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Phenotypic variability in a Spanish family with a *Caveolin-3* mutation

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ABSTRACT

We report a Spanish family affected from a late onset, hand-involved and autosomal dominant distal myopathy associated to *Caveolin-3* mutation. Signs of muscle hyperexcitability and hyperckemia were observed in the youngest relatives but not motor symptoms.

Patients and methods: Neurological examination was performed in all members of the family. Muscle biopsy sample was taken from the proband and DNA genomics was amplified for the two exons of *Cav-3* by the polymerase chain reaction (PCR) in all the affected members and in three asymptomatic relatives.

Results: Signs of muscle hyperexcitability and hyperckemia were observed in the affected members from early ages. Cav-3 expression was greatly reduced in the sarcolemma of the proband's muscle. Genetic studies revealed a G → A transition at nucleotide position 80 in exon 1 of the *Cav-3* gene (c.80G>A), generating a Arg → Gln change at codon 27 (p.R27Q) of the amino acid chain in heterozygous state, while no mutation was found in unaffected members.

Conclusions: Signs of muscle hyperexcitability and hyperckemia at early ages may predict the development of a late onset autosomal dominant hand-involved myopathy associated to *Cav-3* mutation in the family reported herein.

1. Introduction

Caveolin-3 (Cav-3) is an integral membrane protein specifically expressed in smooth, cardiac and skeletal muscle cells, although recently it has been identified in astroglia [1,2]. *Cav-3* gene is located in chromosome 3p25. Monomers of 150 amino acids oligomerize to form a high molecular mass scaffolding network which constitutes the main support of the caveolae in skeletal muscle cells [3].

Five autosomal dominant phenotypes associated with *Cav-3* mutations have been described [3]: Limb-girdle muscular dystrophy (LGMD-1C), rippling muscle disease (RMD), distal myopathy, hyperckemia, and hypertrophic cardiomyopathy. Moreover, a specific *Cav-3* mutation may be associated with different phenotypes within a family [4,5].

We report a Spanish family whose members developed an autosomal dominant hand-involved distal myopathy associated with a *Cav-3* mutation. Interestingly, the youngest members displayed signs of muscle hyperexcitability and hyperckemia before the distal myopathy became apparent clinically.

2. Patients and methods

The pedigree of the family is shown in Fig. 1.

2.1. Case reports

I_1 (proband): This patient is a 77-year-old man. At the age of 45, he started to complain of a slow and progressive weakness in his hands. He had difficulty opening doors using keys, buttoning his shirt, and writing. There was no history of cramps, myalgias, involuntary movements, exercise intolerance or fatigue. At this time, neurological examination revealed thenar and hypothenar muscle atrophy in both hands and moderately reduced muscle strength in intrinsic muscles and finger flexors. These symptoms were more marked in the left hand. Calf hypertrophy and *pes cavus* were also observed (Fig. 2). In addition, tapping on skeletal muscles with a reflex hammer revealed the presence of percussion-induced rapid contractions (PIRC), predominantly on distal muscles of upper limbs. The rest of the neurological examination was normal.

Serum creatin kinase (CK) levels were around 643 IU/L (normal level <180 IU/L). Other biochemical values were normal including lactic and pyruvic acid.

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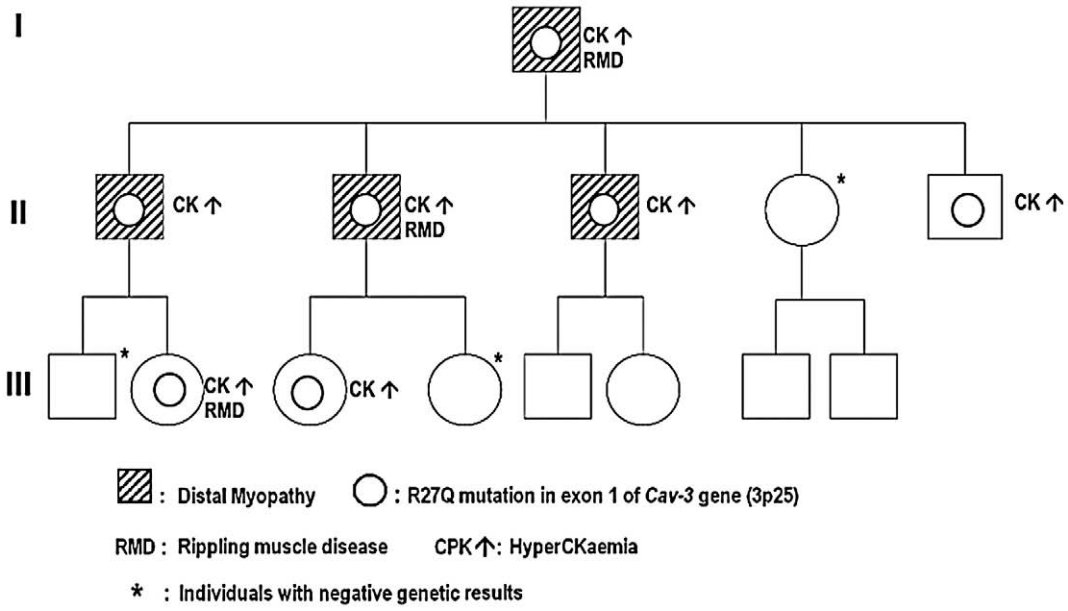

Fig. 1. Pedigree of the family: Crossed symbols depict patients who have developed motor deficit in hands. However, there is no evidence of motor deficit in subjects II_5, III_2 and III_3 who present with hyperckemia and signs of muscle hyperexcitability. Note that *Cav-3* gene mutation was found in the members marked with ◯. I_1: proband. □: male. ○: female.

II_1: This is a 52-year-old man, the oldest son of the proband. A high level of CK (767 IU/L) was detected in a routine analysis at the age of 28. In addition, calf hypertrophy and *pes cavus* were also observed. Nine years later, at the age of 37, he complained of weakness in his hands. At that time, neurological examination showed bilateral hypothenar muscle atrophy and asymmetrical paresis of opponens pollicis and interossei muscles, which was more marked in the left hand. The motor deficit has shown a slight progress until today.

II_2: The second son of the proband, a 51-year-old man, was examined at the age of 26 presenting hyperckemia (1223 U/L), calf hypertrophy and *pes cavus*. In addition, PIRC and percussion-induced muscle mounding (PIMM) in biceps brachii and triceps muscles were also observed. Fifteen years later, paresis in interossei muscles and opponens pollicis appeared being more marked in the left hand.

II_3: This patient is a 45-year-old man. Hyperckemia (452 U/L), calf hypertrophy and *pes cavus* were detected at the age of 21. When he was 42, he presented paresis in interossei muscles and thenar muscle atrophy predominantly in the right hand.

II_5: The youngest son of the proband, a 33-year-old man, presented an asymptomatic hyperckemia (619 IU/L) and *pes cavus* from the age of 9. No evidence of motor deficit has been observed to date.

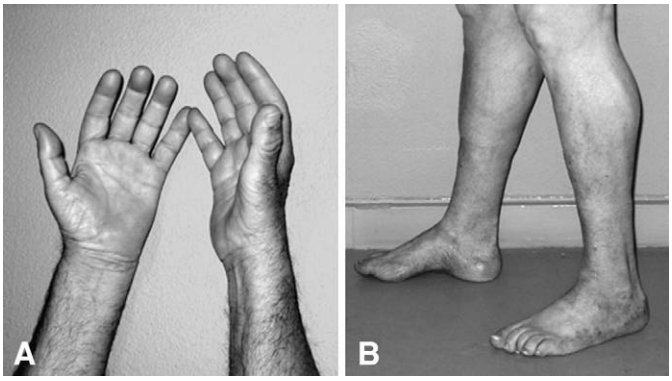

Fig. 2. Proband of the family (I_1): A) Thenar and hypothenar muscle atrophy, predominantly in the left hand. B) Calves hypertrophy and *pes cavus*.

III_2: A 22-year-old woman, the first granddaughter of the proband, was examined at the age of 16, detecting hyperckemia (1177 U/L), PIRC in biceps brachii muscle, calf hypertrophy and *pes cavus*. No evidence of motor deficit has been demonstrated to date.

III_3: A 23-year-old woman presented exercise-induced myalgias and muscle stiffness, predominantly in calves since the age of fifteen. Neurological examination at this age only revealed *pes cavus*, and a high serum CK level (1357 IU/L). Neither muscle hyperexcitability nor motor deficit has been demonstrated.

2.2. Muscle biopsy studies

An open biopsy sample from the left biceps brachii muscle was taken from the proband under local anesthesia and after the patient's informed consent. Routine histochemical stainings were performed on 7 μ transverse sections of frozen muscle with hematoxylin and eosin, modified Gomori trichrome, NADH, SDH and ATPase at basic and acid pH. Immunohistochemistry was performed on gelatinized slides and avindin–biotin complex peroxidase system. Mouse monoclonal antibodies directed against the three different domains of dystrophin (Dys1, Dys2, Dys3), the α, β, γ, δ sarcoglycans, utrophin, dysferlin, (all from Novocastra Laboratories, New Castle upon Tyne, UK) and Cav-3 (Transduction Laboratory; San Diego, CA) were used. A control muscle biopsy was taken from a patient diagnosed with normal muscle biopsy.

For ultrastructural studies, a small fragment was trimmed into tiny rectangular pieces; fixed in 2.5% of glutaraldehyde, postfixed in osmium tetraoxide and embedded in Epon after routine dehydration. Semithin sections were stained with toluidine blue. Ultrathin sections were mounted on copper grids, contrasted with uranyl acetate and lead citrate and examined with a Philips CM100 electron microscope.

2.3. Molecular genetic studies

Total genomic DNA from peripheral blood of the seven patients and three family relatives (all subjects gave their informed consent) was amplified for the two exons of the *Cav-3* gene by the polymerase chain reaction (PCR). Primers and conditions used were those described by Minetti et al. [6] The samples were purified with a QIAquick column PCR purification kit (QIAGEN), and analysed by direct forward and