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Asymptomatic carriers for homozygous novel mutations in the FKRP gene: the other end of the spectrum

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Autosomal recessive limb-girdle muscular dystrophy linked to 19q13.3 (LGMD2I) was recently related to mutations in the fukutin-related protein gene (FKRP) gene. Pathogenic changes in the same gene were detected in congenital muscular dystrophy patients (MDC1C), a severe disorder. We have screened 86 LGMD genealogies to assess the frequency and distribution of mutations in the FKRP gene in Brazilian LGMD patients. We found 13 Brazilian genealogies, including 20 individuals with mutations in the FKRP gene, and identified nine novel pathogenic changes. The commonest C826A European mutation was found in 30% (9/26) of the mutated LGMD2I alleles. One affected patient homozygous for the FKRP (C826A) mutation also carries a missense R125H change in one allele of the caveolin-3 gene (responsible for LGMD1C muscular dystrophy). Two of her normal sibs were found to be double heterozygotes. In two unrelated LGMD2I families, homozygous for novel missense mutations, we identified four asymptomatic carriers, all older than 20 years. Genotype–phenotype correlation studies in the present study as well as in patients from different populations suggests that the spectrum of variability associated with mutations in the FKRP gene seems to be wider than in other forms of LGMD. It also reinforces the observations that pathogenic mutations are not always determinant of an abnormal phenotype, suggesting the possibility of other mechanisms modulating the severity of the phenotype that opens new avenues for therapeutic approaches.

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Introduction

LGMD

The limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of genetically determined progressive disorders of the muscle with a primary or predominant involvement of the pelvic or shoulder-girdle musculature. The clinical course in this group is characterized by normal

intelligence and great variability, ranging from severe forms with onset in the first decade and rapid progression to milder forms with later onset and a slower course.¹

At least 15 genes, five autosomal dominant (AD) and 10 autosomal recessive (AR), responsible for LGMD have already been mapped. Linkage analysis indicates that there is further genetic heterogeneity both for AD and AR-LGMD.² The AD forms are relatively rare and represent less than 10% of all LGMD. The product of the 10 AR genes has been identified: calpain-3 for LGMD2A,³ dysferlin for LGMD2B,⁴ β -sarcoglycan for LGMD2D,^{5,6} β -sarcoglycan for LGMD2E,^{7,8} γ -sarcoglycan for LGMD2C,^{9,10} δ -sarcoglycan for LGMD2F,^{11–13} the sarcomeric protein telethonin

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for LGMD2G,^{14,15} TRIM-32 for LGMD2H,¹⁶ fukutin-related protein for LGMD2I¹⁷ and titin for LGMD2J.¹⁸

LGMD2I, linked to chromosome 19q13.3, was first described in a large consanguineous Tunisian family.¹⁹ In the following year, Brockington *et al*²⁰ identified a new gene, the fukutin-related protein (FKRP) gene that mapped at the same *locus* of the LGMD2I. Mutations in this gene were shown to cause LGMD2I as well as the congenital muscular dystrophy 1C (MDC1C).¹⁷ MDC1C is a severe condition (age at onset in the first few weeks of life and inability to walk), while LGMD2I has a milder and variable course (age at onset from the first to the third decade of life and slower progression), both with preserved intelligence and elevated serum creatine-kinase (CK). Weakness and wasting of the shoulder-girdle muscles, primary restrictive respiratory and cardiac involvement have been reported for both disorders.^{17,20,21}

The FKRP gene was identified through homology to the Fukuyama congenital muscular dystrophy (FCMD) gene that is mutated in the FCMD disorder, a progressive muscular weakness from early infancy and marked central nervous system involvement.²² The FCMD gene encodes the fukutin protein that together with the FKRP protein belongs to the fukutin family. Both genes code for putative glycosyltransferases, Golgi-resident proteins. The FKRP protein is required for the post-translational modification of dystroglycan.²³

The α -dystroglycan is abnormally processed in both disorders. This selective reduction together with the sparing of the β -dystroglycan, and the selective loss in its higher molecular weight (MW) forms, suggest that it is abnormally glycosylated, representing a novel pathogenic mechanism in limb-girdle muscle dystrophy.¹⁷ More recently it has been shown that abnormal glycosylation seems to be the mechanism for several types of muscular dystrophies.²⁴ Moreover, Hewitt and Grewal²⁴ showed in the myodystrophy mouse, a natural model for muscular dystrophy with glycosylation defects, that the altered α -dystroglycan glycosylation reduces its ability to bind to extracellular matrix ligands such as laminin and agrin.

The 12-kb FKRP gene is composed of three noncoding exons and a single large exon that contains part of the 5' untranslated region, the entire open frame and the 3' untranslated region.²⁰ The FKRP gene encodes a 495 amino-acid protein and has a 1488 bp open reading frame. Sequence analysis of FKRP predicts the presence of a hydrophobic transmembrane-spanning region (amino acids 4–28) followed by a 'stem region' and the putative catalytic domain.²⁰ A similar molecular organization is found in several Golgi resident glycosyltransferases.²⁵

In the present work, we identified 20 individuals from 13 LGMD2I families with pathogenic mutations in the FKRP gene among a set of 86 screened Brazilian LGMD genealogies. We have identified nine novel FKRP mutations. The common European C826A mutation was found

in 30% (9/26) of the pathogenic Brazilian alleles. In two unrelated families, where patients were homozygous for novel missense mutations, we identified four asymptomatic sibs.

Subjects and methods

Subjects

We have analyzed 86 Brazilian LGMD families for the FKRP gene. All patients were ascertained in the Human Genome Research Center, Department of Biology, University of São Paulo (IB-USP), Brazil and classified as LGMD according to the criteria reported in Bushby and Beckmann.²⁶ The study was performed following patients' informed consent. In patients from 46 genealogies, from which a muscle biopsy was available for at least one affected member, a positive immunofluorescence (IF) pattern and/or a normal MW Western blot (WB) band was found for dystrophin, the four sarcoglycans, calpain-3, dysferlin and telethonin proteins (data not shown). Two families, with at least three sibs with increased serum CK levels, showed a positive lod scores at the 19q13.3 *locus*, while a third one had been excluded from all known AR-LGMD *loci*. The remaining genealogies were isolated cases not submitted to muscle biopsies.

Methods

Blood samples were obtained from patients and DNA was extracted according to standard protocols.²⁷ For mutation analysis, exon 4 (the only coding) was divided into seven overlapping fragments that were screened by heteroduplex analysis in denaturing high-performance liquid chromatography (dHPLC) or single-strand conformation polymorphism (SSCP) through electrophoresis on an MDETM (FMC Bioproducts) gel. All abnormal fragments in SSCP gel or abnormal profiles in the dHPLC were sequenced in the ABI Prism 377 automatic sequencer machine. The conditions used for the FKRP gene screening are described in Table 1.

The IF and WB methodologies were carried out as described in Vainzof *et al*²⁸ and Ho-Kim *et al*²⁹ using the following antibodies: four sarcoglycans,³⁰ calpain-3,³¹ dysferlin³² and telethonin.³³

Results

Mutations analysis

Pathogenic changes in both alleles of the FKRP gene were found in 15 affected patients from 12 Brazilian genealogies (Table 2). In one additional consanguineous isolated case, a change was found in just one allele. A total of 10 patients, seven of them from consanguineous parents, are isolated cases. The other three genealogies had multiple affected sibs. They had been previously submitted to linkage analysis and showed positive lod scores with the 19q13.3 *locus* and/or were excluded from the other known

Table 1 Primer sequences and screening conditions used in the coding region of the FKRP gene

Primer name	Primer sequences (5'–3')	FKRP gene fragm.	PCR product size (in base pair) (genomic DNA)	Ann. temp. (°C)	PCR obs.	Screening methodology
FKRP-1F	TGGTCTGACAATCAGCTGCT G	1	274	62	—	dHPLC
FKRP-1R	CCGCGTTGTCAAATGCCTCGAA					Temp.: 65°C
FKRP-2F	CCCGTGTACCCGTCCTGGTGC	2	332	65	1%	DHPLC
FKRP-2R	GGCCACCAGACGTGCGCTTCCT				DMSO	Temp.: 66°C
FKRP-3F	CTGCTGGAGCGCATGGTGGAGGCG	3	369	66	5% DMSO	MDE gel
FKRP-3R	GCGTCCCTCGCGCTCAGCCTTCCA					
FKRP-4F	GCAGCTGCTGGAAGTACCTTCGC	4	348	61	5% DMSO	MDE gel
FKRP-4R	CCCAGCAGTGAGCCGCCCTCGAG					
FKRP-5F	TGGAGGCTGCGGGCTGCGCTACTG	5	257	67	—	dHPLC
FKRP-5R	CCACAGGTCCACGTGCAAGTGGT					Temp.: 65°C
FKRP-6/7F	TTCCGCGTGCAGTACAGCGAAAGC	6	313	62	—	dHPLC
FKRP-6R	CCCGAAAAACAAAGGCGAGGTT					Temp.: 64°C
FKRP-6/7F	TTCCGCGTGCAGTACAGCGAAAGC	7	339	62	—	dHPLC
FKRP-7R	AGAGCTTCTCCACATCCAGACA					Temp.: 64°C

Fragm = fragment; ann. temp. = annealing temperature; temp. = over temperature for dHPLC.

Table 2 Description of FKRP mutations in 13 Brazilian families

Family	No. of clinically affected patients from each family	Cons.	Mutation	Protein change	State	Fragment
1	1	Yes	C826A	Leu 276 Ile	Hom	4/4
2	1	Yes	C826A	Leu 276 Ile	Hom	4/4
3	1	Unknown	C826A	Leu 276 Ile	Hom	4/4
4	1	Yes	C826A	Leu 276 Ile	Hom	4/4
5	1	Yes	A545G	Tyr 182 Cys	Hom	3/3
6	1	Yes	A545G	Tyr 182 Cys	Hom	3/3
7	1	No	C878T/C1073T	Thr 293 Ile/Pro 358 Leu	Comp Het	4/5
8	1	Yes	G478T	Val 160 Phe	Hom	3/3
9	1	Yes	G235A/?	Val79Met/?	Het	2/?
10	2	Yes	C400T	Arg 134 Trp	Hom	2/2
11	1	Yes	T899C	Val 300 Ala	Hom	4/4
12	1	No	C826A/G898A	Leu 276 Ile/Val 300 Met	Comp Het	4/4
13	3	No	G235A/G764A	Val79Met/ Trp255X	Comp Het	2/4

Cons. = consanguinity; hom = homozygous; comp het = compound heterozygous; het = heterozygous.

AR-LGMD *loci*. The sibs selected for linkage had either clinical weakness or serum CK elevation. At least one affected patient from each family was submitted to muscle biopsy and showed a positive pattern for dystrophin, the sarcoglycans, telethonin, dysferlin and calpain-3 proteins through IF and/or WB analysis. All novel missense pathogenic changes were not found in 200 chromosomes from 100 normal Brazilian controls.

A total of 10 distinct pathogenic changes, nine novel mutations (eight missense and one stop codon), were found. The C826A change, present in five families (four homozygous and one compound heterozygous), was the most prevalent mutation. With the exception of the A545G and G235A mutations found in more than one

family, the other six missense changes were not recurrent. Interestingly, the same codon (Val 300) was changed twice, resulting in an alanine (T899C) or a methionine (G898A) in two unrelated families. In 12 of the 13 families affected, patients carried missense mutations. The only exception was family 13, with three affected sisters who were compound heterozygotes for two novel mutations: one stop codon (G764A) and one missense (G235A) change (protein consequence: Trp255X and Val79Met, respectively). In an isolated patient (case 9), born from consanguineous parents, we found the pathogenic G235A change inherited from his mother in just one allele. Interestingly, although his parents are first-degree cousins, his father is not heterozygous for this same mutation.

Other nonallelic changes One female patient (case 1) homozygous for the C826A mutation is also a heterozygous carrier of a missense change (R125H) in the caveolin-3 gene, responsible for LGMD1C.³⁴ This patient previously reported,³⁵ has two normal sibs (a brother aged 41 years and a sister aged 29 years) who carry the R125H change in one allele in the caveolin-3 gene. They also carry the C826A mutation in the FKRP gene in heterozygous state.

Polymorphic changes The following polymorphic silence changes, all of them in heterozygous state, were also found: C135T (Ala45Ala) and C585T (Asp195 Asp) in patients and in normal controls; C192T (Pro64Pro) and G606A (Leu202Leu) in two normal controls; C249T (Ala83Ala) and C567T(Pro189Pro) in two unrelated patients.

Clinical findings Among the 20 individuals from the 13 families that carry FKRP mutations, 16 are clinically affected: 12 with a milder LGMD course, two already deceased had a severe Duchenne-like phenotype, and two an intermediate course (Table 3).

Although the course was highly variable, all symptomatic patients lost the ability to walk on heels before the capacity to walk on toes. In all, 12 patients have a relatively milder course. Their mean age at onset and ascertainment were 20.2 + 5.16 and 27.5 + 8.68 years, respectively. Interestingly, in two patients (cases 4 and 5) one of the first symptoms was the loss of neck flexion due to contracture of extensor muscles. In one patient (case 11), the muscle weakness started in both the lower and upper limbs almost

simultaneously. All other patients showed a pattern of proximal muscle involvement starting in the lower limbs and progressing to the upper limbs afterwards. The more severe phenotype was observed in family 13 where the three affected sisters showed hypotonia at birth. The two older sisters had a Duchenne-like course (were confined to a wheelchair at age 11 and 12 years and died of cardiac/respiratory arrest at age 14 and 15 years, respectively). The youngest sister, who is currently 24 years old, is still able to walk short distances with support. Another intermediate phenotype was also seen in the patient with just one identified pathogenic allele (case 9). He is currently 25 years old, was confined to a wheelchair since age 17 years and has presently a pronounced upper limb weakness. Immunohistochemical muscle protein analysis showed a positive pattern for dystrophin, the four sarcoglycans and dysferlin. Screening of mutations in the calpain-3 gene excluded the most frequent mutations in our population.³⁶ One male patient (case 12), although with a very slow muscle weakness progression (age at onset and ascertainment 40 and 44 years, respectively), developed nocturnal hypoventilation. Two families (cases 2 and 13) have an African-Brazilian background while all the others are Caucasians. The main clinical findings are summarized in Table 3.

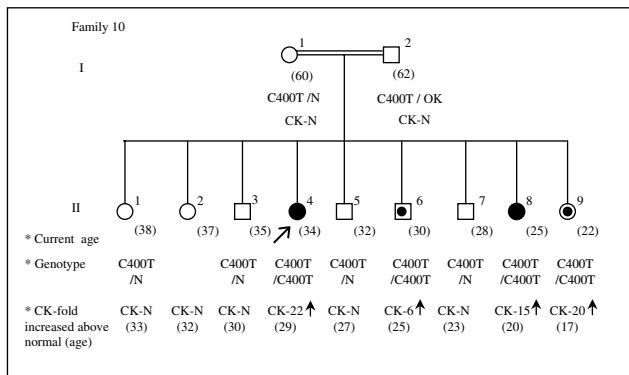
Asymptomatic sibs

In the two unrelated families (families 10 and 11, Table 2) who were identified through linkage analysis, asymptomatic sibs, homozygous for the same mutation present in the affected probands, were found. In family 10, the propositus, currently aged 33 years, referred onset at age

Table 3 Main clinical findings in 16 symptomatic LGMD2I Brazilian patients

Patient	Sex	Age at onset	Age at ascert.	Current age (years)	CK-fold ↑ at ascert.	Calves' hypertrophy	Clinical course	Walking ability
1	F	18	27	37	8	Yes	Mild	Walks with support
2	M	27	43	51	13	Yes	Mild	ND
3	M	19	29	41	19	Yes	Mild	Walks short distances only with support
4	F	11	20	27	13	Yes	Mild	Walks without support
5	M	18	23	32	26	Yes	Mild	Walks without support
6	F	—	15	29	12	No	Mild	Walks; unable to climb stairs
7	F	25	28	39	7	No	Mild	Walks without support
8	F	17	25	30	21	Yes	Mild	ND
9	M	5	13	25	19	No	Intermediate	WC bound at age 17 years
10a	F	26	28	34	22	Yes	Mild	Walks without support
10b	F	19	19	25	15	Yes	Mild	Walks short distances only with support
11	F	14	29	†(33)	6	Yes	Mild	Ambulant when deceased
12	M	40	44	51	6	Yes	Mild	Walks without support
13a	F	2	7	†(15)	74	Yes	DMD-like	WC bound at age 12 years
13b	F	1	5	†(14)	92	Yes	DMD-like	WC bound at age 11 years
13c	F	8	—	24	45 (1 year)	Yes	Intermediate	Walks short distances only with support

Ascertain = ascertainment; † = death; CK-fold ↑ = CK-fold increase above the upper value of normal; ND = no current data.

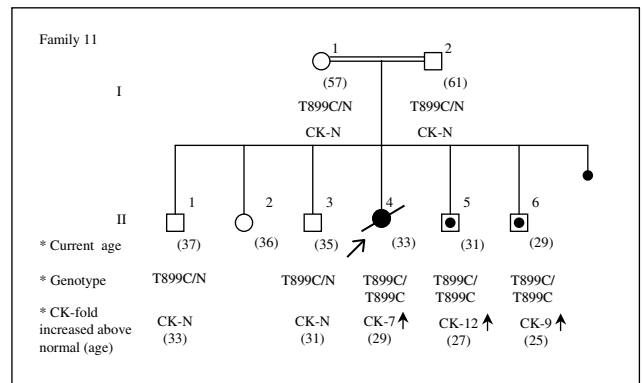


CK-N= normal; CK-↑= increased above the upper value of normal at ascertainment ()

Figure 1 Family 10 carrying the C400T mutation in two asymptomatic and two affected sibs.

26 years. She is the mother of three normal children (aged 10, 7 and 1 years, respectively). At ascertainment, at age 28 years, her serum CK was 22-fold increased above the upper value of the normal. On clinical examination, she could walk on her tiptoes but not on her heels, and although she had more pronounced weakness in her lower limbs she also had difficulties in raising her arms. Her younger affected sister was 19 years at ascertainment. Her CK was increased 15-fold above the upper value of the normal. On clinical examination, she complained of pain in the legs and difficulty in running. She could still walk on her heels, although it was easier for her to walk on her toes. She had calves' hypertrophy, which was more pronounced in one leg. During examination, the two affected sisters reported that they had seven unaffected sibs: four brothers and three sisters (Figure 1). Before consultation, the seven sibs had sent their blood for serum CK determination and linkage analysis. Two of them, a 25-year-old brother (II-6) and a 17-year-old sister (II-9), had increased serum CK (six- and 20-fold, respectively). Linkage analysis revealed that these two sibs with elevated CK shared the same haplotype as the two affected sisters. Subsequently, mutation analysis confirmed that four of them were homozygous for the same C400T mutation. However, surprisingly, clinical and neurological examination performed after the confirmation of mutation analysis, revealed that both (currently aged 31 and 22 years, respectively) had no sign of any muscle weakness. In addition to normal muscle strength (assessed by an experienced physiotherapist), they also showed normal motor ability for running, climbing stairs and jumping.

In family 11, also identified through linkage analysis, the proband was an affected female who died recently at the age of 33 years following a pneumonia. At ascertainment, at the age of 29 years, her serum CK was elevated seven-fold. On clinical examination, she could walk on tiptoes but not on her heels. She could not jump or rise up from the ground without support. Although she noticed a fast



CK-N= normal; CK-↑= increased above the upper value of normal at ascertainment ()

Figure 2 Family 11 carrying the T899C mutation in two asymptomatic and one affected deceased sister.

progression after the age of 26 years, she related her first symptoms (weakness in her legs and arms simultaneously) at age 14 years. She had five normal sibs (Figure 2). Two of her brothers (II-5 and II-6, Figure 2), who also had elevated CK levels at ascertainment (12- and nine-fold above the upper value of the normal, respectively), are completely asymptomatic (at ages 31 and 29 years, respectively), a situation analogous to the previous family. The three sibs with elevated serum CK share the same haplotype and the same homozygous T899C mutation.

Discussion

Although mutations in the FKRP gene can cause clinically different disorders, only patients classified as LGMD were analyzed in the present study. The only family with a more severe course included three sisters, where the two older ones showed a Duchenne-like progression, with wheelchair confinement at ages 11 and 12 years and death because of respiratory/cardiac failure at ages 14 and 15 years, respectively. This family was also the only one where affected patients were compound heterozygotes for one null mutation (with a missense on the other). This observation is in accordance with the observation in German patients, where it has been reported that patients with one null mutation had a Duchenne-like presentation or an intermediate Duchenne-Becker course (Thomas Voit,¹⁸ personal communication). However, it differs from our findings for calpain-3 mutations, since we observed that LGMD2A patients carrying one null mutation were not more severely affected than those carrying two null mutations.³⁶ This difference in genotype–phenotype correlation is still not understood and might be related to the distinct pathogenic mechanism responsible for these two forms of LGMD (apoptotic pathway in LGMD2A³⁷ and glycosylation defect in LGMD2I¹⁷). Interestingly, no patient with two null mutations in the FKRP gene has been reported to date, suggesting that it might be incompatible with survival.¹⁸

Patients from the other 12 families carried only missense mutations in one or both alleles. The C826A missense change, the most common mutation found in European population,¹⁷ was found in 30% (9/26) of Brazilian LGMD2I mutated alleles. The A545G and G235A pathogenic changes were found, each one, in two unrelated families. The remaining six missense changes and one stop codon mutation (G898A, C878T, C1073T, C400T, T899C, G478T and G764A, respectively) are private changes inherited in specific families.

The finding of just one mutated allele in patient 9 inherited from his mother, but absent in his father, was surprising. Since his parents are first-degree cousins, we would expect that he would be homozygous for the G235A mutation inherited from a common origin. The possibility that his father carries another FKRP pathogenic mutation that was not detected because of the limited sensibility of the technique used cannot be ruled out. However, this unusual finding opens some speculative hypothesis. For example, this patient could be a double heterozygote carrying a second pathogenic change in another gene (diallelic inheritance) that would interact with the FKRP mutation resulting in an abnormal phenotype. Alternatively, he could be homozygous for another nonallelic mutation inherited from a common ancestry. In this case, either (i) the FKRP pathogenic change would be necessary to cause an abnormal phenotype, suggesting a triallelic inheritance, or (ii) the FKRP mutation would act as a modifier gene. The finding of FKRP mutations in patients with known or new mutations in other genes might help us to elucidate these hypotheses. Interestingly, Mercuri *et al*³⁸ recently reported that they were unable to find the second mutation in four among 16 FKRP patients, suggesting that mutations exist outside the four FKRP exons.

In the present study, we identified four asymptomatic individuals, in two unrelated genealogies, homozygous for two FKRP mutations. Their affected sisters developed the first symptoms at ages 14, 19 and 26 years, respectively (mean age 19.6 + 6 years). When last examined at ages 22, 29, 30 and 31 years, they showed normal muscle strength and motor ability. However, we cannot rule out the possibility that they may still develop muscle weakness, since one of our patients (case 12) reported onset at age 40 years. In addition, recently, Poppe *et al*³⁹ reported that two among 16 patients in their series complained of first symptoms at ages 35 and 40 years. However, since our patients carry different mutations, their effect on the course of the disease is still unknown and genotype–phenotype correlation studies are of utmost interest.

Spectrum of clinical variability

Clinical heterogeneity in patients carrying the same pathological mutation has been observed for all forms of LGMD, with the exception of LGMD2F where all patients

reported to date seem to have a severe phenotype.^{2,40} Intrafamilial clinical variability was also reported for sibs carrying the C826A mutation.⁴¹ However, the spectrum of clinical variability associated with mutations in the FKRP gene, ranging from severe congenital forms with mental retardation⁴² to asymptomatic carriers, seems to be wider than in other forms of LGMD.

In 10 of the 13 families in the present series, the probands were isolated cases. However, in the three with multiple affected sibs, there was intrafamilial clinical variability. In family 13, the two older sisters, who died in their teens, showed a similar severe Duchenne-like course while the youngest affected sister has a milder phenotype and is still able to walk short distances at age 24 years.

Prandini *et al*⁴¹ reported one LGMD2I family homozygous for the C826A mutation where affected patients showed a discordant clinical course. In the present work, we found four asymptomatic sibs, three males and one female (all older than 22), from two unrelated families, both carrying private novel pathogenic mutations in homozygous state. In family 10, the two clinically affected sisters reported onset at ages 19 and 26 years, respectively, while their two unaffected brothers carrying the same mutation are currently 22 and 30 years old. In family 11, the proband referred onset at age 14 years while her two brothers aged 29 and 30 years are completely asymptomatic. It is of interest that three of four asymptomatic carriers are males. Future analysis of other LGMD2I families will help to clear out if it is a coincidence or a gender difference affecting females more severely than males in this disorder.

The finding of four asymptomatic carriers of FKRP missense mutations caught our attention for another important point: these ‘nonaffected’ sibs would remain undetected if they did not have symptomatic sibs, which raises the possibility that LGMD2I mutations may be underestimated. On the other hand, should we classify a mutation that does not cause muscle weakness in some carriers as pathogenic?

It is also of interest that the finding of a missense change (R125H) in the CAV3 gene in three individuals carrying the C826A change (one homozygous and two heterozygous) does not seem to have any influence in the LGMD2I course or to cause muscular weakness in double heterozygotes.

Relative proportion of LGMD2I among Brazilian autosomal recessive LGMD families

In our population, with the exception of LGMD2H and LGMD2J, all other autosomal recessive forms have been identified among our LGMD patients.⁴³ Among 127 families who were classified based on DNA and muscle protein analysis,⁴³ 13 were found to have mutations in the FKRP gene that correspond to about 10% (13/127). This proportion is lower than that observed in several European

countries where LGMD2I seems to be the most prevalent form of AR-LGMD.¹⁸ However, the current screening of the FKRP gene in other clinical groups (Duchenne-like and CMD patients) will give the real proportion of the FKRP mutations in the Brazilian population.

In summary, understanding the spectrum of severity associated with FKRP mutations ranging from severe MDC1C or Duchenne-like to complete absence of symptoms in some cases remains a great challenge. It supports previous findings for other disease genes, indicating that pathogenic mutations may not be necessarily determinant of a clinical disorder and that other mechanisms or genes may have an important role in modulating the severity of the phenotype. On the one hand, this makes genetic counseling a difficult task since no prognosis can be established. On the other hand, comprehension of the underlying mechanisms can lead to new avenues aiming at future therapeutic approaches.

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