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Genetic heterogeneity within a consanguineous family involving the LGMD 2D and the LGMD 2C genes

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Abstract

The sarcoglycanopathies are a group of autosomal recessive limb girdle muscular dystrophies (AR-LGMD 2) characterised by mutations in gene encoding one of the sarcoglycan subunits. Mutations in SGCA, SGCB, SGCG and SGCD genes are associated with LGMD 2D, 2E, 2C and 2F, respectively. We report three Tunisian patients belonging to the same consanguineous family sharing similar LGMD 2 phenotype but heterogeneous sarcoglycans immunohistochemical patterns. Linkage analysis suggests linkage with the LGMD 2D locus for the two siblings and with LGMD 2C locus for the third patient. Mutation analysis revealed two distinct mutations. A del521T homozygous mutation in exon 6 of the SGCG gene (LGMD 2C), widely distributed in Tunisian patients, was found in one patient, whereas a 157G > A homozygous mutation in exon 2 of the SGCA gene (LGMD 2D) was found in the two siblings. The presence of two distinct genetic forms, LGMD 2C and LGMD 2D in a consanguineous family raises the problem of the complexity of genetic counselling in inbred populations.

Keywords: Limb girdle muscular dystrophy; Genetic heterogeneity

1. Introduction

Sarcoglycanopathies (LGMD 2C–2F) (MIM 253700; MIM 600119; MIM 604286; MIM 601287) [1] are a group of autosomal recessive limb girdle muscular dystrophies caused by primary mutations in genes encoding γ-(SGCG) [2], α-(SGCA) [3], β-(SGCB) [4,5] and δ-sarcoglycans (SGCD) [6]. They are characterized by a progressive weakness of pelvic and shoulder girdle muscles. Usually, mutation in one of the sarcoglycan genes causes the absence of the expression of the corresponding subunit and a secondary reduction that can vary from partial deficiency to total absence of the other three glycoproteins.

AR-LGMDs have a particular high frequency in Tunisia [8], probably related to the high degree of consanguineous marriage. LGMD 2C is the most common genetic form encountered and the majority of AR-LGMD Tunisian patients share the del521T mutation in the SGCG gene, indicating a strong founder effect [2,9,10]. Other AR-LGMD forms such as LGMD 2E [11], LGMD 2B [12] and LGMD 2I [13] have been rarely reported in the Tunisian population.

We report clinical, immunohistochemical muscle biopsy findings, and genetic analysis of three patients belonging to the same consanguineous family with an LGMD 2 phenotype caused by mutations in two different sarcoglycan genes.

2. Patients and methods

2.1. Patient IV2

Patient IV2 is a 21-years-old man who developed since the age of 6 years a progressive pelvic muscle weakness responsible for difficulty in walking and climbing stairs. He became wheelchair bound at 16. Neurological examination at 21 years found a wheelchair bound patient with severe weakness of lower limbs, calves hypertrophy and contracture of knee and ankles articulations. This was associated to a proximally predominant severe weakness of upper limb muscles. The patient was able to perform very limited movements with hand muscle and to move his electric

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wheelchair. CK rate was 1274 IU/l (normal range $\leq$64 IU/l).

2.2. Patients III7 and III8

Patients III7 and III8 were two siblings.

The elder brother, patient III7, was 31 years old when he was first examined in the department. The age of onset has been estimated as 5 years with progressive difficulty in walking and climbing stairs. He became wheelchair bound at 17. Neurological examination found a severe kyphoscoliosis, amyotrophy in four limbs with moderate calves hypertrophy. CK rate was 89.1 IU/l.

His sister, patient III8, was 25 years old at the time of examination. The age of onset was around 5 with difficulty in walking and climbing stairs. She became wheelchair bound at 18. Neurological examination showed severe proximal predominant muscles weakness in four limbs leading the patient in a wheelchair. She was able to move her wheelchair by herself but had difficulty to eat without help. CK rate was 313 IU/l.

2.2.1. Muscle biopsy immunohistochemistry

Serial 8–10 μm cryostat sections of available muscle biopsies from two patients (III8 et IV2) were stained for dystrophin, spectrin, α, β, γ and δ-sarcoglycan expression using the following antibodies; dystrophin (NCL-Dys 1/2, Novocastra), spectrin (NCL-SPEC1, Novocastra), α-sarcoglycan (NCL-a-SARC, Novocastra), β-sarcoglycan (NCL-b-SARC, Novocastra), γ-sarcoglycan (NCL-g-SARC, Novocastra) and δ-sarcoglycan (NCL-d-SARC, Novocastra). The indirect horseradish peroxydase technique was applied using the Extravidin Peroxydase Staining Kit (EXTRA2-Sigma).

2.2.2. Linkage study

After informed consent, blood samples were obtained from patients and the available family members. Genomic DNA was extracted from whole blood using standard procedures. Linkage analysis was carried out with the following microsatellite primers spanning the LGMD 2C locus (D13S232-D13S787-D13S1275-D13S1285-D13S292), the LGMD 2D locus (D17S791-D17S1319-D17S806), the LGMD 2E locus (CA12T-D4S405-D4S395-D4S391) and the LGMD 2F locus (D5S410-D5S640-D5S412).

DNA linkage studies were performed using radioactive and fluorescent genotyping analysis. Fluorescent PCR amplifications were performed with the True Allele PCR kit (Applied Biosystems, Perkin–Elmer) on an ABI 310 automated gene sequencer following the manufacturer's conditions.

2.2.3. Mutations screening

All SGCA and SGCG gene exons have been sequenced on both strands. Primers flanking each exons were from LMDP [14]. Sequencing was performed using an automated ABI 310 sequencer (Applied Biosystems, Perkin–Elmer).

After sequencing process, data were analysed automatically using the ABI PRISM sequencing analyser v3.7 software.

3. Results

Neurological examination showed the same clinical features of severe limb girdle muscular dystrophy with onset during the first decade, progressive course of muscle weakness involving proximal muscles of lower limbs

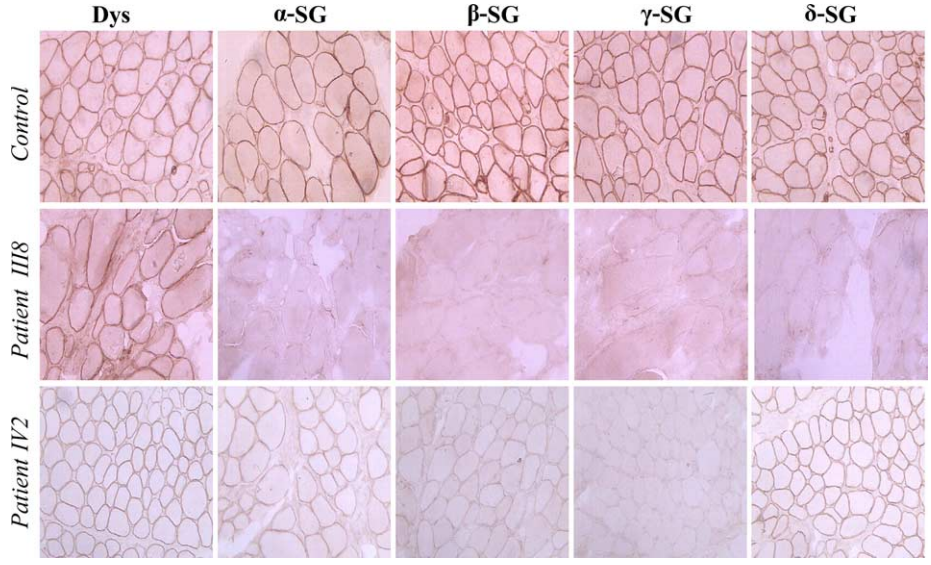

Fig. 1. Immunohistochemical analysis of muscle biopsy sections. Absent staining of the four sarcoglycans (patient III8). Negative γ-SG staining with a relatively preserved staining of α, β, and δ-SG (patient IV2).