Original Article

Limb-Girdle Muscular Dystrophy Type 2B (LGMD2B) caused by Pathogenic Splice and Missense Variants of *DYSF* Gene among Iranians with Muscular Dystrophy

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Abstract

Background: The phenotypic range of limb-girdle muscular dystrophies (LGMDs) varies significantly because of genetic heterogeneity ranging from very mild to severe forms. Molecular analysis of the *DYSF* gene is challenging due to the wide range of mutations and associated complications in interpretations of novel *DYSF* variants with uncertain significance. Thus, in the current study, we performed the NGS analysis and its results are confirmed with Sanger sequencing to find the plausible disease-causing variants in patients with muscular dystrophy and their relatives via segregation analysis.

Materials and Methods: Nine patients with LGMD type 2B (LGMD2B) characteristics were screened for putative mutations by the whole-exome sequencing (WES) test. Either the patients themselves or their parents and first relatives were investigated in the segregation analysis through Sanger sequencing. The majority of variants were classified as pathogenic through American College of Medical Genetics and Genomics (ACMG) guidelines, segregation results, and *in silico* predictions.

Results: Results revealed eight variants in *DYSF* gene, including three splicing (c.1149+4A>G, c.2864+1G>A, and c.5785-7G>A), two nonsense (p.Gln112Ter and p.Trp2084Ter), two missense (p.Thr1546Pro and p.Tyr1032Cys), and one frameshift (p.Asp1067Ilefs), among nine Iranian families. One of the eight identified variants was novel, including p.Asp1067Ilefs, which was predicted to be likely pathogenic based on the ACMG guidelines. Notably, prediction tools suggested the damaging effects of studied variants on dysferlin structure.

Conclusion: Conclusively, the current report introduced eight variants including a novel frameshift in *DYSF* gene with noticeable pathogenic effects. This study significantly can broaden the diagnostic spectrum of LGMD2B in combination with previous reports about *DYSF* mutations and may pave the way for a rapidly high-ranked identification of the accurate type of dysferlinopathy.

Keywords: *DYSF*, gene, limb-girdle muscular dystrophy type 2B, muscular dystrophy

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Introduction

As uncommon muscular dystrophies (MDs), dysferlinopathies are caused by mutations in the *DYSF* gene.[1,2] Dysferlinopathies are divided into two main phenotypes including Miyoshi MD 1 (OMIM: 254130) and limb-girdle MD type 2B (LGMD2B) (OMIM: 253601). The former manifests with distal weakness, and the latter has a noticeable impact on proximal muscles.[3,4] The other reported phenotypes consist

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of promptly progressive distal myopathy with anterior tibial involvement, a proximodistal feebleness, and a pseudometabolic display.[2,5] The fourth most common MD only after myotonic dystrophy type I (DM1) is limb-girdle MD (LGMD), which can emerge at any age. LGMDs will frequently get worse gradually and are mostly diagnosed by progression in proximal limb muscles causing feebleness, which mainly involve pelvic and shoulder girdle muscles (occurrence rate: 0.8–5.7 in every 100,000 individuals).[6-8] The phenotypic spectrum of LGMDs differs widely because of genetic heterogeneity, which ranges from very mild (adolescent) to severe (childhood) form.[9]

DYSF gene with 58 exons located on 2p13.2 cytogenetic region with a length of genomic DNA over 230 kb encodes dysferlin protein (2119 amino acids).[10] Dysferlin is a type II transmembrane protein with various domains including seven protein-binding and lipid-binding C2, multiple Dysf and Fer, and a C-terminal transmembrane domain.[11] Dysferlin plays vital roles in repairing plasma membrane, and lack of dysferlin leads to defective membrane reclosing and weakening of muscle fibers.[12-14] Noticeably, dysferlin is also detected in cytoplasmic vesicles and involved in the t-tubule network.[15]

Due to the overlapping phenotypes of dysferlinopathies, several individuals may be misdiagnosed; thus, a precise diagnosis is needed to be right to the point. The conclusive diagnosis and categorization of LGMDs commonly rely on the age of onset, familial history, the progression of feebleness and muscular atrophies, high levels of serum creatine kinase (CK), electromyography (EMG), magnetic resonance imaging (MRI), and genetic tests.[6,7] Border-breaking advances in high-throughput genetic testing techniques have made whole-exome sequencing (WES) a rapid and cost-effective strategy to investigate the type and pathogenic level of variants in numerous Mendelian disorders.[16,17] Diagnosis of accurate subtypes of dysferlinopathies as complex rare diseases with extreme heterogeneity is simply based on the typical symptoms and signs, requiring prompt and precise molecular testing like WES.

Mutational analysis of *DYSF* is completely challenging because of the extensive mutational range and allied complications in interpretations for novel *DYSF* variants with uncertain significance, specifically splicing and missense variants.[18] Thus, the current study investigated the clinical characteristics, serum creatine phosphokinase (CPK) levels, EMG, and WES results of Iranian patients with LGMD2B in detail. In addition, the Next-generation sequencing (NGS) results are confirmed with Sanger sequencing to validate the disease-causing variants in the probands and their family members through segregation analysis.

Materials and Methods

Subjects

In this study, nine patients with symptoms of muscle weakness at different ages were included based on the neurologist's diagnosis of genetic pathogenicity. Clinical and biochemical examinations of the patients' profiles indicated that the CPK and lactate dehydrogenase (LDH) levels were elevated. Based on the physical examinations and laboratory testing, due to the possibility of a genetic effect and to find the plausible variant of variants, the WES technique was requested for all probands. Informed consents of all participants and the ethical number of the study (IR.IAU.MSHD.REC.1398.099) were obtained. Then, 5 mL of peripheral blood was obtained in ethylenediaminetetraacetic acid (EDTA) tubes from the available family members. DNA was extracted by the standard salting-out protocol. All probands' DNA samples were sent for exome sequencing (Macrogen, Seoul, Korea). Data were then analyzed, and putative variants with disease-causing impact were assessed via online bioinformatic tools and also genomic databases such as Franklin (https://franklin.genoox.com/clinical-db/home), VarSome (https://varsome.com/), and ClinVar (www.ncbi.nlm.nih.gov/clinvar). Furthermore, variant frequencies were considered based on the reported frequencies in genomic databases including 1000 Genomes Project, GnomAD, Iranome, and Exome variant server (EVS). To validate the candidate variants, family cosegregation investigations were done through Sanger sequencing. By the online software Primer3 Plus, forward and reverse primers were designed to detect the candidate variant. For Sanger sequencing, all the final polymerase chain reaction (PCR) products were prepared in a total volume of 25 µL (Kawsar Biotech Co., Tehran, Iran). Sequencing results were analyzed by CLC workbench software version (7.7).

In vitro diagnosis

WES was carried out to uncover the plausible pathogenic mutations in patients as follows. Genomic deoxyribonucleic acid (gDNA) was extracted and purified from each case's blood sample by a filter-based procedure and then quantified. A total of 100 ng volume of gDNA was used for DNA preparation. For this purpose, DNA was fragmented and the targeted enrichment was done using the SureSelect Human All Exon V7 kit (Agilent Technologies, Santa Clara, CA, USA). Libraries were sequenced on the Illumine NovaSeq 6000 platform for 2*100 bp reads with an average coverage of 100× (Eurofins Genomic Services, Bengaluru, India). The sequence read quality assessment was done by performing quality control (QC) with FastQC. Burrows–Wheeler alignment (BWA) algorithm was used with default parameters for alignment to human genome assembly GRCh37 (hg19).

After sequencing, variants with low quality were filtered out at a cut-off of ≥20, and the finalized clean reads were in accordance with the reference human genome (hg19) utilizing BWA software. Single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) were screened by the Genome Analysis Toolkit (GATK) software. Additional analysis was accomplished according to the routine statistics. All acknowledged variations were annotated by ANNOVAR with awareness to recognize candidate variants, including the depth of coverage, percentage of reads with the mutations, allele frequencies, critical splice variation, and the pathogenicity level of mutations in the protein structure.

After WES analysis, the following criteria were used for filtration to obtain disease-related variants:

1. Allelic frequency of variants (minor allele frequency (MAF) >0.01) in GnomAD, 1000G, Iranome, and ExAC population databases
2. Variant position (intergenic, intronic, and upstream and downstream variants of the gene were filtered in the early stages)
3. Variant type (synonymous variants were filtered)
4. Using prediction tools such as MutationTaster, SIFT, PolyPhen, and combined annotation dependent depletion (CADD) and deep neural networks (DANN) scores.

Thus, the variants reported in the MutationTaster database as polymorphism were initially ignored. Variants classified as tolerated and benign in sorts intolerant from tolerant (SIFT) and PolyPhen were then removed. Then, based on the CADD and DANN scores, the variants with higher pathogenicity were selected. 5-Disease databases such as ClinVar, Human gene mutation database (HGMD), VarSome, Franklin, and InterVar were used to confirm the pathogenicity of the variants found.

Sanger sequencing confirmation

After screening the variants by WES, mutational confirmations were performed by designing the primers of specific regions through Sanger sequencing technique. gDNA was extracted from the blood samples of all probands and their families using a standard salting-out protocol for Sanger sequencing.

Results

Clinical phenotypes

The phenotypic and genotypic features of all patients, including the clinical characteristics, WES results, and mutation confirmation by Sanger sequencing, are reported here in detail, and their pedigrees are shown in Figure 1. Among the nine Iranian families, eight variants were finally found in the *DYSF* gene in four categories, including splicing variants c.1149+4A>G, c.2864+1G>A, and c.5785-7G>A, nonsense variants (p.Gln112Ter and p.Trp2084Ter), missense variants (p.Thr1546Pro and p.Tyr1032Cys), and frameshift (fs) variant (p.Asp1067Ilefs). p.Asp1067Ilefs was novel with a damaging impact on dysferlin structure.

Patient with splicing mutation c.1149+4A>G was a 33-year-old symptomatic man with MD and his parents were non-relatives. The age of onset was 26 years. EMG revealed irritable myopathic process, and compatible with lower limbs, proximal myopathy was more prominent on the right side. CPK and LDH were increased. He showed progressive muscular weakness. There was no history of MD in his family. Patient with minor allele of c.5785-7G>A was a 30-year-old woman whose parents were relatives (first cousins [FC]). Symptoms were weakness of limbs, very high CPK, and high LDH, and EMG results showed myopathy. The age of onset was 18 years. A sign of MD history was found in her family. Her parents were healthy and carriers, but among other siblings (three sisters and a brother), two sisters were patients, and the other relatives were not patients. A 40-year-old symptomatic man who had the altered allele of c.2864+1G>A suffered from MD and FC consanguinity relativity. Four of his relatives were affected. Also, this study identified two patients with p.Gln112Ter. One of them was a 23-year-old symptomatic man who suffered from MD. His parents were relatives. Clinical evidence were as follows: elevated serum CK and LDH levels. EMG showed myopathic changes, and skeletal muscle biopsies indicated severe myopathic changes with variation of fiber size, fiber splitting, increased connective tissue, and some necrotic changes. Disease progression was relatively slow. There was a prior history of MD in the family. Patient's mother and one of his aunts were homozygotes for the mutated allele, but patient's father was a carrier heterozygote. The disease was not manifested in the other relatives. Sanger sequencing confirmed the mutation in proband, his father (heterozygote), and his mother (affected homozygote). Another patient with p.Gln112Ter mutation was a 47-year-old man with four fully disabled muscular organs. He showed elevated CPK and LHD levels, and his parents were relatives (FC). A sign of disease history of MD was found in his family. His parents were healthy and carriers; but of his five siblings, a brother and two sisters were patients. The other relatives were healthy. p.Gln112Ter was previously reported related to different dysferlinopathies in many patients who carried either both mutated alleles for the Q112X variant or a mutated allele and another mutation in the compound heterozygosity genotype. It is noteworthy that dysferlin was not found in the skeletal muscle of homozygote persons.[19] Thus, this variant is predicted to cause loss of normal protein function either through protein truncation or by nonsense-mediated mRNA decay.

p.Trp2084Ter was found in a 30-year-old-woman with normal EMG and elevated CPK and LDH levels. Her parents were not relatives. In her available relatives, there was just a patient similar to her, who was the son of her mother's aunt. A 24-year-old symptomatic man with p.Thr1546Pro mutation had EMG results as follows: prominent proximal weakness- brisk reflexes with clonus of Achilles and bilateral Babinski sign. CPK and LDH levels were also elevated. He was suspected to have LGMD with autosomal recessive inheritance. His parents were relatives (FC). There was no history of MD manifestation among his relatives. This alteration substitutes threonine with proline at codon 1546 of dysferlin. Threonine is mildly conserved, and there is a slight physicochemical change between threonine and proline. This variant has not been reported in population databases (no frequency in ExAC) and also in the literature for the individuals with dysferlinopathies symptoms. *In silico* predictors which measure the outcome of missense alterations on protein structure and function are either not available or have conflicting results on the possible effect of this missense variant. Briefly, the supporting evidence is presently inadequate to define the disease-causing role of

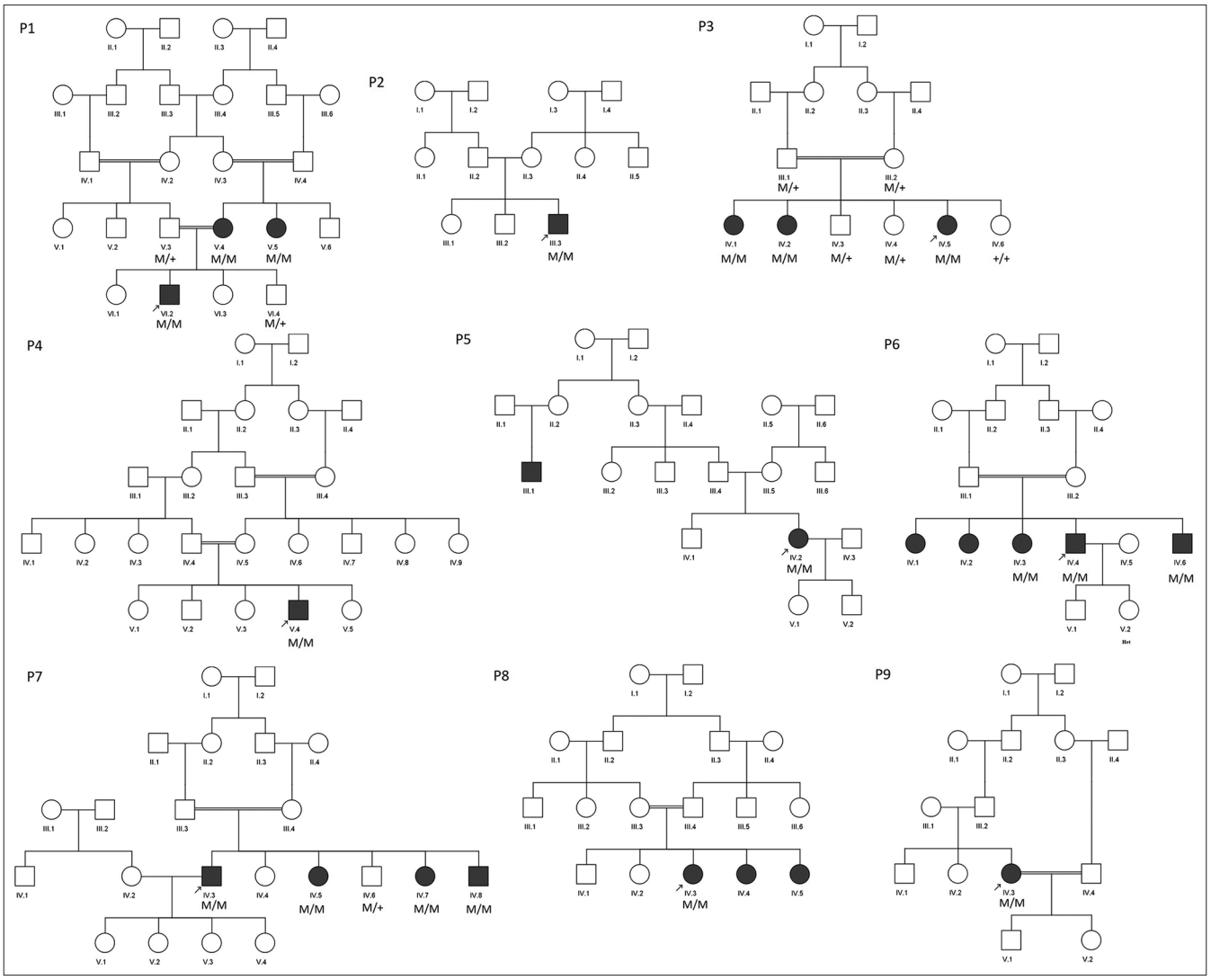

Figure 1: Overview of the pedigrees of the nine families presented in this study. Probands who underwent WES analysis are indicated with an arrow. Segregation status has been shown in available cases. WES = whole-exome sequencing

this variant. The next patient was a 47-year-old woman who showed p.Tyr1032Cys mutation. Her parents were relatives (third cousins [TC]), and the symptoms were myopathy detected by EMG, walking problems for 12 years, weakness in legs and hands, and high CPK. There was no sign of MD history found in her family. Sanger sequencing indicated that her parents were healthy and carriers, and her children were not patients. The tyrosine residue is approximately conserved, and there is an obvious physicochemical difference between Tyr and Cys. This variant is present in population databases as rs756328339 with ExAC frequency of 0.01% and has been found to be homozygous. In combination with another *DYSF* variant, p.Tyr1032Cys is present in many persons affected with dysferlinopathies.[20,21] Finally, the last patient, a 36-year-old symptomatic woman, who suffered from myopathy had p.Asp1067Ilefs mutation (novel variant). Her parents were consanguineous (FC). There was a prior history of MD in her family. Sanger sequencing confirmed that two of her four sisters were homozygote for the mutated allele, but patient's parents were both carriers and healthy. The disease was not present in her other relatives.

Genetic analysis

Filtered variants that resulted from WES analysis (excluding benign, likely benign, non-coding RNA variants) were investigated for final filtering based on the pathogenicity level using Franklin (https://franklin.genoox.com/clinical-db/home), VarSome (https://varsome.com/), and InterVar (https://wintervar.wglab.org/). Three were splicing variants, including c.1149+4A>G, c.2864+1G>A, and c.5785-7G>A. The other five variants were exonic and divided into three types of mutations including stop-gained (p.Gln112Ter and p.Trp2084Ter), missense (p.Thr1546Pro and p.Tyr1032Cys), and fs (deletion: Asp1067Ilefs) [Table 1]. c.1149+4A>G was classified as a variant of uncertain significance (VUS) with PM2 and PP3 evidence in Franklin. This variant was also classified as a pathogenic variant in VarSome (PM4, PP3, and PM2). ClinVar classified it as a VUS. c.2864+1G>A was

Table 1: Variant classification based on ACMG guidelines

Patient	Consanguinity	Chromosome position	DNA change	Type	Protein change	dbSNP	Ex/Int number	Zygosity	VarSome	Franklin	ClinVar
1	FC	Chr2:71730438	c.334C >T	Nonsense	p.Gln112Ter	rs746315830	Ex 4	HOM	Path	Path	Path (ID: 265108)
2	NO	Chr2:71748038	c.1149+4A>G	Splicing	-	rs761302882	Int 12	HOM	Path	VUS	VUS (ID: 288116)
3	FC	Chr2:71797840	c.3199delG	Frameshift	p.Asp1067Ilefs	-	Ex 29	HOM	L.Path	L.Path	Novel
4	FC	Chr2:71883301	c.4636A>C	Missense	p.Thr1546Pro	-	Ex 43	HOM	VUS	VUS	VUS (ID: 1058370)
5	NO	Chr2:71909738	c.6236G>A	Nonsense	p.Trp2084Ter	-	Ex 55	HOM	Path	Path	Path (ID: 1180715)
6	FC	Chr2:71795469	c.2864+1G>A	Splicing	-	rs199954546	Int 26	HOM	Path	Path	Path (ID: 443997)
7	FC	Chr2:71730438	c.334C>T	Nonsense	p.Gln112Ter	rs746315830	Ex 4	HOM	Path	Path	Path (ID: 265108)
8	FC	Chr2:71901320	c.5785-7G>A	Splicing	-	rs753861836	Int 51	HOM	Path	Path	Path (ID: 283474)
9	TC	Chr2:71797738	c.3095A>G	Missense	p.Tyr1032Cys	rs756328339	Ex 29	HOM	Path	Path	Path (ID: 196175)

Ex=exon, FC=first cousins, HOM=homozygote, Int=intron, L.Path=likely pathogenic, Path=pathogenic, TC=third cousins, VUS=variant of uncertain significance dbSNP: Single Nucleotide Polymorphism Database

pathogenic in both Franklin and VarSome (PVS1, PM2, PP5); however, ClinVar defined it to be likely pathogenic/pathogenic. c.5785-7G>A was pathogenic in both Franklin and VarSome (PP3, PP5, PM2); however, ClinVar defined it as likely pathogenic/pathogenic. p.Gln112Ter was identified as a pathogenic variant in Franklin (PVS1, PM2, PP5), VarSome (PVS1, PM2, PP5), InterVar (PVS1, PM2, PP3, PP5), and ClinVar. p.Trp2084Ter was pathogenic in Franklin (PVS1, PM2, PP5), VarSome (PVS1, PM2, PP5), and InterVar (PVS1, PM2, PP3) and likely pathogenic in ClinVar. p.Thr1546Pro was VUS in Franklin (PM2), VarSome (PM2 and PP3), and ClinVar. p.Tyr1032Cys was pathogenic in Franklin (PM2, PP3, and PP5) and VarSome (PM2, PP3, and PP5) and likely pathogenic in InterVar (PM1, PM2, PP3, and PP5), and it had evidence for being both pathogenic and likely pathogenic in ClinVar. Finally, Asp1067Ilefs was documented as a likely pathogenic variant both in Franklin (PVS1 and PM2) and VarSome (PVS1 and PM2). There was no citable publication in ClinVar to refer this variant. The frequency of this variant was not found in the databases, and it might be considered as a novel variant that is reported here for the first time.

In silico predictions

In silico predictions were investigated for all variants including MutationTaster, SIFT, PolyPhen, CADD, DANN, and Provean. Also, achieved frequencies of the variants were considered as Iranome, EVS, GnomAD, and 1000 Genomes Project in Table 2. Homology modeling was also performed to obtain a deeper view on stop-gained p.Gln112Ter variant in comparison with the normal structure. Therefore, in the first step, the normal structure of dysferlin was built based on homology modeling. In the next step, homology modeling was performed for stop-gained allele (mutant) structure too. Dysferlin (2119 amino acids) (UniProt ID: O75923) was modeled through SWISS-MODEL (PDB: 4P42) and also validated with MolProbity and ProSA online software (available at https://prosa.services.came.sbg.ac.at/prosa.php and (https://prosa.services.came.sbg.ac.at/prosa.php). Stop-gained p.Gln112Ter was built by SWISS-MODEL based on PDB: 4IHB structure. Interestingly, superimposition of p.Gln112Ter and normal-modeled dysferlin was done through Chimera version 1.15[22] [Figure 2].

Discussion

Due to the heterogeneity of *DYSF* gene and diagnosis complexity of dysferlinopathies, in this study, we conducted a remarkable screening of disease-causing variants among nine Iranian families with LGMD2B through NGS technique. WES test was performed among the referred patients for genetic testing to find out the putative variants related to their MD and increased levels of CPK and LDH. The final filtered variants were classified into three types including pathogenic, likely pathogenic, and VUS. The current study replicates the previous documents about some pathogenic variants and represents evidence such as segregation analysis by Sanger sequencing

Table 2: The basic information of studied variants including *in silico* predictions in detail

Variant	Prediction tools	VarSome	CADD score	DANN score	PhyloP score	Provean	Allele frequency in databases			
							Iranome (n=800)	EVS (n=6515)	GnomAD (n=141,456)	1000 Genomes
c. 334C >T	DC	Path	41	0.998	2.3	Neutral	Not found	Not found	Not found	Not found
c. 3199delG	DC	L.Path	NA	NA	5.3	NA	Not found	Not found	Not found	Not found
c. 4636A >C	DC	VUS	23.8	0.994	2.99	Neutral	Not found	Not found	Not found	Not found
c. 6236G >A	DC	Path	51	0.996	9.75	NA	Not found	Not found	Not found	Not found
c. 3095A >G	DC	Path	24.8	0.996	9.09	Dam	Not found	Not found	0.000012	Not found
c. 1149+4A >G	NA	Path	23.1	0.90	7.1	NA	Not found	Not found	0.0000159	Not found
c. 2864+1G >A	DC	Path	35	0.99	9.63	NA	Not found	Not found	0.000008	Not found
c. 5785-7G >A	DC	Path	24.5	0.75	0.85	NA	Not found	Not found	0.0000119	Not found

Dam=damaging, DC=disease causing, L.Path=likely pathogenic, NA=not applicable, Path=pathogenic, T=tolerate, VUS=variant of uncertain significance

Figure 2: The superimposition of modeled wild-type dysferlin (white) and p.Gln112Ter (blue) models visualized by Chimera MatchMaker

and *in silico* predictions through homology modeling and computational tools. Briefly, the screened allele alterations showed clinical and computational evidence as pathogenic variants among Iranian patients with LGMD2B phenotypes.

There are remarkable reports which have focused on the pathogenic effects of variants causing LGMD2B. Krahn *et al*.[23] showed that more than 50% of their French patients (134) had two disease-causing mutations, which suggest genetic tests for the diagnosis of primary dysferlinopathy. They provided confirmatory evidence supporting the higher rate of nonsense mutations compared with a lower ratio of damaging missense alterations. p.Gln112Ter (Q112X) was included in their study as a novel mutation. Cacciottolo *et al*.[19] documented a novel report on primary dysferlin gene mutations leading to dysferlin deficiency. They conducted a mutation examination for the *DYSF* gene among 65 patients with LGMD/Miyoshi myopathy (MM) (including Italians, Turkish, and Argentinians). They introduced 65 different mutations consisting of 38 novel mutations. Segregation studies were also performed on 31 patients with homozygous alleles to confirm the pathogenicity of the mutations. They stated that there is no mutational hot-spot region; however, the mutations spread along the entire *DYSF* gene. They found nine nonsense mutations including K32X, Q112X, Q231X, Q606X, Q612X, Q750X, Q832X, C1398X, and W1869X. Interestingly, most of these stop-gained mutations have occurred after Q residues. These Gln residues might be related to an unknown molecular mechanism of mutation in this gene. Zhang *et al*.[24] designed a study on the expression of dysferlin in blood monocytes, which supported primary dysferlinopathy in eight Chinese patients. They found 13 mutations in the *DYSF* gene. Their study replicated the disease-causing Gln112Ter in a patient (heterozygote genotype), whose daughter did not have the mutation. Kergourlay *et al*.[25] identified the splicing damages caused by mutations in the *DYSF* gene. They reported c.1149+4A>G and c.3349–10G>A for the first time. They categorized c.1149+4A>G in intron 12 with alteration of the

wild-type donor site, typically affecting splicing. They stated that the activation of an intronic cryptic donor site and potential alteration of splicing lead to exon 12 skipping. Notably, Malek *et al*.[26] reported a *DYSF* gene pathogenic mutation in a 34-year-old Iranian male patient as a novel splice variant in intron 26 (c.2864+1G>A). They reported that two members of the patient's family were affected and their parents were relatives.

Huang *et al*.[21] demonstrated p.Tyr1032Cys pathogenicity in functional assays on antibody fragments, including western blotting, immunofluorescence microscopy, and immunoprecipitation. Rosales *et al*.[20] examined 21 patients with dysferlinopathies (Hispanic, Asian-Indian, African-American, and Caucasian non-Hispanic). They found 27 various mutations in the *DYSF* gene; five of them were reported for the first time. They also focused on introducing new diagnostic features of dysferlinopathies through direct immunofluorescence with overexpression by western blot. p.Tyr1032Cys was replicated by them as a pathogenic mutation. Another study by Chakravorty *et al*.[27] evaluated the clinical and genomic status of 207 genetic myopathies among 207 clinically well-defined inherited Indian patients with different ethnicities. The study classified individuals with p.Tyr1032Cys having dysferlinopathy-specific features like lack of dysferlin stain in muscle immunohistochemistry (IHC), difficulty running, walking problems, calves wasting, and biceps lump, and the most affected muscles are gastrocnemius, iliopsoas, hip adductors, hamstrings, and quadriceps.

There are few studies in the literature that focused on the treatment of LGMD2B. Gushchina *et al*.[28] proved the therapeutic potential of rhMG53 (recombinant human MG53) protein in treating LGMD2B. Another interesting therapy is exon skipping, which utilizes the antisense oligonucleotides.[29,30] This splicing process repairs the open reading frame, leading to a truncated protein production which has normal function yet.[31] Vallecillo-Zúniga *et al*.[32] showed that as a soluble carbohydrate-binding protein, galectin-1 (Gal-1) regulates disease pathogenicity by either altering the expression of integral myogenic protein or stabilizing the mechanical membrane. A recent study by Pozsgai *et al*.[33] reviewed the reports from animal studies and suggested the dual-vector treatment (AAV.DYSF.DV) for LGMD2B by producing complete functional dysferlin with no toxicity and confirming the potential translation of gene delivery for patients with dysferlin deficiency. Human studies also gave positive results on using the dual-vector delivery to small forearm muscles. Based on these reports, it is clear that treatment of LGMD2B has a potential focus on transcription-editing oligonucleotide-based therapies.

In conclusion, the present study identified eight variants of *DYSF* gene among Iranians with MD manifestations, who were referred by a neurologist to genetic investigations. The studied variants, classified as pathogenic, likely pathogenic, and VUS via segregation studies, disease manifestations, and *in silico* predictions such as SIFT, PolyPhen2, MutationTaster, and MutationAssessor, included p.Gln112Ter, p.Asp1067Ilefs, p.Thr1546Pro, p.Trp2084Ter, p.Tyr1032Cys, c.1149+4A>G, c.2864+1G>A, and c.5785-7G>A. Based on the discussed studies focusing on transcription-editing oligonucleotide-based therapies and the findings of the present study, screening the splicing variants of *DYSF* gene is essential for an Iranian suspected to have LGMD2B symptoms. The screening of these variants by WES and Sanger sequencing replicated the previous studies which concluded that the genetic tests play a remarkable role in the rapid and confident diagnosis of *DYSF* pathogenic variations causing LGMD2B.

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Conflicts of interest

There are no conflicts of interest.

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