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Genetic variability in Iranian limb-girdle muscular dystrophy type 2B patients: An evidence of a founder effect

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Abstract

Background: Dysferlinopathies are a group of autosomal recessive limb-girdle muscular dystrophies (LGMDs) caused by mutations in *DYSF* (#603,009). This gene encodes a transmembrane protein called dysferlin. Since there are few reports on Iranian dysferlinopathy patients, we tried to identify the *DYSF* mutations in affected individuals of Iran.

Methods: Eight unrelated Iranian families have been selected for this study. Sanger sequencing followed by haplotype analysis was performed to identify individual variations in *DYSF* sequence. Identified variants were analyzed, and their pathogenicity was interpreted according to the recommendations of the American College of Medical Genetics and Genomics.

Results: We identified two new mutations in *DYSF*, the first one is a nonsense mutation c.2419C > T (p.Gln807*), which eliminates downstream part of the protein. Another novel mutation is c. (1,053 + 1_1,054-1)_(1,397 + 1_1,398-1)del, which causes deletion of the DNA segment from exon 12 to exon 15.

Conclusion: Two of the other six families are from the same ethnicity and share the same mutation and haplotype patterns, suggesting a founder mutation. Genetic analysis of dysferlinopathy can prevent a wrong diagnosis of myositis for these patients.

KEYWORDS

DYSF, founder effect, novel mutations, haplotype analysis, Iran

1 | INTRODUCTION

Dysferlinopathies are a group of autosomal recessive muscular disorders including limb-girdle muscular dystrophy type 2B (LGMD2B), Miyoshi myopathy (MM), and distal myopathy (Nguyen et al., 2007). LGMD2B is a progressive muscle weakness which affects predominantly pelvic and shoulder girdle muscles, usually starting in the second decade of life (Khadilkar, Faldu, Patil, & Singh, 2017).

Dysferlinopathies are caused by mutations in *DYSF* consisting of 55 exons located on chromosome 2p13. The gene encodes a protein called dysferlin. Dysferlin is a sarcolemmal protein which has an important role in membrane repair and vesicle trafficking (Liu et al., 1998; Urtizberea et al., 2008).

Autozygosity mapping is a powerful method in gene tracking of autosomal recessive disorders in inbred families like what we have in Iran (Carr et al., 2013). It is often used in heterogeneous genetic disorders including LGMD to bypass

TABLE 1 clinical features of studied patients in the present study

Family	Age at onset (yrs)	Loss of ambulation (yrs)	Calf hypertrophy	Ankle contractures	Abnormal pulmonary function	Cardiac involvement	Winging scapulae	Scoliosis	Lordosis	Serum CK (U/L)	Muscle biopsy	EMG with myopathic features
19,315	15	Ambulant at age 20	Yes	No	No	No	No	No	No	11,726	Dysferlinopathy	Yes
19,342	18	Ambulant at age 21	No	No	No	No	No	Yes	Yes	12,000	Dysferlinopathy	Yes, BMD[a]
17,132	19	29–28	No-No	NAa-NA	No-No	No-No	Yes-No	No-Yes	Yes-Yes	3,900	Dysferlinopathy	Yes
16,935	13–19	29–30	No-No	No-No	No-No	No-No	No-No	No-No	No-No	4099–6506	LGMD	Yes-Yes
17,298	35–23	43–25	Yes-Yes	No-No	No-No	No-No	No-No	No-No	No-No	4,500	Dysferlinopathy	Yes-Yes
16,938	20–17–18	44-Ambulant at ages of 33 and 28	No-No-No	Yes-No-No	Yes-Yes-No	Yes-No-Yes	Yes-No-No	Yes-Yes-No	Yes-Yes-Yes	3,000	LGMD	LGMD
17,124	22-NA	Ambulant at ages of 33–33	No-No	No-No	No-Yes	No-Yes	No-No	No-No	Yes-Yes	4586–3850	Myositis	Chronic myopathy in lower limbs- Miyoshi myopathy
16,941	22–14	27–24	No-No	Yes-Yes	No-No	No-No	No-No	No-No	No-Yes	3000–2368	Dysferlinopathy	Yes

CK, creatine phosphokinase.
[a]Becker Muscular Dystrophy.

TABLE 2 previously reported mutations found in *DYSF*[a] in the present study

Family	Mutation at DNA level	Mutation at protein level	Intron/exon number	SIFT	polyphen	CADD (Phred score)	Provean	UMD results (Pathogenicity)	Mutation taster	Mutant allele	Ethnicity of family
19,315	c.2706dupC	p.Lys903Glnfs*4	Exon 26	—	—	35	—	—	Disease causing	Homozygous	Boyer-Ahmad
19,342	c.2706dupC	p.Lys903Glnfs*4	Exon 26	—	—	35	—	—	Disease causing	Homozygous	Boyer-Ahmad
17,132	c.3112C > T	p. Arg1038*	Exon 29	—	—	39	—	—	Disease causing	Homozygous	Sirjan
16,935	c.3225delT	p.Phe1075Leu*fs**45	Exon 30	—	—	35	—	—	Disease causing	Homozygous	Arak
16,938	c.5804C > T	p.Pro1935Leu	Exon 52	Damaging (0.001)	Probably damaging (0.997)	34	Deleterious (−9.13)	Presumably pathogenic (65)[b]	Disease causing	Homozygous	Ardebil
17,124	c.5633T > C	p.Leu1878Pro	Exon 50	Damaging (0.000)	Probably damaging (0.993)	32	Deleterious (−6.34)	Presumably pathogenic (70)	Disease causing	Homozygous	Tehran

[a]NM_003494.3.
[b]The closer the score is to 100, the more it will be pathogenic.

sequencing of all suspected genes for easier finding of the disease-causing mutations.

In this study, we aimed to detect mutations in eight Iranian families which presumably have LGMD2B and characterized them with autozygosity mapping and Sanger sequencing.

2 | MATERIAL AND METHODS

2.1 | Subjects

Twenty-five patients from eight unrelated Iranian families were referred to the Kawsar Human Genetics Research Center [KHGRC]. The affected male patients were ruled out for dystrophinopathy based on clinical, pathological, and molecular analysis of dystrophin gene. Clinical diagnosis of patients was made when they had weakness and atrophy of the pelvic and shoulder girdle muscles, and raised serum creatine phosphokinase concentration. Age at onset of dysferlinopathy is usually in the second decade of life. All examination and clinical procedure were made by neurologists.

The study protocol was approved by Ethics Committee of the Pasteur Institute of Iran (No: 91/0201/10425). Informed consent was received from all participants before sampling, and written informed consent was also obtained from the participants for the publication.

2.2 | Muscle biopsy

Fresh muscle biopsy samples were frozen instantly in isopentane precooled by liquid nitrogen. The samples were assayed by a standard panel of histochemical procedures (Dubowitz, 1973). Immunohistochemical studies were performed using monoclonal antibodies against dystrophin, dysferlin, and beta-spectrin. Beta-spectrin was applied as a positive control. All antibodies were purchased from Novocastra Laboratories (Newcastle, UK). Peroxidase method was used as a detection system.

2.3 | Molecular genetics studies

DNA was extracted by salting out procedure (Miller, Dykes, & Polesky, 1988). Autozygosity mapping was done by a multiplex set of four short tandem repeat (STR) markers flanking the *DYSF* (NM_003494.3). DNA sequencing, interpretation of data, and fragment analysis were performed as described previously (Mojbafan et al., 2016). Long-range polymerase chain reaction (PCR) was done using primers flanking the deleted region. Cycling conditions were as follows: 92°C for 2 min followed by 30 cycles of 92°C for 10 s, annealing temperature of 62°C for 15 s, and extension temperature of 68°C for 10 min. A final 7 min elongation step was performed at 68°C. The sequences of primers are available upon request.

2.4 | In silico analysis

Pathogenic effects of variants and mutations were evaluated using different softwares, such as SIFT (Kumar, Henikoff, & Ng, 2009), polyphen-2 (Polymorphism Phenotyping v2) (Adzhubei et al., 2010), PROVEAN (Protein Variation Effect Analyzer) (Choi & Chan, 2015), CADD (Combined Annotation Dependent Depletion) (Kircher et al., 2014), and mutation taster (Schwarz, Cooper, Schuelke, & Seelow, 2014).

3 | RESULTS

Clinical and histopathological characteristics of patients are listed in Table 1. The consanguinity rate of our studied families was 88%, and their haplotype analysis showed homozygosity in the *DYSF*. Sanger sequencing of the gene in all eight families showed seven different mutations, two of which were not described previously (Tables 2 and 3). Pedigrees of all families are presented in Figure 1(a–g). Two non-consanguineous families (19,315 and 19,342) shared the same haplotypes (Figure 2) and the same frameshift mutation (c.2706dupC) which led to a premature termination of translation.

Three of the seven mutations were deletions/duplications causing the frameshift and two were nonsense mutations resulting in a premature stop codon. The other two mutations were missense ones (Tables 2 and 3).

One of the novel mutations detected in our study was a nonsense mutation (c.2419C > T, p.Gln807*) in exon 24, another new mutation was found in the family 16,941 with two affected members, which was a multi-exon deletion. Since one of the STR markers, located in intron 13, did not produce any fluorescent peak in the affected individuals (designated

TABLE 3 Novel mutations found in *DYSF* in the present study

Family	Mutation at DNA level	Mutation at protein level	Intron/exon number	CADD (Phred score)	Mutation taster	Mutant allele	ACMG interpretation	Ethnicity of family
17,298	c.2419C > T	p.Gln807*	Exon 24	40	Disease causing	Homozygous	Pathogenic	Mashhad
16,941	c.(1,053 + 1_1054−1)_(1,397 + 1_1398−1)del	-	Deletion of exons 12–15	-	-	Homozygous	Pathogenic	Kermanshah

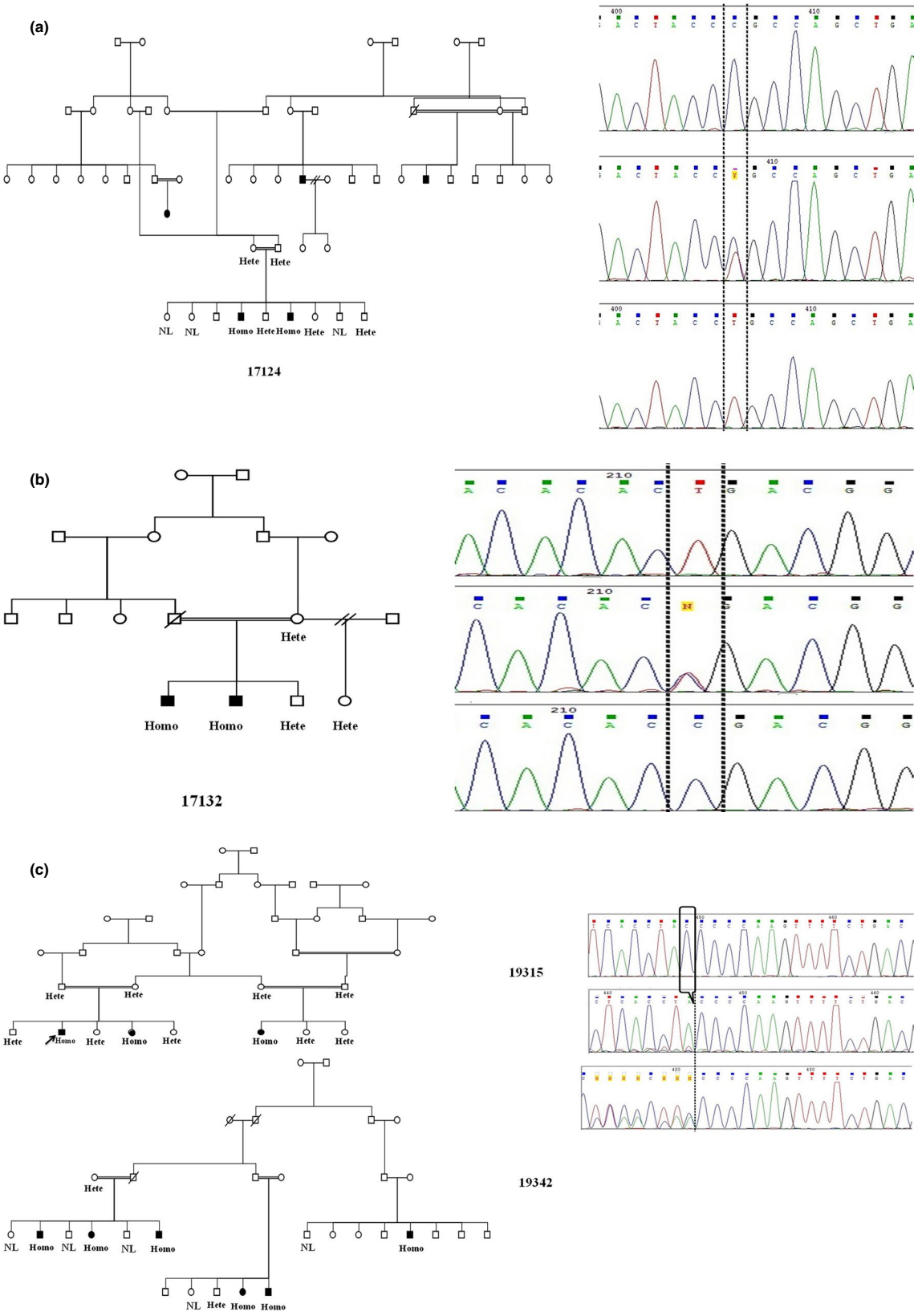

FIGURE 1 (a–g) Pedigrees of eight families with dysferlinopathy. *Squares*—males, *circles*—females, *filled symbols* affected individuals, o*pen symbols*—unaffected individuals. *Double bars* represent consanguineous unions. All family members tested in this project were abbreviated as Homo, Hete, and NL. Homo represents homozygote, Hete represents Heterozygote, and NL stands for Normal regarding to the mutation found in the family. Sequencing results are corresponding to the individuals in the pedigree who were shown by Homo, Hete, and NL

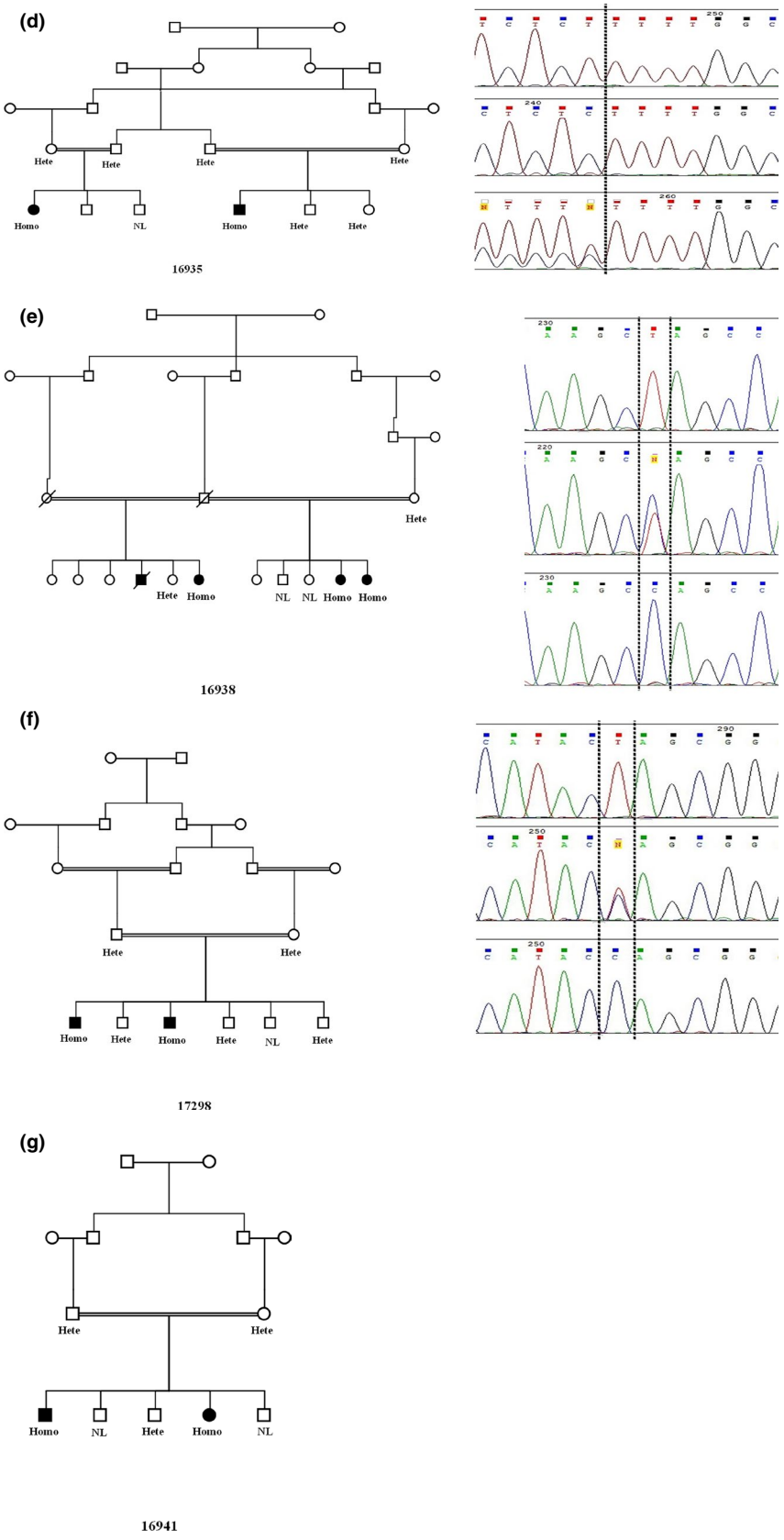

FIGURE 1 Continued

as DNW, "Does Not Work", in Figure 3) and showed a homozygous peak in the affected members' parents, we suspected the presence of a possible deletion in this region (Figure 3). PCR performed in order to sequence the whole *DYSF* showed that all exons except 12–15 yield amplification products in the affected members. We repeated the PCR

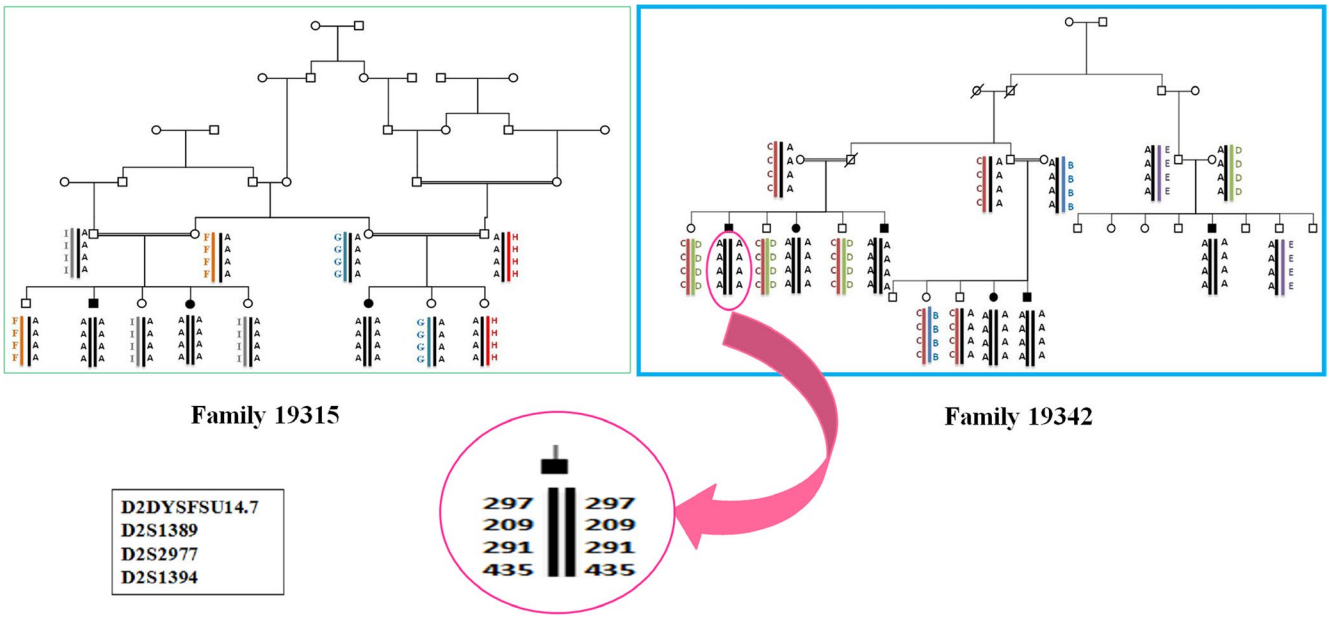

FIGURE 2 Two families, 19,315 and 19,342, shared the same haplotypes. Electropherogram peaks are shown in different colors and alphabetic letters for simplicity. All affected individuals of both families have haplotype "A" in homozygous form. This represents markers D2DYSFSU14.7, D2S1389, D2S2977, and D2S1394 with resulting peaks of 297, 209, 291, and 435 in the above mentioned families. D2DYSFSU14.7 marker was not previously reported, so we gave it this new name. Number 2 in the markers' name is the number of the chromosome on which *DYSF* is located, S denotes STR, U means upstream, and the following numbers denote distance from the gene (e.g., 8.05×10^5 base pairs)

of these exons with several primer pairs, various annealing temperatures, and cycle numbers, but it did not yield any amplification products suggesting a possible deletion of these exons. Further analysis of patient's DNA had shown that the multiple primer sets flanking exons 12–15 failed to produce PCR product. Finally, a multiplex PCR with internal control, using exon 55, indicated that exons 12–15 have been deleted in the patients compared to a healthy control individual and patient's parents (Figure 4).

Next, we used a long-range PCR to confirm this multi-exon deletion. We designed a primer set flanking exons 12 and 15 of *DYSF* for targets located in the middle of exons 11 and 16 which produced a predicted amplification product. The length of this region in a control individual was about 18.5 kb. We also used a primer pair near exon 13 as an internal control. The mutation in the affected individuals of family 16,941 caused a deletion of about 16.5 kb fragment including exons 12–15 and may additionally include some fragments of neighboring introns. As expected, exon 13 was deleted in the patient's gene and therefore did not produce any bands; however, amplification of regions flanking exons 11 and 16 showed an about 2 kb PCR product which confirmed this deletion (Figure 5). When DNAs from parents heterozygous for this deletion were used as templates, amplification products with correct size were detected in both internal control and the deleted region. At the same time, in DNAs of control individuals without multi-exon deletion, we revealed only the internal band, while the region of 18.5 kb was too long to be amplified in control individual (Figure 5).

Immunohistochemical staining of muscle samples from a patient belonging to family 16,941 showed round and dispersed atrophic fibers with a wide variation in the fiber size when it was stained with hematoxylin and eosin (H & E) (Figure 6a). Endomysial connective tissue was significantly enlarged, which might be associated with slight adipose tissue replacement typical for dystrophic changes. Further immunohistochemical analyses were carried out using monoclonal antibodies against beta-spectrin as a positive control (Figure 6b), and dysferlin (Figure 6c). The loss of sarcolemmal labeling of all muscle fibers against dysferlin antibody confirmed our genetic results (Figure 6c). As it is mentioned in Table 1, muscle biopsy studies of the patient from family 17,298 showed the same features like family 16,941 which were compatible with dysferlinopathy.

4 | DISCUSSION

The prevalence of dysferlinopathy is not known but it has been reported in different countries and ethnicities (Gomez-Diaz et al., 2012; Magri et al., 2012; Moore et al., 2006; de Paula et al., 2002; Sveen, Schwartz, & Vissing, 2006; Tagawa et al., 2003; Walter et al., 2013). Autosomal recessive disorders like LGMDs are more prevalent in countries with high rate of consanguineous marriages including Iran, Turkey, and India (Dincer et al., 2000, 1997; Fatehi et al., 2015). Since *DYSF* is large, it is difficult to identify frequent mutations in this gene and different studies revealed that no hotspot is evident in this gene and all mutations are spread throughout the gene; but a

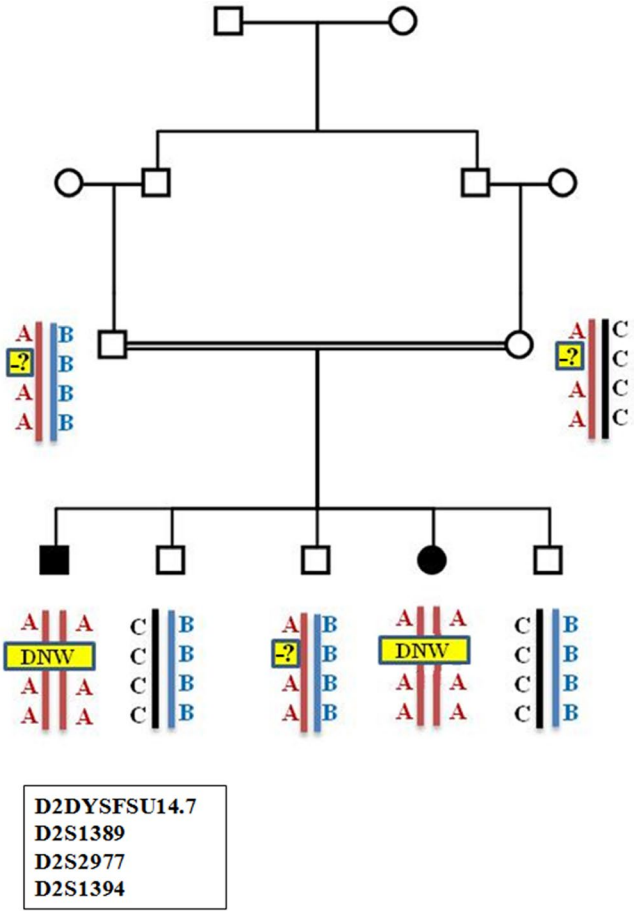

FIGURE 3 STR markers surrounding *DYSF* in family 16,941. D2DYSFSU14.7 is a novel STR marker, where U means upstream. The numbers denote distance from the gene (e.g., 8.05×10^5 base pairs). Marker D2S1389 is located in intron 13 which does not produce any peak (DNW = Does Not Work). This marker reveals a homozygous peak in DNA from parents and in the heterozygote sibling

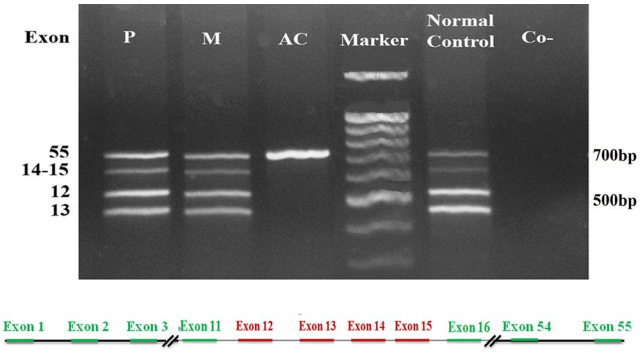

FIGURE 4 Evaluating exon deletion of exon 12 to exon 15 in *DYSF* on agarose gel by multiplex PCR. The deleted exons are shown in red in the schematic image. The PCR product size of each exon (s) is as follows: exon 12: 527 bp, exon 13:458 bp, exon 14–15: 641 bp, exon 55: 730 bp. The ladder used in this figure is a 100 bp ladder. P: Father, M: Mother, AC: Affected child, Co-: Negative control, PCR: Polymerase chain reaction

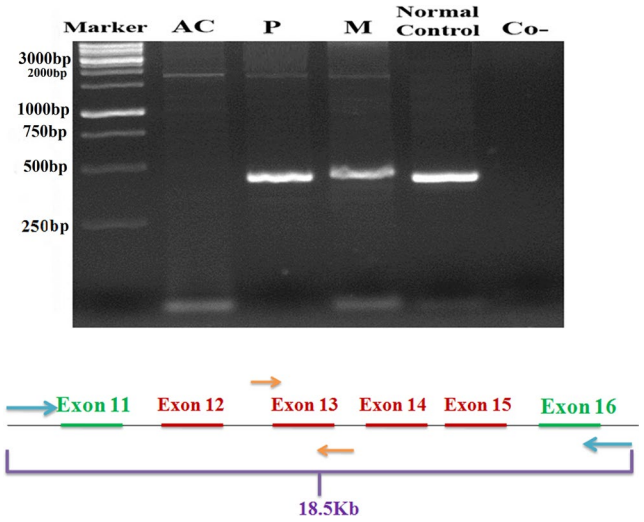

FIGURE 5 Evaluating deletion of exon 12 to exon 15 in *DYSF* on agarose gel by long-range PCR. Arrows show the location of primers: a primer pair (shown in orange) is designed for amplifying exon 13, while the other primer set is localized in a sequence surrounding exons 11 and 16 (shown in light blue). P: Father, M: Mother, AC: Affected child, Co-: Negative control, PCR: polymerase chain reaction

study in affected individuals of Span could define a founder variant of Arg1905* (Vilchez et al., 2005), and another study showed that c.937 + 1G > A, c.1566C > G, c.2997G > T, and c.3373delG mutations account for half of all the mutations identified in Japanese LGMD2B patients (Hayashi et al., 2010). In Iran, it was just one genetic study on LGMD2B patients which was performed on nine families, and because of small sample size, no frequent mutation could be identified (Fatehi et al., 2015). Another investigation on muscle biopsy of 100 Iranian individuals who were suspected to have neuromuscular disorders revealed that only one patient in this study group suffered from dysferlinopathy (Dubowitz, 1973). In the present study, we have examined 25 LGMD2B patients from eight families, and detected novel mutations in two of eight. Previously reported mutations in *DYSF* were described in Italy (c.2706dupC) (Angelini, Grisold, & Nigro, 2011; Cacciottolo et al., 2011), France, and Japan (c.3112C > T, c.3225delT) (Krahn et al., 2009; Nguyen et al., 2005; Takahashi et al., 2013). Two other missense mutations (c.5804C > T and c.5633T > C) have been described in *DYSF* database (http://www.umd.be/DYSF/) as probable pathogenic mutations. These mutations have not been reported in Iranian population so far, and the results of in silico analyses shown in Table 2 revealed that they are deleterious.

One of the novel mutations described here was a nonsense mutation c.2419C > T (p.Gln807*) causing premature termination of translation producing a truncated protein that potentially can be removed from the cell by nonsense-mediated decay (NMD). According to the joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) for the interpretation of DNA sequence variants [28],

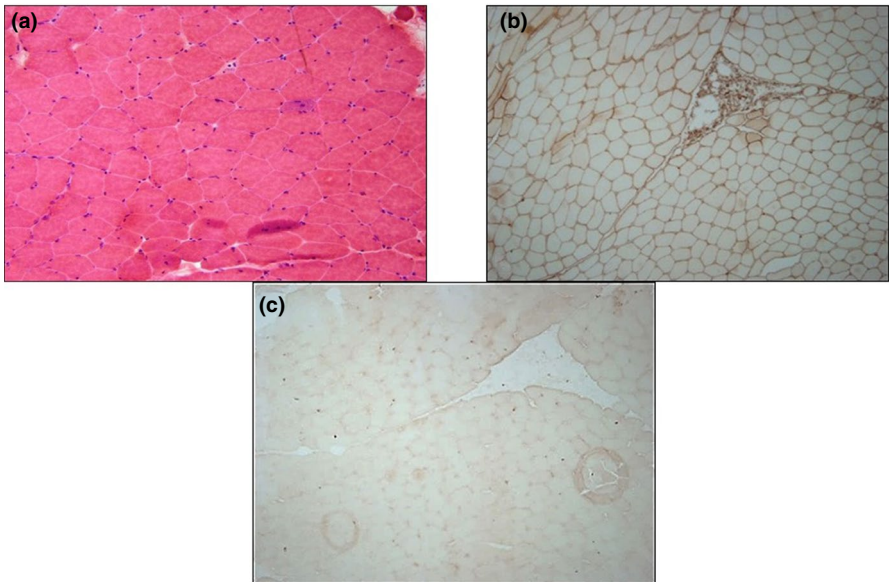

FIGURE 6 Immunohistochemical analysis of patient's muscle biopsy from family 16,941. (a) Dystrophic pattern of staining with hematoxylin and eosin (H & E). (b) Sarcolemmal labeling of almost all muscle fibers by beta-spectrin. (c) The loss of sarcolemmal labeling of all muscle fibers by dysferlin antibody

this novel variant demonstrates its pathogenicity because: 1. Null variants, such as nonsense mutations, are a strong evidence of pathogenicity (PVS1). 2. Absence of this variant in controls in different databases such as Exome Sequencing Project, 1,000 Genomes Project, or Exome Aggregation Consortium can be considered as a moderate proof for pathogenicity (PM2). 3. Haplotype analysis showed co-segregation of this variant with the disease in the family which provides an additional evidence for the pathogenicity of this variant (PP1). 4. Multiple lines of computational evidence support a deleterious effect on the gene product (PP3). 5. The phenotype of the patients in the family is compatible with LGMD2B (PP4).

The second novel mutation was c.(1,053 + 1_1054-1)_(1,397 + 1_1398-1)del, which causes deletion of DNA segment from exon 12 to exon 15. This deletion leads to a frameshift and truncation of dysferlin from 2,080 to 358 amino acids. This multi-exon deletion can potentially produce a truncated protein, which can be also discarded from the cell by NMD. According to the ACMG guideline, a single or multi-exon deletion may provide a strong evidence of pathogenicity (PVS1). Other evidence for pathogenicity includes PM2, PP1, and PP4 which have been described above. Functional analysis of the patients in both families, 17,298 and 16,941, by immunohistochemistry also showed lack of sarcolemmal labeling of all muscle fibers by dysferlin antibody confirming our genetic results.

Two families from Lurs of Boyer-Ahmad, an ethnicity in the south-west of Iran, had the same mutation (c.2706dupC) and the same haplotype (Figure 2). These data point to a possible founder effect for *DYSF* in this ethnicity. Larger sample size from patients of this ethnic group should be analyzed to confirm this hypothesis. Another evidence of founder mutation effect in LGMD2B patients (c.2779delG) was previously reported in Caucasus Jews isolate which was originated from an Iranian patient (Leshinsky-Silver et al., 2007).

In family 17,124, the initial diagnosis based on the immunohistochemistry data was myositis which can mislead the neurologist from the correct diagnosis and treatment. The genetic data could help the patient to avoid a wrong treatment by corticosteroids and immunosuppressive agents. Few researches have been done in Iranian population in the field of *DYSF* genetic analysis and diagnostics. This study contributes to our knowledge of the genetic abnormalities in the *DYSF* and improves our understanding of LGMD2B mechanism. Despite the fact that *DYSF* is a large gene lacking a hotspot, further studies in Iranian population may help to identify possible frequent mutations or founder effect in different ethnicities. We also could not find genotype–phenotype correlation in our studied patients; more sample size may help us to define the possible correlation in LGMD2B patients.

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We thank all patients and their families for contributing in this study.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Marzieh Mojbafan wrote the draft of manuscript and designed the primers and STR markers and did all PCRs. Tina Shirzadeh and Fatemeh Zafarghandi Motlagh carried out the analysis of some data. Andrei Surguchov edited the draft. Yalda Nilipour contributed in muscle biopsy study. Sirous Zeinali supervised the project, helping in the all process. All authors read and approved the paper.

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REFERENCES

Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., … Sunyaev, S. R. (2010). A method and server for predicting damaging missense mutations. *Nature Methods*, *7*(4), 248–249. https://doi.org/10.1038/nmeth0410-248

Angelini, C., Grisold, W., & Nigro, V. (2011). Diagnosis by protein analysis of dysferlinopathy in two patients mistaken as polymyositis. *Acta Myol*, *30*(3), 185–187.

Cacciottolo, M., Numitone, G., Aurino, S., Caserta, I. R., Fanin, M., Politano, L., … Nigro, V. (2011). Muscular dystrophy with marked Dysferlin deficiency is consistently caused by primary dysferlin gene mutations. *European Journal of Human Genetics*, *19*(9), 974–980. https://doi.org/10.1038/ejhg.2011.70

Carr, I. M., Bhaskar, S., O' Sullivan, J., Aldahmesh, M. A., Shamseldin, H. E., Markham, A. F., … Alkuraya, F. S. (2013). Autozygosity mapping with exome sequence data. *Human Mutation*, *34*(1), 50–56.https://doi.org/10.1002/humu.22220

Choi, Y., & Chan, A. P. (2015). PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*, *31*(16), 2745–2747. https://doi.org/10.1093/bioinformatics/btv195

de Paula, F., Vainzof, M., Passos-Bueno, M. R., de Cássia M Pavanello, R., Matioli, S. R., V B Anderson, L., … Zatz, M. (2002). Clinical variability in calpainopathy: What makes the difference? *European Journal of Human Genetics*, *10*(12), 825–832. https://doi.org/10.1038/sj.ejhg.5200888

Dincer, P., Akcoren, Z., Demir, E., Richard, I., Sancak, O., Kale, G., … Topaloglu, H. (2000). A cross section of autosomal recessive limb-girdle muscular dystrophies in 38 families. *Journal of Medical Genetics*, *37*(5), 361–367. https://doi.org/10.1136/jmg.37.5.361

Dincer, P., Leturcq, F., Richard, I., Piccolo, F., Yalnizoàlu, D., De Toma, C., … Topaloàlu, H. (1997). A biochemical, genetic, and clinical survey of autosomal recessive limb girdle muscular dystrophies in Turkey. *Annals of Neurology*, *42*(2), 222–229. https://doi.org/10.1002/ana.410420214

Dubowitz, V. (1973). Histological and histochemical stains and reactions. Muscle Biopsy, A Modern Approach.

Fatehi, F., Nafissi, S., Urtizberea, J. A., Blanck-Labelle, V., Lévy, N., Krahn, M., … Attarian, S. (2015). Dysferlinopathy in Iran: Clinical and genetic report. *Journal of the Neurological Sciences*, *359*(1–2), 256–259.https://doi.org/10.1016/j.jns.2015.11.009

Gómez-Díaz, B., Rosas-Vargas, H., Roque-Ramírez, B., Meza-Espinoza, P., Ruano-Calderón, L. A., Fernández-Valverde, F., … Coral-Vázquez, R. M. (2012). Immunodetection analysis of muscular dystrophies in Mexico. *Muscle and Nerve*, *45*(3), 338–345. https://doi.org/10.1002/mus.22314

Hayashi, S., Ohsawa, Y., Takahashi, T., Suzuki, N., Okada, T., Rikimaru, M., … Sunada, Y. (2010). Rapid screening for Japanese dysferlinopathy by fluorescent primer extension. *Internal Medicine*, *49*(24), 2693–2696. https://doi.org/10.2169/internalmedicine.49.3771

Khadilkar, S. V., Faldu, H. D., Patil, S. B., & Singh, R. (2017). Limb-girdle muscular dystrophies in India: A review. *Annals of Indian Academy of Neurology*, *20*(2), 87–95. https://doi.org/10.4103/aian.AIAN_81_17

Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J., Cooper, G. M., & Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nature Genetics*, *46*(3), 310–315. https://doi.org/10.1038/ng.2892

Krahn, M., Beroud, C., Labelle, V., Nguyen, K., Bernard, R., Bassez, G., … Levy, N. (2009). Analysis of the DYSF mutational spectrum in a large cohort of patients. *Human Mutation*, *30*(2), E345–E375. https://doi.org/10.1002/humu.20910

Kumar, P., Henikoff, S., & Ng, P. C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nature Protocols*, *4*(7), 1073–1081. https://doi.org/10.1038/nprot.2009.86

Leshinsky-Silver, E., Argov, Z., Rozenboim, L., Cohen, S., Tzofi, Z., Cohen, Y., … Sadeh, M. (2007). Dysferlinopathy in the Jews of the Caucasus: A frequent mutation in the dysferlin gene. *Neuromuscular Disorders*, *17*(11–12), 950–954. https://doi.org/10.1016/j.nmd.2007.07.010

Liu, J., Aoki, M., Illa, I., Wu, C., Fardeau, M., Angelini, C., … Brown, R. H. (1998). Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nature Genetics*, *20*(1), 31–36. https://doi.org/10.1038/1682

Magri, F., Bo, R. D., D'Angelo, M. G., Sciacco, M., Gandossini, S., Govoni, A., … Comi, G. P. (2012). Frequency and characterisation of anoctamin 5 mutations in a cohort of Italian limb-girdle muscular dystrophy patients. *Neuromuscular Disorders*, *22*(11), 934–943. S0960-8966(12)00148-4

Miller, S. A., Dykes, D. D., & Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, *16*(3), 1215. https://doi.org/10.1093/nar/16.3.1215

Mojbafan, M., Tonekaboni, S. H., Abiri, M., Kianfar, S., Sarhadi, A., Nilipour, Y., … Zeinali, S. (2016). Linkage study revealed complex haplotypes in a multifamily due to different mutations in CAPN3 gene in an Iranian Ethnic Group. *Journal of Molecular Neuroscience*, *59*(3), 392–396. https://doi.org/10.1007/s12031-016-0772-1

Moore, S. A., Shilling, C. J., Westra, S., Wall, C., Wicklund, M. P., Stolle, C., … Mendell, J. R. (2006). Limb-girdle muscular dystrophy in the United States. *Journal of Neuropathology and Experimental Neurology*, *65*(10), 995–1003. https://doi.org/10.1097/01.jnen.0000235854.77716.6c

Nguyen, K., Bassez, G., Bernard, R., Krahn, M., Labelle, V., Figarella-Branger, D., … Lévy, N. (2005). Dysferlin mutations in LGMD2B, Miyoshi myopathy, and atypical dysferlinopathies. *Human Mutation*, *26*(2), 165. https://doi.org/10.1002/humu.9355

Nguyen, K., Bassez, G., Krahn, M., Bernard, R., Laforêt, P., Labelle, V., … Eymard, B. (2007). Phenotypic study in 40 patients with dysferlin gene mutations: High frequency of atypical phenotypes. *Archives of Neurology*, *64*(8), 1176–1182. https://doi.org/10.1001/archneur.64.8.1176

Schwarz, J. M., Cooper, D. N., Schuelke, M., & Seelow, D. (2014). MutationTaster2: Mutation prediction for the deep-sequencing age. *Nature Methods*, *11*(4), 361–362. https://doi.org/10.1038/nmeth.2890

Sveen, M. L., Schwartz, M., & Vissing, J. (2006). High prevalence and phenotype-genotype correlations of limb girdle muscular dystrophy type 2I in Denmark. *Annals of Neurology*, *59*(5), 808–815. https://doi.org/10.1002/ana.20824

Tagawa, K., Ogawa, M., Kawabe, K., Yamanaka, G., Matsumura, T., Goto, K., … Hayashi, Y. K. (2003). Protein and gene analyses of dysferlinopathy in a large group of Japanese muscular dystrophy patients. *Journal of the Neurological Sciences*, *211*(1–2), 23–28. https://doi.org/10.1016/s0022-510x(03)00041-8

Takahashi, T., Aoki, M., Suzuki, N., Tateyama, M., Yaginuma, C., Sato, H., … Itoyama, Y. (2013). Clinical features and a mutation with late onset of limb girdle muscular dystrophy 2B. *Journal of Neurology, Neurosurgery and Psychiatry*, *84*(4), 433–440. https://doi.org/10.1136/jnnp-2011-301339

Urtizberea, J. A., Bassez, G., Leturcq, F., Nguyen, K., Krahn, M., & Levy, N. (2008). Dysferlinopathies. *Neurol India*, *56*(3), 289–297. https://doi.org/10.4103/0028-3886.43447

Vilchez, J. J., Gallano, P., Gallardo, E., Lasa, A., Rojas-García, R., Freixas, A., … Illa, I. (2005). Identification of a novel founder mutation in the DYSF gene causing clinical variability in the Spanish population. *Archives of Neurology*, *62*(8), 1256–1259. https://doi.org/10.1001/archneur.62.8.1256

Walter, M. C., Reilich, P., Thiele, S., Schessl, J., Schreiber, H., Reiners, K., … Lochmüller, H. (2013). Treatment of dysferlinopathy with deflazacort: A double-blind, placebo-controlled clinical trial. *Orphanet Journal of Rare Diseases*, *8*, 26. https://doi.org/10.1186/1750-1172-8-26

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