Brief Report

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Reporting one very rare pathogenic variation c.1106G>A in *POMT2* gene

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SUMMARY Dystroglycan (DG) is a major cell membrane glycoprotein, which is encoded by the *DAG1* gene. α-DG is one of DG subunits, belongs to O-mannosylated protein of mammals and was identified in brain, peripheral nerves and muscle. Dystroglycanopathies are a group of heterogeneous congenital muscular dystrophies, which can result from defective α-DG mannosylation. First line of α-DG glycosylation is catalyzed by protein O-mannosyltransferase family (PMT). In this study, the mutation was identified in the *POMT2* gene, which encodes O-mannosyltransferase 2 protein and its mutations can be contributed to dystroglycanopathies. A very rare missense mutation in the *POMT2* gene (NM_013382: exon9: c. 1106G>A) was identified by next generation sequencing (NGS) and was subsequently confirmed using Sanger sequencing in both affected siblings. There was no report of this mutation in the literature, therefore, the significance was uncertain. Our findings confirmed the pathogenicity of mutation and expanded the mutation spectrum of *POMT2*, which will be helpful in further molecular evaluations of muscular diseases.

Keywords dystroglycanopathy, alpha-dystroglycan, rare mutation, *POMT2*

1. Introduction

Protein glycosylation is a complex process which takes place in the ER and Golgi apparatus. This post translational process maintains main protein features such as stability, conformation and function. Glycans attach to protein in two distinctive sites: N-glycans are attached to asparagine residues and O-glycans to threonine and serine residues of the protein. These glycosylated proteins can be distinguished by the sugar subunits, such as mannose, N-acetylgalactosamine and fucose (*1*).

Dystroglycan (DG) is a major cell membrane glycoprotein that links cytoskeleton of muscles and nerve cells to the extracellular matrix (ECM) and plays a major role in dystrophin associated protein complex. *DAG1* gene encodes DG which is cleaved into two subunits, α-DG and β-DG, as a post translational proteolytic process. α-DG is a kind of O-mannosylated cell surface protein in mammals, which was identified in brain, peripheral nerves and muscle. Protein O-mannosylation is a vital complex process and conserved from bacteria to humans but not found in plants or nematodes. It is an essential protein modification for central nervous system (CNS) development and function. Defective α-DG mannosylation results in congenital muscular dystrophy (CMD) with CNS malfunction, generally named dystroglycanopathies (*2,3*).

Dystroglycanopathies are a group of heterogeneous CMD, often characterized by variable neurological and ocular involvement and are inherited as an autosomal recessive pattern. The relevant genes are *POMT1, POMT2, POMGNT1, LARGE, GTDC2, B4GAT1, B3GALNT2, DPM(1,2,3), DOLK, POMK, GMPPB, FKTN, FKRP, ISPD, TMEM5* and *DAG1* that all encode a series of enzymes involved in glycosylation of α-DG (*4,5*).

Hypoglycosylation of α-DG can lead to dystroglycanopathies including progressive muscular dystrophy and eye involvement. First line of α-DG glycosylation is catalyzed by protein O-mannosyltransferase family (PMT), O-mannosyltransferase 2 protein and its homolog O-mannosyltransferase 1. PMTs were originally discovered in yeast and tend to be evolutionarily conserved. Co-expression of *POMT1* and *POMT2* enzymes is essential for sufficient enzymatic activity.

O-mannosyltransferase 2 protein, an integral ER membrane protein, is encoded by *POMT2* gene on chromosome 14 (14q24.3). This gene expands to 46 kb of the entire genome and has 21 exons (*2,6,7*).

POMT2 mutations can result in three distinct forms of muscular dystrophy-dystroglycanopathies, including severe congenital muscular dystrophy with brain and eye involvement (type A2, formerly named Walker-Warburg Syndrome (WWS) or muscle-eye-brain disease, MIM number# 613150), a less severe congenital muscular dystrophy with mental retardation (type B2, MIM number# 613156) and mild adult onset limb-girdle muscular dystrophy (type C2, MIM number# 613158) (*8*).

2. Materials and Methods

The patient was 10-year-old presented with poor growth, microcephaly, mental retardation, muscle weakness, contractures and movement and speech impairment. She had an older sister (11-year and 6-month- old) presenting with similar symptoms. Both children were the product of full term C/S delivery with normal birth weight, head circumference and normal APGAR scores.

Brain computerized tomography (CT) findings included periventricular calcification foci, ventricular dilation, cerebral atrophy and microcephaly in patient I (Figure1A, 1B).

Other physical examination indicated low head circumference in both cases (42 cm, < 2.5 percentile in younger and 43 cm, < 5 percentile in older sister). Face muscle weakness with severe drooling was obvious. They showed stiff neck, scoliosis and chest deformity (Figure 1C) and additionally, the younger sister had a sign of hip dislocation. Ophthalmologic studies showed astigmatism in both siblings, while the younger one had strabismus eyes with normal vision. The viral markers of both cases were negative. Elevated serum creatine kinase and seizures were reported in both cases.

Whole exome sequencing (WES) was performed on younger affected sib in order to capture, enrich and sequence all exons of protein coding genes in addition to other critical genomic segments. Next generation sequencing was carried out using illumine Hiseq 2000 machine to sequence 100 million reads approximately and standard illumine protocol for paired-end 99 nucleotide sequencing. The test platform assessed > 95% of the target regions with sensitivity of above 99%.

The next generation sequencing (NGS) data was analyzed using various online Bioinformatics tools. WES reads was aligned against human genome using BWA aligner and genome variants were identified by GATK, which were annotated with the use of ANNOVAR software. In order to confirm pathogenic variants, standard bioinformatics software such as CADD-Phred, SIFT, Polyphen, Phastcons, Mutation Assessor, I-Mutant 2.0, and Mutation taster were used.

The novel pathogenic variant should be confirmed by Sanger sequencing in the next step of investigation. Whole blood samples of all family members were collected in EDTA tubes to extract DNA using QIAamp DNA blood Mini kit (Germany) according to protocol. The genomic DNA concentration was assayed by NanoDrop One (Thermoscientific, USA) and stored at -20°C until use.

Polymerase chain reaction (PCR) and Sanger sequencing were then performed on patients, non-affected sister, parents and one of their cousins, in

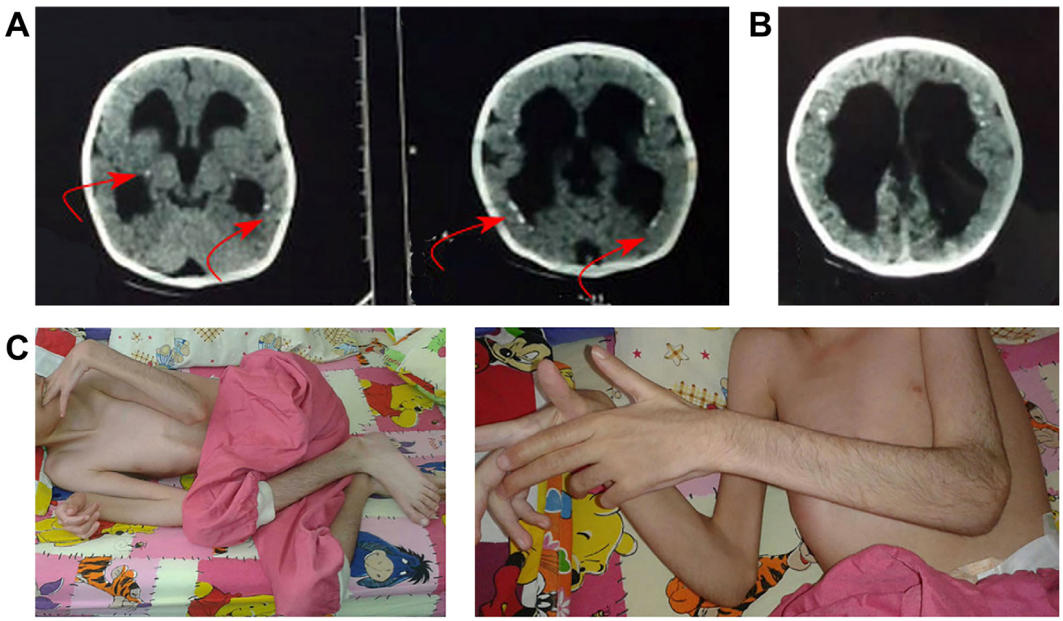

Figure 1. Brain CT scan. (A) Numerous periventricular calcified nodules (red arrows). **(B)** Periventricular Dilation. **(C)** Clinical presentation: The proband was 10 years old with poor growth, contractures, movement impairment, severe drooling, scoliosis and chest deformity.

both directions on the resulting PCR products using ABI BigDye terminator cycle sequencing kit (Applied Biosystems®, USA).

3. Results and Discussion

NGS data analysis of younger patient revealed one extremely rare deleterious homozygous missense mutation in *POMT2* gene (NM_013382: exon9: c.1106G>A: p.R369H).

Mutations of *POMT2* gene are shown to cause dystroglycanopathies with various organ involvements and severity. The mutation was confirmed using Sanger sequencing. Sanger confirmation revealed two mutant homozygous alleles in two affected siblings and two wild type alleles in healthy sib. Their parents and cousin were heterozygous carriers of mentioned mutation. Therefore, the inheritance pattern must be autosomal recessive and the mutation is segregated in this family (Figure 2).

According to mutation taster results, this variation is disease causing leading to a single base exchange. 1106 G>A nucleotide alteration affects amino acids sequence (The replacement of Arg by His) (*9*). Conservation analysis of this amino acid was evaluated using comparative multiple amino acid sequence alignment of POMT2 protein across various animal kingdoms and revealed a high level of Arginine conservation during evolution (Figure 3A). Mutation taster uses Phastcons values to determine the grade of nucleotide conservation. Phastcons is a program for evaluating conserved elements by multiple alignment of genome sequence of 46 different species. The scores closer to 1 reflect a higher conservation of the nucleotide. In this case Phastcon score was 0.998, reflecting strong nucleotide conservation.

I-Mutant 2.0 is a tool to predict protein stability changes upon various point mutations. As figure 3b shows protein stability will be decreased upon replacement of Arginine 369 with Histidine. The result also shows the effect of other amino acid substitutions in the 369 residue (Figure 3B).

STRING online software v11.0 shows *POMT2* gene in close co-operation with various partners, which have critical functions in glycosylation of α-DG (Figure 4).

O-Man glycosylation pathway was originally discovered in yeast. Mammalian homologs were later recognized as POMT1/POMT2. Co-expression of these two multi-pass transmembrane enzymes is essential for the catalysis of the first step in O-Man glycosylation of α-DG. Dolichol phosphate-β-D-mannose (Dol-P-Man) is used as a donor substrate to catalyze the attachment of α-linked mannose to serine and threonine residues in ER lumen and further elongation in the Golgi apparatus. This process is critical for accurate structure and function of α-DG (*2*).

Yoshida *et al*, identified muscle-eye-brain disease with defective O-mannosyl glycan in 2001 and introduced a novel pathological pathway for muscular dystrophy and neuronal migration disorders (*10*). Investigations have shown that mammalian POMT2 is an ER integral membrane protein and is expressed in all tissues but predominantly in testis (*11*).

POMT2 mutations were first identified in severe

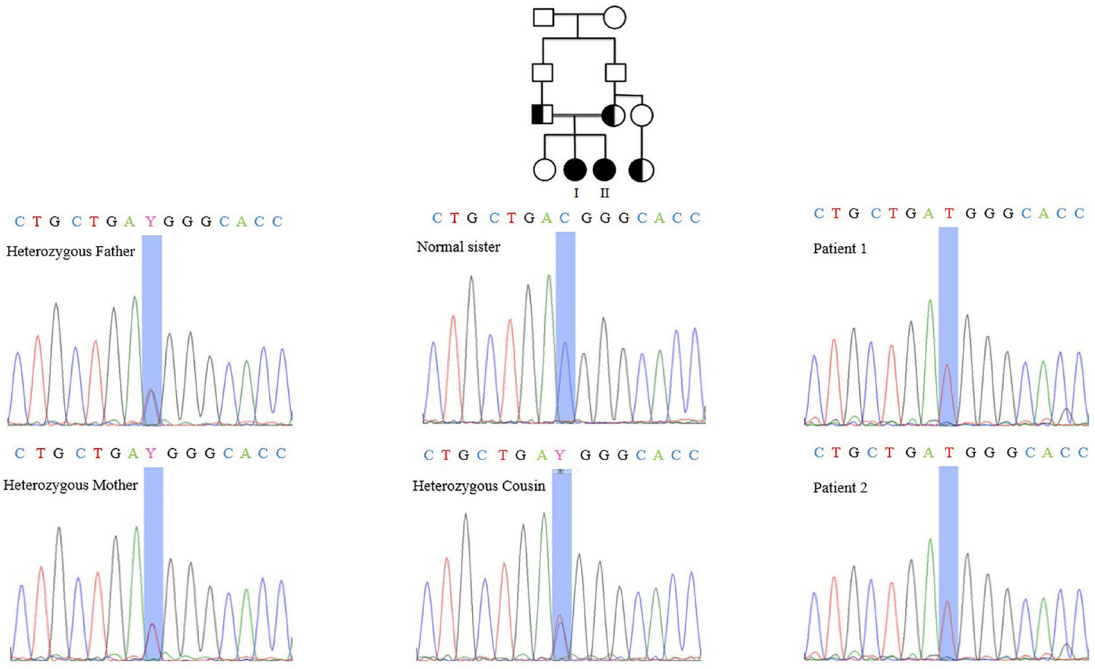

Figure 2. Family pedigree and Sanger sequencing chromatogram. Both Parents and cousin are heterozygous for c.1106G>A. Non-affected sister and two affected sisters are homozygous for wild type and mutant alleles, respectively. The autosomal recessive pattern of inheritance seems obvious in the pedigree.

Figure 3. (A) Comparative amino acid sequence alignment of POMT2 protein across various animal kingdoms shows the conservation of arginine amino acid during evolution. The conserved residue is marked in the rectangle. (B) I-Mutant V2.0 analysis of protein stability revealed the reduction of POMT2 protein stability upon the substitution of Arg 369 with His. This program also predicted the effect of Arg 369 substitution with all other amino acids on protein stability. As is clear in the picture, most of the alterations can reduce protein stability.

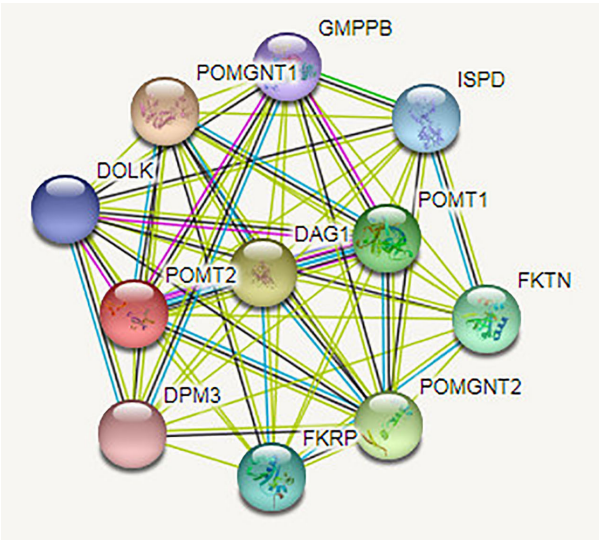

Figure 4. STRING online software v11.0 shows the close interactions of various proteins in the process of α-DG glycosylation. All mentioned genes play a role in coding the series of enzymes involved in glycosylation of α-DG.

WWS (currently labelled as severe congenital muscular dystrophy with brain and eye involvement). However, various clinical severities of dystroglycanopathies from severe congenital muscular dystrophy to mild adult onset limb-girdle muscular dystrophy have been reported so far.

Dystroglycanopathies are an autosomal recessive group of muscular dystrophy, in which functional glycosylations of α-DG decline or are completely absent. α-DG, a critical extracellular component of dystrophin-glycoprotein, is glycosylated by a series of proteins and disruption of each step will interfere with protein function and the attachment to ECM ligands such as laminin, neuexin and agrin (*12,13*).

Here, a novel rare mutation of *POMT2* was identified in a family with two affected siblings (NM_013382: exon9: c. 1106G>A: p.R369H). Sanger sequencing was performed on all family members to confirm NGS data results. The mentioned mutation was confirmed in the family as an autosomal recessive pattern of inheritance. The homozygous A mutant allele in two patients, the homozygous G wild-type allele in normal sib, and the heterozygous G/A alleles in their parents and cousin were confirmed.

c.1106G>A single nucleotide variant was reported in dbSNP with reference SNP: rs398124260 and T allele global minor allele frequency is 0.0000 on average. It is classified as an unknown significant variant and no patients have been identified for this variant so far.

Using STRING software V11.0 to identify *POMT2* functional partner, revealed its interaction with all O-mannosylation biosynthesis genes. Defective function of each enzyme due to gene mutation can lead to distinct forms of dystroglycanopathies with a broad spectrum of clinical phenotypes that range from severe congenital muscular dystrophy with brain and eye

involvement to a benign limb girdle form. In the current study, Muscular and CNS abnormalities in addition to ocular involvement were apparent in two patients, early in life. These characteristics help us to classify mentioned patients in type A2 of muscular dystrophy-dystroglycanopathy (severe congenital muscular dystrophy with brain and eye involvement).

Homozygous c.1106G>A mutation in two affected sibs leads to substitution of the evolutionary highly conserved arginine 369 with a histidine and various bioinformatics analysis provide evidence of pathogenicity for the mentioned mutation. This amino acid substitution (Arg369His) is located at the highly conserved domains mannosyl-IP3R-RyR (MIR), which encompasses position 334-514 in the protein. This domain was first identified in three proteins, mannosyltransferase, Inositol 1,4,5-trisphosphate receptor (IP3R) and Ryanodine receptor (RyR), and so named MIR. It had been reported in fungi that a hydrophilic region of PMT facing the ER lumen played an important role in protein function and it encompassed the triplet of the MIR domain. However, the exact functions of MIR domain have not been completely identified (*14,15*). Two pathogenic missense mutations (p.V373F and p.G353S) were reported in the vicinity of the current mentioned mutation (R369H), which are located in MIR domain (*8,16*). On the basis of this evidence it can be concluded that this mutation is pathogenic in the mentioned family.

In conclusion, this study revealed the pathogenicity of a single nucleotide variant in *POMT2* gene (rs398124260), which expands the knowledge of pathogenic mutations of *POMT2* to identify the underlying cause of muscle problems and such studies can be useful in performing genetic counseling and prenatal diagnosis accurately.

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