Child Neurology: Mucopolysaccharidosis IIID

Evidence From Ultrastructural and Genomic Study

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Abstract

Mucopolysaccharidosis IIID (MPS IIID/Sanfilippo syndrome D, OMIM # 252940) is an autosomal recessive lysosomal storage disorder (LSD) and the rarest form of the mucopolysaccharidosis (MPS) III subtypes. It is caused by sequence variations in the gene encoding lysosomal enzyme N-acetyl glucosamine-6-sulphatase (GNS). Deficiency of GNS impairs catabolism of glycosaminoglycans causing accumulation of heparan sulphate within lysosomes of various tissues, which is visualized as membranous cytoplasmic bodies (MCBs) on electron microscopy. The recognition of this ultrastructural feature in a muscle biopsy instigated genetic evaluation for LSD in our case resulting in the detection of a novel pathogenic GNS gene variant. The patient also exhibited intellectual disability since childhood, reduced vision due to pigmentary retinopathy, and behavioral abnormalities without other systemic features of MPS. In this study, we report a patient of Indian origin with MPS IIID based on a novel pathogenic variant c.1078 G>T (p.G360C) in the GNS and the presence of MCBs in muscle biopsy, characterized by several novel findings including the occurrence of pigmentary retinopathy, which extends the clinical spectrum of MPS IIID.

Clinical Case

A 23-year-old woman presented with insidious-onset painless bilateral progressive visual impairment from 12 years of age. She was born to healthy consanguineous parents (second-degree) with no family history of reduced vision or neurologic symptoms. She struggled academically due to intellectual disability, exhibited behavioral abnormalities including irritability with anger outbursts, and preferred limited social interaction but ultimately achieved independence in self-care and was able to do domestic work. Her birth and developmental milestones were otherwise normal. Systemic examination was normal including no neurocutaneous markers. Initially, she had difficulty with night vision, followed by daylight vision. At age 23 years, bilateral visual acuity was 6/60. Fundus examination showed retinitis pigmentosa (RP). The rest of the neurologic examination was unremarkable. Specifically, there was no weakness in the muscles of head/neck, trunk, and limbs.

Visual evoked potential showed right poor waveform, prolonged P100 (167.7 ms, N < 108 msec), and absent left P100 potential. Brainstem auditory and somatosensory evoked potentials were normal. MRI showed mild cortical and cerebellar atrophy (Figure 1A). A mitochondrial disease was considered, and therefore, quadriiceps muscle was biopsied. Biopsied tissue was archived in Human skeletal muscle disease Biobank at our institution with prior consent, and the same was retrieved for the study.

Hematoxylin and eosin (H&E)–stained cryosections of the muscle biopsy showed a rare myofiber with cytoplasmic small irregular eosinophilic deposits and clear vacuoles in the periphery (eFigure 1A, links.lww.com/WNL/C992). Oxidative enzyme stains for succinate dehydrogenase and nicotinamide adenine dinucleotide–tetrazolium reductase (NADH-Tr)
showed a mild increase in activity in the peripheral regions of a few fibers. One percent toluidine blue–stained resin sections highlighted a couple of fibers with vacuoles and lipid droplets in the perinuclear and peripheral regions (Figure 1B; eFigure 1B). The corresponding areas on electron microscopy (EM) revealed several membrane-bound multilayered concentric lamellar structures with granular core called membranous cytoplasmic bodies (MCBs) (Figure 1C; eFigure 1C), characteristic of certain forms of lysosomal storage disorders (LSDs), particularly mucopolysaccharidosis (MPS) and gangliosidosis. These have been described in other tissues including the brain, liver, kidney, and skin.1,2 A careful search through the literature failed to find documented reports of MCBs in muscle tissue. The possibility of MPS/GM1 gangliosidosis was nevertheless considered based on the ultrastructural observations, which instigated a genetic evaluation for LSD.

Clinical exome sequencing (MedGenome labs, Bangalore) showed a novel homozygous missense c.1078 G > T, p.G360C variation in N-acetyl glucosamine-6-sulphatase (GNS) (transcript ENST00000543646.5) in the proband (Figure 1D), further validated by Sanger sequencing (eFigure 1D, links.lww.com/WNL/C992). Both parents and her brother were heterozygous carriers (eFigure 1D). The brothers’ heterozygous status provides evidence for the variant’s pathogenicity because it segregates within the family according to mendelian expectations. This variation is conserved across species and predicted to be disease-causing by in silico analysis using Mutation taster and Rare Exome Variant Ensemble list (REVEL, score 0.972) and deleterious with Combined Annotation–Dependent Depletion (CADD, score 31). This variant was not found in 1000 Genomes Project, Exome Aggregation Consortium (ExAC), Genome Aggregation Database (gnomAD), and Indian database, Indigenomes, and hence, it is a novel variant. No variants were identified in mitochondrial genome sequencing.

Biochemical investigations such as serum/plasma lactate, plasma ammonia, mitochondrial respiratory enzyme assays, and β-galactosidase enzyme level were normal. The leukocyte GNS enzyme activity was significantly low [0.1 nmol/24 h/mg; (reference range: mean ± SD – 4–16 (13 ± 1.4)]. Based on these features, a diagnosis of MPS IIID was made.

**Figure 1** MRI, Pathologic Findings, and Inheritance Pattern in a Patent With MPS IIID

(A) Axial FLAIR MRI of the brain showing mild cerebellar atrophy (arrow). (B) Resin section from skeletal muscle stained with 1% toluidine blue showing lightly stained areas in the periphery of myofiber with numerous clear vacuoles ([arrow], magnification = ×100; scale bar = 10 μm). (C) Electron micrograph showing portion of myofiber with several single membrane-bound membranous cytoplasmic bodies (MCBs) with multilayered concentric lamellae with granular core (asterisks) in the subsarcolemmal region of the myofibers (>5,000). (D) Pedigree.
Table 1  Clinical Features of Mucopolysaccharidosis (MPS) Subtypes

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<tr>
<th>MPS subtypes</th>
<th>Neurologic features</th>
<th>Systemic features*</th>
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<tbody>
<tr>
<td>MPS I</td>
<td>Intellectual impairment, behavioral changes, cervical myelopathy</td>
<td>Skeletal deformations, coarse facial features, hepatosplenomegaly, cardiac diseases, respiratory diseases, ocular disorders</td>
</tr>
<tr>
<td>MPS II</td>
<td>Intellectual impairment, behavioral changes, seizures, cervical myelopathy</td>
<td>Skeletal deformations, coarse facial features, hepatosplenomegaly, cardiac diseases, respiratory diseases, ocular disorders—corneal clouding and retinopathy are rare</td>
</tr>
<tr>
<td>MPS III</td>
<td>Intellectual impairment, behavioral changes, seizures, sleep disturbance, cervical myelopathy</td>
<td>Hernia, sensorineural hearing loss, retinopathy, and dental caries. Corneal clouding is rare</td>
</tr>
<tr>
<td>MPS IV</td>
<td>Learning disability</td>
<td>Skeletal deformations, valvular heart diseases, dental caries, restrictive respiratory disease, corneal clouding</td>
</tr>
<tr>
<td>MPS VI and VII</td>
<td>Learning disability</td>
<td>Skeletal deformations, coarse facial features, corneal clouding, optic atrophy, short stature, cardiomyopathy, hernias</td>
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*Cardiac diseases include cardiomyopathy, valvular heart disease, and ischemic heart disease. Ocular disorders include corneal clouding, open-angle and closed-angle glaucoma, optic atrophy, refractive errors, hypertelorism, and exophthalmos. Skeletal deformation includes dysostosis multiplex, thoracolumbar gibbus, odontoid hypoplasia, and pitting tibia.

Discussion

Mucopolysaccharidoses are devastating autosomal recessive disorders caused by the accumulation of glycosaminoglycans (GAG). The deficiency of the lysosomal enzyme that breaks down heparan sulphate (HS) results in MPS III disorders, with various subtypes including MPS IIIA (heparan N-sulfatase, SGSH), MPS IIIB (alpha-N-acetylglucosaminidase, NAGLU), MPS IIIC (heparan-alpha-glucosaminide N-acetyltransferase, HGSNAT), and MPS IIID (N-acetyl glucosamin-6-sulphatase, GNS). Among the subtypes, MPS I is the most common overall, while MPS IIIC and IIID (1 in 1,000,000 live births) are the rarest forms according to the National MPS Society. However, region and ethnic background may affect the phenotype of MPS. For example, of patients in Asia, mainly from China, Japan, and South Korea, most reported were subtype MPS II. In Europe, the incidence of MPS II was lower than that of MPS I and MPS III. In Germany, Netherlands, and Estonia specifically, MPS III was the most common MPS subtype. In this study, we report an adult patient from India with intellectual disability since childhood, vision loss, RP, and MCBs on muscle biopsy. She was diagnosed with MPS IIID based on genetic analysis and low GNS enzyme activity. Notably, there have been no prior reports of genetically proven MPS IIID from the Indian subcontinent, while MPS IIID has been described in a study based on skin fibroblasts from a European patient of East Indian origin.

Mucopolysaccharidosis is characterized by neurologic symptoms, facial dysmorphism, cardiac diseases, skeletal abnormality, and respiratory and ocular disorders (Table 1). The clinical manifestations overlap substantially across subtypes. Patients typically exhibit normal development for the first few years of life. Neurologic symptoms, including developmental (primarily speech) delay, intellectual impairment, behavioral changes in the form of agitation, aggression, and hyperactivity, sleep disturbances, and seizures, generally start between the ages of 2 and 6 years. Disease progression is characterized by the development of spasticity, recurrent falls leading to loss of independent ambulation, feeding difficulties ultimately requiring enteral feeding, speech loss, recurrent respiratory infections, and mortality in adolescence. Systemic manifestations include pigmentary retinopathy, optic atrophy, sensorineural hearing loss, coarse facial features (broad nose with flared nostrils, flat nasal bridge, prominent supraorbital ridges, large rounded cheeks, synophrys, hirsutism, thick lips, macroGLOSSIA, and frontal bossing), abdominal and inguinal hernia, femoral head osteonecrosis, osteopenia, and hepatosplenomegaly. MPS IIIC and IIID have a slightly milder course with a longer survival compared with MPS IIIA/IIIB. The clinical course in our patient was marked by normal development through her first decade. However, she exhibited poor scholastics leading to a diagnosis of intellectual disability and preferred limited social interaction. Starting at age 12 years, she developed significant ocular manifestations including vision loss and RP. Pigmentary retinopathy, as seen in our patient, is a novel clinical feature in MPS IIID. While pigmentary retinopathy was reported in patients with MPS IIIA and MPS IIC, it was not seen in a cohort of 15 patients with MPS IIID by Valstar et al. or other published cases of MPS IIID. The differential diagnoses for pigmentary retinopathy include other MPS, Niemann-Pick disease, Gaucher disease, multiple sulfatase deficiency, fucosidosis, mannosidosis, and mitochondrial disorders.

The first screening test for MPS is a quantitative assay for urinary GAG. If elevated, individual GAG species can be identified by mass or electrophoretic spectroscopy. In MPS III, HS is elevated. To identify the subtypes, leukocyte or fibroblast enzyme activity assay is desirable. Molecular genetic analysis will identify the underlying gene defect. In our patient, blood leukocyte GNS enzyme activity was performed after genetic testing and was low.
HS is the most variable GAG, and its functional role depends on its structural variability, allowing it to form conjugates with various protein moieties including heparan sulphate proteoglycans (HSPGs). HSPGs are widely expressed on the basal lamina and surface of skeletal muscle cells. Inappropriate catabolism of HS destroys muscle development and physiologic function causing weakness. Improper HS breakdown leads to accrual within cell lysosomes, which form the characteristic MCBs detected on EM. MCBs are a characteristic feature of MPS and GM1/GM2 gangliosidosis. The biopsied muscle from our patient highlighted MCBs, which gave the preliminary clue to diagnosis of MPS, although MCBs have not been previously reported in skeletal muscle fibers of patients with MPS IIID. This case highlights the role of ultrastructural morphodiagnosis in peripheral tissue biopsy.

Brain and spine imaging in MPS can show enlarged Virchow-Robin spaces, focal or confluent T2/FLAIR white matter hyperintensities, communicating hydrocephalus, brain atrophy, cervical spinal canal stenosis with or without cord compression, and abnormalities of skull and vertebrae. Brain atrophy is most common in MPS II and III. Likewise, mild cortical and cerebellar atrophy was noted in our patient with MPS IIID phenotype. Spine MRI was not performed.

MPS IIID is caused by homozygous variants in the GNS located on chromosome 12, first described in 2002 by Mok et al. Subsequent cases reported by various groups have identified 23 different alterations overall including point variants, deletions, and large rearrangements with similar clinical presentation and age at onset in the first decade of life. In this study, we describe a novel homozygous missense c.1078 G>T (p.G360C) variation in exon 9 of the GNS. Sanger sequencing confirmed homozygosity in patient, while parents and younger brother were heterozygous carriers (Figure 1D). The biallelic loss of function was supported by almost complete loss of GNS enzyme activity.

Treatment for MPS III includes enzyme replacement therapy, substrate reduction therapy, gene therapy, hematopoietic stem cell therapy, and enzyme enhancement therapy. In 2017, promising data from a mouse model for MPS IIID treated with adeno-associated virus 9 –mediated gene therapy suggest gene therapy may be a future treatment option. The novel variants, functional validation, and further studies on pathophysiology are required to pave the way for developing additional treatment strategies.

The novel findings of this report are the occurrence of retinitis pigmentosa, lack of systemic features of MPS, presence of MCBs in muscle biopsy, and a novel GNS pathogenic mutation in a patient with MPS IIID. A high index of suspicion is desirable in patients with intellectual disability, behavioral changes, hyperactivity, retinitis pigmentosa even without coarse facial features, skeletal abnormalities, or organomegaly. Awareness of pathologic findings in peripheral tissue biopsies can guide targeted gene testing and diagnosis.

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Appendix Authors

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