ELECTRONIC LETTER

Genotype-phenotype correlations in 35 Brazilian families with sarcoglycanopathies including the description of three novel mutations

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The limb-girdle muscular dystrophies (LGMDs) are genetically determined progressive disorders clinically defined by the primary involvement of the pelvic and shoulder girdle musculature.[1] Among autosomal recessive LGMDs (LGMD 2), sarcoglycanopathies (LGMD 2C-2F) represent a subgroup caused by mutations in one of the genes that encode γ-, α-, β-, and δ-sarcoglycans (SGs), respectively.[2–6] The SGs are transmembrane glycoproteins which, together with sarcospan, dystrophin, dystroglycans, syntrophins, and α-dystrobrevin, constitute the dystrophin-glycoprotein complex (DGC).[7–10] In addition to the four SGs that comprise the SG-sarcospan subcomplex of the DGC in striated muscle,[11] a fifth SG, named ε-SG, is expressed in a wide variety of tissues.[12 13] In smooth muscle, ε-SG replaces α-SG as an integral component of a unique SG-sarcospan complex composed of ε-, β-, γ- and δ-SGs and sarcospan.[14] Interestingly, it has been shown that mutations in ε-SG cause myoclonus-dystonia syndrome, an autosomal dominant non-degenerative central nervous system disorder.[15]

It is well recognised that the DGC acts as a linker between the cytoskeleton of the muscle cell and the extracellular matrix, providing mechanical support to the plasma membrane during myofibre contraction.[9 16 17] Besides this structural function, there is now increasing evidence that the DGC might play a role in cellular communication, as highlighted by its interaction with signalling molecules such as calmodulin, nitric oxide synthase, and Grb2.[10 17] So far, 41, 20, 10, and six distinct pathogenic mutations have been found in the α- (LGMD 2D), β- (LGMD 2E), γ- (LGMD 2C), and δ-SG (LGMD 2F) genes, respectively (Leiden Muscular Dystrophy pages, www.dmd.nl). Severe clinical Duchenne-like presentations tend to be more common among these patients, with onset occurring early in childhood and confinement to a wheelchair before the age of 16[18–23]; however, milder courses have also been described in LGMD 2C-2E patients who harbour missense mutations.[4 24–26] Also intriguing is the fact that a few frequent alterations, like c.229C>T (R77C) in the α-SG (LGMD 2D)[22 27–31] and c.521delT in the γ-SG (LGMD 2C) genes,[26] have been associated with both severe and mild forms. Therefore, the establishment of precise genotype-phenotype correlations has proven a challenge for most researchers working on the sarcoglycanopathies.

We have previously reported 23 kindreds affected by one of the sarcoglycanopathies.[6 22 26 30 32–36] In the present study, we identified another 12 LGMD 2C-2F families among 20 who were screened for the coding region of the α-, β-, γ- and δ-SG genes. Here we report our main genotype-phenotype correlation findings in these 35 Brazilian families and we discuss possible alternative mechanisms that might be associated with the variability of the clinical course.

MATERIALS AND METHODS

Patients

Forty-three unrelated families ascertained in the Centro de Estudos do Genoma Humano, Departamento de Biologia,

Key points

- In the present paper, 20 novel unrelated LGMD families, as well as three others in which only one of the pathogenic alleles had been identified, were submitted to a mutation screening for the coding regions of the α- (LGMD 2D), β- (LGMD 2E), γ- (LGMD 2C), and δ-SG (LGMD 2F) genes.
- Among 13 distinct pathogenic mutations found in 15 families, three are described here for the first time: (1) α-SG/c.329G>T (R110L) (LGMD 2D), present in a DLMD patient previously shown to be heterozygous for α-SG/c.229C>T (R77C); (2) β-SG/c.595-598delAATG (LGMD 2E), also associated with a severe phenotype in a homozygous patient; (3) β-SG/c.2T>C (LGMD 2E), found in the homozygous state in two brothers, each showing a different degree of disease severity. This last change probably results in a truncated protein, which was not detected in the muscle of any of these patients.
- Considering the 35 Brazilian families described so far, each of the LGMD 2C-2F forms account for 23%, 40%, 23%, and 14% of our sample, respectively. This relative balance among the different sarcoglycanopathies in Brazil contrasts with the proportions found in other countries and probably reflects the high degree of intermarriage and increased consanguinity in our population.
- Although 18 distinct SG mutations are segregating in these 35 LGMD 2C-2F families, three of them (α-SG/c.229C>T (R77C) (LGMD 2D), γ-SG/521delT (LGMD 2C), and δ-SG/c.656delC (LGMD 2F)) account for more than 60% of the disease alleles. These findings suggest that any screening of sarcoglycanopathies in Brazil should start with these mutations.
- Immunohistochemistry analysis in most of our patients showed a drastic decrease of the primarily affected SG together with an instability of the entire complex, irrespective of the type of mutation. Interestingly, this pattern is associated exclusively with a severe clinical course in LGMD 2E and 2F and with both mild and severe presentations in LGMD 2C and 2D.
- Two distinct missense mutations in the α-SG gene, c.850C>T (R284C) and c.739G>A (V247M), seem to result in partial preservation of the SG complex and in a mild phenotype, even in the heterozygous state.
- Precise genotype/phenotype correlation within the sarcoglycanopathies is still a challenging task, which will only be achieved through the identification of all the factors and genes that interact with SGs to produce the final phenotype.

Table 1 Main findings in the 14 most recent families in which we identified SG mutations

LGMD form	Family	Patient	Age at onset*	Age at ascertainment	Age at last examination	Wheelchair bound at age	Mean CK at ascertainment†
2C	LG92	a	8	11	11	–	15
	LG93	a	7	17	17	12	6
		b	7	19	19	13	11
	LG94	a	7	10	10	–	81
		b	7	8	8	–	62
	LG95	a	7	14	14	–	78
2D	LG7	a	15	21	31	–	8
		b	16	33	35	–	23
		c	12	31	33	–	18
		d	12	26	28	–	15
	LG81	a	4	10	18	12	370
	LG83	a	8	12	26	15	8‡
	LG85	a	6	10	10	9	20
		b	5	5	5	–	44
	LG86	a	7	35	35	–	24
		b	4	41	41	39	6
	LG87	a	1	10	10	–	42
2E	LG88	a	4	22	22	7	8
	LG89	a	4	20	20	11	13
	LG90	a	1	12	16	16	35
		b	–	8	13	–	31
	LG91	a	1	11	14	10	66
		b	8	8	8	–	100
	LG102	a	7	22	22	12	4

*Age in years. †CK-fold increased above normal. ‡Serum CK levels were measured only at last examination.

IB-USP were included in the present study. All of the patients have clinical and laboratory findings as well as family history compatible with the diagnosis of LGMD 2.[31] Mutation analysis was previously reported for 23 families, including three (LG7, LG81, and LG102, table 1) in which only one of the disease causing mutations had been identified.[22 32 33] Another 20 families were selected based on the following criteria: (1) nine showed a deficient labelling for at least one of the SGs on immunohistochemical analysis of muscle samples, performed as previously described[22]; (2) 11 families, in which muscle analysis was not possible, were selected based on the observation of a severe clinical course accompanied by at least one of the following observations: (i) the presence of at least one affected female in the family (among the isolated female cases, karyotyping was performed when possible); (ii) affected offspring of consanguineous marriage. The clinical course was classified as severe or mild if the loss of ambulation occurred before or after the age of 16, respectively. All studies were performed following informed consent.

Mutation analysis

Blood samples were obtained from the patients and, when possible, from their parents and sibs. DNA was extracted according to standard protocols.[37] The sequence of the primers used in the mutation screening of the coding regions of the α-, β-, γ-, and δ-SG genes are available at the Leiden Muscular Dystrophy Pages (www.dmd.nl) and the PCR conditions are described, respectively, in Carrié *et al*,[28] Bönnemann *et al*,[33] McNally *et al*,[26] and Nigro *et al*.[6] The PCR products were submitted to at least one of the following procedures: (1) restriction analysis, (2) single strand conformational polymorphism (SSCP) analysis through electrophoresis on a MDE gel at a constant 8 W for 10-16 hours at 18°C, (3) direct sequencing of both strands using the BigDye Terminator Cycle Sequencing kit on an ABI PRISM 377 Automated DNA Sequencer. The finding of novel pathogenic mutations was validated through the screening of 50 normal subjects from our population and the observation of a consistent segregation pattern in first degree relatives of the patient.

RESULTS AND DISCUSSION

Novel mutations

Twenty novel unrelated LGMD families, as well as three others in which only one of the pathogenic alleles had been identified (LG7, LG81, and LG102, table 2),[22 32 33] were submitted to mutation screening for the coding regions of the α- (LGMD 2D), β- (LGMD 2E), γ- (LGMD 2C), and δ-SG (LGMD 2F) genes. Thirteen distinct pathogenic mutations in the α- (LGMD 2D), β- (LGMD 2E), and γ-SG (LGMD 2C) genes were found in 15 of them, including the three kindreds above mentioned (LG7, LG81, and LG102). The main clinical findings and the mutations identified are described in tables 1 and 2, respectively. Three of these kindreds (LG83, LG89, and LG90) harbour alterations never described before. One affected female (LG83) was found to be heterozygous for a G to T transversion in exon 4 of the α-SG (LGMD 2D) gene, resulting in the substitution of an arginine, a basic amino acid, to an uncharged leucine residue at position 110 of the protein (R110L). In spite of being a compound heterozygote for two missense mutations (R77C/R110L)[19] (this paper), she shows a severe phenotype with loss of ambulation occurring before the age of 16. This is consistent with the fact that the arginine residue at position 110 of α-SG is conserved not only among all the mammals in which this protein was characterised (mouse, rabbit, and hamster) but also in human, mouse, and pufferfish ϵ-SG and in the alpha/epsilon-sarcoglycan-like protein of *Drosophila melanogaster*. Interestingly, arg77 is conserved in all these instances with the exception of mouse α-SG. Also, immunohistochemical analysis of α-, β-, and γ-SGs showed the absence of these proteins in the muscle of this patient (table 3). Although missense mutations in the α-SG gene (LGMD 2D) were initially associated with milder phenotypes,[3 27 32] several subsequent studies showed that such a strict correlation does not hold true in a great number of patients.[23 28 38 39] R77C itself has been found in LGMD 2D homozygous patients with different levels of clinical severity.[22 27–31]

The other two novel mutations identified lie in the β-SG (LGMD 2E) gene. One of them, c.595-598delAATG, leads to a

Table 2 Summarised data on the 35 Brazilian families affected by sarcoglycanopathies

LGMD form	Family	No of patients	Mutations		Consequence		Consanguinity	Phenotype	References
			Allele 1	Allele 2	Allele 1	Allele 2			
2C	LG42	2M:1F	c.521delT	c.521delT	Frameshift	Frameshift	No	Mild	19,22,26,29
	LG55	1M:3F	c.521delT	c.521delT	Frameshift	Frameshift	Yes	Severe	19,22,26,29
	LG96	1M	c.521delT	c.521delT	Frameshift	Frameshift	Yes	Severe	19,22,26,29
	LG97*	1F	c.521delT	c.521delT	Frameshift	Frameshift	Yes	?¶	22,26
	LG92	1F	c.521delT	c.521delT	Frameshift	Frameshift	?§	?	19
	LG93*	1M:2F	c.521delT	c.521delT	Frameshift	Frameshift	Yes	Severe	–
	LG94	2F	c.521delT	c.521delT	Frameshift	Frameshift	No	?	–
	LG95	1F	c.521delT	c.521delT	Frameshift	Frameshift	?	?	–
2D	LG16	1M:4F	c.229C>T	c.229C>T	R77C	R77C	Yes	Mild	19,22,29,32
	LG30	2F	c.229C>T	c.229C>T	R77C	R77C	No	Mild	19,22,29,32
	LG56	1M:1F	c.229C>T	c.229C>T	R77C	R77C	?	?	19,22,29
	LG82	1F	c.229C>T	c.229C>T	R77C	R77C	Yes	?	19,22
	LG84	1M:1F	c.229C>T	c.229C>T	R77C	R77C	Yes	Mild	19,22
	LG98	1F	c.229C>T	c.229C>T	R77C	R77C	?	Mild	19,22,29
	LG99	1M	c.229C>T	c.229C>T	R77C	R77C	Yes	Severe	19,22,29
	LG7	2M:2F	c.229C>T‡	c.850C>T	R77C	R284C	No	Mild	19,22,32
	LG81*	1F	c.229C>T‡	c.92T>C	R77C	L31P	No	Severe	22
	LG83	1F	c.229C>T	c.329G>T	R77C	R110L	No	Severe	19
	LG86	1M:1F	c.229C>T	c.739G>A	R77C	V247M	No	Mild	–
	LG78	3M	c.724G>T	c.850C>T	V242F	R284C	No	Mild	34
	LG87	1M	c.724G>T	c.724G>T	V242F	V242F	Yes	?	19
	LG85	2F	c.269A>G	c.269A>G	Y90C	Y90C	Yes	Severe	–
2E	LG3	2M:1F	c.272G>C	c.272G>C	R91P	R91P	?	Severe	19,22,29,33
	LG100*[1]	1F	c.299T>A	c.299T>A	M100K	M100K	Yes	Severe	22,33
	LG101	1F	c.465-466delAG	c.465-466delAG	Frameshift	Frameshift	?	Severe	19,22,29,33
	LG102	1F	c.323T>G‡	c.622-2A>G	L108R	Aberrant splicing	No	Severe	19,22,29,33
	LG88*	1F	c.299T>A	c.383^384ins376-383	M100K	Frameshift	No	Severe	–
	LG89*	1M	c.595-598delAATG	c.595-598delAATG	Frameshift	Frameshift	Yes	Severe	–
	LG90	2M	c.2T>C	c.2T>C	?†	?	Yes	Mild/severe	19
	LG91	1M:1F	c.622-2A>G	c.622-2A>G	Aberrant splicing	Aberrant splicing	Yes	Severe	19
2F	LG26	1M:2F	c.656delC	c.656delC	Frameshift	Frameshift	Yes	Severe	6,19,22,29
	LG43	1M:2F	c.656delC	c.656delC	Frameshift	Frameshift	Yes	Severe	6,19,22,29
	LG68	1M	c.656delC	c.656delC	Frameshift	Frameshift	Yes	Severe	6,19,22,29
	LG33*	1F	c.656delC	c.656delC	Frameshift	Frameshift	Yes	Severe	6,22
	LG103	1F	c.784C>A	c.784C>A	E262K	E262K	Yes	Severe	19,22,36,42

*Families in which immunohistochemical analysis was not performed. †See text. ‡Mutations previously identified.[22 32 33] §Cannot be excluded. ¶Not followed up until the age of 16.

frameshift followed by a premature stop codon 48 bases downstream and, therefore, is predicted to cause a truncated protein. This affected patient (LG89), born to consanguineous parents and homozygous for this deletion, shows a typical Duchenne-like phenotype (table 1). Through the sequencing of exon 4 of β-SG, we confirmed heterozygosity for the disease allele in his mother and found that his healthy sister carries two copies of the normal allele.

The third novel mutation is a homozygous base pair substitution in the translation initiation codon of β-SG (LGMD 2E), c.2T>C, found in two affected sibs (LG90). It was also detected in the heterozygous state in their mother. This transition is predicted to abolish initiation of translation at the normal mRNA position, possibly leading to the use of the next methionine codon in the appropriate context, that is, in the presence of a purine in position –3 and of a G at nucleotide +4,[40] as the start site. In this specific case, the reading frame would be maintained, but the encoded protein would miss the first 99 amino acid residues. On the other hand, Parfait *et al*[41] reported a similar mutation in the human flavoprotein gene associated with the production of an unstable transcript. Whatever the consequences of this substitution, the final result is the absence of the β-SG protein together with a deficiency of the other SGs in the muscle of both patients (fig 1, table 3). Interestingly, there is marked variability in the clinical course in these two brothers. While the oldest was confined to a wheelchair at the age of 16, the youngest, currently aged 13, reports only pain in his legs and is still able to jump, climb stairs, and rise from the floor without support. Actually, he was considered unaffected by his mother and the diagnosis was made based on grossly raised serum CK levels and muscle protein analysis (tables 1 and 3, fig 1).

Relative proportions of the four sarcoglycanopathies in Brazil and the spectrum of mutations

Altogether, we have been able to identify 35 Brazilian families affected by sarcoglycanopathies so far (table 2). Each of the LGMD 2C-2F forms were found to account, respectively, for 23%, 40%, 23%, and 14% of this sample. This is the best estimate of the relative proportion of each sarcoglycanopathy in our country to date, since it includes most of our familial and isolated cases. Interestingly, these results suggest that the four sarcoglycanopathies are well represented in Brazil, in contrast to what has been observed in other countries. While in Europe and North America the great majority of patients deficient for the sarcoglycan proteins are affected by LGMD 2D,[18 39 42] LGMD 2C represents almost 100% of the sarcoglycanopathies in northern Africa.[43] In addition, LGMD 2F seems to be very rare all over the world.[18 23 30] Probably, the relative balance among the different forms in Brazil may be attributed to the high degree of intermarriage in our population and increased consanguinity, particularly for some groups. Indeed, the rate of consanguinity in LGMD 2F is 100%, compared to values between 43% and 75% in the other three forms. Therefore, the higher prevalence of LGMD 2F among Brazilian sarcoglycanopathies may reflect both a founder effect as well as the inbred nature of this sample.

Besides these 35 kindreds, no SG mutation was found in another eight unrelated patients showing a severe course, including one in whom muscle analysis showed deficiency of

Table 3 Immunohistochemical data on 28 Brazilian families affected by sarcoglycanopathies

			Immunohistochemical pattern*				
LGMD form	Family	Patient	Dystrophin	α-SG	β-SG	γ-SG	δ-SG
2C	LG42	a	+	±	±	-	NP
		b	+	±	±	-	±
		c	+	±	±	-	NP
	LG55	a	+	-	±	-	±
	LG96	a	+	±	±	-	±
	LG92	a	+	±	±	-	±
	LG94	a	+	±	±	-	±
		b	+	±	±	-	±
	LG95	a	+	-	±	-	±
2D	LG16	a	+	-	-	±	-
		b	+	-	-	±	-
	LG30	a	+	-	-	±	-
	LG56	a	+	-	-	±	-
		b	+	-	-	±	-
	LG82	a	+	-	NP	NP	NP
	LG84	a	+	-	-	±	-
	LG98	a	+	-	-	NP	NP
	LG99	a	+	-	-	-	-
	LG7	a	+	±	NP	±	NP
	LG83	a	+	-	-	-	NP
	LG86	a	+	±	±	±	±
	LG78	a	+	±	+	+	+
	LG87	a	+	-	-	±	-
	LG85	a	+	-	±	±	±
2E	LG3	a	+	-	-	-	-
	LG101	a	+	-	NP	±	-
	LG102	a	+	-	-	-	NP
	LG90	a	+	±	-	±	±
		b	+	±	-	±	±
	LG91	a	+	-	NP	NP	NP
2F	LG26	a	+	-	-	-	-
	LG43	a	+	-	-	-	-
	LG68	a	+	-	-	-	-
	LG103	a	+	-	-	±	-

*+ = positive staining, ± = partial deficiency, - = total deficiency, NP = not performed.

the four SGs. In this last case, the pathogenic mutation(s) may be located in unexplored non-coding regions in one of the SG genes. Alternatively, it might represent a secondary effect of alteration(s) in another known or unknown gene encoding a protein of the DGC. With regard to the other seven families, in which muscle analysis was not performed, the diagnosis of a distinct form of LGMD cannot be excluded.

Out of the 18 distinct SG mutations that are segregating in the 35 Brazilian LGMD 2C-2F families, seven were found to be recurrent (table 2) and three of them account for more than 60% of the disease alleles. These are c.521delT in the γ-SG gene, c.656delC in the δ-SG gene, and c.229C>T (R77C) in α-SG, which were detected in 100%, 80%, and 64% of the LGMD 2C, 2F, and 2D alleles, respectively. The alterations γ-SG/c.521delT and δ-SG/c.656delC have probably been introduced into our population by African ancestries, since all patients in each of these two subgroups share a common haplotype.[26 35] On the other hand, α-SG/c.229C>T is associated with at least three distinct haplotypes in Brazil.[32] Actually, this is the most frequent α-SG alteration, corresponding to 14-32% of the LGMD 2D alleles in other populations,[23 27 28 38 39] and this cytosine, which lies in a CpG island, has been considered a mutational hot spot.[28] Nevertheless, the percentage of c.229C>T among the LGMD 2D alleles in our country is apparently the highest reported so far, possibly reflecting the inbred nature of our sample.

Among the remaining 15 mutations identified by us, the great majority lie either in the α- (LGMD 2D) or in the β-SG (LGMD 2E) genes, while only one lies in δ-SG (LGMD 2F). As in most of the published case reports,[18 23 27 28 38 39] we found only missense mutations associated with LGMD 2D. They are spread across five exons and all of them result in amino acid substitutions localised in the extracellular domain of the protein, which corresponds to its largest portion. With regard to β-SG (LGMD 2E), although missense, frameshift, and splicing mutations were detected, 75% of them lie in exons 3 and 4 (table 2).

Genotype-phenotype correlations: an overview

As shown in table 2, clinical classification, possible in 28 out of the 35 families, showed that 67% of the patients have a severe phenotype. This proportion reaches 100% or nearly 100% in the LGMD 2F and 2E subgroups, respectively. One could assume that this finding is a consequence of our selection criteria, since, among the families that were not submitted to muscle analysis, we picked only those showing a severe clinical course. However, upon exclusion of the seven kindreds without immunohistochemical results from our sample (table 2), the proportion of Duchenne-like patients in the four subgroups remains almost the same, except for LGMD 2D. Results reported by other researchers also suggest that mild outcomes are more prevalent within LGMD 2D as compared to the other sarcoglycanopathies.[18 23] To some extent, this has been attributed to the predominance of missense mutations in LGMD 2D in contrast to the prevalence of null mutations in the other forms[2 5 6 22 23 27 28 38 39 42 44–48] (this paper). However, the issue of genotype-phenotype correlation in sarcoglycanopathies is not that simple, as previously mentioned and further explained below.

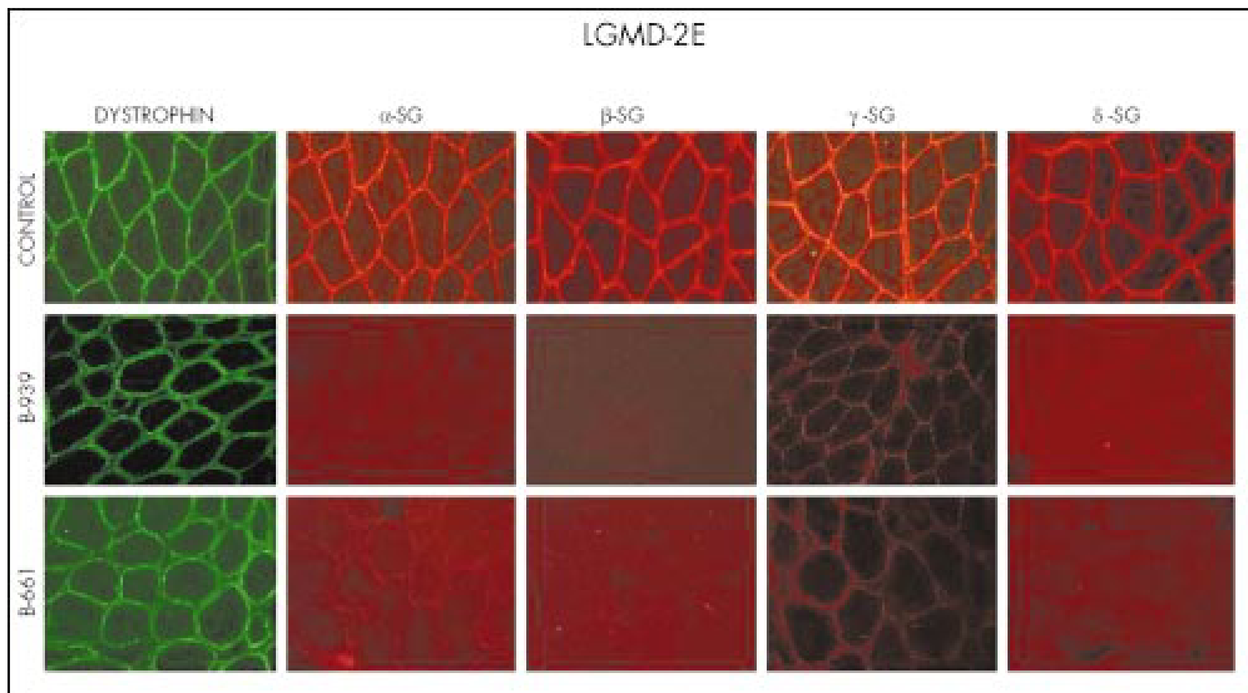

Figure 1 Immunohistochemical analysis for dystrophin and the four sarcoglycan proteins in the two sibs from LG90 showing a concordant pattern, with a total absence of β-SG and traces of α-, γ-, and δ-SGs in the sarcolemma of muscle fibres. B-661 = patient 1 (oldest), B-939 = patient 2 (youngest).

Immunohistochemistry analysis in 13 patients (11 families) harbouring null mutations in both alleles of one of the SG genes showed a drastic decrease of the primarily affected SG together with an instability of the entire complex, usually correlated with a severe phenotype (tables 2 and 3). As stated above, one of our families (LG42, table 2) provides an exception to this general rule, since the three affected sibs, homozygous for the mutation γ-SG/c.521delT (LGMD 2C), present a mild phenotype, despite the complete absence of this protein accompanied by a drastic decrease of the other three SGs in their muscle (tables 2 and 3).[19 22 26 29] Another exception is represented by the variability in the clinical course observed in the two sibs homozygous for the mutation c.2T>C in the β-SG (LGMD 2E) gene (LG90), both showing an identical immunohistochemical profile for the SG complex, as already explained in this paper (see "Novel mutations").

Most of our patients with missense changes in both alleles also show a dramatic reduction of the primary protein together with variable deficiency of the secondary SGs. This pattern is associated exclusively with a severe clinical course in LGMD 2E and 2F and, intriguingly, with both mild and severe presentations in LGMD 2D (tables 2 and 3). Interestingly, within this last subgroup, clinical variability among patients homozygous for the mutation α-SG/c.229C>T (families LG16, LG30, LG56, LG82, LG84, LG98, and LG99) seems to correlate with the residual amount of γ-SG in the muscle, despite the absence of the other three SGs in all of them (tables 2 and 3).[29] This also illustrates another previous conclusion of our group,[29 36] that the α, β, and δ subunits of the SG complex might be more closely associated and that γ-SG might interact more directly with dystrophin.

In our sample, the only exceptions to this general rule of absence of the primary protein together with deficiency of the other SGs are the changes R284C (c.850C>T) and V247M (c.739G>A), both in α-SG (LGMD 2D). Affected subjects from families LG7, LG78, and LG86, who are compound heterozygotes for either one of these mutations (table 2), show only partial reduction of α-SG in muscle (table 3), and the staining pattern for the other three SGs in one of these families (LG78) is as strong as in the healthy controls.[34] Both mutations present in the second disease allele in each of these kindreds (either R77C or V242F, table 2) result in the absence of α-SG and deficiency of the other three SGs in the muscle when in homozygosity (fig 2, table 2).[29] Therefore, the relative integrity of the SG complex and the mild phenotype in patients from families LG7, LG78, and LG86 are most probably associated with the α-SG/R284C and α-SG/V247M changes. Indeed, these results are supported by reports of other groups.[27 28 38] Crosbie *et al*[49] have also described a patient with sarcoglycanopathy and relative preservation of the SG complex in the muscle. Interestingly, although there is no information concerning the clinical course, this case is associated with homozygosity for a nonsense mutation in the γ-SG gene.

Recent reports describing the disruption of exonic splicing enhancers (ESEs) by point mutations[50–54] can shed some light on the apparent contradiction in the association of homozygous or compound heterozygous missense mutations with severe clinical presentations. These elements serve as binding sites in the pre-mRNAs for a family of specific serine/arginine rich (SR) proteins, which, in turn, act as splicing factors.[53] Now recognised to be present in constitutive or alternative exons of a growing number of genes, ESEs are required for efficient splicing of these exons. Since ESEs are typically short nucleotide motifs, single nucleotide changes have the potential to disrupt them, resulting in the skipping of the respective exon during pre-mRNA splicing.[50–54] Depending if the length of the skipped exon is a multiple of three nucleotides or not, the mutation will result either in the deletion of an entire exon encoded segment of the protein or in a frameshift, respectively. It is worth mentioning that the disruption of ESEs leading to the skipping of exons has already been confirmed in a great proportion of patients affected by neurofibromatosis type 1[51] and ataxia telangiectasia[50] and in one familial case of breast cancer associated with the *BRCA1* gene.[52] Besides, this mechanism is responsible for the skipping of exon 7 of the survival motor neurone 2 (*SMN2*) gene, and, therefore, contributes to the development of the spinal muscular atrophy phenotype in subjects harbouring mutations in the *SMN1* gene.[54]

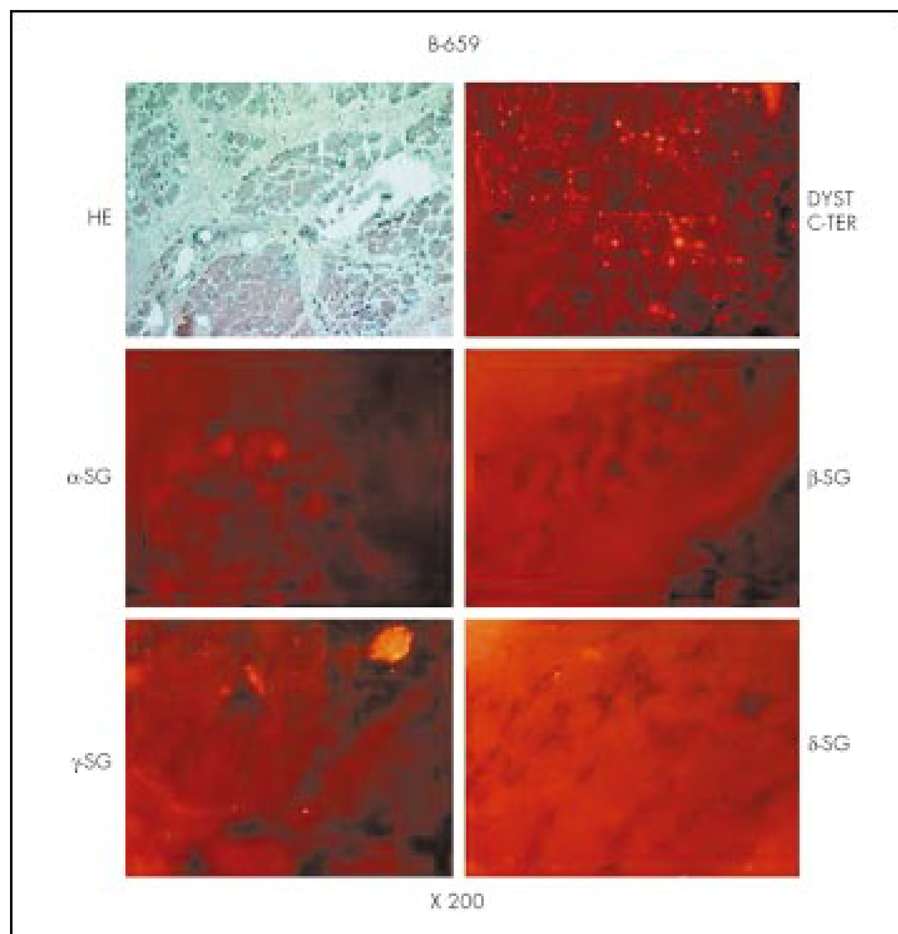

Figure 2 Histological (HE staining) and immunohistochemical analysis for dystrophin and the four sarcoglycan proteins in the affected patient from LG87, showing total absence of α-, β-, γ-, and δ-SGs in the sarcolemma of muscle fibres.

Based on the observations above, one could hypothesise that some of the SG mutations supposed to be missense have in fact a major effect on the final product. This could explain the drastic disruption of the entire SG complex in muscle as well as the severe outcome observed in a number of these patients, but not the mild phenotype observed in others with this same immunohistochemical profile. As there are multiple functional ESEs and their consensus sequences are quite degenerated, this subject deserves careful exploration.

An alternative explanation for the association between SG missense mutations and severe courses is that the amino acids involved in such changes are essential to the signalling role of the DGC. However, the fact that all four SGs are deficient in the muscle of most of these patients suggests that the mechanism(s) leading to these phenotypes most likely involve(s) the structural rather than the signalling role of the DGC.

In conclusion, this paper describes three novel mutations associated with sarcoglycanopathy as well as the spectrum of mutations and their resulting phenotype in 35 Brazilian families affected by this group of diseases. At a practical level, our findings imply that any screening of sarcoglycanopathies in Brazil should start with mutations α-SG/c.229C>T (LGMD 2D) and γ-SG/c.521delT (LGMD 2C), followed by δ-SG/c.656delC (LGMD 2F), since they were found to correspond, respectively, to 26%, 23%, and 11% of the disease alleles. As repeatedly described, in only a few cases have we have been able to establish a correlation between the type of mutation, the immunohistochemical pattern of the sarcoglycans in the muscle, and the resulting phenotype. Undoubtedly, this challenging task will only be achieved through the identification of all the factors and genes that interact with SGs to produce the final phenotype.

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