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Clinical, genetic, and pathologic characterization of *FKRP* Mexican founder mutation c.1387A>G

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Abstract

Objective

To characterize the clinical phenotype, genetic origin, and muscle pathology of patients with the *FKRP* c.1387A>G mutation.

Methods

Standardized clinical data were collected for all patients known to the authors with c.1387A>G mutations in *FKRP*. Muscle biopsies were reviewed and used for histopathology, immunostaining, Western blotting, and DNA extraction. Genetic analysis was performed on extracted DNA.

Results

We report the clinical phenotypes of 6 patients homozygous for the c.1387A>G mutation in *FKRP*. Onset of symptoms was <2 years, and 5 of the 6 patients never learned to walk. Brain MRIs were normal. Cognition was normal to mildly impaired. Microarray analysis of 5 homozygous *FKRP* c.1387A>G patients revealed a 500-kb region of shared homozygosity at 19q13.32, including *FKRP*. All 4 muscle biopsies available for review showed end-stage dystrophic pathology, near absence of glycosylated α-dystroglycan (α-DG) by immunofluorescence, and reduced molecular weight of α-DG compared with controls and patients with homozygous *FKRP* c.826C>A limb-girdle muscular dystrophy.

Conclusions

The clinical features and muscle pathology in these newly reported patients homozygous for *FKRP* c.1387A>G confirm that this mutation causes congenital muscular dystrophy. The clinical severity might be explained by the greater reduction in α-DG glycosylation compared with that seen with the c.826C>A mutation. The shared region of homozygosity at 19q13.32 indicates that *FKRP* c.1387A>G is a founder mutation with an estimated age of 60 generations (~1,200–1,500 years).

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Glossary

CMD = congenital muscular dystrophy; **DSHB** = Developmental Studies Hybridoma Bank; **EF** = ejection fraction; **H&E** = hematoxylin and eosin; **HRC** = Haplotype Reference Consortium; **IF** = immunofluorescence; **IRB** = institutional review board; **LGMD2I** = limb-girdle muscular dystrophy type 2I; **SNP** = single nucleotide polymorphism; **WGA** = wheat germ agglutinin; **α-DG** = α-dystroglycan; **β-DG** = β-dystroglycan.

Dystroglycanopathies are muscular dystrophies resulting from hypoglycosylation of α-dystroglycan (α-DG), a protein in the dystrophin-glycoprotein complex.[1–3] More than 17 genes are required for proper α-DG functional glycosylation; *FKRP* is one of the most commonly mutated genes.[4] It was recently shown that FKRP functions as a ribitol 5-phosphate transferase.[5]

FKRP mutations result in highly variable phenotypes, ranging from severe congenital muscular dystrophy (CMD) to mild limb-girdle muscular dystrophy type 2I (LGMD2I).[6,7] The most common founder mutation (c.826C>A, p.Leu276Ile) is associated with an LGMD2I phenotype.[8,9] Muscle biopsies from patients with LGMD2I show mild to moderate dystrophic changes and highly variable partial reduction in immunostaining for glycosylated α-DG.[10]

In 2007, a novel homozygous *FKRP* mutation (c.1387A>G, p.Asn463Asp) was identified in 2 Mexican American girls; the authors suggested a possible founder mutation.[11] The 2 patients had hypotonia at birth and never achieved the ability to stand or walk. Both girls had a marked reduction in glycosylated α-DG and decreased laminin α2 (merosin) immunostaining.[11] A third Mexican patient homozygous for *FKRP* c.1387A>G had a slightly milder clinical course with independent ambulation between 14 and 24 months of age[12]; no muscle biopsy immunostaining was reported.

We have identified 6 additional patients homozygous for the *FKRP* c.1387A>G variant and 3 compound heterozygous patients with the *FRKP* c.1387A>G and the *FKRP* c.826C>A mutations. Here, we describe the clinical and pathologic features of these cases and provide genetic evidence that c.1387A>G is a founder mutation originating in pre-Columbian central Mexico.

Methods

Patient data collection

Standardized clinical data were collected for all patients known to the authors with a c.1387A>G mutation in *FKRP*. Patients were identified through diagnostic testing in the Department of Pathology at The University of Iowa, personal communications, or through patient participation in the Iowa Wellstone Center dystroglycanopathy natural history study (clinical trials identifier NCT00313677). The clinical teams involved in the patients' care abstracted the clinical data, including results from genetic testing, from the medical records using a standardized data collection form, and the deidentified information was collated centrally.

Genotype and haplotype analysis

The *FKRP* mutations were identified or confirmed through clinical testing in Clinical Laboratory Improvement Amendments-certified laboratories. Identification of the *FKRP* mutation for patient 4 was initially done through whole-exome sequencing using Broad dual-barcoded library construction followed by the Illumina Rapid Capture Exome enrichment kit with 38 Mb target territory (29 Mb baited).

Genome-wide single nucleotide polymorphism (SNP) genotyping was performed on genomic DNA using Illumina Human Infinium Omni2.5Exome-8 v1.3 BeadChips. Samples were processed on an Illumina iScan system using standard Illumina protocols, and genotypes were called with Illumina GenomeStudio software. Genotypes were cleaned using PLINK 1.9 software,[13] yielding an average genotyping rate of 99.8% and between 2,594,691 to 2,602,822 genotypes per sample. Phased haplotypes for chromosome 19 were computed from unphased genotypes using the Eagle2 software and the Haplotype Reference Consortium (HRC r.1.1) reference panel of human haplotypes, executed on the Michigan Imputation Server.[14] The age of the founder mutation was estimated using the Gamma method assuming a correlated "tree-like" genealogy applied to the genetic length of the ancestral segment lengths surrounding the *FKRP* mutation.[15] Ancestry inference combined patient genotypes with 1000 Genomes Project Illumina Omni2.5 genotypes merged from ALL.chip.omni_broad_sanger_combined.20140818.snps.genotypes.vcf.gz (ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/supporting/hd_genotype_chip). The merged genotype set was pruned for linked SNPs using PLINK indep-pairwise functionality with the arguments 1000 50 0.2, keeping 135,080 unlinked SNPs for subsequent ancestry analysis using the program ADMIXTURE with $K = 3$ ancestral populations.[16]

Muscle biopsy evaluation

All available muscle biopsies from these patients were reviewed and re-evaluated (K.A.J. and S.A.M.). Frozen sections of skeletal muscle were evaluated at The University of Iowa using standard hematoxylin and eosin (H&E) staining and immunofluorescence (IF). Immunostaining was performed using the following antibodies: dystrophin, carboxy terminus (rabbit polyclonal ab15277; Abcam, Cambridge, UK); α-DG (clone IIH6; Developmental Studies Hybridoma Bank (DSHB), The University of Iowa), β-DG (clone 7D11; DSHB), and merosin (laminin α2) (clone 5H2; Millipore

Sigma, Massachusetts, US). Secondary antibodies used included goat anti-rabbit immunoglobulin G (IgG), goat anti-mouse IgM, or goat anti-mouse IgG all labeled with Alexa-Fluor488 (Life Technologies, Carlsbad, CA). Immunostains were analyzed in a blinded manner by standard fluorescence microscopy. The intensity of staining with each antibody was graded from zero (absent) to 3+ (normal expression). Control human skeletal muscle was included with research patient material on each glass slide immunostained in the study.

Western blotting

Pooled cryosections cut from selected muscle biopsies were used for Western blotting at The University of Iowa. Wheat germ agglutinin (WGA) glycoprotein preparations were performed and samples run on 3%–15% gradient gels as previously described.[17,18] Antibodies used for blotting included IIH6 (gift from Kevin P. Campbell, The University of Iowa) and AF6868 (R&D Systems, Minneapolis, MN). Blots were imaged on an Odyssey infrared fluorescence imaging system (Li-Cor Biosciences, Lincoln, NE).

Standard protocol approvals and patient consents

The University of Iowa institutional review board approved this study (IRB# 201703860). Initial sequencing for patient 4 was approved by the NIH/NINDS Institutional Review Board (IRB# 12-N-0095). Informed consent was obtained from all participants who had muscle biopsy tissue stored in the Iowa Wellstone Center Tissue Repository. Letters of agreement were obtained from all collaborating clinicians.

Data availability

Study data for the primary analyses presented in this manuscript are available upon reasonable request from the corresponding and senior author.

Results

Clinical

Clinical data were collected on 6 patients from 5 families (patients 1–6) who are homozygous for the c.1387A>G *FKRP* mutation and 3 patients from 2 families with compound heterozygous *FKRP* mutations, c.1387A>G and c.826C>A (patients 7–9). Genotypes and clinical data are summarized in table 1. Homozygous c.1387A>G mutations were found in a seventh patient (patient 0) through clinical testing in The University of Iowa's Molecular Pathology Laboratory, but we were unable to obtain clinical information. This seventh homozygous c.1387A>G patient was only included in the genetic analysis.

The average current age of the homozygous patients is 9.3 years (range 4–19 years). All are of Hispanic ethnicity; some individuals reported a history of family members emigrating from central Mexico (figure 1A). Three of the 6 patients are male. All patients for whom details of early course are available had onset of symptoms (hypotonia and delayed motor milestones) before age 1 year. All patients learned to sit, but most (5 of the 6) patients never walked independently. Many started using a wheelchair by age 1–2 years. One patient walked at 2.5 years but required a wheelchair fulltime at age 8 years. Cognition is normal to mildly impaired; brain imaging (MRI or CT) and vision are normal. Most patients are currently speaking in sentences. Creatine kinase levels were >10× normal (average 11,695 IU/L). All 3 of the patients who underwent echocardiogram had normal ejection fractions (EFs) at ages 3, 9, and 19 years.

The average current age of the 3 patients with compound heterozygous mutations in *FKRP* (c.1387A>G and c.826C>A) is 19.3 years (range 7–29 years). They met initial developmental milestones on time (sitting, walking, and talking) when details were known. They presented with hypotonia, difficulty with stairs, and muscle hypertrophy in childhood from age <2 years to <10 years. The youngest patient (7 years) is still ambulatory. The other 2 patients became wheelchair dependent at ages 12 and 16 years. Cognition is normal. Ejection fraction on echocardiogram was normal for the youngest patient (EF 59% at age 7 years) but was decreased for the 2 other patients (EFs 44% at age 22 years and 35–40% at age 21 years).

Genetic analysis

All homozygous *FKRP* c.1387A>G patients report Hispanic ethnicity. Two compound heterozygous *FKRP* c.1387A>G/c.826C>A patients (siblings) reported Hispanic ethnicity, with the mother carrying *FKRP* c.1387A>G. The other patient with the *FKRP* c.1387A>G/c.826C>A genotype reported a father with Hispanic ethnicity. All 3 homozygous *FKRP* c.1387A>G cases in the literature also reported Hispanic ethnicity. Reported family origins of current and published cases localize to central Mexico (figure 1A). Genome-wide SNPs from 5 unrelated homozygous c.1387A>G patients (patients 0, 3, 4, 5, and 6) and 1 compound heterozygous c.1387A>G/c.826C>A patient (patient 9) were compared with 1000 Genomes Project populations with varying degrees of continental Native American, European, and African admixture. The genomic ancestry of *FKRP* c.1387A>G patients showed largely Native American fractions (37%–74%) followed by European (22%–53%), consistent with cosmopolitan Mexican ancestry (figure 1B). Patient 9, with c.1387A>G/c.826C>A genotype, had a European ancestry fraction of 72%, consistent with 1 Hispanic parent. Three additional homozygous *FKRP* c.826C>A patients showed predominantly European ancestry (patients A, B, and C, figure 1B). Fine-scale heterozygosity analysis surrounding the *FKRP* locus on chromosome 19 revealed a ~500-kb region of shared homozygosity between the *FKRP* c.1387A>G patients, and the decay of haplotype sharing (figure 1C) indicated that c.1387A>G is a founder mutation. In the 3 homozygous *FKRP* c.826C>A patients of European ancestry, a smaller ~150-kb region of shared homozygosity confirmed that c.826C>A is also a founder mutation. Phased haplotypes from the compound heterozygous *FKRP* patient (c.1387A>G/

Table 1 Summary of clinical data

Patient	1	2[a]	3[a]	4	5	6	7[b]	8[b]	9
Allele 2[c]	c.1387A>G	c.1387A>G	c.1387A>G	c.1387A>G	c.1387A>G	c.1387A>G	c.826C>A	c.826C>A	c.826C>A
Age (y)/sex	4/M	4/F	6/M	9/M	14/F	19/F	7/F	22/M	29/M
Ethnicity	H	H	H	H	H	H	H	H	H/C
Consanguinity	Y	N	N	Y	U	N	N	N	N
Age at onset	<2 y	9 mo	4 mo	Birth	8 mo	<2 y	<2 y	<2 y	2–10 y
First sitting	5 mo	<1 y	Normal age	6 y	1 y	8 mo	Normal age	Normal age	<1 y
First walking	NA	NA	NA	NA	NA	2.5 y	1 y	1 y	9 mo
First words	18–21 mo	1 y	Normal age	1 y	Normal age	18 mo	Normal age	1 y	1 y
Cognitive function	Mild impairment	Normal	Mild impairment	Normal	Normal	Mild impairment	Normal	Normal	Normal
Age FT wheelchair	NA	1 y	2–3 y	1 y	NA	8 y	NA	12 y	16 y
Respiratory support	None	None	Nocturnal NIV at 5 y	Cough assist	None	Trach/vent at 14 y	None	None	NIV at 24 y
CK	2,800[e]	26,810 (RR: 20–200)	22,170 (RR: 20–200)	2,657 (RR: 4–87)	U	4,038 (RR: 28–170)	14,451[e]	U	U
Brain imaging	Normal CT	Normal MRI	Normal MRI	Normal MRI	U	Normal MRI	U	Normal MRI	U
EF[d] (age)	64% (3 y)	U	U	64% (9 y)	U	61% (19 y)	59% (7 y)	44% (22 y)	35–40% (21 y)
Muscle bx age	NA	NA	2 y	2 y	10 mo	4 y	NA	NA	9 y

Abbreviations: Bx = biopsy; C = Caucasian; CK = creatine kinase; EF = ejection fraction; FT wheelchair = full-time wheelchair use; H = Hispanic; NA = not applicable; NIV = noninvasive ventilation; normal age = specific age is not known but considered within a normal range; U = unknown.
[a] Patients 2 and 3 are siblings.
[b] Patients 7 and 8 are siblings.
[c] Allele 1 for all patients is c.1387A>G.
[d] EF measured by echocardiogram.
[e] CK reference range unknown. RR: CK reference range in U/L.

c.826C>A) revealed a compound diplotype of the 2 founder mutations (figure 1D) and confirmed that these 2 founder mutations occurred on different ancestral chromosomes. The range of physical lengths of the c.1387A>G ancestral segments were 0.98 Mb in patient 3, 1.38 Mb in patient 5, 1.52 Mb in patient 6, 2.32 Mb in patient 0, and 4.48 Mb in patient 4. The estimated age of the c.1387A>G founder mutation was 59.9 generations (95% confidence interval 10.8–123.5), which is ~1,200–1,500 years old, assuming 20- to 25-year average generation spans.

Muscle biopsy histopathology

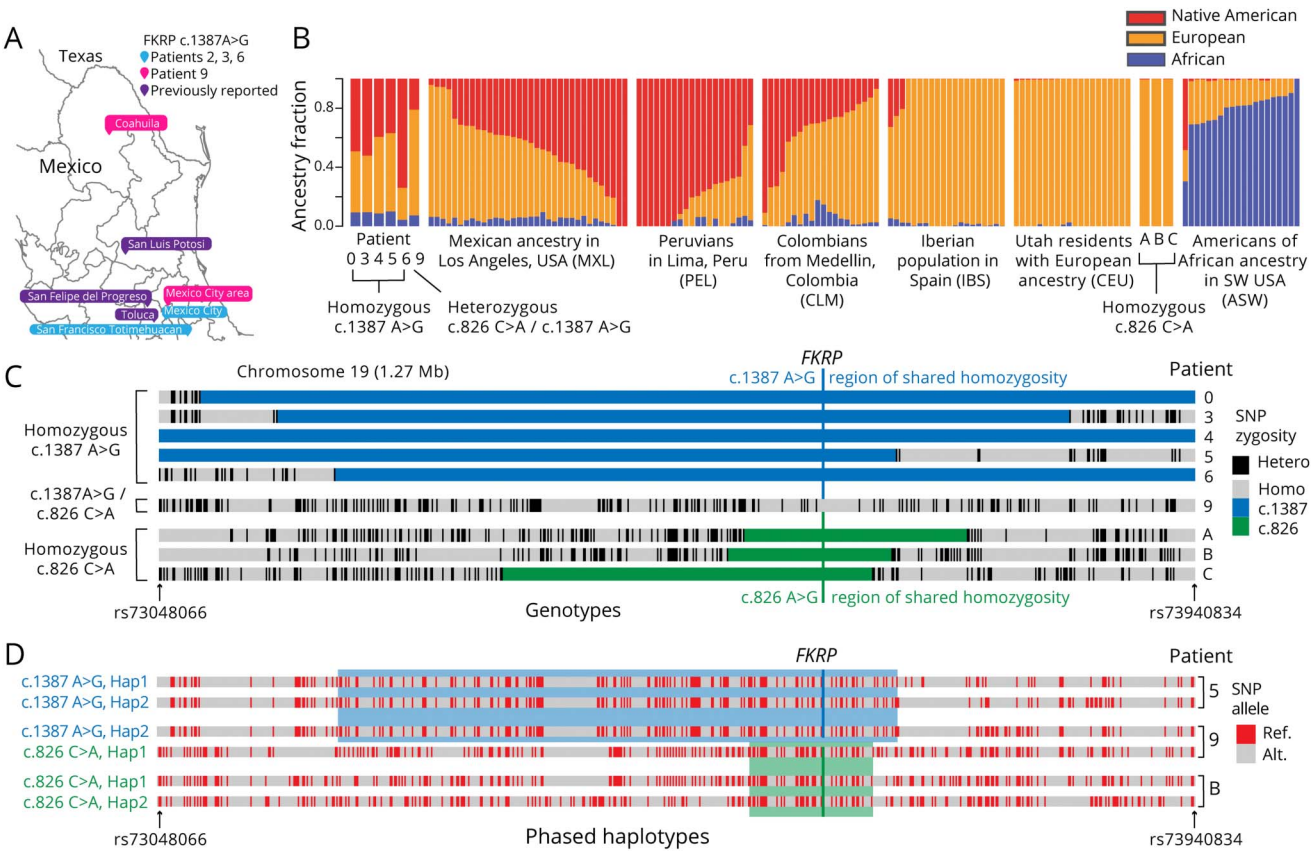
Muscle biopsies from patients with *FKRP* c.1387A>G mutations (4 homozygous and 1 compound heterozygous c.1387A>G/c.826C>A) were reviewed and compared with the muscle biopsy from a patient homozygous for the *FKRP* c.826C>A common founder mutation (patient D; biopsy at age 25 years). The average age at muscle biopsy for homozygous *FKRP* c.1387A>G patients was 2.2 years. The compound heterozygous patient had a muscle biopsy at age 9 years.

Muscle biopsies from the patient homozygous for c.826C>A and the patient compound heterozygous for c.1387A>G and c.826C>A (patient 9) showed similar mild to moderate dystrophic changes on H&E (figure 2, A and B, respectively). These included increased fiber size variation with scattered atrophic and hypertrophic fibers, necrotic fibers undergoing myophagocytosis, and grouped regeneration. In contrast, muscle biopsies from 4 patients homozygous for c.1387A>G (patients 3–6) showed severe dystrophic pathology on H&E staining including marked endomysial fibrosis and fatty replacement, large variation in fiber size with atrophic and very large hypertrophic fibers, conspicuous myonecrosis/myophagocytosis, and grouped regeneration (figure 2, C and D). Some biopsies could be classified as "end stage" because of the extensive loss of muscle fibers.

Immunostaining

IF staining was evaluated centrally in a blinded manner (K.A.J. and S.A.M.). Table 2 outlines IF staining quantification results. The 4 patients homozygous for c.1387A>G (patients

Figure 1 Comparative ancestry and *FKRP* haplotype sharing



(A) Map of reported family origins of patients homozygous for *FKRP* c.1387A>G. Blue markers represent patients 2, 3 (siblings), and 6, pink markers represent patient 9's distant grandparents, and purple markers represent 3 previously reported homozygous *FKRP* c.1387A>G cases. (B) Global ancestry proportions estimated with ADMIXTURE (K = 3) for *FKRP* patients 0, 3, 4, 5, 6, 9, A, B, and C, compared with 1000 Genomes Project samples from unrelated Native Americans (MXL, 34 samples; PEL 20 samples; CLM 20 samples), Europeans (IBS, 20 samples; CEU 20 samples), and African Americans (ASW, 20 samples). Continental ancestry fraction is shown as Native American (red), European (orange), and African (blue). (C) Heterozygosity for 701 SNPs from chr19:46,664,561-47,933,257 (hg19), with shared homozygous regions for c.1387A>G highlighted in blue and c.826C>A in green. (D) Phased haplotypes from patient 9 (heterozygous c.1387A>G/c.826C>A), patient 5 (c.1387A>G), and patient B (c.826C>A) with red/gray indicating the allele at each SNP position and the minimally shared homozygous regions highlighted in blue/green. SNP = single nucleotide polymorphism.

3–6) all showed largely decreased to absent glycosylated α-DG positivity (0–1+) with the IIH6 antibody, and mild variable decreases in β-DG, dystrophin, and merosin. The patient heterozygous for c.1387A>G/c.826C>A (patient 9) and a patient homozygous for c.826C>A (patient D) both showed a much more variable pattern of α-DG glycosylation loss with some fibers retaining a normal staining intensity (0–3+). These biopsies also showed mild variable decreases in β-DG, dystrophin, and merosin. Representative images of IF staining are shown in figure 3 with normal control (figure 3, A–D), homozygous c.826C>A (patient D; figure 3, E–H), and 2 of the homozygous 1387A>G patients (patients 3 and 4; figure 3, I–L and M–P, respectively).

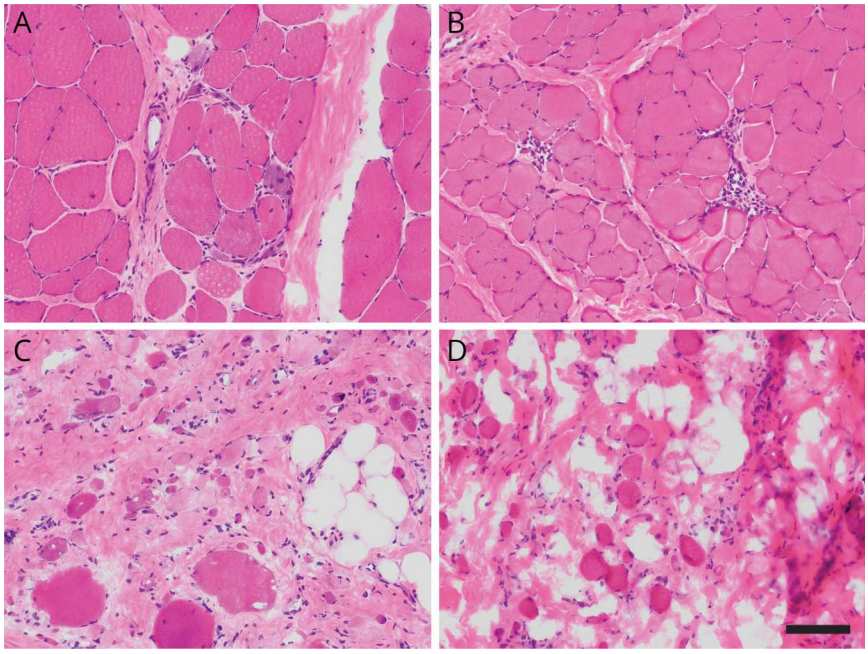
Western blotting

Gradient gel separation of WGA preparations derived from frozen muscle biopsies showed that each patient with *FKRP* mutations has reduced molecular weight α-DG (figure 4). These same patients have lost α-DG functional glycosylation as demonstrated by the absence or near absence of IIH6 binding. The smaller molecular weight of α-DG in the 2 patients homozygous for *FKRP* c.1387A>G suggests that this mutation results in a greater degree of α-DG hypoglycosylation.

Discussion

We present the clinical features, genetic analysis, and muscle pathology of 6 individuals from 5 unrelated families who are homozygous for *FKRP* c.1387A>G and 3 compound heterozygous patients from 2 unrelated families for *FKRP* c.1387A>G and c.826C>A. Our results expand on the phenotype of the 3 previously reported cases with this founder mutation. Five of our cases presented with a typical CMD phenotype and never walked independently, consistent with previous reports.[6,11,12] The remaining patient presented before age 2 years with delayed acquisition of motor skills, and although the patient acquired independent walking, this was lost by age 8 years. None of our patients had overt abnormalities in eye or brain development, reported in some cases of *FKRP*-related CMD with different mutations.[19–22] Based

Figure 2 Histopathology



(A) Representative image of muscle biopsy from a patient homozygous for the European common mutation in *FKRP* c.826C>A (patient D) showing mild to moderate dystrophic changes. (B) Representative image of muscle biopsy from patient 9 (heterozygous for c.1387A>G and c.826C>A) showing similar changes to the biopsy in part A. (C and D) Representative images from patients 3 and 4 (both homozygous for c.1387A>G) showing a very severe dystrophic, nearly end-stage histopathology. Scale bar = 100 μm, equivalent for all photomicrographs.

on all known cases to date, *FKRP* c.1387A>G mutations do not appear to be associated with the more severe muscle-eye-brain phenotype.

In contrast, the patients with compound heterozygous mutations (c.1387A>G/c.826C>A) had a milder and more slowly progressive course; all achieved walking at the expected age of 1 year. This is consistent with what is reported in other patients who have compound heterozygous mutations with 1 c.826C>A allele.[8]

Cardiac function may be impaired in both CMD and LGMD2I because of *FKRP* mutations.[23–25] None of the patients homozygous for *FKRP* c.1387A>G who underwent testing in our series have cardiomyopathy, but they were young at the last echocardiogram (ages 3, 9, and 19 years) and may develop abnormal cardiac function later in life. Two of the 3 compound heterozygous patients had decreased EF on echocardiogram, ages 21 and 22 years at the time of evaluation. The probability of cardiomyopathy in patients with *FKRP* mutations increases with age, most commonly occurring in adulthood,[23–25] and therefore, patients should be appropriately screened.

Three of the 6 patients homozygous for the *FKRP* c.1387A>G mutation use some sort of respiratory support in our series, despite the young average age of the cohort, and a single patient with compound heterozygous mutations started using noninvasive ventilation at age 24 years. These observations emphasize the importance of monitoring respiratory status in these patients.

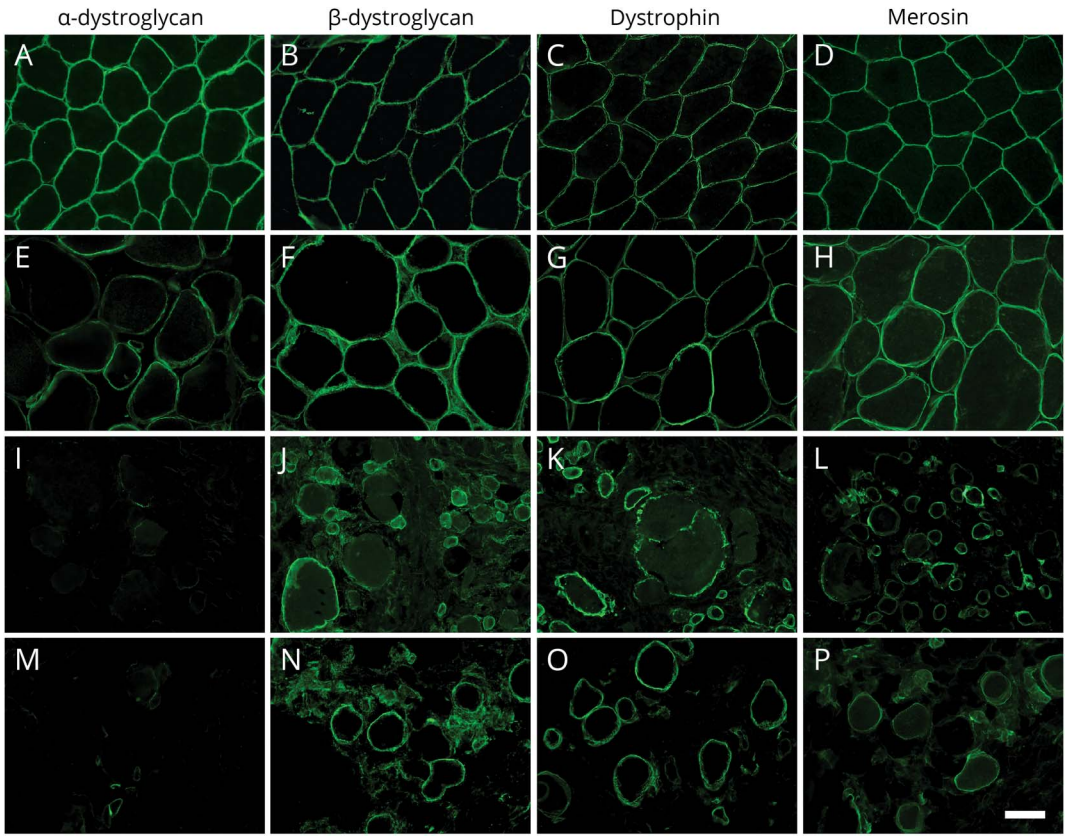
Microarray analysis confirms that the *FKRP* c.1387A>G allele is part of a ~500-kb shared homozygous segment on

Table 2 Immunofluorescence staining quantification

Patient number	3	4	5	6	9	D[a]
Genotype	c.1387A>G	c.1387A>G	c.1387A>G	c.1387A>G	c.1387A>G/c.826C>A	c.826C>A
α-DG (IIH6)	0–1+	0–1+	0–1+	0–1+	0–3+	0–3+
β-DG	1–3+	2–3+	3+	2	2–3+	3+
Dystrophin	2–3+	3+	2–3+	3+	2–3+	2–3+
Merosin	1–3+	2–3+	3+	3+	2–3+	3+

Abbreviation: α-DG = α-dystroglycan.
In this scoring system, zero = absent and 3+ = normal.
[a] Patient D is representative of a homozygous c.826C>A, LGMD2I patient. The biopsy is included for comparative purposes.

Figure 3 Immunofluorescence staining



(A–D) Normal control muscle staining patterns for each of the antibodies. (E–H) Representative images from patient D homozygous for the European common mutation (c.826C>A). (I–L) Representative images from patient 3 homozygous for c.1387A>G. (M–P) Representative images from patient 4 homozygous for c.1387A>G. Scale bar = 50 μm, equivalent for all photomicrographs.

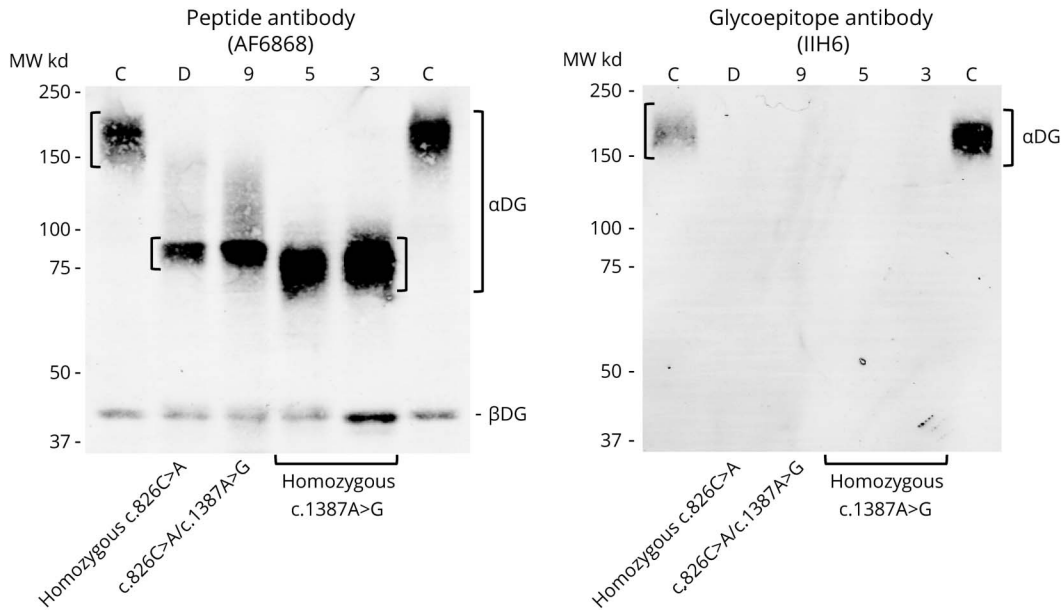
chromosome 19. The size of the homozygous interval and haplotype analysis indicate that the allele originated from a common ancestor approximately 60 generations ago. Compared with the smaller ~150-kb homozygous segment associated with the c.826C>A allele of European origin, the c.1387A>G allele has a more recent origin, but still likely predates European settlement in the Americas. Family history information for the cases reported here (and previously[11,12]) suggests an origin in central Mexico (figure 1A).

The common European founder mutation (c.826C>A, p.Leu276Ile) is found in many European populations, and the mutation is thought to have occurred once in a common ancestor.[6,7,26,27] *FKRP* c.826C>A has an increased prevalence in Scandinavian countries,[28] leading to the speculation that the founder mutation occurred in the Scandinavian population. Other founder mutations have also been reported as summarized in table 3.[29–31]

Muscle biopsies of patients homozygous for *FKRP* c.1387A>G showed severe dystrophic histopathology, and immunostaining showed greatly decreased to absent fully glycosylated α-DG, consistent with other dystroglycanopathies manifesting a CMD phenotype.[6] The muscle biopsies also showed variable, slightly reduced expression of merosin, which is comparable to findings in 2 previously reported homozygous *FKRP* c.1387A>G cases.[11] By Western blotting, α-DG glycosylation was reduced to a greater degree in patients homozygous for *FKRP* c.1387A>G compared with patients with either compound heterozygous mutations c.1387A>G/c.826C>A or homozygous *FKRP* c.826C>A mutations. It has been previously suggested that there is a relationship between the level of α-DG glycosylation and clinical phenotype in patients with *FKRP* mutations[32]; however, this relationship could not be confirmed in other studies.[33–35] Our finding of further decreased α-DG glycosylation in those homozygous for c.1387A>G compared with those with milder phenotypes supports the interpretation that the degree of α-DG hypoglycosylation is relevant for the severity of the phenotype.

The characterization of the phenotype associated with homozygous *FKRP* c.1387A>G mutations provided in this report adds to those previously described and will aid in the diagnosis and counseling regarding prognosis of patients with this rare mutation. Genetic analysis indicates that this mutation is of Mexican origin, and further genetic analysis of the specific population of origin may be of interest. In addition,

Figure 4 Western blotting



The antipeptide antibody (AF6868) shows greatly reduced molecular weight for α-DG in each of the patients with *FKRP* mutations. Fully glycosylated control (C) α-DG is >150 kd, whereas the α-DG from homozygous c.1387A>G patients (3 and 5) ranges from ~65–90 kd, and the α-DG from homozygous c.826C>A (D) or compound heterozygous c.1387A>G/c.826C>A (9) patients ranges from ~75–90 kd. The smaller molecular weight α-DG observed in homozygous c.1387A>G patients suggests a greater degree of hypoglycosylation than that of c.826C>A patients. Each patient with *FKRP* mutations has lost functional glycosylation and no longer binds the anti-glycoepitope antibody (IIH6). The AF6868 antibody binds to epitopes on both α-DG and β-DG. The β-DG bands show the relative amounts of protein loaded in each lane. Lanes were loaded equivalently in both gels. These images are representative of blots performed 3 or 4 times for each patient sample.

our findings are consistent with the idea that the more severe clinical phenotype associated the c.1387A>G mutation is explained, in part, by the greater reduction of α-DG glycosylation relative to other genotypes examined; however, clarity on this issue requires additional study.

Author contributions

A.J. Lee and K.A. Jones: study concept/design, analysis/interpretation of data, and drafting/revision of the manuscript. R.J. Butterfield: study design, acquisition/analysis/interpretation of data, and revision of the manuscript. M.O. Cox: analysis/interpretation of data and revision of the manuscript. C.G. Konersman, C. Grosmann, J.E. Abdenur, M. Boyer, B. Beson, C. Wang, J.J. Dowling, M.A. Gibbons, and A. Ballard: acquisition of data and revision of the manuscript. J.S. Janas, R.T. Leshner, S. Donkervoort, C.G. Bönnemann, and D.M. Malicki: acquisition of data and revision of the manuscript. R.B. Weiss: study design, analysis/interpretation of data, and drafting/revision of the manuscript. S.A. Moore: study concept/design, analysis/interpretation of data, and revision of the manuscript. K.D. Mathews: study concept/design, analysis/interpretation of data, and revision of the manuscript.

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Table 3 Founder mutations in *FKRP*

Mutation	Protein change	Population	Phenotype	Author (year)
c.826C>A	p.Leu276Ile	European[a]	LGMD2I	Frosk et al. (2005)[26]
c.1364C>A	p.Ala455Asp	Tunisian	CMD with brain involvement	Louhichi et al. (2004)[30]
c.545A>G	p.Tyr182Cys	Chinese	Asymptomatic	Fu et al. (2016)[31]
c.1100C>T	p.Ile367Thr	South African Afrikaner	LGMD2I	Mudau et al. (2016)[29]
c.1387A>G	p.Asn463Asp	Mexican	CMD without brain involvement	

Abbreviations: CMD = congenital muscular dystrophy; LGMD2I = limb-girdle muscular dystrophy type 2I.
Reported founder mutations in *FKRP*. Phenotypes described are for patients homozygous for these mutations.
[a] Genetic analysis was performed in the Hutterites, and a shared region was also identified in samples of other European populations. Further research showed the highest prevalence of *FKRP* c.826C>A in Scandinavian populations.[28]

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