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Early onset LGMDR19 with unusual features related to *GMPPB* gene in South Indian siblings with variable phenotype

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Abstract

Guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (*GMPPB*) is a cytoplasmic enzyme that catalyzes the synthesis of GDP-mannose, a crucial substrate for several glycosylation pathways. Reports of pathogenic variants in the *GMPPB* gene are infrequent. As of April 2023, 109 cases with pathogenic variants in the *GMPPB* gene have been reported worldwide. Here, we present two siblings born of consanguineous parentage from Southern India. This is a retrospective study on genetically confirmed siblings with *GMPPB* pathogenic variants. The siblings, a 15-year-old girl, and a 13-year-old boy, presented with progressive limb-girdle weakness and cataracts from early childhood. The girl had exertion-induced breathlessness and mental subnormality. Creatine kinase levels were 5750 and 4320 IU/L. An echocardiogram of the heart revealed global hypokinesia, moderate left ventricular dysfunction, and mild mitral regurgitation with dilation of the left atrium and left ventricle in the girl child. Exome sequencing showed a homozygous pathogenic variant (NM_021971.4 (*GMPPB*): c.358A>G (p.Met120Val) in Exon 4 of *GMPPB* gene in both the siblings. Thus, we report an unusual early onset LGMD phenotype of GMPPB-related disorder with cardiac involvement and cataracts from a single family.

Keywords: GMPPB, Limb-girdle muscular dystrophy, Congenital myasthenic syndrome, India

INTRODUCTION

Limb-girdle muscular dystrophy (LGMD) are inherited and genetically diverse group of muscle disorders with progressive muscle loss/ hypertrophy, weakness, and severe dystrophy due to pathogenic variants in genes involved in the integrity of muscle.[1-3] One of the most recently discovered genes causing LGMD is the guanosine diphosphate mannose (GDP-mannose) pyrophosphorylase B (*GMPPB*) coding gene.[4] Carss *et al*., in 2013, first described the pathogenic variant in *GMPPB* gene, leading to reduced α-DG glycosylation.[4] As of April 2023, 109 cases with pathogenic variants in *GMPPB* gene have been reported worldwide. Adult-onset LGMDR19, congenital muscular dystrophy (CMD), congenital myasthenic syndrome (CMS), isolated rhabdomyolysis, epilepsy, intellectual disability, ocular involvement (Walker-Warburg syndrome), heart and brain abnormalities are all described phenotypes associated with pathogenic variants in *GMPPB*.[4,5-7] Hitherto unreported, we present two interesting siblings, a 15-year-old girl and her 13-year-old brother, with LGMD phenotype, cataracts and cardiac involvement in the girl due to a homozygous *GMPPB* pathogenic variant.

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METHODS

Subjects and clinical evaluation

This is a retrospective study done at a quaternary neurology hospital. Detailed clinical, laboratory, and imaging features were recorded. The pedigree is shown in Figure 1. Genetic analysis was done by next-generation sequencing. An institutional Ethics Committee was obtained for this study. Informed consent was obtained from the parents to unmask the faces in the images and videos for publication.

Genetic analysis

DNA Extraction: Genomic DNA was extracted from the peripheral blood sample by the Qiagen blood kit method (Qiagen, Hilden, Germany). The DNA quantity and quality were assessed by Nanodrop spectrophotometer and by agarose gel electrophoresis. For the DNA samples that pass the first step of quality estimation, a second quantification step was performed by Qubit Fluorometric Quantification (Thermo Fisher Scientific).

Exome Sequencing and data analysis: Exome sequencing libraries were prepared using 37 Mb capture probe sets which include protein-coding genes and the mitochondrial genome using Twist Biosciences Human core exome kit. Fragmentation and adapter ligation of the total DNA was performed according to the manufacturer's protocol to produce 250-bp to 400-bp fragments. Unique indices were used to multiplex samples, and the library was PCR amplified. The Quality control of the exome Library was checked using Bioanalyzer (Agilent Inc.) and was quantified by Qubit (Thermo Fisher Scientific). Exome Capture was performed by Hybridization method utilizing a 37 Mb capture probe set. Fragments were captured on streptavidin magnetic beads, followed by a series of wash steps to eliminate non-specific and un-hybridized fragments. The resulting libraries were amplified, and quality was analyzed using Bioanalyzer. The final enriched libraries post quality assessments were sequenced on Illumina NextSeq 550 platform using 2x150 bp read chemistry according to NextSeq 550 platform (Illumina: San Diego, CA, USA). The raw data generated post-sequencing in *.bcl format was converted to FASTQ format. The quality check of FASTQ formatted file was performed using FASTQC/NGS Tool kit. Quality control passed FASTQ formatted files were aligned with the human reference genome hg19 GRCh37 by BWA v0.7.17 based on Burrow Wheeler algorithm. The resultant output, the BAM files, were checked for quality using samtools and further used for downstream analysis. InDel

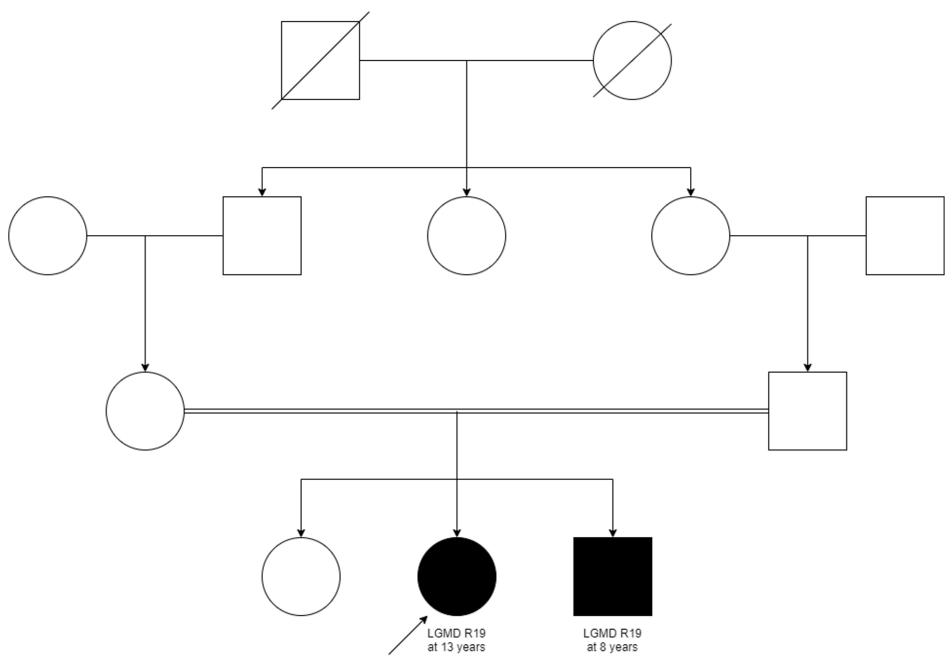

Figure 1. Pedigree chart of the patient siblings.

correction and Variant calling were executed using the GATK toolkit to produce a variant file in VCF format. Duplicate removal using Picard was done. Highly confident variants having DP>10x and GQ>20 were selected for the annotation step. The VCF file was further annotated using snpEff package. Databases used for annotation were from multiple sources like 1000Genome Phase 3, Clinvar, gnomAD, dbSNP 151, ExAC, EVS, Genome Asia project, PhyloP (46ways, 100ways), Phast Cons(46ways,100ways), and several publicly available variant impact in-silico prediction tools. Further variant filtration was performed based on the above annotations to filter out clinically relevant variants. A literature search was also performed to see if any of these variants have been reported as pathogenic variants earlier in associated studies using Human Gene Pathogenic Variant Database (HGMD) and ClinVar. Phenotype information obtained from the clinical assessment was correlated with sequencing data to narrow down the most promising variants. The pathogenicity of the variants was assessed based on the 2015 American College of Medical Genetics (ACMG) guidelines. Variants that have very low allele frequencies across the populations, especially in South Asian populations, were filtered. The resultant variants after the above-mentioned procedures were analyzed for disease association.

Segregation analysis by Sanger sequencing: Sanger sequencing was done in all the family members (both parents, affected siblings, and one unaffected sibling)**.** PCR was performed for DNA (from all family members) using primers designed to amplify the genomic region spanning the targeted variant. We designed sequencing primers to target the 340-base pair (bp) region in exon 4 of the GMPPB gene. The primers were designed usingprimer3 and checked for primer dimer and self-dimers using multiple primer analyzer (Thermo Fisher Scientific Inc) followed by performing in-silico PCR on UCSC genome browser. PCR products from the samples were electrophoresed to look for quality and amplification accuracy on 1-1.5% agarose gel. Post- PCR cleanup was performed to remove the unutilized primers, unused dNTPs and other contents of PCR master mix using JetSeq- Clean Beads (Bioline). The purified amplicons were subjected to cycle sequencing. The purified PCR amplicons were subjected to bidirectional Sanger sequencing which was performed on Seq Studio Genetic Analyzer (Thermo Fisher Scientific) using Big Dye Terminator v3.1 kit as per manufacturer's instructions (Thermo Fisher Scientific). The raw data file .abi was visualized using Finch TV and the sequence was blatted on UCSC browser to compare with the reference sequence. Further, the variant in the sequence was analyzed based on ACMG criteria, and the inheritance pattern was checked.

RESULTS

Clinical findings

Patient 1

This is a 15-year-old girl born to consanguineous parents presented during October 2020, with progressive limb girdle weakness predominantly of lower limbs from early childhood. She was born as a preterm baby weighing 1100 grams and was admitted to the neonatal intensive care unit for 8 days. She had delayed motor and language milestones with speaking bisyllables at two years and walking independently at four years of age. Since then, she had frequent falls and inability to run fast. There has been worsening of weakness for the past 6 years requiring assistance to rise from floor and climb stairs. She also had excessive fatigability of limbs. At age eleven, she developed progressive visual impairment due to cataract, which was operated. There was exertional dyspnea and palpitations on walking for about 500 meters or climbing one flight of stairs for two months. There was no history of bulbar and facial weakness. On examination, there was raised jugular venous pulse, calf hypertrophy, and tendoachilles contractures. The cranial nerve examination was normal. Muscle strength assessment revealed mild to moderate weakness, according to the modified Medical Research Council (MRC) Scale: deltoid – 3, biceps brachii – 3, pectoralis major - 3, triceps brachii - 4, iliopsoas and gluteus maximus – 3, gluteus medius - 3, quadriceps femoris - 4, hamstrings - 3 and gastrocnemius - 4. Gait of the patient is shown in the Supplementary Video 1.

Investigations showed an elevated creatine kinase (CK) level of 5750 U/L. Fundus showed a greyish hue of the retina bilaterally, shunt vessels on the optic disc on right side, and mild retinal edema inferior to optic disc on the right (Figure 2e). An echocardiogram of heart showed global hypokinesia and dilatation of the left ventricle with moderate systolic dysfunction, moderate mitral regurgitation, and an ejection fraction of 44% (Figure 2d).

Patient 2

This younger sibling of Patient 1 was an 11-year-old boy with progressive limb girdle weakness, predominantly of lower limbs, since early childhood. He had delayed motor milestones and could never run fast. At age seven, he had blurred vision and decreased visual acuity due to cataracts which were operated. Examination showed low-set ears, tendoachilles contractures, calf hypertrophy (Figure-2 b, c), and a waddling gait. The muscle power according to modified MRC were: deltoid - 4, biceps brachi - 3, triceps - 4, iliopsoas and gluteus maximus - 2, quadriceps femoris - 4, and hamstrings - 3. Distal muscle power was normal. The gait of the patient is shown in Supplementary Video 2. Investigations showed an elevated CK level of 4320 U/L. The electrocardiogram and echocardiogram were normal.

Based on the above clinical and investigation findings, a recessive form of LGMD was suspected, and exome sequencing was performed to confirm the diagnosis.

Genetic testing of Patient 1 and Patient 2

Clinical exome sequencing revealed a homozygous pathogenic missense variant [NM_021971.4 (*GMPPB*)] : c.358A>G (p.Met120Val) in exon 4 of *GMPPB* gene. The p.Met120Val variant is novel (not in any individuals) in 1kG All, gnomAD as well as in our in-house database (consisting of data from 7718 chromosomes). The gene *GMPPB* has a low rate of benign missense variation as indicated by a high missense variants Z-Score of 1.13. The gene *GMPPB* contains 19 pathogenic missense variants, indicating that missense variants are a common mechanism of disease in this gene. The p.Met120Val missense variant is predicted to be damaging by both SIFT and PolyPhen2. The methionine residue at codon 120 of *GMPPB* is conserved in all mammalian species. The nucleotide c.358 A>G in *GMPPB* is predicted to be conserved by GERP++ and PhyloP across 100 vertebrates. Sanger sequencing was done for the five family members, which showed that the two affected siblings (patients 1 and 2) are homozygous for the variant. The variant is absent in the unaffected sibling, and the parents are heterozygous carriers for the *GMPPB* variant.

Further, the clinical phenotype of the proband and the sibling matches with that of the disorder caused by pathogenic variants in the gene *GMPPB*, confirming the diagnosis of autosomal recessive

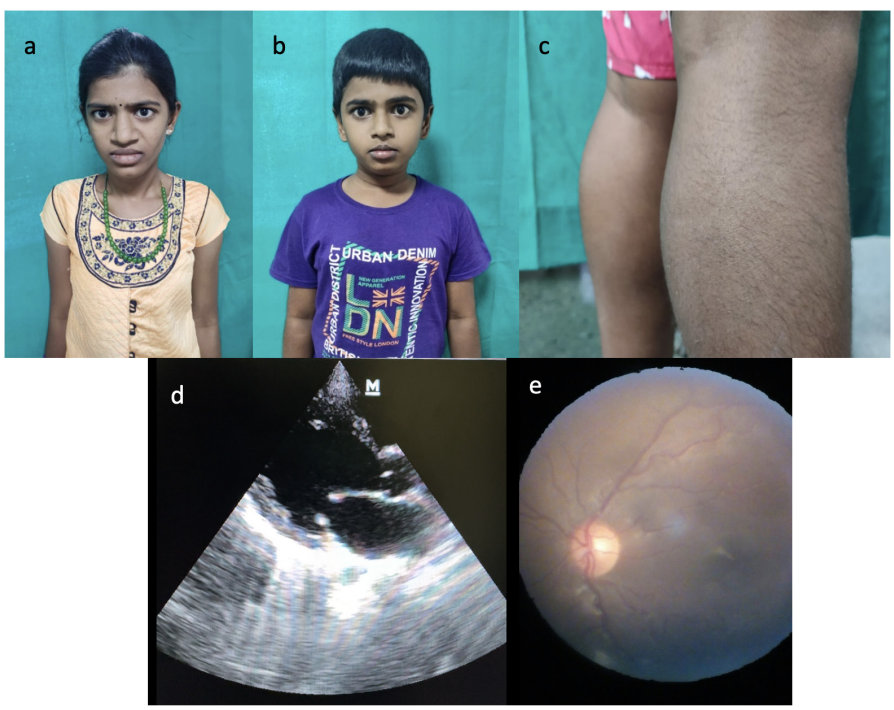

Figure 2. a. Patient 1, b. Patient 2, c. calf hypertrophy in Patient 2, d. echocardiogram of Patient 1 showing enlargement of left ventricle and atrium, e. Fundus image of Patient 1 showing greyish hue of the retina with shunt vessels on the optic disc and mild retinal edema inferior to optic disc on the right.

LGMDR19. For these reasons, this variant has been classified as pathogenic by ClinVar (Accession: SCV003194727).

Though repetitive nerve stimulation (RNS) did not show any decremental response, both siblings were started on pyridostigmine 60 mg (3 tablets per day for girl child and 2 tablets per day for the boy) and Salbutamol 2mg per day twice daily for 2 years. In the follow-up during January 2023, there was a distinct improvement in the motor capabilities. A subjective improvement of 20-30% with the ability to climb stairs and get onto a bicycle by self was noted. The speed of walking had improved, and fatigue had reduced. There was moderate improvement in the girl and mild improvement in the boy. Telephonic follow up was done in December 2023. Patient 1 had expired of cardiac failure while Patient 2 was maintaining his improvement.

DISCUSSION

In this report, we describe two siblings born to consanguineous parents with *GMPPB* pathogenic variant related to early onset with variable phenotypes. They had an early onset progressive limb-girdle syndrome, cardiomyopathy (elder child), cataracts, and elevated CK levels. It is important to identify these LGMD phenotypes with neuromuscular junction impairment as these patients partially respond to myasthenic medications. GMPPB is an enzyme that catalyzes GDP-mannose synthesis, required for several glycosylation processes such as -DG O-mannosylation in most human tissues, notably the muscle and brain.[4] A subset of dystroglycanopathy, also known as secondary dystroglycanopathy, is related to pathogenic variants in genes involved in the glycosylation of α-dystroglycan (α-DG), which is a peripheral membrane protein and a component of dystrophin-associated glycoprotein complex.[4,8]

Pathogenic variants in more than 15 genes are associated with secondary dystroglycanopathy.[9] The majority of the pathogenic variants were first identified in individuals with congenital muscular dystrophies, which damage not only

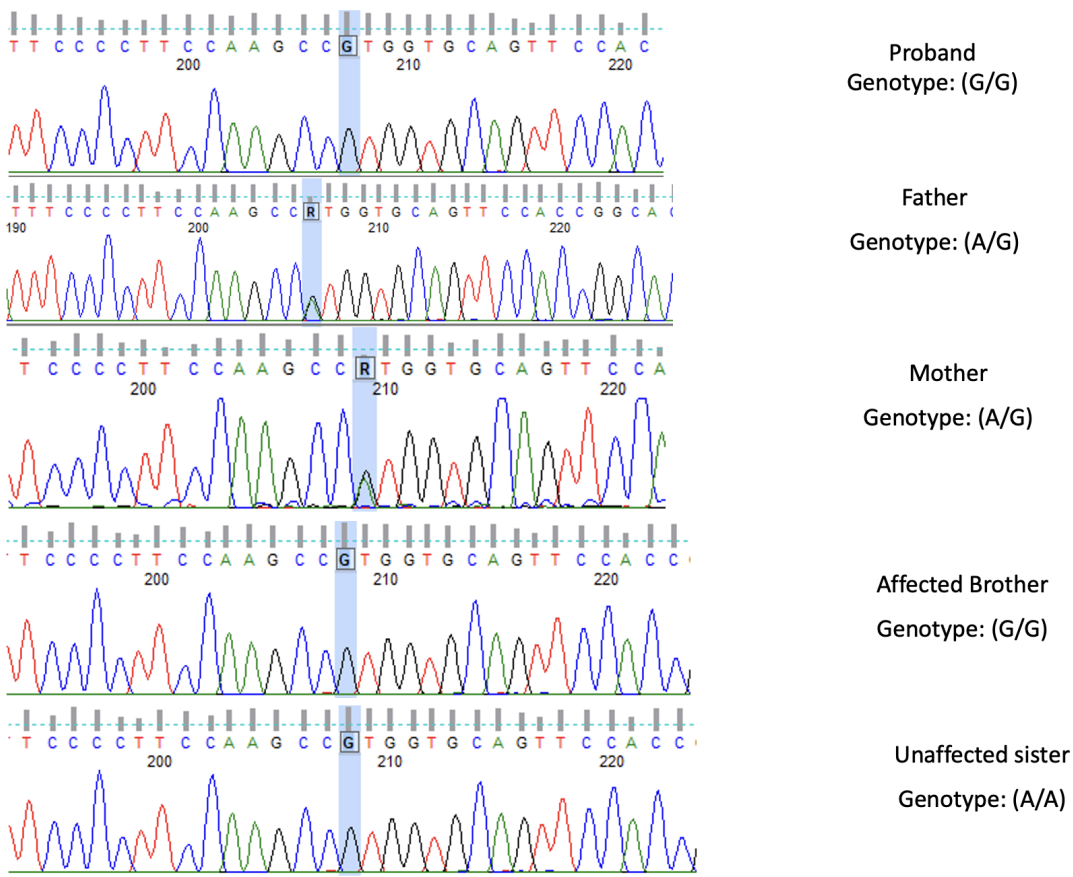

Figure 3. Spectropherogram of the Sanger sequencing.

muscle but are also connected with eye and brain abnormalities and have onset by the age of 6 months, comparable to our patients, who had progressive limb girdle weakness beginning in early childhood.[9] The neurological phenotype, along with the complicated phenotypes of the muscle defects, is observed as early as less than 10 years of age.[10] The two most important indicators of dystroglycanopathy are persistently elevated CK and decreased immunostaining for DG in muscle biopsies.[6] Additionally, some individuals show decremental response of compound muscle action potential (CMAP) on repetitive nerve stimulation.[6]

LGMDR19 is the most prevalent phenotype of *GMPPB* pathogenic variant, followed by CMS.[9] According to Belaya et al., certain patients with *GMPPB* pathogenic variants have CMS.[7] The most common manifestations of *GMPPB* gene pathogenic variants are intellectual disability, hypotonia, cataract, epilepsy, and cardiomyopathy.[4,9] In the current report, both siblings had bilateral cataracts, hypotonia, elevated CK, and cardiomyopathy in the girl, similar to the previous studies but did not have epilepsy and/or intellectual disability. According to one study, the pathogenesis for multisystem involvement is *GMPPB* was responsible for muscle and neural development in zebrafish, and embryos with depleted *GMPPB* demonstrated significant motor dysfunction.[11] Pathogenic variants in *GMPPB* cause CMS with brain and ocular problems at the severe end of the disease range. At the mild end of the continuum, *GMPPB* pathogenic variants cause LGMD phenotype.[7] The homozygous variants such as c.553C > T, c.308C > T, and c.810_813delinsTGGC have been reported from Mexican, Egyptian, and Iranian ancestries, respectively. The variant c.79G>C is the most common pathogenic variant among the compound heterozygous variants (49 distinct pathogenic variants), followed by c.860G > A[17] and c.859C>T(10).[10] The common variant c.79G >C causes early-onset LGMD with or without intellectual impairment or epilepsy.[12-13] Both our patients presented with a homozygous pathogenic c.358A>G (p.Met120Val) variant in exon 4 of the *GMPPB* gene, which was first reported by the University of Wuerzburg in September 2018.[14]

Thus, we describe a novel mutation in *GMPPB* gene in two siblings who presented with delayed milestones, limb-girdle fatigable weakness with cataracts, and cardiomyopathy in the elder sibling. This expands the phenotypic spectrum of a novel pathogenic variant in *GMPPB*. Genetic confirmation of LGMD is required since GMPPB-related LGMD shows a response to treatment with myasthenic medications.

DISCLOSURE

Data availability: Anonymized data id available with the corresponding author on request.

Sources of support: None

Conflicts of interest: None

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