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ORIGINAL ARTICLES

Mild limb girdle muscular dystrophy R9 phenotype caused by novel compound heterozygous FKRP gene mutation

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Fukutin-related protein (FKRP) mutations cause a broad spectrum of muscular dystrophies, from a relatively mild limb-girdle muscular dystrophy type 9 (LGMDR9) to severe congenital muscular dystrophy (CMD). This study aims to report two siblings belonging to a non-consanguineous Tunisian family harboring a novel compound heterozygous *FKRP* variant and presenting a mild LGDMR9 phenotype. For mutation screening, massive parallel sequencing was performed, followed by Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) to validate the existence of the discovered variants. The absence of alpha-dystroglycan was determined by immunohistochemistry. Brain and thigh magnetic resonance imaging (MRI) were performed to detect thigh and brain abnormalities. The two siblings had a late age at onset and clinical examination showed that the pelvic girdles had a predominantly proximal and symmetrical distribution of weakness without cardiac or respiratory involvement. They both had a modified Gardner-Medwin Walton Scale mGMWS grade of 4 and a modified Rankin Scale (mRS) score of 1. The DNA sequencing revealed a novel deletion of exons 2 and 3 in one allele and a missense mutation c.1364C > A, which has been reported to be responsible for congenital muscular dystrophy and mental retardation on the second allele. The simultaneous presence of the two variations in the two cases suggests that the variants segregate with the pathophysiology.

Key words: FKRP, Dystroglycanopathy, α-Dystroglycan, LGMDR9, limb girdle muscular dystrophy

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Introduction

Fukutin-related protein (FKRP) is a ribitol 5-phosphate transferase implicated in the glycosylation of alpha dystroglycan (α-DG), a crucial element of the dystrophin glycoprotein complex (DGC) which serves to stabilize the sarcolemma in the muscle cell [1]. Mutations in *FKRP* lead to the loss of muscle stability, yielding to a group of muscular disorders such as Walker-Warburg syndrome (WWS), congenital muscular dystrophy type 1C (MDC1C), muscle-eye-brain disease (MEB) and limb girdle muscular dystrophy type 9 (LGMDR9) [2]. LGMDR9 is described as the milder form of FKRP-related myopathy. It manifests between early infancy and adulthood. The severity of LGMDR9 varies according to the phenotype,

ranging from a Duchenne-like phenotyp e at an early age to a mild phenotype with late onset or even an asymptomatic phenotype [3]. LGMDR9 patients are often characterized by muscle weakness affecting the hips more than the shoulders, elevated creatine kinase (Ck) levels, hypotonia and calves hypertrophy [4]. Furthermore, Severe restrictive respiratory failure and dilated cardiomyopathy have been observed in few LGDMR9 patients [4]. To date, more than 100 distinct *FKRP* mutations have been identified, among which 60 are responsible for LGMDR9 [5]. In the present study, we report two Tunisian siblings from a non-consanguineous family having a mild LGMDR9 phenotype and carrying a novel heterozygous *FKRP* gene mutation: exon 2 and exon 3 deletion. We also report the involvement of the c.1364C > A variant in this mild phenotype. This latter has reportedly been associated with congenital muscular dystrophy associated with mental retardation and central nervous system abnormalities in Tunisian patients [6].

Patients and methods

Clinical assessment

In this study, we report on two siblings from a non-consanguineous south Tunisian family. A scale developed by the Medical Research Council (MRC) was used to measure muscle strength [7]. Disease severity was determined using the Modified Gardner-Medwin Walton Scale (m-GMWS) [8] and the Modified Rankin Scale (mRS). For each patient, two-minutes' walk test (2MWT) [9] and 4 stairs climb test (4SC) [10] were performed to measure the functional ability. To assess the cognitive function, Mini Mental State Examination (MMSE) [11] and the Frontal Assessment Battery (FAB) [12] were performed to evaluate the cognitive function.

The two siblings had a full evaluation of blood testing, including (Ck) levels. They also had electroneuromyography (EMG), electrocardiography (ECG), transthoracic echocardiography (TTE), and respiratory functional exploration. (RFE).

Cerebral and thigh MRI were performed for the proband 1. For Cerebral MRI, axial diffusion, axial flair, T1 and T2 weighted sequences were performed. Magnetic resonance imaging (MRI) 1.5 Tesla was performed with thigh slices. 3 millimeters thick slices were performed in Axial and Coronal T1-weighted (Echo Time (TE)/Repetition Time (TR): 11/640), T2 (TE/TR). 92.9/5900) and T2 Short -time inversion recovery (STIR) (TE/TR: 40/3400).

Muscle biopsy and immunohistochemistry

After getting proband 1 permission, biopsies from the deltoid muscle were taken under local anesthesia, then they were frozen at - 80°C. Afterwards, frozen muscle biopsies underwent histological staining such as hematoxylin phloxine saffron (HPS) and modifed Gomori trichome.

Immunohistochemistry was performed on frozen sections of deltoid muscle biopsy using the following antibodies: α- Dystroglycan (α-DG) (sc-53987), dystrophin (sc-33697), utrophin (sc-33700).

DNA Extraction

After informed consent and following the manufacturer's instructions, genomic DNA was extracted from 0.2 mL of peripheral blood taken from the patients and their parents using the "Qlagen QIAamp DNA Blood Mini Kit". Qubit 4.0 fluorometric (Thermo Fisher Scientific, USA) was used to quantify the isolated DNA.

FKRP gene analysis

We performed massive parallel sequencing. The DNA Target Capture was done Using SeqCap EZ Choice (exons +/- 50 Pb). The following genes were chosen for the gene panel: ANO5, CAPN3, CAV3, DMD, DYSF, FKRP, SGCA, SGCG. Libraries constructions were performed with Kapa Nimblegen technology. The samples were subjected to paired-end sequencing using the Illumina (2*150 bp) NextSeq 500TM platform. The Bioinformatics analysis were performed by the in-house nextflow pipeline of the Hospices Civils de Lyon, which follows the recommendations furnished by the Broad Institute. The sequences were aligned and compared to the human reference genome (GRCh37/hg19). The Variants were filtered by their occurrence (present in less than 3 patients of the run) in the exonic regions and intron/exon junction. Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) were employed in order to verify the variants that could potentially be responsible for the disease.

In Sanger bidirectional capillary sequencing followed PCR amplification on ABI13500XM. We used the following primers for the amplification:

- *FKRP-4F*: TCTGCAGACCGCCCTTCGCG;
- *FKRP-4R*: GCGACATCCCTCCACCCCTC.

The produced sequences were compared to the human reference genome (CRCh37/hg19) with the SeqScape v3 software (LifeTechnologies).

In MLPA, we used The P116 SGC SALSA kit from MRC Holland and the data analysis was done using Coffalyser software.

Results

Clinical description

Proband 1

A 39-year-old man presented to our department with muscle weakness that had been gradually progressing for 10 years, mainly affecting the pelvic girdle but also affecting the shoulder and axial girdles. The gait was acquired at the age of 1, without any problem in practicing sport in childhood or adolescence. Currently, the gait is waddling, climbing the stairs is limited to 4 stairs with rails and a weakness in carrying heavy loads has been developed. Neurological examination revealed a marked Gowers' sign, a normal calf aspect and slight hyperlordosis. In the upper limbs, there was no apparent deficit of the triceps or bicipital or long supinator muscles. However, there was a deficit in the deltoid muscle. According to the Medical Research Council (MRC) manual muscle testing scale, his muscle strength was 3 in the deltoid muscle, 3 in the psoas muscle, 4 in the quadriceps, and 4 in the tibial hamstring muscle. The gluteals, gluteus minimus, gluteus medius and adductors strength was also 3. The neck flexors and extensors were not involved, but there was an axial involvement

Table I. Clinical and paraclinical data of each patient.

Patient	Proband 1	Proband 2
Gender	M	F
Age	39	44
Age at onset	27	29
Electroneuromyography	Myogenic pattern	Myogenic pattern
Electrocardiography **RFE** **TTE**	Normal Normal Normal	Normal Normal Normal
CK	3615	4000
Modified Gardner Medwin Walton scale(mGMWS) **Modified ranking scale (mRS)**	Grade 4 Grade 1	Grade 4 Grade 1
2 min walk distance (meter)	143	121
4 stairs climb test (second)	16	18
Mini Mental State Examination (MMSE)	30	30
Frontal assessement Battery (FAB)	18	18

CK: creatine phosphokinase, F: Female, M: Male, RFE: Respiratory Functional Explorations, TTE: transthoracic echocardiography.

and difficulty in rising from a lying to a sitting posture without assistance from the hands. All the reflexes were preserved except the left patellar. (Clinical and paraclinacal data of the two probands are summarized in (Tab. I).

Proband 2

A 44-year-old woman who has had a gait issue, recurrent falls, myalgia and exertional fatigue for the last 14 years. She progressively starts to develop running and climbing stairs difficulties, lower limbs stiffness, frequent falls and difficulties in combing her hair. In addition, she mentioned swallowing disorders after ingesting solids. The current physical exam revealed a waddling gait, a marked Gowers sign, calves hypertrophy and lumbar hyperlordosis. All the reflexes were present coupled with significant decrease in muscular strength in the pelvic and scapular girdle's muscles. Indeed, muscle strength was 3 in sternocleidomastoid, deltoid, psoas, Gluteus and adductors. For the two probands, cardiac and respiratory functions were normal. In addition, MMSE and FAB tests scores were normal (Tab. I).

Muscle Biopsy and immunohistochemistry

The muscle biopsy of the proband 1 revealed fibers size inequality with the coexistence of small atrophic fibers, rounded or angular, dispersed randomly. Many segmented and vacuolated fibers coexist side by side. A few regenerative fibers coexist with rare myophagic necrotic fibers. There is no perivascular or intraparenchymal inflammatory infiltration (Fig. 1A). Gömöri trichrome stain (Fig. 1B) reveals many bordered vacuoles in the fibers. These latter have larger diameter. Some sarcoplasms contain basophilic aggregates. The Oxidative methods reveal a good expression of the mitochondrial respiratory chain enzymes (complex I, II, and IV: NADH, SDH, and COX, respectively). Immunohistochemistry shows and a nor-

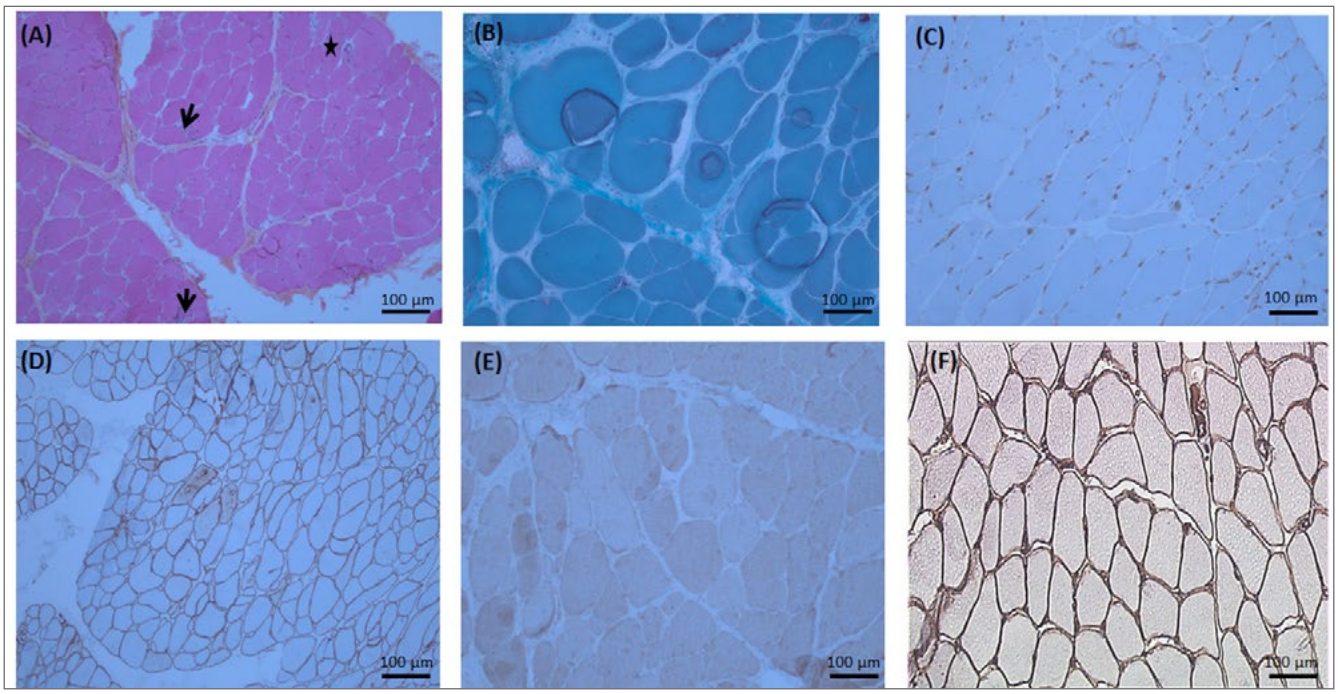


Figure 1. Histopathology and Immunohistochemical staining of deltoid muscle biopsy. (A) Hematoxilin phloxine saffron staining showing a great fibers size variability with rounded atrophic fibers and hypertrophic fibers. Somme fibers are necrotic undergoing macrophagic resorption (arrow), other fibers present rimmed vacuoles (asterix). (B) Gömöri trichrome stain revealing the presence of rimmed vacuoles and segmentation of many fibers. Immunohistochemical reaction showing normal expression of utrophine (C) and dystrophin (D) and a total absence of α-DG (E). (F) positive control showing a normal presence of α-DG in a muscle biopsy.

mal expression of utrophine (Fig. 1C), dystrophin (Fig.1 D) and an absence of α-DG expression (Fig.1 E) compared with a positive control (Fig.1 F).

FKRP gene analysis

High-throughput sequencing analysis detected two heterozygous variants in *FKRP* gene:

- one missense mutation: NM_024301.4 (*FKRP):* c.1364C > A located in exon 4;
- deletion of exons 2 and 3: NM_ 024301.4 (*FKRP):* c.(-253+1_-252-1)_(-40+1_-39-1).

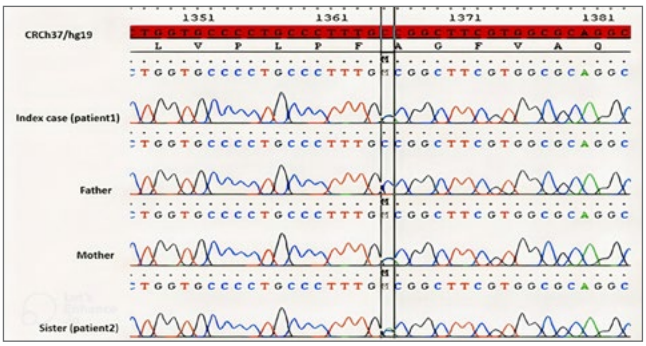


Figure 2. Sanger electropherogram of the proband 1, the proband 2 and the mother samples shows the substitution of cystosine by adnenin in a heterozygous form at the position 1369. The electropherogram of the father sample shows the absenc e of this substitution and the presence of cytosine in a homozygous form at the position 1369.

c.1364C > A mutation (ALA455ASP)

Sanger sequencing confirmed the presence of this variant in the two probands and their mother in a heterozygous state. However, in the father sample, we noted the absence of this missense mutation (Fig. 2) supplementary figures). ALA455ASP is located in the catalytic domain (C-terminal domain) of FKRP. This mutation modifies the protein's conformation, catalytic activity, and recognition sites for α-DG [6].

Exon 2 and exon 3 deletion

MLPA analysis revealed the reduction of signal for the *FKRP2* and *FKRP3* probes (exons 2 and 3 probes) in the probands samples and the father sample. This was confirmed in the ratio chart and the data table generated by coffalyser software (Fig. 3 and Fig. 4 in supplementary figures). In these subjects samples, the ratio (or dosage quotient) was below 0.7 and close to 0.5 which stand for a heterozygous deletion of the exon 2 and 3. In the mother sample, no reduced signal in exon 2 and exon 3 probes was noted. The ratio was approximately 1 which is interpreted as a normal copy number.

Cerebral and thigh MRI

Proband 1 thigh MRI indicated amyotrophy with fatty degeneration of all the thigh muscles that mostly affected the hamstring muscles (Fig. 5A). No hypoplasia or cerebellar cysts was noted in T1 sagittal and T2 axial cerebral MRI (Fig. 5B and C). However, we noted the presence of a mega cisterna magna.

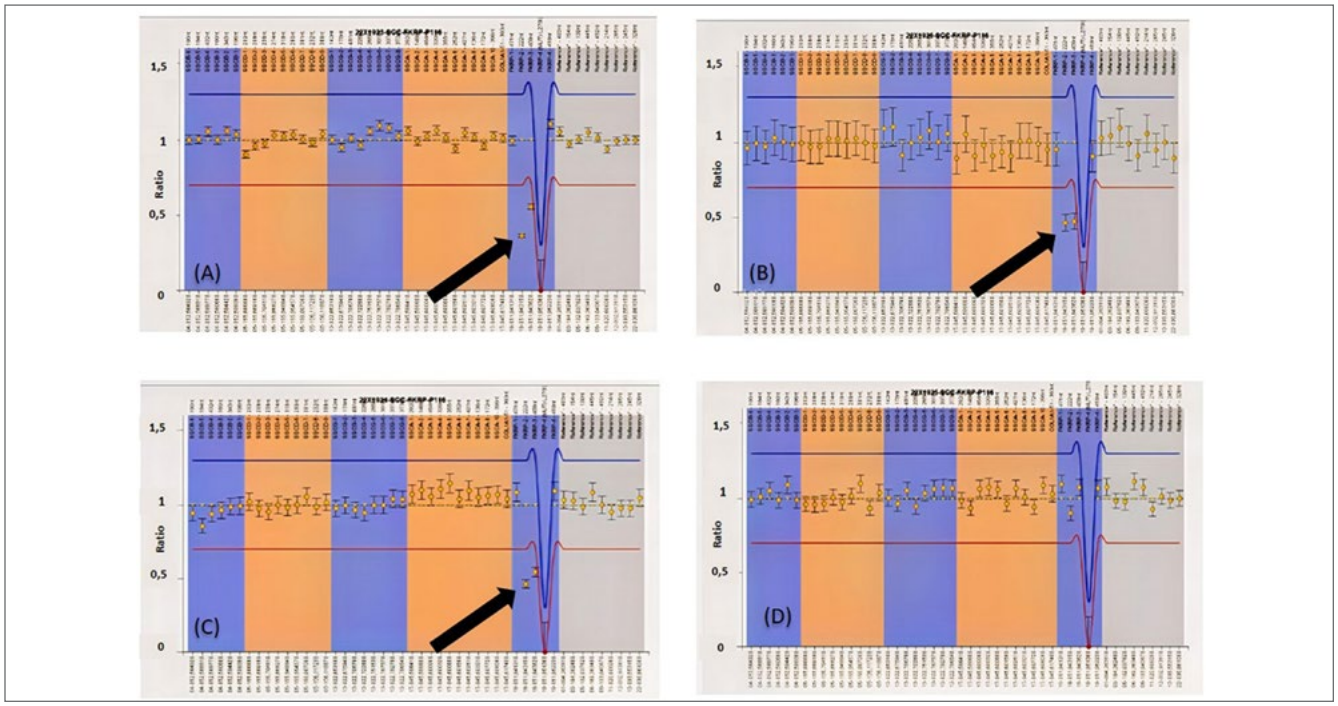


Figure 3. Ratio chart produced using MLPA analysis software Coffalyser.net. A probe ratio below 0.7 indicates a heterozygous deletion,whereas a ratio of 1 indicates a normal copy number. The ratio chart of the proband 1 (A), the proband 2 (B) and the father (C) shows 2 consecutive data points (shown in yellow) below the 0.7 ratio mark, indicating the deletion of the two exons 2 and 3 in these samples. (D) Ratio chart of the mother sample showing the absence of data point below 0.7 ratio mark which indicates the absence of the deletion of exon 2 and 3.

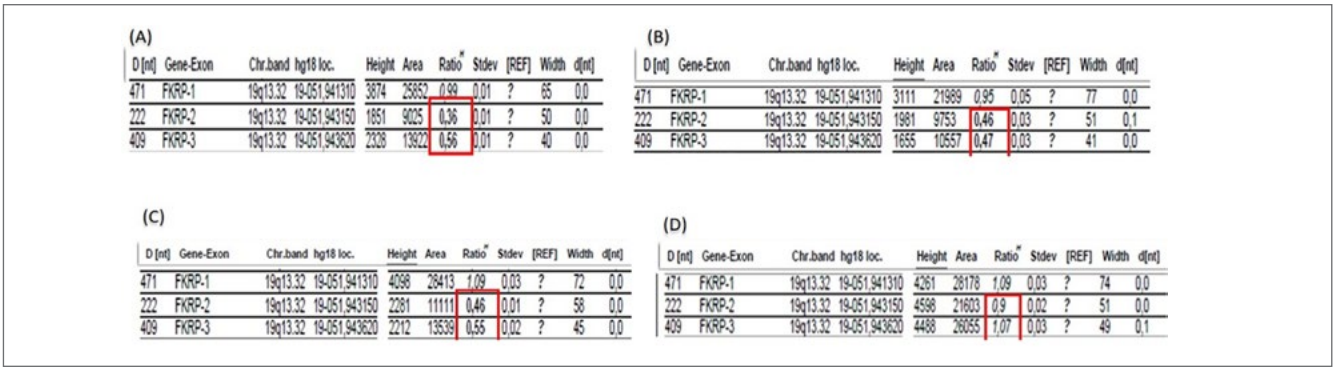


Figure 4. Ratio values of the proband 1 sample (A, proband 2 sample (B), the father sample (C) and the mother sample (D) generated by coffalyser software.

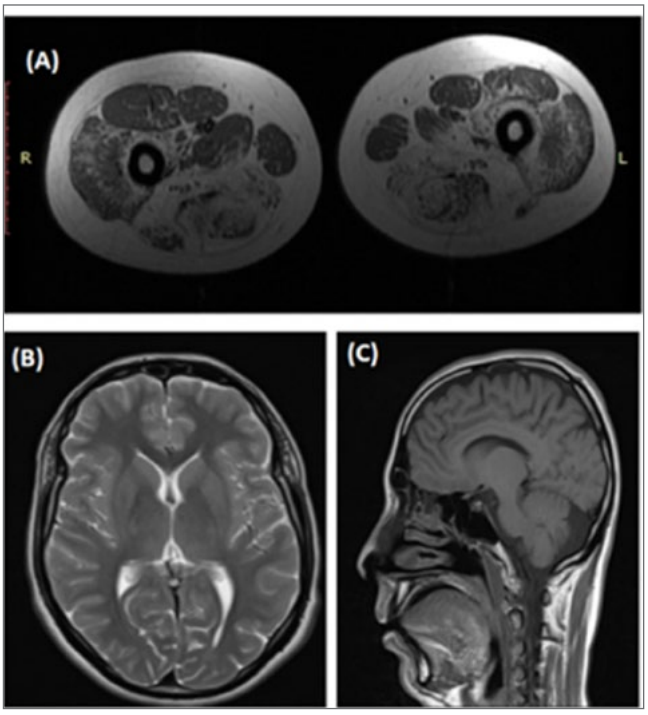


Figure 5. Proband 1 cerebral and thigh MRI. (A) Axial T1 weighted thigh MRI. (B) Axial T2-weighted sequences of brain MRI (C) Sagittal T1 weighted brain MRI.

Discussion

Mutations in the *FKRP* have been linked to a wide range of muscular dystrophy, ranging from a severe congenital form (MDC1C) [13] to a considerably mild LGMDR9 phenotype [14]. Driss et al. were the first to identify and describe LGMDR9 in a large consanguineous Tunisian family [15,16]. Moreover, c.1364C > A (ALA455ASP) variant in *FKRP* has also been described for the first time in a Tunisian family [6].

The c.1364C > A variant has been reported to be responsible of CMD associated with central nervous system abnormalities and mental retardation In Tunisian patients [6] and Moroccan patients [17,18]. Patients who had this variant in homozygous state were severely affected. They had dysphagia, severe facial weakness, severe restrictive respiratory insufficiency, macroglossia, associated with cerebral cysts and hypoplasia[6]. In this study, we report the involvement of this variant in a mild LGMDR9 phenotype.

The second DNA mutation identified in the other allele is a novel deletion of exon 2 and 3. To the best of our knowledge, no CNV of this gene has been reported in the literature. The consequences of this deletion are impossible to predict because these exons are not coding (5'UTR) and the coding region is restricted to exon 4 [19]. These areas most likely include transcription factor binding sites and 5' UTR mutations can regulate transcript levels and mRNA translation rates by generating DNA binding sites or RNA-based cis-regulatory motifs [20]. Moreover, numerous studies showed that 5'UTR mutations leads to numerous diseases by perturbing uORFs and reducing protein abundance [21-24].

Additionally, the presence of the two variants in the two patients demonstrates that the variants segregate with the pathology, which is another argument in favor of their causality. We also speculate that this novel CNV variant in *FKRP* is responsible for a milder phenotype. In this study, we confirmed the presence of the novel variant by MLPA. This confirms the value of the MLPA technique in the diagnosis of neuromuscular disorders and supports previous research on the subject. The first known application of MLPA in the analysis of Duchenne muscular dystrophy (DMD) dates back to 2005 [25]. Since then, MLPA has been increasingly utilized globally to screen for dystrophin gene deletion or duplication in both male patients and female carriers [26,27]. Afterwards, it was shown that MLPA is a fast and reliable method for identifying copy number changes in sarcoglycanopathies [28]. Our findings are consistent with these studies and demonstrate that MLPA is a practical, efficient and affordable method for screening large deletions and duplications in LGMD2 frequent genes.

In the two siblings, the age at onset was close to what has been reported in LGMDR9 patients with late onset [3,29]. In addition, the distribution of weakness was mainly proximal and symmetrical in the pelvic girdles, which is consistent with what has been reported in LGMDR9 phenotype in the literature[4,3].LGMDR9 patients are commonly characterized by calf hypertrophy which is observed in proband 2 [30,4,31]. However, the proband 1 had a normal calf aspect which is also reported in this phenotype [32,3].

The Proband 2 in our study had dysphagia, which is a very rare

feature of autosomal recessive LGMD, even in its advanced stages, according to Argov et al. [33]. Despite, in the same study, Arkov et al. mentioned exceptionally the presence of dysphagia in FKRP related LGMDR9 and LGMDR5-γ - sarcoglycan-related.
Dilated cardiomyopathy and severe restrictive respiratory insufficiency have been reported in some Caucasian patients with LGMDR9 [34,35]. In Margeta et *al.* study, Cardiac pathology has even exceeded skeletal muscular pathology [35]. Additionally, c.1364C > A variant has been associated with a higher risk of developing cardiac problems [36]. This was not observed in our patients and their actual ECG, RFE and TTE are normal.
For the two probands, we performed 2MWT to measure the functional capacity and walking ability. According to the literature, many factors can influence the walking distance of each person (it depends on the age, gender, height and weight) [37]. Based on the meta analysis reported by Bohannon et *al.*, the normative value of the walking distance performed by men in the age range between 30 and 39 is 202 m and by women in the age range between 40 and 49 is 180 m [38]. In our study the distance performed by the two probands in these two age ranges were lower than the normative values which is explained by motor deficit and muscle weakness in lower limbs.
Brain MRI abnormalities are common in patients with FKRP associated muscular dystrophy presenting at birth or in early childhood [17]. Referring to the literature, patients harboring homozygous c.1364C > A (ALA455ASP) mutation had significant hypoplasia and cerebellar cysts [6,17]. In proband 1 in our study, no hypoplasia and or cerebellar cysts has been noted. Moreover, previous study showed homozygous c.1364C > A variant and other homozygous FKRP gene mutations were associated with mental retardation in Turkish and Tunisan patients [6,17]. In our study the two patients harboring the c.1364C > A variant in heterozygous compound form had a normal cognitive functions confirmed by MMSE and FAB tests.

Conclusions

We suggest that the novel deletion of exon 2 and 3 can be included in the repertoire of variants in *FKRP* associated with LGMDR9. Our findings support the theory that the severity in the clinical course of LGMDR9 in compound heterozygous individuals is mostly determined by the second pathogenic mutation.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflicts of interest.

Authors contribution

Ikhlass Belhassen conceived the methodology and wrote the manuscript. Rita Menassa and Laurence Michelle Calemard performed the genetic analysis. Salma Sakka supervised the clinical study and revised the manuscript. Nathalie Streichenberger performed the histopathological study. Dorra Ben Ayed and Nadia Bouattour performed data curation.
Chokri Mhiri and Mariem Dammak supervised, reviewed, and validated the final manuscript.

Ethical consideration

The study protocol was approved by the ethics committee of Habib Bourguiba University Hospital.

References

1 Awano H, Saito Y, Shimizu M, Sekiguchi K, Niijima S, Matsuo M, et al. FKRP mutations cause congenital muscular dystrophy 1C and limb-girdle muscular dystrophy 2I in Asian patients. J Clin Neurosci: 2021;92:215-221. https://doi.org/10.1016/j.jocn.2021.08.014.

2 Jensen SM, Müller KI, Mellgren SI, Bindoff LA, Rasmussen M, Ørstavik K, et al. Epidemiology and natural history in 101 subjects with FKRP-related limb-girdle muscular dystrophy R9. The Norwegian LGMDR9 cohort study (2020). Neuromuscul Disord 2023;33:119-132. https://doi.org/10.1016/j.nmd.2022.11.005.

3 Hong D, Zhang W, Wang W, Wang Z, Yuan Y. Asian patients with limb girdle muscular dystrophy 2I (LGMD2I). J Clin Neurosci 2011;18:494-499. https://doi.org/10.1016/j.jocn.2010.08.010.

4 Boito CA, Melacini P, Vianello A, Prandini P, Gavassini BF, Bagattin A, et al. Clinical and Molecular Characterization of Patients With Limb-Girdle Muscular Dystrophy Type 2I. Arch Neurol 2005;62:1894. https://doi.org/10.1001/archneur.62.12.1894.

5 Alhamidi M, Brox V, Stensland E, Liset M, Lindal S, Nilssen Ø. Limb girdle muscular dystrophy type 2I: No correlation between clinical severity, histopathology and glycosylated α-dystroglycan levels in patients homozygous for common FKRP mutation. Neuromuscul Disord 2017;27:619-626. https://doi.org/10.1016/j.nmd.2017.02.015.

6 Triki C, Quijano-Roy S, Richard P, Makri S, Romero NB, Guicheney P, et al. New FKRP mutations causing congenital muscular dystrophy associated with mental retardation and central nervous system abnormalities. Identification of a founder mutation in Tunisian families. Neurogenetics 2004;5:27-34. https://doi.org/10.1007/s10048-003-0165-9.

7 Hermans G, Van den Berghe G. Clinical review: intensive care unit acquired weakness. Crit Care Lond Engl 2015;19:274. https://doi.org/10.1186/s13054-015-0993-7.

8 Fanin M, Angelini C. Muscle pathology in dysferlin deficiency. Neuropathol Appl Neurobiol 2002;28:461-470. https://doi.org/10.1046/j.1365-2990.2002.00417.x.

9 Andersen LK, Knak KL, Witting N, Vissing J. Two- and 6-minute walk tests assess walking capability equally in neuromuscular diseases. Neurology 2016;86:442-445. https://doi.org/10.1212/WNL.0000000000002332.

10 Mcdonald CM, Henricson EK, Abresch RT, Florence JM, Eagle M, Gappmaier E, et al. THE 6-MINUTE WALK TEST AND OTHER ENDPOINTS IN DUCHENNE MUSCULAR DYSTROPHY: LONGITUDINAL NATURAL HISTORY OBSERVATIONS OVER 48 WEEKS FROM A MULTICENTER STUDY. Muscle Nerve 2013;48:343-356. https://doi.org/10.1002/mus.23902.

11 Bellaj T, Jemaa SB, Romdhane NA, Dhiffallah M, Ali NB, Bouaziz M, et al. VERSION ARABE DU MINI MENTAL STATE EXAMINATION (A-MMSE) : FIDÉLITÉ, VALIDITÉ ET DONNÉES NORMATIVES. Tunis Med 2008;86.

12 Jemaa SB, Bellaj T, Romdhane NA, Chérif A, Zakraoui NO, Bouaziz M. LA FRONTAL ASSESSMENT BATTERY (FAB) : FIDÉLITÉ, VALIDITÉ ET ÉTALONNAGE D'UNE FORME ARABE.

13 Brockington M, Blake DJ, Prandini P, Brown SC, Torelli S, Benson MA, et al. Mutations in the Fukutin-Related Protein Gene (FKRP) Cause a Form of Congenital Muscular Dystrophy with Secondary Laminin a2 Deficiency and Abnormal Glycosylation of a-Dystroglycan. Am J Hum Genet 2001.

14 Brockington M, Yuva Y, Prandini P, Brown SC, Torelli S, Benson MA, et al. Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. Hum Mol Genet 2001;10:2851-2859. https://doi.org/10.1093/hmg/10.25.2851.

15 Driss A, Amouri R, Ben Hamida C, Souilem S, Gouider-Khouja N, Ben Hamida M, et al. A new locus for autosomal recessive limb-girdle muscular dystrophy in a large consanguineous Tunisian family maps to chromosome 19q13.3. Neuromuscul Disord NMD 2000;10:240-246. https://doi.org/10.1016/s0960-8966(00)00099-7.

16 Driss A, Noguchi S, Amouri R, Kefi M, Sasaki T, Sugie K, et al. Fukutin-related protein gene mutated in the original kindred limb-girdle MD 2I. Neurology 2003;60:1341-1344. https://doi.org/10.1212/01.wnl.0000065886.82930.c5.

17 Quijano-Roy S, Martí-Carrera I, Makri S, Mayer M, Maugenre S, Richard P, et al. Brain MRI abnormalities in muscular dystrophy due to FKRP mutations. Brain Dev 2006;28:232-242. https://doi.org/10.1016/j.braindev.2005.08.003.

18 Trovato R, Astrea G, Bartalena L, Ghirri P, Baldacci J, Giampietri M, et al. Elevated serum creatine kinase and small cerebellum prompt diagnosis of congenital muscular dystrophy due to FKRP mutations. J Child Neurol 2014;29:394-398. https://doi.org/10.1177/0883073812474951.

19 Sveen M-L, Schwartz M, Vissing J. High prevalence and phenotype-genotype correlations of limb girdle muscular dystrophy type 2I in Denmark. Ann Neurol 2006;59:808-815. https://doi.org/10.1002/ana.20824.

20 Lim Y, Arora S, Schuster SL, Corey L, Fitzgibbon M, Wladyka CL, et al. Multiplexed functional genomic analysis of 5' untranslated region mutations across the spectrum of prostate cancer. Nat Commun 2021;12:4217. https://doi.org/10.1038/s41467-021-24445-6.

21 Ryczek N, Łyś A, Makałowska I. The Functional Meaning of 5′UTR in Protein-Coding Genes. Int J Mol Sci 2023;24:2976. https://doi.org/10.3390/ijms24032976.

22 Damjanovich K, Langa C, Blanco FJ, McDonald J, Botella LM, Bernabeu C, et al. 5'UTR mutations of ENG cause hereditary hemorrhagic telangiectasia. Orphanet J Rare Dis 2011;6:85. https://doi.org/10.1186/1750-1172-6-85.

23 Whiffin N, Karczewski KJ, Zhang X, Chothani S, Smith MJ, Evans DG, et al. Characterising the loss-of-function impact of 5' untranslated region variants in 15,708 individuals. Nat Commun 2020;11:2523. https://doi.org/10.1038/s41467-019-10717-9.

24 Plassmeyer SP, Florian CP, Kasper MJ, Chase R, Mueller S, Liu Y, et al. A Massively Parallel Screen of 5'UTR Mutations Identifies Variants Impacting Translation and Protein Production in Neurodevelopmental Disorder Genes. MedRxiv Prepr Serv Health Sci November 2023:2023.11.02.23297961. https://doi.org/10.1101/2023.11.02.23297961.

25 Schwartz M, Dunø M. Improved Molecular Diagnosis of Dystrophin Gene Mutations Using the Multiplex Ligation-Dependent Probe Amplification Method. Genet Test 2004;8:361-367. https://doi.org/10.1089/gte.2004.8.361.

26 Janssen B, Hartmann C, Scholz V, Jauch A, Zschocke J. MLPA analysis for the detection of deletions, duplications and complex rearrangements in the dystrophin gene: potential and pitfalls. Neurogenetics 2005;6:29-35. https://doi.org/10.1007/s10048-004-0204-1.

27 Deepha S, Vengalil S, Preethish-Kumar V, Polavarapu K, Nalini A, Gayathri N, et al. MLPA identification of dystrophin mutations and in silico evaluation of the predicted protein in dystrophinopathy cases from India. BMC Med Genet 2017;18:67. https://doi.org/10.1186/s12881-017-0431-6.

28 Wildförster V, Dekomien G. Detecting copy number variations in autosomal recessive limb-girdle muscular dystrophies using a multiplex ligation-dependent probe amplification (MLPA) assay. Mol Cell Probes 2009;23:55-59. https://doi.org/10.1016/j.mcp.2008.11.002.

29 Audhya IF, Cheung A, Szabo SM, Flint E, Weihl CC, Gooch KL. Progression to Loss of Ambulation Among Patients with Autosomal Recessive Limb-girdle Muscular Dystrophy: A Systematic Review. J Neuromuscul Dis 9:477-492. https://doi.org/10.3233/JND-210771.

30 Poppe M, Cree L, Bourke J, Eagle M, Anderson LVB, Birchall D, et al. The phenotype of limb-girdle muscular dystrophy type 2I. Neurology 2003;60:1246-1251. https://doi.org/10.1212/01.wnl.0000058902.88181.3d.

31 Vieira NM, Schlesinger D, de Paula F, Vainzof M, Zatz M. Mutation analysis in the FKRP gene provides an explanation for a rare cause of intrafamilial clinical variability in LGMD2I. Neuromuscul Disord 2006;16:870-873. https://doi.org/10.1016/j.nmd.2006.08.007.

32 Kang PB, Feener CA, Estrella E, Thorne M, White AJ, Darras BT, et al. LGMD2I in a North American population. BMC Musculoskelet Disord 2007;8:115. https://doi.org/10.1186/1471-2474-8-115.

33 Argov Z, de Visser M. Dysphagia in adult myopathies. Neuromuscul Disord NMD 2021;31:5-20. https://doi.org/10.1016/j.nmd.2020.11.001.

34 Mercuri E, Brockington M, Straub V, Quijano-Roy S, Yuva Y, Herrmann R, et al. Phenotypic spectrum associated with mutations in the fukutin-related protein gene. Ann Neurol 2003;53:537-542. https://doi.org/10.1002/ana.10559.

35 Margeta M, Connolly AM, Winder TL, Pestronk A, Moore SA. Cardiac pathology exceeds skeletal muscle pathology in two cases of limb-girdle muscular dystrophy type 2I. Muscle Nerve 2009;40:883-889. https://doi.org/10.1002/mus.21432.

36 Kefi M, Amouri R, Chabrak S, Mechmeche R, Hentati F. Variable Cardiac Involvement in Tunisian Siblings Harboring FKRP Gene Mutations. Neuropediatrics 2008;39:113-115. https://doi.org/10.1055/s-2008-1081465.

37 Bohannon RW. Normative reference values for the two-minute walk test derived by meta-analysis. J Phys Ther Sci 2017;29:2224-2227. https://doi.org/10.1589/jpts.29.2224.

38 Bohannon RW. Normative reference values for the two-minute walk test derived by meta-analysis. J Phys Ther Sci 2017;29:2224-2227. https://doi.org/10.1589/jpts.29.2224.