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Short Communciation

A novel mutation in SGSH causing Sanfillipo type 3A Mucopolysaccharidoses in an Indian family

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ABSTRACT

Mucopolysaccharidoses (MPS) type III also termed as Sanfillipo syndrome, involves defect in enzymes required for degradation of heparan sulphate. We report a clinical case of MPS-III later followed by genetic investigation for MPS-III genes *SGSH*, *NAGLU*, *HGSNAT* and *GNS*. It allowed us to identify a novel and likely pathogenic variant p. G205R in *SGSH*. Protein based Inslico prediction and protein modelling suggests aberration of helical structure of SGSH protein and reduced binding affinity for its substrate.

1. Introduction

Mucopolysaccharidoses (MPSs) are group of rare and inherited lysosomal storage disorders. These are biochemically characterized by abnormal accumulation of partially degraded glycosaminoglycans (GAGs) fragments in urine, blood and cerebral spinal fluid. Each MPS disorder (I-IX) is caused by the deficiency of a specific lysosomal enzyme which is required for the GAGs degradation that leads to accumulation of GAGs in different body tissues [1].

Sanfillipo syndrome (MPS-III), results from deficiency of different lysosomal enzymes involved in degradation of mucopolysaccharide heparan sulfate [2,3]. MPS-III includes four subtypes on the basis of the different lysosomal enzymes involved in the degradation of GAGs. The four enzymes involved namely heparan sulfamidase, α-*N*-acetylglucosaminidase, acetyl–CoA: α-glucosaminide *N*-acetyltransferase and *N*-acetylglucosamine-6-sulfate sulfatase are responsible for the stepwise degradation of heparan sulfate. MPS III-A or Sanfillipo syndrome type A occurs when the activity of heparan sulfamidase is lost or reduced to a great extent [2,3]. In this case report, we describe a novel mutation in *SGSH* gene identified in a patient who manifested mainly neuronal phenotype along with cardiac, skeletal and skin manifestations. This is the first genetic study for MPS-III reported from India.

2. Case report

A 16 year old girl born to consanguineous parents presented to us with seizures for past 1 year duration. She had normal neonatal period and developmental history till the age of 4 years. In subsequent years she manifested behavioral abnormalities involving hyperactivity, aggressiveness and palilaia (repetition of spoken words/phrases) followed by speech regression and by the age of 12 she had stopped speaking even monosyllabic words. At age 7 she developed weakness of both lower limbs, and at age 14 she required support for walking. From last one year she developed repeated convulsive episodes characterized by unresponsiveness, lip smacking without tonic posturing of limbs lasting for 3–4 min. Among other associated illnesses, day time sleepiness, dysphagia and recurrent respiratory tract infection. No history of similar illness was noticed in any of the family member. On examination, we observed following abnormalities, Coarse facies (Fig. 1A), Glossitis and macroglossia; silky hair and white nail; Café au lait macule over left iliac region extending to left thigh and cubitus valgus (Fig. 1B). Central nervous examination had shown generalized muscle wasting, hyperreflexia with plantar extensor (right) and absent abdominal reflex. Her gait could not be assessed and she performed stereotypic sideways movement of the head. Hepatomegaly with no spleenomegaly; grade-3 pan systolic murmur with abnormal breathing patterns was other significant findings. Among paraclinical tests following abnormailities were noted, slight elevated ammonia level in serum (70 μg/dL). Moderate mitral valve and mild tricuspid regurgitation in echocardiograph, oar shaped ribs (chest X-ray Fig. 1C), mild scoliosis with convexity to the right was noted in X-ray lumbosacral spine (Fig. 1D). Electroencephalogram recordings were inconclusive. Magnetic resonance imaging (MRI) of brain had shown moderate diffuse cortical atrophy, mild diffuse T2 FLAIR hyperintensity in bilateral periventricular region

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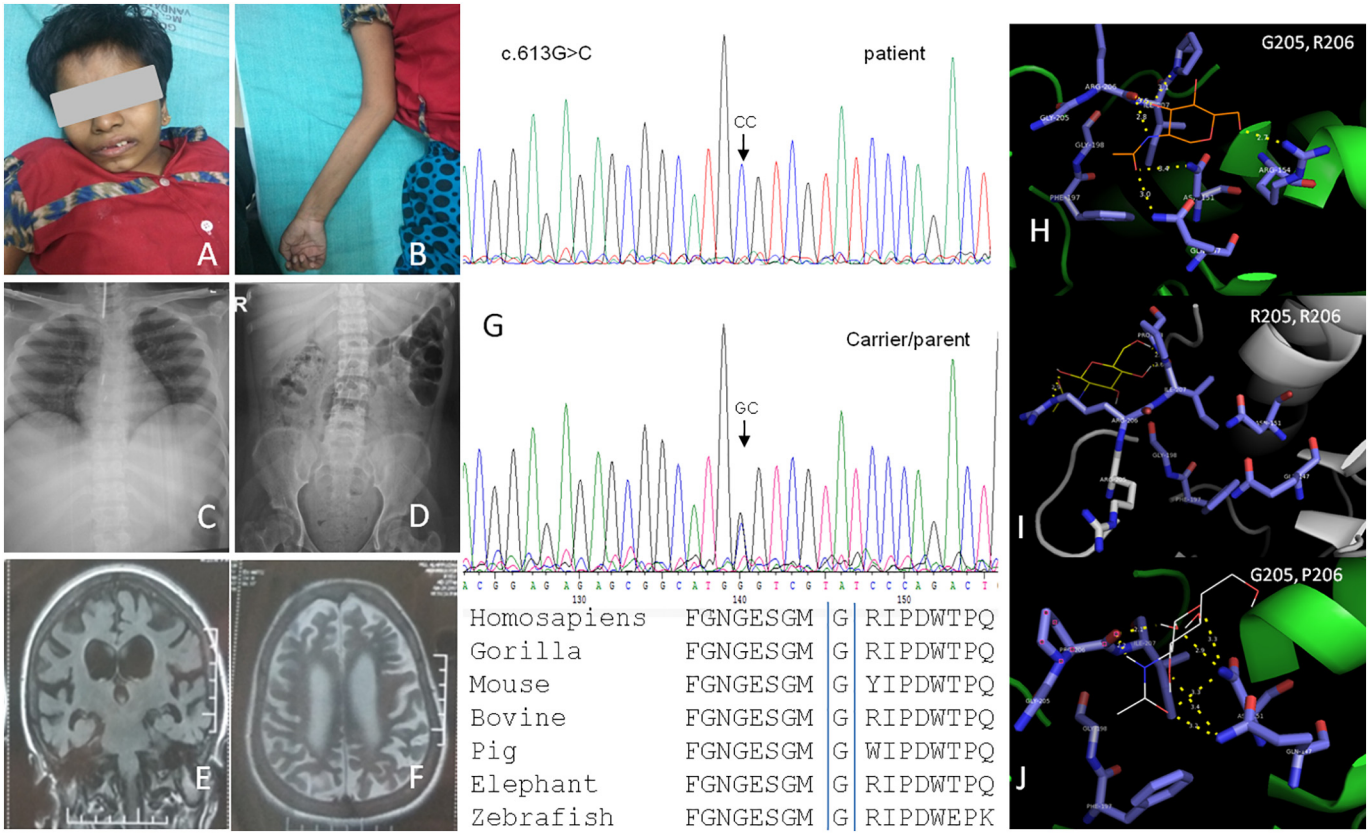

Fig. 1. Clinical and genetic findings in the affected patient. Patient showing A) coarse facies, B) wide carrying angle, C) X-ray chest showing Oar shaped ribs, D) X-ray lumbosacral spine shows scoliosis with right convexity, E) brain MRI coronal section T1 weighted image showing periventricular hyperintensities, F) brain MRI Axial image showing cortical atrophy, G) sequencing electeropherogram showing homozygous variant (arrow) in patient and heterozygous carrier state in parents DNA sample, sequence alignment of G205R variant across species showing conserved residue. H) image represents the docked NAG in the binding pocket of wildtype (G205,R206) *SGSH* (4mhx). Substrate makes polar contact with residue N151,Q147,R154,R206 which also includes the site of glycosylation (Asn151). I) residue G205 was mutated to Arg. This affected the binding of NAG, because of the bulky side chain; it may lose the bond with N151 residue which is responsible for glycosylation. The distortion of the loop was observed as the residues were seemed to be displaced when compared to wild type *SGSH*. J) Residue R206 was mutated to Pro, the binding of NAG was observed with same residues as in wild type but with small difference in bond lengths.

(Fig. 1E and F).

Based on the observed clinical features; coarse facies, behavioral abnormality, speech regression, neuro-cognitive impairment, hepatomegaly, cardiac involvement and skeletal abnormalities a diagnosis of MPS type III was made. Patient's urine sample was tested positive for Toluidine blue spot test that further suggested our diagnosis. Hence, DNA based genetic testing was undertaken for genes implicated with MPS-III for patient and parents.

Unfortunately patient's condition deteriorated further and later we were informed of her demise due to respiratory illness by her family.

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

3. Results and discussion

For genetic analysis, peripheral blood sample was used to extract Genomic DNA using modified salting out procedure [4]. The entire coding region and intron-exon boundaries of the *SGSH* gene were amplified by Polymerase Chain Reaction with appropriate primer pairs (8 exons and 9 primer pairs, supplementary table1). PCR amplicons were sequenced using a standard sequencing protocol and subjected to capillary electrophoresis on ABI 3730 DNA Analyser. NM_000199.4 was used as a reference sequence for cDNA and protein nomenclature, and also for variant reported in this case study.

After the analysis of all the exons of *SGSH* gene in patient, we found a variant in homozygosis: c.613G > C (p. G205R), and in heterozygous state among parents (Fig. 1G). This variant has not been previously reported in the ExAC, 1000 Human 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). According to different In-Silico prediction tools (PolyPhen-2, Mutation Taster and SIFT) c.613G > C (p. G205R) is predicted to be probably damaging with the score 1. Based on ACMG (The American college of Medical Genetics and Genomics) guidelines, the variant is likely pathogenic due to its location in hotspot region, where R206P is already a reported pathogenic allele located in active site of the enzyme. In Addition to *SGSH*, we also sequenced coding regions of other MPS-III loci (NAGLU; MPS-IIIB, *HGSNAT*; MPS-IIIC and *GNS*; MPS-IIID) to rule out possibilities of other disease causing variants and modifier variations [2,5]. *HGSNAT* was sequenced partially (all 18 exons except Exon2, Exon6, Exon7, Exon8, eExon9 and exon11), this however covered 27 of 36 reported mutations.

Sanfillipo syndrome has been very well studied all over the world. All the studies are based on clinical phenotypes, biochemical profilling and genetic analysis, but in India all the reported cases have no genetic information. This is a very first genetic study reported from India till date [6].

There is a treatment available for MPS I, II, and VI known as enzyme replacement therapy (ERT) which aims at delivering the functional enzyme to patients which appears to be effective in slowing down the progression of the disease. However, ERT for sanfillipo syndrome (MPS III A) is still unavailable or under trials [7], (https://mpssociety.org).

We found only c.613G > C (p. G205R) mutation in homozygous

state in *SGSH* gene, apart from this no mutation was found in other concerned genes. This also suggests that mutation found is independently capable of causing the particular disease. This mutation was not found in the major databases (ExAC, 1000G, and HGMD) and reported literature proposing it to be a novel variant.

We also performed the In-silico analysis of the variant G205R to conform our result. Insilico docking of *SGSH* (4mhx) protein with substrate NAG (N-Acetylglucasoamine), variant G205R revealed the disturbance in the loop near the glycosylation site Asn151, that might affect the polar contacts between the substrate and the residues involved in binding along with the glycosylation site itself (Asn151) (Fig. 1H and I). Whereas the adjoining variant R206P shows, only the change in Phi-Psi angles of residues in the binding pocket but not affecting the binding of substrate as well as glycosylation site (Fig. 1J). The structural effect of G205R matches with structural disturbances caused by L146P [8,9].

We propose that the found variant (c.613G > C) is novel and reported for the first time from India and independently responsible for MPS type III-A, since the clinical phenotypes of the patient correlate with the reported phenotypes. More studies and functional validations may be needed for this novel variant.

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Conflict of interest

Nothing to report for any author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2018.04.003.

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