European Journal of Human Genetics (1998) 6, 396–399

http://www.stockton-press.co.uk/ejhg

SHORT REPORT

Severe limb girdle muscular dystrophy in Spanish gypsies: further evidence for a founder mutation in the γ-sarcoglycan gene

Adriana Lasa[1], Federica Piccolo[2], Carles de Diego[1], Marc Jeanpierre[2], Jaume Colomer[3], Maria José Rodríguez[1], Jon Andoni Urtizberea[4], Montserrat Baiget[1], Jean Claude Kaplan[2] and Pia Gallano[1]

[1]*Unitat de Genètica Molecular, Hospital Sant Pau, Barcelona, Spain*
[2]*INSERM 129, Institut Cochin de Génétique Moléculaire, Paris, France*
[3]*Servei de Neurologia, Hospital Sant Joan de Deu, Barcelona, Spain*
[4]*Association Française centre les Myopathies, Evry, Paris, France*

Limb-girdle muscular dystrophy type 2C (LGMD2C) is an autosomal recessive muscular dystrophy with primary γ-sarcoglycan deficiency, generally associated with a severe clinical course. γ-sarcoglycan, a 35 kDa dystrophin-associated protein, is encoded by a single gene on chromosome 13q12. Six different mutations have been described in that gene, and it has been proved they are the origin of the disease. One of these mutations (C283Y), a G→A transition in codon 283, was recently and exclusively identified in Gypsy patients from different European countries. We report the study of 11 LGMD2C unrelated Gypsy families (nine Spanish and two Portugese). The muscle biopsies of these patients showed a drastically decreased immunostaining with α and γ-sarcoglycan antibodies. All the patients were homozygous for C283Y missense mutation, and all affected chromosomes (patients and heterozygous relatives) carried the allele 5 (112 bp) of the intragenic microsatellite D13S232. Unexpectedly, this allele is most frequent in the Caucasian population but not in the normal Gypsy population. The clinical severity of all patients demonstrates that the C283Y missense mutation in a homozygous state causes a severe LGMD2C (DMD-like). The elevated number of families ascertained let us assume that LGMD2C is prevalent in the Gypsy population, and that all the families have inherited a founding mutation.

Keywords: LGMD2C; founder mutation; endogamy

Correspondence: Dr P Gallano, Unitat de Genètica Molecular, Hospital Sant Pau, Av. Padre Claret 167, 08025 Barcelona, Spain. Tel: +343 291 9361; Fax: +343 291 9494; E-mail: pgallano@santpau.es
Received 23 October 1997; revised 12 January 1998; accepted 16 January 1998

Introduction

The autosomal recessive limb girdle muscular dystrophies represent a heterogeneous group of progressive disorders mainly affecting the pelvic and shoulder girdle musculature. The clinical course is characterised by great variability, ranging from severe forms with onset in the first decade and rapid progression, resembling Xp21 Duchenne dystrophy (DMD), to milder forms with later onset and slower progression resembling Xp21 Becker dystrophy (BMD).[1] These autosomally recessive inherited forms are now classified as limb-girdle muscular dystrophies type 2 (LGMD2)[2] and represent a less common cause of muscular dystrophy.

Sarcoglycan, a major component of the DAG complex,[3,4] is composed of at least four transmembrane glycoproteins: α-sarcoglycan (formerly called 50 kDa DAG or adhalin),[5,6], β-sarcoglycan (43 DAG),[7,8] γ-sarcoglycan (35 DAG)[9] and δ-sarcoglycan (35 kDa).[10] The muscular dystrophies that arise from mutations in α-, β-, γ- and δ-sarcoglycan genes are classified as the LGMD type 2D, E, C and F, respectively.[2] All patients with sarcoglycan related dystrophies are deficient in α-sarcoglycan in addition to their individual gene related sarcoglycan deficiency, so that initial immunohistochemical testing for α-sarcoglycan deficiency in all muscle biopsy specimens from patients with normal dystrophin provides a preliminary method of identifying this group of sarcoglycanopathies.[2,11]

The LGMD2C, linked to 13q12,[12] is prevalent in northern Africa[13] and only very few cases have been described in other populations.[9,14–16] Recently, Piccolo *et al*.[17] published a unique missense mutations (C283Y) in the γ-sarcoglycan gene in seven unrelated LGMD2C Gypsy families from different countries of Western Europe. This mutation was in complete linkage disequilibrium with allele 5 (112 bp) at the intragenic D13S232 marker indicating a founder effect. We report here 11 additional unrelated Gypsy families (nine from Spain, two from Portugal) clinically resembling Duchenne muscular dystrophy. All the affected patients were homozygous for the missense mutation C283Y in the γ-sarcoglycan gene.

Materials and Methods

Clinical and Immunological Studies

Nine Spanish and two Portugese unrelated Gypsy families, with a total of 13 patients (10 boys and three girls) were studied according to different criteria. All patients showed proximal muscle weakness with an onset of symptoms in the first decade of life. A Gower's manoeuvre and elevated serum creatine kinase were present in each of the children. Ten of the 13 patients were wheelchair-bound at around the age of 12. The remaining three patients were too young to evaluate the progression of the disease. Family history revealed that seven patients were isolated cases whilst in the other four families there were affected relatives. Eight families were definitely consanguineous, and in the remaining three families, distant consanguinity could not be excluded because the maternal and paternal ancestors came from the same region.

Duchenne muscular dystrophy was excluded on the basis of autosomal inheritance, and presence of normal dystrophin staining on immunocytochemical analysis. α and γ-sarcoglycan immunostaining performed in seven biopsies was drastically reduced. In the remaining patients, biopsies could not be performed due to family refusal. The antibodies used were the following: monoclonal antidystrophin DYS1 and DYS2 antibodies (Novocastra, Newcastle upon Tyne, UK); monoclonal anti-α and anti-γ-sarcoglycan antibodies (Novocastra, Newcastle upon Tyne, UK).

Genetic Analysis

Genomic DNA was extracted according to the method of Miller *et al*.[18] The presence of C283Y mutation in the γ-sarcoglycan gene was screened by PCR amplification followed by RsaI restriction as described by Piccolo *et al*.[17] Genotyping at the D13S232 intragenic polymorphic microsatellite was performed using the primers listed in the Genome Database (GDB). The amplified products were mixed with formamide 95%, denatured and loaded onto a 6% urea polyacrilamide gel. The DNA samples were transferred to a nylon membrane (Hybond-N^+, Amersham, Amersham Place, UK) and hybridised with a modified 5' (CA)n probe using the ECL labelling system (Amersham, Amersham Place, UK).

Results and Discussion

The 13 patients analysed, belonging to 11 families, were established as homozygous for the C283Y mutation, a G□ transition in codon 283 of the γ-sarcoglycan gene. Sixteen asymptomatic parents were studied, all found to be heterozygous for this mutation. The molecular screening of this mutation in 14 relatives showed 11 hetorozygous carriers and three non-carriers (Figure 1). The presence of this mutation was excluded in 28 chromosomes from unrelated control Spanish Gypsies, and in 90 non-Gypsy chromosomes.

The nine Spanish Gypsy families with LGMD2C live in different parts of the country and they are not known to be related to each other. The analysis of D13S232 marker, revealed that all the mutated chromosomes carried allele 5. This allele is most frequent in the Caucasian population (GDB allele frequency: 0.39). We analysed 55 Spanish Gypsy control chromosomes in order to see the alleles distribution of marker D13S232

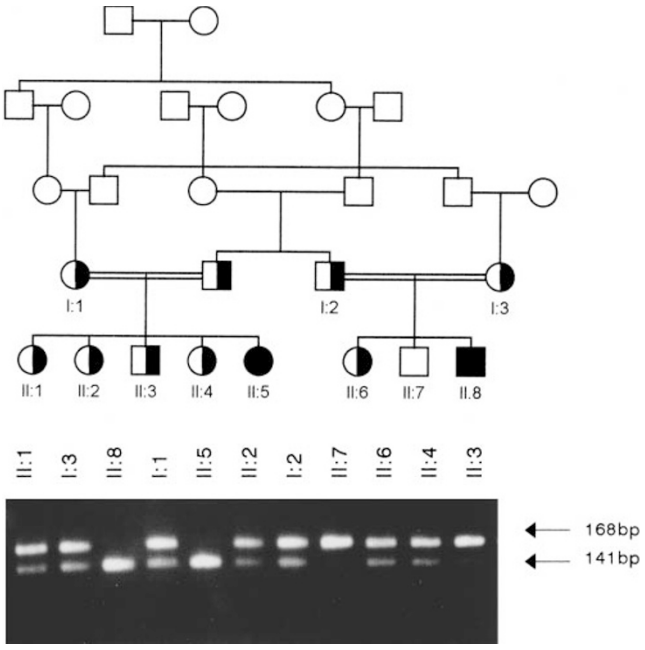

Figure 1 *Detection of mutation C283Y in the γ-sarcoglycan gene in one LGMD2C Spanish Gypsy family by PCR amplification followed by RsaI restriction. Since the mutation creates a new RsaI restriction site, this enzyme generates two restriction bands of 141 and 27 bp instead of the 168 bp normal band. Each lane represents a family member as indicated in the pedigree. The arrows indicate the normal (168 bp) and the mutated band (141 bp).*

Table 1 Allele frequencies of the intragenic marker D13S232

Allele (bp)	*Caucasian population*[a]	*LGMD2C*[b] *Gypsies*	*Gypsies control*[c]
1 (126)	0.17	0.0	0.07
2 (124)	0.03	0.0	0.02
3 (122)	0.02	0.0	0.02
4 (120)	0.09	0.0	0.09
5 (112)	0.39	1	0.25
6 (110)	0.18	0.0	0.40
7 (108)	0.11	0.0	0.15

[a]GBD; [b]53 chromosomes; [c]55 chromosomes.

in this population (Table 1). Allele 6 (110 bp) was present in 40% of the chromosomes while allele 5 appeared in only 5% of them.

The Gypsy population still forms a closed isolated society, maintaining its own language, customs and culture over time.[19] Since their arrival in Spain, they have continued to be genetically isolated up to the present day.[20] An estimated 7 to 9 million live in Europe today,[21] and the Spanish Gypsy population represents the largest Gypsy community in Western Europe with approximately half a million people living all over the country.[19] Like other isolated populations, Gypsies have an elevated level of endogamy, with a subsequent high rate of consanguinity. The proportion of consanguineous couples in the Spanish Gypsy population is 19.5 times (29.3%) that of non-Gypsies (1.5%).[22] This level of parental consanguinity leads to a high proportion of homozygotes for recessive conditions in the offspring and, consequently, to a high rate of recessive disorders.

The discovery of a private γ-sarcoglycan gene mutation in Gypsies[17] induced us to think that, due to the importance of the Gypsy community in Spain, it would be worth screening all patients suffering from severe LGMD for this mutation. In fact, prior to undertaking the molecular analysis we did not know the ethnic origin of the patients. The finding of the C283Y mutation in each of the 11 families prompted us to check their origin, and to find that they were all Gypsies. It should be noted that the C283Y mutation is as yet the only reported missense mutation in the γ-sarcoglycan gene.[9,14,17]

The severe phenotype observed in 10 of our 13 LGMD2C Gypsy patients with homozygous C283Y mutation, was in accordance with the clinical features observed by Piccolo *et al*.[17] The remaining three patients were too young to evaluate the severity of the disorder. These results let us assume that homozygous C283Y mutation causes a severe LGMD2C resembling DMD.

All the chromosomes with the C283Y mutation analysed in this study carried allele 5 of the D13S232 microsatellite marker. This is the most frequent allele (0.39) in the Caucasian population (Table 1). It is interesting to note that although all the Gypsy mutated chromosomes carried allele 5, it is not the most representative of this population.

Since Spanish Gypsies are integrated in the health care system, the simple diagnostic test employed to detect C283Y mutation may help in the accurate diagnosis, carrier detection and direct prenatal diagnosis of the disease in this population. The avoidance of muscle biopsy in children is an important feature of care for this community in Spain, because the parents very often refuse to give consent for this distressing event.

Acknowledgements

We wish to thank Drs F. Astiazarán, J.L. Macarrón, T. Martínez, S. Martínez Gil, M. Molina, J. Narbona, M.A.

Ramos and J.M. Trejo for providing samples and clinical information from their patients, and Dr J. Bertranpetit for his valuable discussion about Spanish Gypsy population data. This work has been supported by the Fundació La Marató TV3 1012/97 and FIS 98/0040-01.

References

1 Van der Kooi AJ, Barth PG, Busch HFM *et al*: The clinical spectrum of limb girdle muscular dystrophy. A survey in the Netherlands. *Brain* 1996; **119**: 1471–1480.
2 Bushby K: Towards the classification of the autosomal recessive limb-girdle muscular dystrophies. *Neuromusc Disord* 1996; **6**: 439–441.
3 Ozawa SL, Yoshida M, Suzuki A, Mizuno Y, Hagiwara Y, Noguchi S: Dystophin-associated proteins in muscular dystrophy. *Hum Mol Genet* 1995; **4**: 11711–11716.
4 Matsumura K, Campbell KP: Dystrophin-glycoprotein complex: its role in the molecular pathogenesis of muscular dystrophies. Muscle and Nerve 1994; **17**: 2–15.
5 Roberds SL, Leturcq F, Allamand V *et al*: Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. *Cell* 1994; **78**: 625–633.
6 Jeanpierre M, Carrié A, Piccolo F *et al*: From adhalinopathies to alpha-sarcoglycanopathies: an overview. *Neuromusc Disord* 1996; **6**: 463–465.
7 Bönnemann CG, Modi R, Noguchi D *et al*: β-sarcoglycan (A3b) mutations cause autosomal recessive muscular dystrophy with loss of the sarcoglycan complex. *Nat Genet* 1995; **11**: 266–273.
8 Lim LE, Duclos F, Broux O *et al*: β-sarcoglycan characterisation and role in limb-girdle muscular dystrophy linked to 4q12. *Nat Genet* 1995; **11**: 257–285.
9 Noguchi S, McNally EM, Ben Othmane K *et al*: Mutations in the dystrophin-associated protein γ-sarcoglycan in chromosome 13 muscular dystrophy. *Science* 1995; **270**: 819–822.
10 Nigro V, Piluso G, Belsito A *et al*: Identification of a novel sarcoglycan gene at 5q33 a sarcolemmal 35 kDa glycoprotein. *Hum Mol Genet* 1996; **5**: 1179–1186.
11 Dubowitz V: The muscular dystrophies. Clarity or chaos? *New Engl J Med* 1997; **36**: 650–651.
12 Azibi K, Bachner L, Beckmann S *et al*: Severe childhood autosomal recessive muscular dystrophy with the deficiency of the 50 kDa dystrophin-associated glycoprotein maps to chromosome 13q12. *Hum Mol Genet* 1993; **2**: 1423–1428.
13 El Kerch F, Sefiani A, Azibi K *et al*: Linkage analysis of families with severe childhood autosomal recessive muscular dystrophy in Morocco indicates genetic homogeneity of the disease in North Africa. *J Med Genet* 1994; **31**: 342–343.
14 McNally EM, Duggan D, Gorospe JR *et al*: Mutations that disrupt the carboxyl-terminus of γ-sarcoglycan cause muscular dystrophy. *Hum Mol Genet* 1996; **5**: 1841–1847.
15 McNally EM, Passos-Bueno MR, Carsten CG *et al*: Mild and severe muscular dystrophy caused by a single γ-sarcoglycan mutation. *Am J Hum Genet* 1996; **59**: 1040–1047.
16 Duggan DJ, Gorospe JR, Fanin M, Hoffman EP, Angelini C: Mutations in the sarcoglycan genes in patients with myopathy. *N Engl J Med* 1997; **336**: 618–624.
17 Piccolo F, Jeanpierre M, Leturcq F *et al*: A founder mutation in the γ-sarcoglycan gene of Gypsies possibly predating their migration out of India. *Hum Mol Genet* 1996; **5**: 2019–2022.
18 Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res* 1988; **16**: 1215.
19 Fraser A: *The Gypsies*. Blackwell: Oxford, UK and Cambridge, USA, 1995.
20 de Pablo R, Vilches C, Moreno ME, Rementería MC, Solís R, Kreisler M: Distribution of HLA antigens in Spanish Gypsies: a comparative study. *Tissue Antigens* 1992; **40**: 187–196.
21 Liegeois JP: Roma, Gypsies, Travellers. Council of Europe Press: Strasbourg, 1994.
22 Martínez-Frías ML, Bermejo E: Prevalence of congenital anomaly syndromes in Spanish Gypsy population. *Med Genet* 1992; **29**: 483–486.