Neuromuscular Disorders 19 (2009) 779–783

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Rippling muscle disease and cardiomyopathy associated with a mutation in the *CAV3* gene

Michela Catteruccia [a], Tommaso Sanna [b], Filippo Maria Santorelli [c], Alessandra Tessa [c], Raffaella Di Giacopo [a], Donato Sauchelli [a], Alessandro Verbo [d], Mauro Lo Monaco [a], Serenella Servidei [a,*]

[a] Department of Neuroscience, Institute of Neurology, Catholic University, Largo Agostino Gemelli 8, 00168 Rome, Italy
[b] Institute of Cardiology, Catholic University, Rome, Italy
[c] Molecular Medicine, IRCCS Bambino Gesù Hospital, Rome, Italy
[d] Institute of General Surgery Catholic University, Rome, Italy

ARTICLE INFO

Article history:
Received 14 May 2009
Received in revised form 8 August 2009
Accepted 27 August 2009

Keywords:
Caveolin-3
Rippling muscle disease
Dilated cardiomyopathy

ABSTRACT

Caveolin-3, the myocyte-specific isoform of caveolins, is preferentially expressed in skeletal, cardiac and smooth muscles. Mutations in the *CAV3* gene cause clinically heterogeneous neuromuscular disorders, including rippling muscle disease, or cardiopathies. The same mutation may lead to different phenotypes, but cardiac and muscle involvement rarely coexists suggesting that the molecular network acting with caveolin-3 in skeletal muscle and heart may differ. Here we describe an Italian family (a father and his two sons) with clinical and neurophysiological features of rippling muscle disease and heart involvement characterized by atrio-ventricular conduction defects and dilated cardiomyopathy. Muscle biopsy showed loss of caveolin-3 immunosignal. Molecular studies identified the p.A46V mutation in *CAV3* previously reported in a German family with autosomal dominant rippling muscle disease and sudden death in few individuals. We suggest that cardiac dysfunction in myopathic patients with *CAV3* mutations may be underestimated and recommend a more thorough evaluation for the presence of cardiomyopathy and potentially lethal arrhythmias.

1. Introduction

Rippling muscle disease (RMD) is a rare muscle disorder characterized by symptoms and signs of increased muscular irritability as percussion induced rapid contractions (PIRC), percussion induced muscle mounding (PIMM) and rippling phenomenon in addition to muscle stiffness, hyperCKemia and muscular hypertrophy. RMD is usually transmitted as an autosomal dominant trait and is associated with mutations in the caveolin-3 gene (*CAV3* on 3p25).

CAV3 encodes caveolin-3, the myocite-specific isoform of caveolin that is preferentially expressed in skeletal, cardiac and smooth muscle [1]. Caveolins, 21–25 kDa integral membrane proteins, are the principal components of caveolae that are 50–100 nm vesicular invaginations of the plasma membrane [2]. These structures participate in vesicular trafficking events and signal transduction by acting as scaffolding proteins for specific lipids and lipid-modified signaling molecules [3].

Mutations in *CAV3* are associated with four different, but sometimes overlapping neuromuscular diseases as limb girdle muscular dystrophy type 1C (LGMD-1C) [4], rippling muscle disease (RMD) [5], asymptomatic hyperCKemia [6], and distal myopathy [7]. Moreover, *CAV3* mutations have been also identified in association with cardiac diseases as familial hypertrophic cardiomyopathy (HCM) [8], long-QT syndrome type 9 (LQT9) [9] and sudden infant death syndrome (SIDS) [10].

Different phenotypes may be linked to the same mutation, but up to now the coexistence of muscular and cardiac dysfunction have been only rarely described and not clearly demonstrated, suggesting that the molecular network acting with caveoline-3 in skeletal muscle and heart may differ.

Here we report an Italian family with autosomal dominant RMD associated with the p.A46V mutation in *CAV3* with both muscular and cardiac involvement.

2. Patients and methods

The pedigree of the family here described is detailed in Fig. 1.

All patients underwent a standardized clinical interview and a careful physical examination. Venous blood samples were drawn for genetic analysis, and routine biochemical profile comprehensive of CPK dosage.

Electromyography, muscle magnetic resonance and muscle biopsy were performed in patient 1. Electrocardiogram (EKG) and

* Corresponding author. Tel.: +39 06 30154303; fax: +39 06 35501909.
E-mail address: s.servidei@rm.unicatt.it (S. Servidei).

0960-8966/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2009.08.015

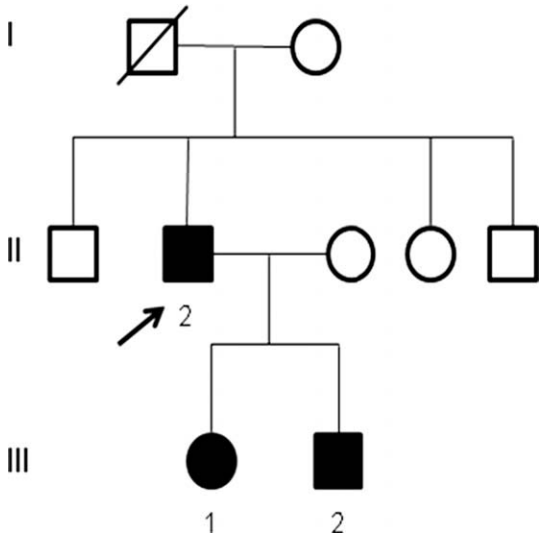

Fig. 1. Family tree, autosomal dominant pattern of inheritance; the arrow (II-2) indicates patient 1 in the report.

echocardiography were recorded in all patients with standard techniques. Cardiac magnetic resonance was carried out in patient 1 because a suspect of cardiac disease was elicited by the results of the preliminary non-invasive evaluation.

2.1. Muscle magnetic resonance

Muscle magnetic resonance (MR) was conducted on 1.5 Tesla MR system. The protocol used for musculoskeletal system included FSE and F-STIR sequences in transverse plane.

2.2. Cardiac magnetic resonance

Cardiac magnetic resonance (CMR) was performed on 1.5 Tesla MR system, including both functional and delayed gadolinium contrast enhancement technique.

2.3. Muscle biopsy

A frozen-deltoid muscle specimen was examined by standard histochemical and histoenzymatic techniques. Immunohistochemical analysis was performed with the following monoclonal antibodies: anti-caveolin-3 (anti-Cav-3 mAb, clone 26 raised against amino-acid residues 3–24; BD Transduction Laboratories, Lexington, KY, USA), anti-dystrophin (N-terminus, intermediate and C-terminus; Novocastra Laboratories), anti-merosin (Novocastra Laboratories), anti-α-sarcoglycan (Novocastra Laboratories), anti-γ-sarcoglycan (Novocastra Laboratories), anti-α-dystroglycan (Novocastra Laboratories), detected by biotinylated anti-mouse IgG (Amersham Biosciences, Little Chalfont, UK) and streptavidin-fluorescein (Amersham Biosciences).

2.4. Molecular analysis

Genomic DNA was prepared from peripheral blood samples from patients according to standard protocols. Sequencing of *CAV3* (Genbank Accession Number NM_033337.1) coding regions and flanking intronic sequences was performed on PCR products after amplification of genomic DNA using oligonucleotide primers and PCR conditions described in Traverso et al. [11], and cycle-sequencing adopting the BigDye 3.1 chemistry (Applied Biosystems, Foster City, CA). Following the consensus guidelines for mutation nomenclature (www.hgvs.org/mutnomen), DNA changes are numbered according to cDNA sequence, with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. For protein changes the initiation codon is codon 1.

3. Results

Patient 1 (II:2) was a 50-year-old man who experienced exercise induced muscle cramps, pain and stiffness since early childhood. He come however to medical attention in July 2006 for atypical chest pain and elevated serum CK (2–4 times normal). An ischemic heart disease was suspected and excluded. He was admitted at our Department of Neurology in November 2006. At neurological examination he had diffuse muscular hypertrophy, more marked in the lower limbs (quadriceps, biceps femoris and calf muscles), localized mounding and rapid contractions on percussion of muscle bellies and rippling phenomenon. Muscle strength was normal except on neck flexors (3.5 on the MRCs). Serum level of electrolytes, complete blood cell count and thyroid function were normal. Serum CK was 748 UI/l (normal range 30–230), LDH and transaminases were mildly elevated. Venous lactic acid at rest and after exercise test on cycloergometer was normal. Electromyography showed myopathic signs and electrically silent waves of contraction elicited by mechanical stimulation. Sensory and motor nerve conduction velocities were normal. An extensive cardiac evaluation was performed. EKG revealed nonspecific abnormalities of cardiac repolarization. 24-h-EKG-Holter monitoring showed episodes of 2nd degree AV block (type I), sporadic supraventricular and ventricular premature beats and non-diagnostic ST depression during sinus tachycardia. Treadmill stress test did not show EKG signs of exercise induced ischemia at higher workload. An echocardiogram revealed left ventricular dilatation, left atrial dilatation, normal left ventricular ejection fraction (60%) but mild reduction of systolic myocardial velocity at Tissue Doppler Imaging (check wave: 7 cm/s).

To better characterize muscle and heart involvement, muscle MR and biopsy and cardiac MR were performed.

3.1. Muscle MRI

MRI of pelvis, thighs and legs in patient 1 showed diffuse, symmetric, marked fatty infiltration without edema of selected muscles both of the anterior and the posterior compartments. MRI signal abnormalities were detected in hip adductors, hip abductors, rectus femoris and hamstring muscles, but the most affected muscles were tensor fascia lata, quadratus femoris and semitendinosus (Fig. 2a and b, arrows). Vastus medialis, vastus intermedius and vastus lateralis were completely spared. Milder changes were seen in almost all leg muscles with a relative sparing of gastrocnemius lateralis and soleus (Fig. 2c).

3.2. Cardiac MRI

CMR in patient 1 showed thickness of the interventricular septum to upper limits, moderate biventricular enlargement, biventricular segmental abnormalities of systolic function and delayed enhancement areas without coronary pattern distribution. Delayed contrast-enhanced sequences detected a linear subendocardial late gadolinium enhancement (LGE) of the basal anterior septal wall and patchy, multi-focal enhancement of the left ventricular mid wall (Fig. 3).

3.3. Muscle biopsy

Biopsy specimen from deltoid muscle of patient 1 demonstrated increased variability in fibre size with scattered round shaped