A first missense mutation in the δ sarcoglycan gene associated with a severe phenotype and frequency of limb-girdle muscular dystrophy type 2F (LGMD2F) in Brazilian sarcoglycanopathies

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Abstract

Among the heterogeneous group of autosomal recessive limb-girdle muscular dystrophies (AR LGMDs), the sarcoglycanopathies (LGMD2C-2F) represent a subgroup characterised by defects in the γ, α, β and δ sarcoglycan genes, respectively. Genotype-phenotype correlations in these forms of AR LGMD are important to enhance our understanding of protein function. Regarding LGMD2F, only two homozygous frameshift mutations have been reported to date in patients with a severe phenotype. In the present report, through screening 23 unrelated AR LGMD patients, we identified three subjects with LGMD2F, two with a previously reported frameshift mutation and the other homozygous for a new missense mutation in the δ sarcoglycan gene. Interestingly, this new mutation is also associated with a severe clinical course. In addition, our results suggest that this form of severe AR LGMD is not very rare in our population.

Keywords: sarcoglycanopathies; limb-girdle muscular dystrophies; δ sarcoglycan; linkage disequilibrium

The sarcoglycanopathies, a subgroup of autosomal recessive limb-girdle muscular dystrophies (AR LGMDs), are characterised by progressive muscle weakness affecting both upper and lower limbs. In the last few years, four sarcoglycanopathies, named LGMD2C, LGMD2D, LGMD2E and LGMD2F, have been identified. They were found to be caused by mutations in the γ (13q12), α (17q21.1), β (4q12), and δ (5q33) sarcoglycan genes, respectively. The sarcoglycans are transmembrane glycoproteins which, together with dystrophin, dystroglycans, and syntrophins, form the "dystrophin-glycoprotein complex" (DGC).

A vast repertory of missense, nonsense, and frameshift mutations have been found in the α, β, and γ sarcoglycan genes in patients with AR LGMD, but linkage disequilibrium was only found for the Δ521-T mutation in the γ sarcoglycan gene. Until now, it has not been possible to establish a clear genotype-phenotype correlation.

The LGMD2F form was first reported in four Brazilian families, where all the affected patients share a homozygous frameshift mutation in exon 7 of the δ sarcoglycan gene (del656C), resulting in the premature truncation of the translatable protein. This mutation is apparently in linkage disequilibrium with alleles of two microsatellite markers of the 5q33-34 region. Recently, Duggan et al. described a girl with a homozygous nonsense mutation in codon 165 (R165X) of the δ sarcoglycan gene. All the LGMD2F patients reported so far show a DLMD phenotype, with complete absence of the whole sarcoglycan complex in muscle.

The aims of the present report are: (1) to describe the haplotype associated with the del656C mutation in Brazilian families; (2) to identify other mutations in Brazilian patients affected by this form of sarcoglycanopathy; and (3) to estimate the frequency of the LGMD2F form among our DLMD families.

Twenty-five unrelated DLMD families were included in the present study: two large families for linkage analysis (LG26 and LG43) and 23 small pedigrees for identification of LGMD2F patients. Clinical data on patients from families LG26, LG33, LG43, and LG68 are detailed elsewhere. Among the 21 new cases, it was possible to perform muscle biopsy in 10, who showed a negative staining pattern for α sarcoglycan.

A total of 15 microsatellite markers of the 5q33-34 region were tested in the two families in which we first detected linkage (LG26 and LG43). Among them, we observed that the affected patients from both families share the same homozygous haplotype for markers D5S487 and D5S412. The frequencies of the alleles from these two markers that are segregating with the disease allele were estimated in normal chromosomes. Allele 1 (188 bp) of D5S412 was present in only one in 80 chromosomes in the healthy population. Interestingly, allele 10 (236 bp) of D5S487 was not found in 204 normal chromosomes from our population, including whites and African-Negroids. Interestingly, this allele, which is apparently being reported here for the first time, is 17 bp smaller than the smallest allele (allele 8=253 bp) published so far. In order to exclude the possibility that our control sample was not representative, we have genotyped it for marker D13S232, since the 122 bp allele from this locus is in linkage disequilibrium with...
LGMD2C. Among 144 tested chromosomes, three had the 122 bp allele. The obtained frequency of 0.021 is consistent with that found in white controls (0.023), suggesting therefore that our control sample is representative of our population. Therefore, among the 15 markers tested that are segregating with the disease gene, it was found that allele 1 of DSS412 is rare, while allele 10 of DSS487 is very rare in the control population, supporting evidence of a strong linkage disequilibrium between each of these alleles and the pathogenic mutation del656C. Based on these findings, we tested DSS412 and DSS487 in 23 small DLMD families (with one or two affected sibs). We found only the two previously reported unrelated patients, from kindreds LG33 and LG68, to be homozygous for alleles 1 and 10 of markers DSS412 and DSS487, respectively. As previously shown, these patients have the same homozygous missense mutation (del656C) found in the LG26 and LG43 affected subjects, confirming the strong linkage disequilibrium between this mutation and the haplotype 1/10-1/10 (DSS412/DSS487).

Single strand conformation polymorphism (SSCP) analysis for exons 2 to 8 of the δ sarcoglycan gene was performed in the remaining 21 DLMD patients. One affected girl showed a different migration pattern for exon 8 compared to the normal population. The sequencing of this exon detected a C→A transition at position 784 of the cDNA, which causes the substitution of a glutamic acid for a lysine at residue 262 (E262K) of the translatable protein. This mutation was confirmed after digestion of the PCR product with TaqI, since it abolishes a restriction site for thisendonuclease. In addition, the parents and the normal sibs of this patient were found to be heterozygous for the E262K mutation (fig 1). This affected girl of normal intelligence, currently 15 years old, belongs to a consanguineous white kindred (LG71) and has three normal sibs. Her parents report that she had a normal neuropsychomotor development. At the age of 8, she had difficulty in walking and climbing stairs, followed by pain and cramps. At the age of 11, when first seen by us, she had hypertrophy of the calves and a Gowers sign. She was confined to a wheelchair at 12 years and at present she is unable to raise her arms, thus showing a severe DLMD course. A recent electrocardiogram (ECG) showed hypertrophy and involvement of the left ventricle. Muscle biopsy showed a dystrophic pattern, with negative immunofluorescence staining for dystrophin, a total absence of δ sarcoglycan, accompanied by a total absence of α and β sarcoglycans, but only a partial deficiency of γ sarcoglycan.

To our knowledge, the substitution of one amino acid, in the present case the glutamic acid in position 262, is the first missense mutation found in the δ sarcoglycan gene. Interestingly, it causes a phenotype as severe as that presented by the other LGMD2F patients with truncating mutations. This glutamic acid residue, which is localised in the δ sarcoglycan extracellular domain two residues upstream of the cysteine cluster, is very conserved among different species and it is also present in the corresponding region of the γ sarcoglycan. This cysteine rich region, which is a conserved region among all the sarcoglycan family members, seems to participate in intra- and intermolecular disulphide bond formation. Therefore, the mutation described here occurs in an important domain of the δ sarcoglycan, interfering with the conformation of the tertiary structure of this molecule. These observations, together with the fact that this change was not found among 100 chromosomes from the healthy population, strongly suggest that the E262K mutation is pathogenic. This mutation is associated with a haplotype distinct from the one found in the other LGMD2F patients, in which the del656C mutation was identified. Interestingly, all the families with del656C have an African ancestry, in contrast to the family with the E262K mutation, who is white. Therefore, these data suggest that the del656C might have originated in Africa and spread in the Brazilian population or alternatively that this haplotype predisposes to the occurrence of this mutation. In this respect, it will be important to verify if the del656C is also found in association with different haplotypes.

Considering the 23 small DLMD Brazilian families screened for mutations in the δ sarcoglycan gene, we estimated that the frequency of cases resulting from mutations in this locus is 13% (3/23) in our population. If we take into account only the 11 families in which α sarcoglycan deficiency could be confirmed, this frequency is even greater, in accordance with the proportion of nearly 30% of δ sarcoglycan mutations among the four sarcoglycanopathies recently estimated in a large Brazilian sample. Therefore, the LGMD2F form is apparently not rare among our DLMD families, as compared to the data recently reported by Dugan et al. in which a frequency of 4% (2/54) was estimated for LGMD2F among families with α sarcoglycan deficiency.
It will be interesting to verify if the mutation E262K is present in other patients, particularly of white ancestry, with LGMD2F. This will be important to elucidate the origin of mutations in this gene as well as to improve our understanding of the function of \( \delta \) sarcoglycan.

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