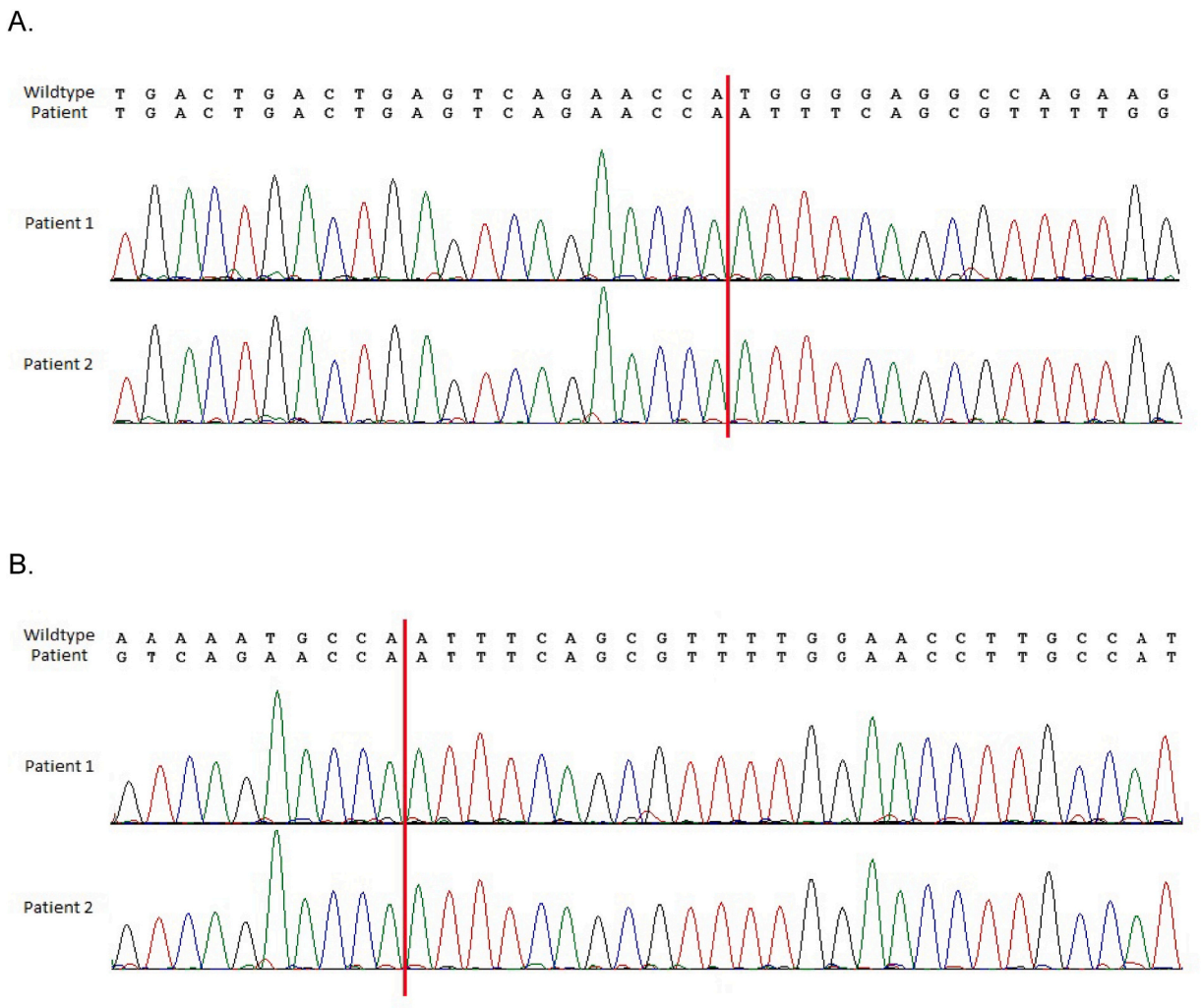

Fig. 2. Sanger sequencing traces identifying the 5′ (A) and 3′ sequences (B) of the deletion breakpoint. The red lines indicate the breakpoint locations according to HGVS nomenclature.

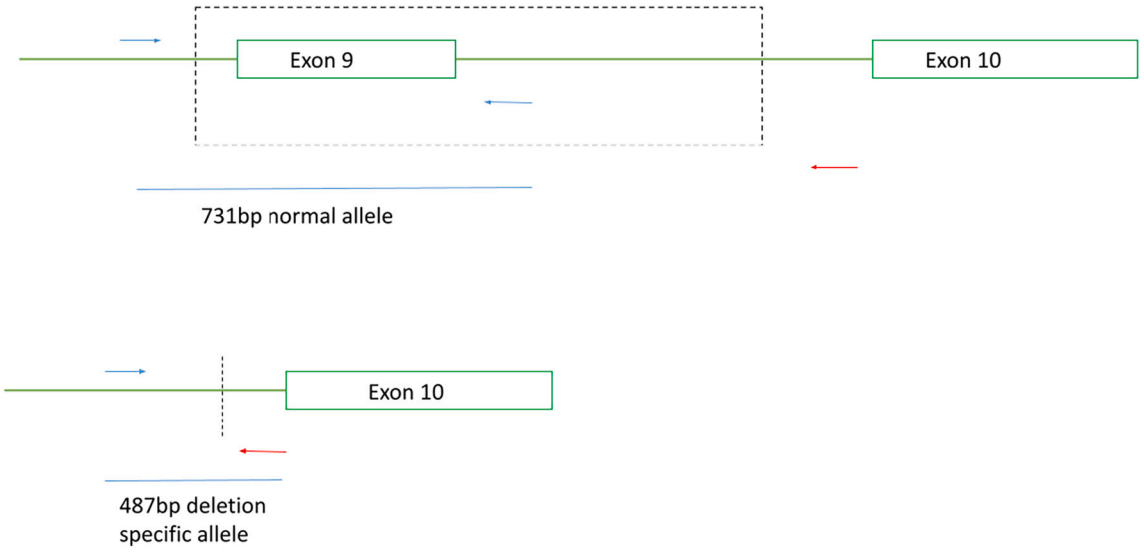

Fig. 3. Schematic for allele specific PCR. For the allele specific PCR exon 9 was amplified using primers flanking the exon (forward primer (blue) is 5′-GTAGTCACCTGAGATGGCCTTTG; reverse primer (blue) 5′ – GGGTGCATGGGGGAGGTGGCCAGTGAGGG – 3′.) The resulting PCR reaction is 731 base pairs (bp) and would indicate the presence of exon 9. In the presence of a deletion of exon 9, a 487 bp product is amplified using a separate 3′ primer (5′-GGCAGCGATGCCTTCCAGAAACAT – 3′.

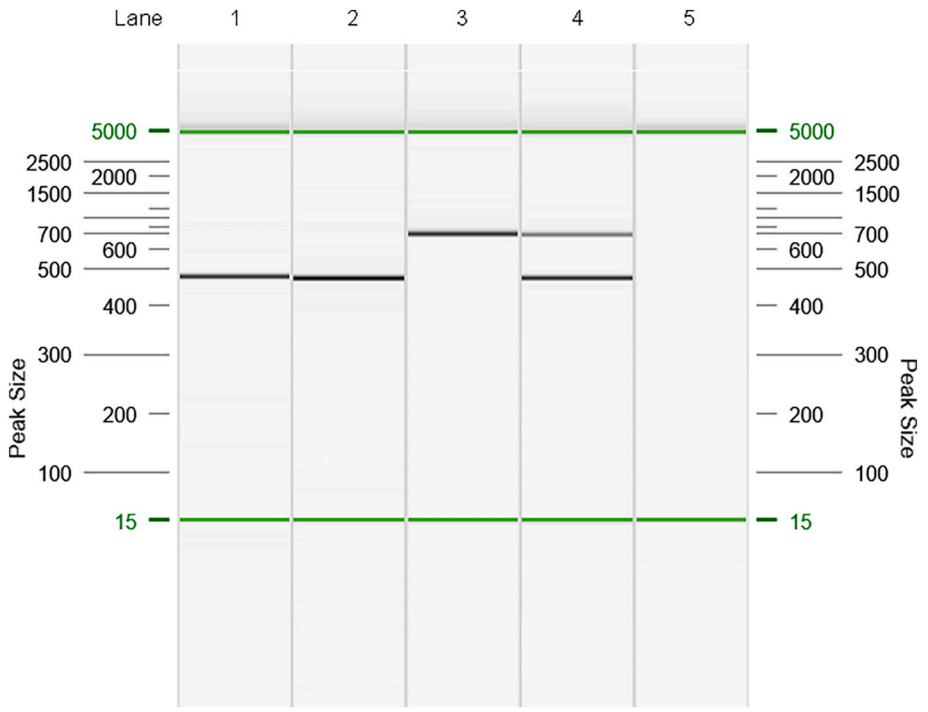

Fig. 4. Allele specific PCR results. Lanes 1 and 2 are the index cases, lane 3- normal control, lane 4 is a synthetic mixture of control and patient, lane 5 – blank.

4. Discussion

The gold-standard for diagnosis of Morquio A syndrome is detection of reduced *N*-acetylgalactosamine-6 sulfatase activity, however molecular analysis is useful to confirm a biochemical diagnosis and assist with genetic counseling and future prenatal testing and carrier testing. Molecular testing via NGS initially was unable to amplify an area containing exon 9 of the *GALNS* gene, which is typically well covered with their selective capture and sequencing of the protein coding region. The laboratory noted that this method of targeted gene sequencing cannot confirm large deletions over 10 bp in size. Repeat molecular analysis of the *GALNS* gene by Sanger sequencing and an allele-specific PCR, identified a homozygous deletion of exon 9 (c.899-397_1003-1862del).

Our patients are sisters from the same non-consanguineous parents. Chromosomal microarray on patient 1 confirmed the reported non-consanguinity. A 2014 review of Morquio A syndrome genotypes for patients of various ethnicities reported that for patients with at least one identified *GALNS* gene alteration, 48% were homozygous [7]. A study of 68 patients of East Indian descent showed homozygous variants in 82% (56 of 68), 28 of which did not have a known family history of consanguinity [8]. Various mechanisms could explain allele homozygosity including uniparental isodisomy (UPD), allelic drop out (a technical artifact), and gross rearrangements. UPD has only been reported once in the literature for *GALNS* [9].

The study by Bidchol et al. 2014 of 68 patients of Indian descent reported 40 distinct variants, but did not report deletions of any size [8]. Indian founder mutations have not been described in the literature. Deletions in addition to duplications and other complex rearrangements are not commonly reported in the literature, which could be due to under detection of these variant types [10]. The deletion characterized in this report was included in the recent review by Zanetti et al. 2021 [11].

The clinical presentation of Morquio A syndrome is variable ranging from a severe and rapidly progressive early-onset form to a slowly progressive later-onset form. Historically, disease severity for Morquio A syndrome focused primarily on height and growth parameters. *GALNS* variants that are predicted to severely affect protein function, such as deletions and nonsense variants are commonly seen in patients with rapidly progressive growth retardation [7]. Tomatsu et al., 2005 assessed the genotype-phenotype correlation of 148 variants using information about variant homozygosity, residual enzyme activity, and predicted change to protein structure. The two large deletions were associated with a severe phenotype [12]. Overall, genotype-phenotype correlation and therefore disease severity can be difficult to determine. Our patients have the classical phenotype on the more severe end of the spectrum.

NGS is increasingly utilized as a first-tier test for lysosomal storage diseases via targeted gene panels or whole exome/genome analysis. The inability of this testing to detect large deletions or rearrangements, especially in the heterozygous state, could lead to a delayed or even missed diagnosis. For example, if the deletion described in this case was *in cis* with missense change in *GALNS*, NGS would label the patient as a carrier rather than affected. Our case highlights the benefit of biochemical analysis for lysosomal storage disorders in cases with equivocal or incomplete molecular analysis.

Ethical approval statement

No ethical approval was required as the testing in child was performed according to medical indication, as part of standard of care.

Patient consent

Patient consent was verbally obtained.

Author contributions

Kathryn DeLong and Dr. Annette Feigenbaum provided clinical evaluation and counseling to the patients and their family. They contributed to the development and editing of the manuscript.

Andrew Lay provided the molecular laboratory work including mapping the deletion breakpoint and development of the allele specific PCR. He also developed Figs. 1 and 2.

Drs. Wood and Pollard provided analysis for the GALNS enzyme

testing and *GALNS* molecular analysis. They also contributed to the development and editing of the manuscript.

Conflict of interest

The authors have declared no competing interest.

Data availability

Data will be made available on request.

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References

[1] Online Mendelian Inheritance in Man, OMIM®, Johns Hopkins University, Baltimore, MD, May 30, 2018. MIM Number: 612222. World Wide Web URL, https://www.omim.org/entry/612222.

[2] Online Mendelian Inheritance in Man, OMIM®, Johns Hopkins University, Baltimore, MD, April 30, 2020. MIM Number: 253000. World Wide Web URL, https://www.omim.org/entry/253000.

[3] M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, K. Stephens, A. Amemiya, GeneReviews®, University of Washington, Seattle, 2019.

[4] A.M. Montaño, S. Tomatsu, A. Brusius, M. Smith, T. Orii, Growth charts for patients affected with Morquio A disease, Am. J. Med. Genet. A 146A (10) (2008 May 15) 1286–1295, https://doi.org/10.1002/ajmg.a.32281. PMID: 18412124.

[5] R. Schrover, K. Evans, R. Giugliani, I. Noble, K. Bhattacharya, Minimal clinically important difference for the 6-min walk test: literature review and application to Morquio A syndrome, Orphanet J. Rare Dis. 12 (1) (2017 Apr 26) 78, https://doi.org/10.1186/s13023-017-0633-1. PMID: 28441951; PMCID: PMC5405472.

[6] O.P. van Diggelen, H. Zhao, W.J. Kleijer, H.C. Janse, B.J. Poorthuis, J. van Pelt, J. P. Kamerling, H. Galjaard, A fluorimetric enzyme assay for the diagnosis of Morquio disease type A (MPS IV A), Clin. Chim. Acta 187 (2) (1990 Feb 28) 131–139, https://doi.org/10.1016/0009-8981(90)90339-t. PMID: 2107987.

[7] A. Morrone, A. Caciotti, R. Atwood, K. Davidson, C. Du, P. Francis-Lyon, P. Harmatz, M. Mealiffe, S. Mooney, T.R. Oron, A. Ryles, K.A. Zawadzki, N. Miller, Morquio A Syndrome-Associated Mutations: A Review of Alterations in the GALNS Gene and a New Locus-Specific, 2014.

[8] A.M. Bidchol, A. Dalal, H. Shah, S. Nampoothiri, M. Kabra, N. Gupta, S. Danda, K. Gowrishankar, S.R. Phadke, S. Kapoor, M. Kamate, I.C. Verma, R.D. Puri, V. H. Sankar, A.R. Devi, S.J. Patil, P. Ranganath, S.J. Jain, M. Agarwal, K.M. Girisha, GALNS mutations in Indian patients with mucopolysaccharidosis IVA, Am. J. Med. Genet. A 164A (11) (2014) 2793–2801, https://doi.org/10.1002/ajmg.a.36735.

[9] S. Catarzi, L. Giunti, F. Papadia, O. Gabrielli, R. Guerrini, M.A. Donati, M. Genuardi, A. Morrone, Morquio A syndrome due to maternal uniparental isodisomy of the telomeric end of chromosome 16, Mol. Genet. Metab. 105 (2012) 438–442.

[10] A. Caciotti, R. Tonin, M. Rigoldi, L. Ferri, S. Catarzi, C. Cavicchi, E. Procopio, M. A. Donati, et al., Optimizing the molecular diagnosis of GALNS: novel methods to define and characterize Morquio A syndrome-associated mutations, Hum. Mutat. 36 (2014) 357–368.

[11] A. Zanetti, F. D'Avanzo, M. AlSayed, A.C. Brusius-Facchin, Y. Chien, R. Giugliani, E. Izzo, D.C. Kasper, H. Lin, S. Lin, L. Pollard, A. Singh, R. Tonin, T. Wood, A. Morrone, R. Tomanin, Molecular basis ofmucopolysaccharidosis IVA (Morquio A syndrome): a review andclassification ofGALNSgene variants and reporting of 68 novelvariants, Hum. Mutat. 42 (2021) 1384–1398, https://doi.org/10.1002/humu.242701398|ZANETTIET (AL).

[12] S. Tomatsu, A.M. Montaño, T. Nishioka, M.A. Gutierrez, O.M. Peña, G.G. Tranda Firescu, P. Lopez, S. Yamaguchi, A. Noguchi, T. Orii, Mutation and polymorphism spectrum of the GALNS gene in mucopolysaccharidosis IVA (Morquio A), Hum. Mutat. 26 (6) (2005 Dec) 500–512, https://doi.org/10.1002/humu.20257. PMID: 16287098.