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CASE REPORT

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A novel frameshift deletion in *NAGLU* causing sanfilipo type III-B in an Indian family

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Key Clinical Message

Mucopolysaccharidoses are group of inherited lysosomal storage disorder. Two siblings of a family manifested behavioral abnormalities; hepatosplenomegaly and hypotonia of infantile onset were found to have a novel homozygous frameshift variation, p.Leu280TrpfsTer19 in *NAGLU*. This variant was predicted to cause the loss of TIM-barrel and alpha-helical region of *NAGLU* protein.

KEYWORDS

MPS IIIB, Mucopolysaccharidoses, *NAGLU*

1 | INTRODUCTION

Mucopolysaccharidoses (MPSs) are a group of inherited lysosomal storage disorders which is characterized by abnormal accumulation of partially degraded glycosaminoglycan (GAGs) fragments in urine, blood, and cerebral spinal fluid.[1,2] MPS disorders are caused by the deficiency of a specific lysosomal enzyme which is required for the GAG degradation. Sanfilipo syndrome (MPS III), results from deficiency of different lysosomal enzymes involved in degradation of heparan sulfate.[1,2] MPS III includes four subtypes on the basis of the different lysosomal enzymes involved in the degradation of GAGs.[2-4] The four enzymes involved namely *sulfamidase* (MPS Type III-A), α- *N-acetylglucosaminidase* (MPS Type III-B), *acetyl-CoA:a-glucosaminide N-acetyltransferase* (MPS Type III-C), and *N-acetylglucosamine-6-sulfate sulfatase* (MPS Type III-D) are responsible for the stepwise degradation of heparan sulfate (Figure 1A).[2,4]

2 | CASE REPORT

The index case, a boy who was born full term, first child of healthy consanguineous parents from an agrarian community from rural Andhra Pradesh, India (Figure 1B) presented at clinic (to VC) with a history of febrile seizures at the age of 2 years (2014). Since birth, he was apparently normal until the age of 6 months and then parents noticed gradual distension of abdomen but further no attention was paid for a medical checkup by the parents. At subsequent period of life, he exhibited delayed developmental milestones and behavioral abnormalities. Physical examination of proband earlier had shown coarse facial features (Figure 1C), hypotonia, and hepatosplenomegaly. In view of hepatosplenomegaly, a diagnosis of mucopolysaccharidoses was considered. Echocardiography did not show any cardiac anomaly, skeletal survey was normal except for changes of mild dysostosis multiplex in pelvic

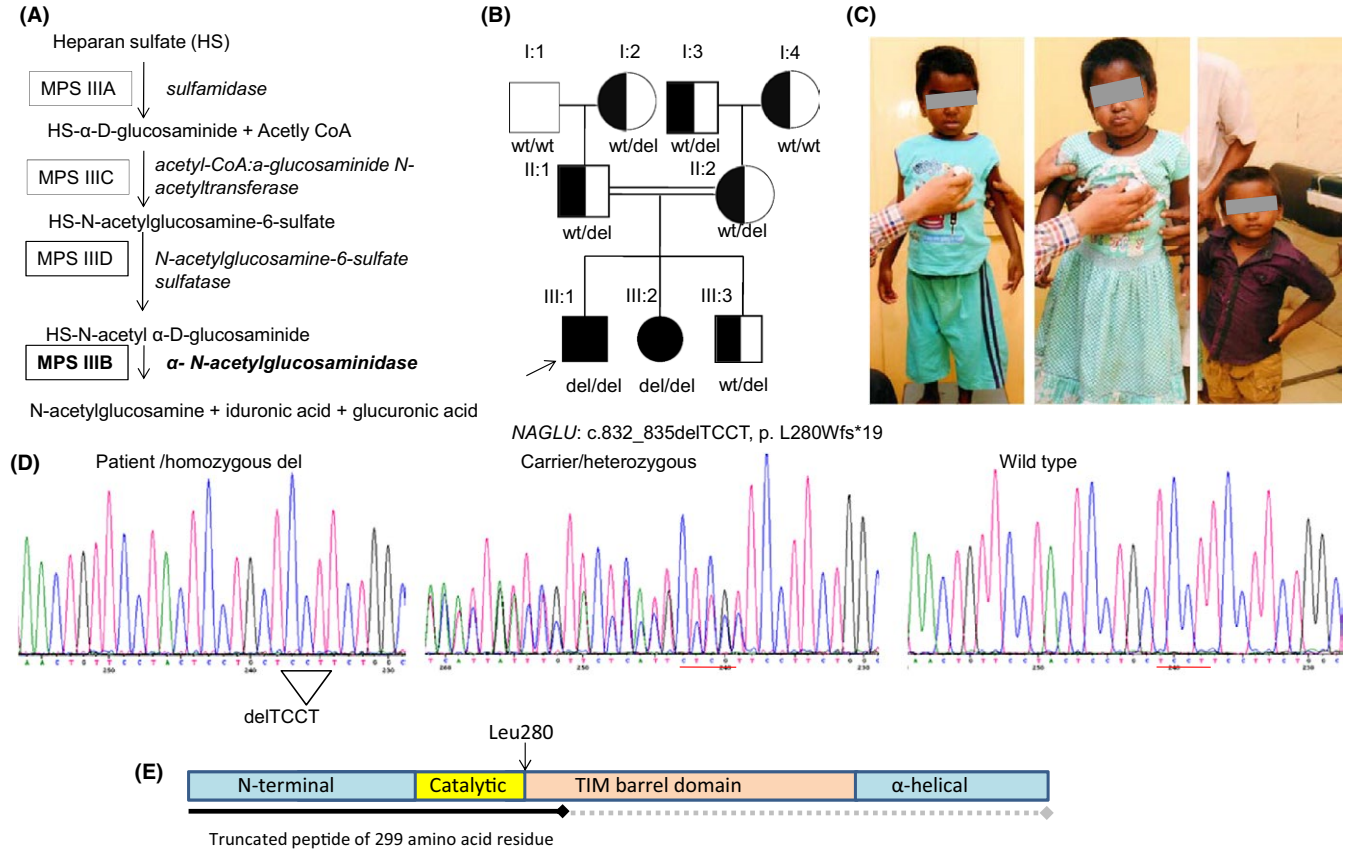

FIGURE 1 A, Steps for heparan sulfate degradation responsible for Sanfilipo Syndrome [MPS Type III]. B, Pedigree chart of the Family comprising of three generations (I- paternal and maternal grandparents, II- parents, and III- proband, affected sibling and unaffected sibling); C, From Left, patient-1 a 6-y-old boy, patient-2 a 5-y-old girl showing coarse facies, and the unaffected sibling, a 4-y-old boy; D, Sequencing electeropherogram showing frameshift deletion variation in patient, the wild type as a reference and the traces of sequences in heterozygous carrier; E, schematic of structure of the protein showing predicted truncated length with mutation at L280 position. (Dark filled symbols in pedigree cart indicate diseased individual. Half-filled symbol indicates carrier of the mutation)

X-ray. Urine MPS spot test identified faint band of dermatan sulfate upon electrophoresis. Further, patient (at the age of 2 years) was referred for MPS-I and MPS IIIB to a laboratory (CDFD, Centre for DNA Fingerprinting and Diagnostics). Based on availability of the tests at CDFD, biochemical investigations for enzyme activity had shown reduced activity for α-*N-acetylglucosaminidase* (0.13 nmol/h/mg, reference range; 0.7-1.7 nmol/h/mg). Immediately after the confirmation of diagnosis MPS IIIB, patient's siblings, a sister (1 year of age, III-2) and a brother (at 1 month of age, III-3) were also tested for α-*N-acetylglucosaminidase* and its activity was found reduced in girl sibling (0.113 nmol/h/mg) and normal for brother (0.819 nmol/h/mg). Three of the siblings have been followed up since 2014 by VC. Proband's younger sister, now age 5 years, manifested similar features at onset as of his brother and also the behavioral abnormalities and delayed developed milestones. She had coarse facial features (Figure 1C), hypotonia, and hepatosplenomegaly. She has now started showing behavioral abnormalities. His youngest sibling, age 4 years had no history of delayed developmental milestones or behavioral abnormalities and examination had revealed the absence of abnormalities alike his siblings. Proband (III-1) has started showing hyperactivity, aggressiveness, and loss of bowel control recently, other than that both the affected siblings had normal social interaction and sleeping patterns.

The entire family was counseled for conducting genetic investigations of the underlying problem. Samples of the available family members were sent to CSIR-IGIB (MF lab) for genetic investigations of *NAGLU* (MPS IIIB gene).

3 | METHOD

Genetic investigations: Sanger sequencing was conducted for the screening of mutations in *NAGLU* gene for the patients, while sample of the unaffected sibling, the parents, and grandparents (Figure 1B) were taken for validation of any identified variation. For genetic analysis, peripheral blood sample was used to extract the Genomic DNA using modified salting out procedure. The entire coding region of

NAGLU (six exons, including intron-exon boundaries) was covered by 10 pairs of primers (Table S1). PCR amplicons were sequenced using a standard sequencing protocol and subjected to capillary electrophoresis on ABI 3730 DNA Analyser. NM_000263 was used as a reference sequence for cDNA and protein nomenclature, and also for variant reported in this case study.

4 | RESULTS

The sequence analysis of all the exons of *NAGLU* gene has shown a frameshift deletion c.832_835delTCCT; (p. Leu280TrpfsTer19), Figure 1D; a variant in homozygosis in both the affected patients in exon 5. This variant is reported in ExAC database with a frequency of 0.000008237, as one heterozygous allele (accessed in May, 2018). Elsewhere, this variant is absent in other clinical and population genetic databases like 1000 Genome Project, Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac), Single Nucleotide Polymorphism database (dbSNP; https://www.ncbi.nlm.nih.gov/projects/SNP), and Clinvar. This variant being a frameshift deletion is causing the protein to truncate at 299 amino acid residue, thereby showing a deleterious effect due to the loss of more than half of the protein structure that would likely involve the junction of catalytic domain and TIM barrel domain (Figure 1E) of the *NAGLU* protein.[5] The proband's unaffected sibling, both the parents and one paternal and maternal grandparent were carrier for this variant (Figure 1B). Parents have seek the management of the disease at the clinic intermittently, however, with no option for curable treatment for the disease in their children, the patients have been lost in the follow-up with the clinic and parents have been trying to seek various other alternative treatment options.

5 | DISCUSSION

The patient was initially presented to us with a history of febrile seizure and physical examination of medical illness revealed the presence of coarse facies and hepatosplenomegaly hence, it pointed toward possibility of MPS. Samples of both the cases were referred for biochemical investigations of MPS types but based on availability of the tests, investigations of MPS-I, and MPS IIIB was only conducted. As this patient was confirmed for MPS IIIB biochemically, and further genetically confirmation was carried out for MPS IIIB gene sequencing. Patient has been receiving treatment for general symptomatic complaints and was under follow-up intermittently. Now patient is not visiting clinic any further and parents have opted for alternate medicine system (like Ayurveda).

The novel mutation p. Leu280TrpfsTer19 reported in our patients is also the first genetically confirmed case of *NAGLU* (MPS III-B) in India. The frameshift deletion is leading to protein truncation after 299 amino acids. As this deleterious mutation is proposed to be present in junction of catalytic domain and TIM barrel domain of *NAGLU* protein it may affect the stability or binding of the ligand at the catalytic site.[5,6]

As per the previous studies, there are various disease (MPS IIIB) causing variants in *NAGLU* and among these variants, frameshift and protein truncating variants reported in *NAGLU* gene have a very severe presentation and progression of MPS IIIB.[7] The earlier reported protein truncating mutations, that is, c.217_221dup5 (p.Val75fs), 503del10, Trp675X, Glu706X, and Arg297X shows severe phenotype where the median age of onset of 3 years,[7-9] however, the survival age differs with greater variability for these variants. The mutation found in this study has been identified for the first time in homozygous state for c.832_835delTCCT; (p. Leu280TrpfsTer19) in both of our patients and shows an early onset of the diseases as early as 6 months. The overall ability to speak and walk is preserved in our patients, the reported loss of ability to speak is (7.5 years, median) and loss of ability to walk (12 years, median),[7] and the median age for cardiac anomaly is 8.5 years.[10] Since our patients age is younger for development of these abnormalities, further clinical assessment will be required in the follow-up period. The clinical data present with us along with the genetic correlation has lead to the confirmation of MPS IIIB.

In India, there is a problem of inadequacy of health care resources in the rural areas and the lack of knowledge of such rare diseases in general, makes identification of true cases difficult. Further, due to the lack of treatment availability even at tertiary health centers makes it further difficult to understand the clinical and genetic spectrum of these diseases. Further, exploration of Indian MPS IIIB patients will be required by genetic evaluation which will allow and facilitate genetic counseling and disease management.

The study was approved by Human Ethics committee of CSIR-IGIB for GOMED (MLP1601). Informed written consent was obtained from each subject.

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CONFLICT OF INTEREST

Nothing to report for any author.

AUTHOR CONTRIBUTION

SJ: carried out genetic investigations and manuscript writing; VC: identified clinical case and clinical evaluation; MF: designed the study and wrote the manuscript writing.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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