Discovery of a new c.1834dup variant in a boy individual presenting with the POMGNT1-associated muscular dystrophy-dystroglycanopathy

Hamed Esmaeil Lashgarian [1], Hamidreza Khodadadi [2*], Masumeh Jalalvand [3], Maryam Zand [4], Amirmasoud Jalalvand[5], Leila Abkhooie [3]

1. Associate Professor, Department of Medical Biotechnology, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
2. Assistant Professor, Hepatitis Research Center, Department of Medical Biotechnology, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
3. Assistant Professor, Department of Medical Biotechnology, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
4. Department of Biotechnology and Molecular Medicine, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran.
5. Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

ABSTRACT

Muscular dystrophy-dystroglycanopathy refers to a group of autosomal recessive neurodegenerative disorders resulting from homozygous or compound heterozygous mutations in the gene encoding POMGNT1 O-mannose β-1,2-N-acetylglucosaminyltransferase. The clinical presentation of this form of muscular dystrophy typically includes early-onset muscle weakness, gait ataxia, microcephaly, and growth delay. A case study was conducted on an 8-year-old Iranian male displaying symptoms such as microcephaly, seizures, hydrocephalus, cerebellar abnormalities, glaucoma, growth delay, and lissencephaly. The parents of the affected child were found to be heterozygous for the POMGNT1 gene. Within the scope of this investigation, a novel duplication (dup1834c) was identified in exon 21 of the POMGNT1 gene. The presence of dup1834c in the POMGNT1 gene was verified through Sanger sequencing in the affected individual and other family members affected by the disease.

Keywords: *Muscular dystrophy-dystroglycanopathy, POMGNT1, Next generation sequencing (NGS)*

1. INTRODUCTION

Muscular dystrophies encompass a diverse group of genetic disorders characterized by progressive muscle weakness due to skeletal muscle destruction (1). Various muscle disorders have been categorized depending on the age at which symptoms first appear, the seriousness of the condition, and which muscles are affected. Duchenne and Becker muscular dystrophies, for example, are genetic conditions that share a common gene but have different levels of severity due to distinct mutations, resulting in complete loss of a protein in Duchenne muscular dystrophy and a less severe deficiency in Becker muscular dystrophy (2).

Furthermore Myotonic dystrophy (DM) is the most common form of adult muscular dystrophy, consisting of two primary subtypes: DM1 and DM2, both of which are autosomal dominant conditions characterized by myotonia, progressive muscle weakness, and cardiac irregularities. DM1 is associated with expansions of CTG repeats in the DMPK gene, whereas DM2 is linked to expansions of CCTG repeats in the CNBP gene (3-5). Autosomal-dominant Facioscapulohumeral muscular dystrophy (FSHD) represents the third most prevalent muscular dystrophy, characterized by progressive muscle weakness that predominantly impacts the facial, scapular, and upper arm muscles, with potential ramifications on additional muscle groups such as the lower limbs and torso. FSHD typically emerges during adolescence, although occurrences during infancy, albeit less frequent, have also been documented. The etiology of this condition stems from genetic processes that entail epigenetic de-repression of the DUX4 gene, culminating in muscular debilitation and impairment (6-8).

Alpha- and beta-dystroglycans are involved in the formation of a membrane complex that mediates the binding between the extracellular matrix (ECM) and the cytoskeleton. Recent years have seen a growth in understanding through biochemical and genetic research regarding the significance of post-translational modifications (PTM) in the functionality of dystroglycan. Among these crucial modifications, glycosylation stands out. When dystroglycan is glycosylated, it functions as a component of the extracellular receptor; however, mutations in various genes can lead to deficiencies in dystroglycan glycosylation. Consequently, such deficiencies contribute to the manifestation of various genetic disorders (9).

Mutations in genes such as POMT1, POMT2, FKRP, FKTN, LARGE, and POMGNT1 have been implicated in dystroglycanopathies, a collection of congenital muscular dystrophies marked by deficient glycosylation of alpha-dystroglycan (10). Particularly, genetic alterations in the POMGNT1 gene have been associated with autosomal recessive retinitis pigmentosa-76 (RP76). These genetic variations have the potential to result in early onset muscle-eye-brain disease and non-syndromic retinitis pigmentosa, broadening the clinical spectrum of these hereditary conditions (11). In this study, we introduce a supplementary case featuring a homozygous novel variant located in the POMGNT1 gene.

2. Methods

2.1. Case presentation

In this study, the affected person is an 8-year-old Iranian boy who referred to the Maternal Medical Genetics Center in Lorestan province with symptoms of microcephaly, seizures, hydrocephalus and cerebellar abnormalities, glaucoma and growth delay, lissencephaly for WES test data analysis. The proband also had parents and a healthy 14-year-old brother, for a more accurate diagnosis, blood samples were taken from the parents and brother, and the DNA sample obtained from their blood was analyzed by Sanger sequencing.

2.2. Next generation sequencing (NGS) and analysis

Following the blood test, genomic DNA was isolated and measured from the peripheral blood sample through a salting out technique. A total of 0.1 microgram of genomic DNA was employed as the starting material for DNA sample processing in each sample. PCR sequencing was executed within our standard procedure to assess genetic abnormalities in impacted individuals. Generation of sequencing libraries was conducted utilizing the Agilent SureSelect Human All ExonV7 kit (Agilent Technologies, CA, USA) following the manufacturer's guidelines, with x-index codes allocated for distinguishing sequences from individual samples. Initially, genomic DNA underwent fragmentation employing a hydrodynamic shear system (Quaris, Massachusetts, USA) to yield fragments ranging from 180 to 280 bp. Subsequent to this, any remaining overhangs were subjected to modifications facilitated by exonucleases and polymerases, succeeded by end-blunting enzymes. Post adenylation of the 3' termini of DNA fragments, adapter oligonucleotides were affixed. DNA fragments possessing ligation adapters at both termini were selectively amplified in a PCR process. Enriched libraries underwent additional amplification in a PCR step for the incorporation of marker tags in readiness for hybridization. The resultant products were purified utilizing the AMPure XP system (Beckman Coulter, Beverly, USA) and assessed for quantity via the Agilent High Sensitivity DNA Assay on an Agilent Bioanalyzer 2100 system. Subsequently, validated libraries were loaded onto Illumina NovaSeq 6000 sequencers. Upon completion of sequencing, data quality control, analysis, and interpretation were executed on a G9 generation HP server operating on a Unix-based system. The whole genome sequencing outcomes were assessed. Then POMGNT1 protein was modeled by using homology method Phyer-2 web server. The energy optimization was carried out by UCSF Chimera software, and mutant structures were created using the Mutation tester. Sanger sequencing validated the pathogenic variants of POMGNT1 as detected through exome sequencing. The BigDye Terminator Cycle Sequencing Kit v3.1 from Thermo Fisher Scientific was utilized for the fluorescent labeling of amplicons. Subsequently, Sanger sequencing was conducted on the ABI PRISM 3500 Genetic Analyzer by Applied Biosystems following the manufacturer's instructions.

3. Results

The acquisition and examination of sequencing data pertaining to the POMGNT1 gene within the patient case were successfully executed. Consequently, the homozygous presence of the pathogenic variant dup1834c in

exon 21 of the POMGNT1 gene was detected in the patient's DNA sample. For the parents and brother of the proband, Whole Exome Sequencing revealed that all three individuals are carriers of the same pathogenic variant in exon 21, displaying heterozygosity for the POMGNT1 gene (Fig 1).

This specific variation has not been previously observed in cases of muscular dystrophydystroglycanopathy, thereby signifying a novel mutation elucidated by this investigation. Significantly, this variant was not cataloged in either public databases or our internal repository, such as ESP, ExAC, 1000Genome project, and Iranome databases. Authentication through Sanger sequencing, the gold standard methodology, confirmed the presence of this variant, which was segregated within the affected family. Consequently, the homozygous dup1834c mutation within the POMGNT1 gene appears to be the primary pathogenic element in the dystrophy patient under examination.

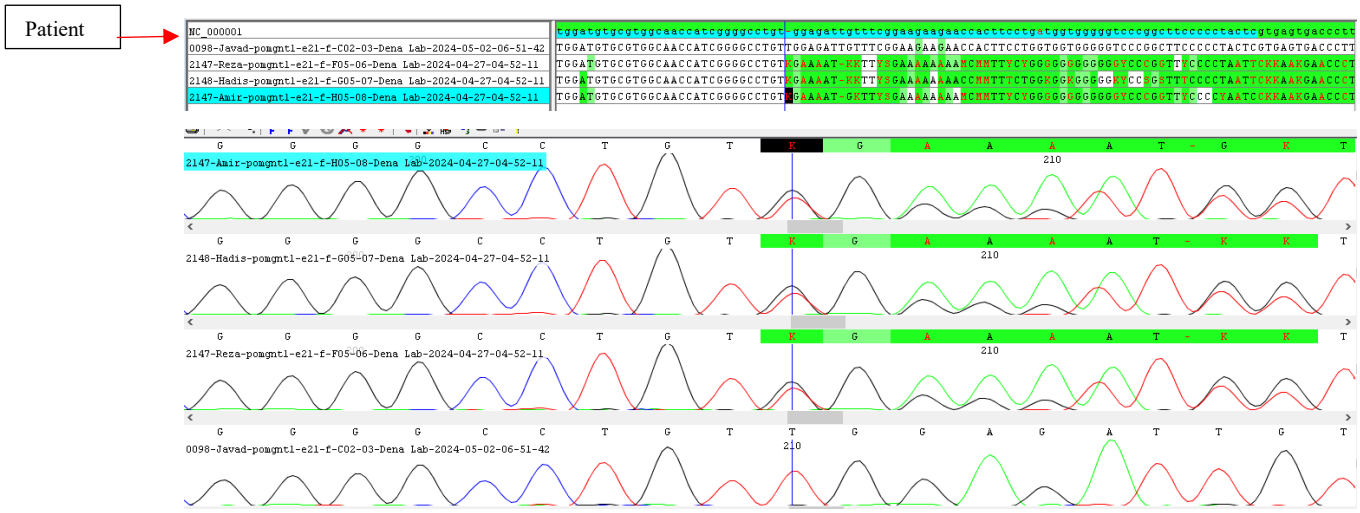

Fig 1: Sanger DNA sequencing of POMGNT1gene. A novel missense mutation (c.1834dup) in exon 21.

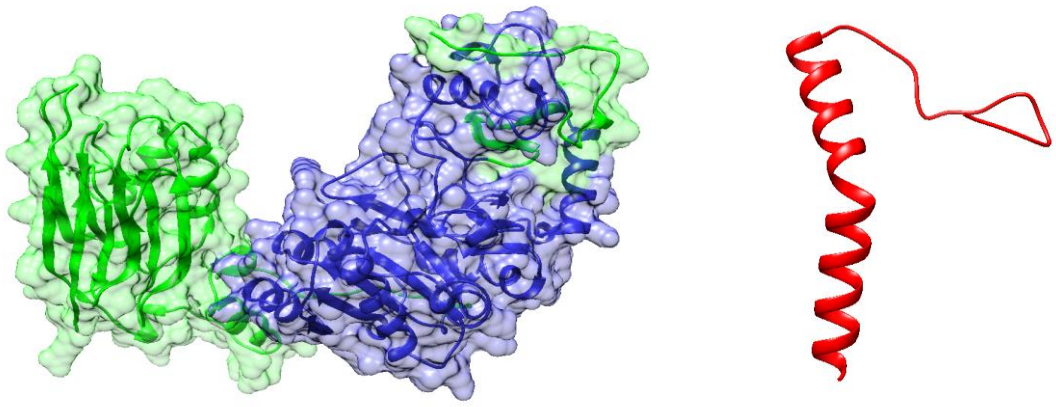

Fig 2: POMGNT1 protein was modeled by using homology method Phyer-2 web server. The energy optimization was performed by UCSF Chimera software. The Mutation tester was used to create mutant structures.

4. Discussion

α-Dystroglycan (α-DG) is of paramount importance in establishing a connection between the basement membrane and the cytoskeleton, a process that is fundamental for both neurological development and muscle integrity. The appropriate glycosylation of α-DG is indispensable for upholding its structural and functional soundness within the brain and skeletal muscles. The linkage of α-DG to extracellular matrix constituents such as agrin, perlecan, neurexin, and laminin heavily relies on O-mannosyl glycans, specifically the cores M1, M2, and M3. Within the endoplasmic reticulum, the O-mannosylation mechanism, facilitated by POMT1 and POMT2, is pivotal in the creation of these crucial glycan configurations that play a vital role in enabling α-DG to maintain tissue integrity and facilitate the transmission of extracellular signals (12-15). During the transference of O-mannosylated α-DG to the Golgi apparatus, the enzymatic domain of POMGNT1 triggers the commencement of GlcNAc transference to the O-mannose, resulting in the development of cores M1 and M2 (16). The recognition of cores M1 and M3 by the carbohydrate-binding function of the stem domain enhances the bonding capacity between the enzymatic domain and α-DG, thus facilitating the glycosylation of the adjacent O-mannosyl residue in the vicinity of the core M1 region (15). Core M3, a vital component synthesized by POMGNT2, B3GALNT2, and POMK in the endoplasmic reticulum, undergoes further alteration by LARGE in the Golgi apparatus, emphasizing a harmonized enzymatic process for the creation of distinct O-mannosyl glycan structures on α-DG (16, 17). Fukutin (FKTN) functions as a ribitol 5-phosphate transferase that plays a role in essential glycosylation mechanisms crucial for the functioning of α-dystroglycan (α-DG). It engages in a molecular complex with POMGNT1, which is necessary for the glycosylation process of core M3 facilitated by various proteins, thereby influencing the glycosylation of α-DG. Genetic alterations in FKTN and fukutin-related protein (FKRP) are associated with various types of muscular dystrophies, such as Walker–Warburg syndrome (WWS), muscle–eye–brain disease (MEB), and different forms of limb–girdle muscular dystrophies. The reduced glycosylation of α-DG resulting from these mutations is the underlying cause of the pathogenesis of these autosomal recessive muscular dystrophy-dystroglycanopathy (MDDG) conditions, emphasizing the significant contribution of FKTN in the glycosylation processes linked to severe congenital muscular dystrophies characterized by brain and eye abnormalities, cognitive impairment, and less severe limb–girdle forms (12, 18-21). Situated on chromosome 1p34.1, the POMGNT1 gene (NM_017739.4) encodes the protein O-mannose beta-1,2-N-acetylglucosaminyl, known for its significant expression in the brain, nerve, and skeletal muscle tissues [33]. A total of 7666 SNPs and 10 transcripts, encompassing 60 exons on the reverse strand, have been documented for this gene (https://www. ebi.ac.uk). The POMGNT1 protein (UniProtKB—Q8WZA1) functions as a glycosyltransferase featuring a catalytic domain (residues 300–646) that interacts with O-mannosylated proteins as substrates through hydrophobic interactions. Furthermore, it possesses a stem domain (residues 92–250) that binds to the O-mannosyl glycan via hydrogen bonds with Arg129, Asp179, and Arg207. Alterations in these critical amino acids can impede the activity of the protein's membrane-bound form. Notably, Arg129 displays high conservation across mammalian species, possibly reflecting its essential function (22, 23). Mutations in the POMGNT1 gene, whether homozygous or compound heterozygous, have been identified as the causative factor

for the hypoglycosylation of α-DG. Currently, a total of 92 mutations within the POMGNT1 gene have been documented in the Human Gene Mutation Database (HGMD) available at https://www.hgmd.cf.ac.uk/ac/gene.php?gene=POMGNT1. Within the context of this research endeavor, an 8-year-old boy from Iran was outlined as a case study, showcasing a homozygous novel dup1834c p.Trp612LeufsTer75 variant located in the carbohydrate-binding catalytic domain of the POMGNT1 gene. In this investigation, the study delineated the significance of Whole Exome Sequencing (WES) in the detection of a new found variant responsible for muscular dystrophy. The discovery of novel genes or variants is imperative for genetic counseling, tailored healthcare, prenatal screening, and preimplantation genetic testing (PGT). Our deduction is that the aforementioned variant [dup1834c] located in exon 21 of the POMGNT1 gene alters the functionality of the catalytic domain, impeding its interaction with the O-mannosylglycan and potentially interfering with the enzymatic function of the protein. Our findings indicated that the dup mutation in the POMGNT1 gene has created a new stop codon, leading to premature termination of the protein. The truncated protein remains unfolded and non-functional, resulting in the death of this 8-year-old boy. Nevertheless, additional research endeavors, including functional analyses and cellular models, are indispensable to authenticate this newly identified variant.

REFERENCES

[1] *Politano L, Santorelli FM. Editorial for the Genetics of Muscular Dystrophies from the Pathogenesis to Gene Therapy Special Issue. MDPI; 2023. p. 926.*
[2] *Bindoff L, Gilhus NE. The genetic basis of muscle disease. Tidsskrift for den Norske Laegeforening: Tidsskrift for Praktisk Medicin, ny Raekke. 2003;123(18):2588-92.*
[3] *Liao Q, Zhang Y, He J, Huang K. Global prevalence of myotonic dystrophy: an updated systematic review and meta-analysis. Neuroepidemiology. 2022;56(3):163-73.*
[4] *Soltanzadeh P. Myotonic dystrophies: a genetic overview. Genes. 2022;13(2):367.*
[5] *Misra AK, Nanda S, Mukherjee J, Kumar S, Agasti N. Myotonic Dystrophy Type 1–An Atypical Presentation Having Symmetric Parkinsonism and Early Proximal Muscle Involvement. Annals of Indian Academy of Neurology. 2023;26(2):201-3.*
[6] *Guruju NM, Jump V, Lemmers R, Van Der Maarel S, Liu R, Nallamilli BR, et al. Molecular diagnosis of facioscapulohumeral muscular dystrophy in patients clinically suspected of FSHD using optical genome mapping. Neurology: Genetics. 2023;9(6):e200107.*
[7] *Mul K. Facioscapulohumeral muscular dystrophy. CONTINUUM: Lifelong Learning in Neurology. 2022;28(6):1735-51.*
[8] *Fecek C, Emmady PD. Facioscapulohumeral muscular dystrophy. 2020.*
[9] *Taniguchi-Ikeda M, Morioka I, Iijima K, Toda T. Mechanistic aspects of the formation of α-dystroglycan and therapeutic research for the treatment of α-dystroglycanopathy: A review. Molecular aspects of medicine. 2016;51:115-24.*
[10] *Song D, Dai Y, Chen X, Fu X, Chang X, Wang N, et al. Genetic variations and clinical spectrum of dystroglycanopathy in a large cohort of Chinese patients. Clinical genetics. 2021;99(3):384-95.*
[11] *Patel A, Cui R, Odom JV, Leys M. Case Series on Autosomal Recessive Non-Syndromic Retinitis Pigmentosa Caused by POMGNT1 Mutations with a Report of a New Variant. Journal of Clinical Medicine. 2023;12(24):7549.*
[12] *Kondo Y, Okajima T. Inhibitory machinery for the functional glycosylation of dystroglycan. Journal of Biochemistry. 2023:mvad003-mvad.*
[13] *Okuma H, Hord JM, Chandel I, Venzke D, Anderson ME, Walimbe AS, et al. N-terminal domain on dystroglycan enables LARGE1 to extend matriglycan on α-dystroglycan and prevents muscular dystrophy. elife. 2023;12:e82811.*

[14] *Quereda C, Pastor À, Martín-Nieto J. Involvement of abnormal dystroglycan expression and matriglycan levels in cancer pathogenesis. Cancer Cell International. 2022;22(1):395.*

[15] *Villones Jr LL, Ludwig A-K, Kumeta H, Kikuchi S, Ochi R, Aizawa T, et al. Exploring the In situ pairing of human galectins toward synthetic O-mannosylated core M1 glycopeptides of α-dystroglycan. Scientific reports. 2022;12(1):17800.*

[16] *Imae R, Kuwabara N, Manya H, Tanaka T, Tsuyuguchi M, Mizuno M, et al. The structure of POMGNT2 provides new insights into the mechanism to determine the functional O-mannosylation site on α-dystroglycan. Genes to Cells. 2021;26(7):485-94.*

[17] *Povolo L, Tian W, Vakhrushev SY, Halim A. Global view of domain-specific O-linked mannose glycosylation in glycoengineered cells. Molecular & Cellular Proteomics. 2024:100796.*

[18] *Gaertner A, Burr L, Klauke B, Brodehl A, Laser KT, Klingel K, et al. Compound Heterozygous FKTN Variants in a Patient with Dilated Cardiomyopathy Led to an Aberrant α-Dystroglycan Pattern. International Journal of Molecular Sciences. 2022;23(12):6685.*

[19] *Wood A, Lin C, Li M, Nishtala K, Alaei S, Rossello F, et al. FKRP-dependent glycosylation of fibronectin regulates muscle pathology in muscular dystrophy. Nature Communications. 2021;12(1):2951.*

[20] *Kuwabara N, Imae R, Manya H, Tanaka T, Mizuno M, Tsumoto H, et al. Crystal structures of fukutin-related protein (FKRP), a ribitol-phosphate transferase related to muscular dystrophy. Nature communications. 2020;11(1):303.*

[21] *Ortiz-Cordero C, Bincoletto C, Dhoke NR, Selvaraj S, Magli A, Zhou H, et al. Defective autophagy and increased apoptosis contribute toward the pathogenesis of FKRP-associated muscular dystrophies. Stem Cell Reports. 2021;16(11):2752-67.*

[22] *Kuwabara N, Manya H, Yamada T, Tateno H, Kanagawa M, Kobayashi K, et al. Carbohydrate-binding domain of the POMGnT1 stem region modulates O-mannosylation sites of α-dystroglycan. Proceedings of the National Academy of Sciences. 2016;113(33):9280-5.*

[23] *Xu C, Zhang M, Bian L, Li Y, Yao Y, Li D. N-glycosylated SGK196 suppresses the metastasis of basal-like breast cancer cells. Oncogenesis. 2020;9(1):4.*