Neuromuscular Disorders 18 (2008) 572–578

www.elsevier.com/locate/nmd

Caveolinopathy – New mutations and additional symptoms ☆

Ahmed Aboumousa [a,g], Jessica Hoogendijk [a,f], Richard Charlton [a], Rita Barresi [a], Ralf Herrmann [b], Thomas Voit [c], Judith Hudson [a], Mark Roberts [d], David Hilton-Jones [e], Michelle Eagle [a], Kate Bushby [a], Volker Straub [a,*]

[a] *Institute of Human Genetics, University of Newcastle upon Tyne, International Centre for Life, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK*
[b] *Department of Paediatrics and Paediatric Neurology, University Hospital Essen, Germany*
[c] *Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Université Pierre et Marie Curie Paris VI, France*
[d] *Department of Neurology, Withington Hospital, Manchester, UK*
[e] *Department of Clinical Neurology, West Wing, John Radcliffe Hospital, Oxford, UK*
[f] *Department of Neurology, Rudolf Magnus Institute of Neurosciences, University Medical Centre Utrecht, The Netherlands*
[g] *Department of Neurology, Kasr Al-Aini Faculty of Medicine, Cairo University, Egypt*

Received 14 February 2008; received in revised form 1 May 2008; accepted 6 May 2008

Abstract

Mutations in the caveolin-3 gene (CAV3) can lead to a broad spectrum of clinical phenotypes. Phenotypes that have so far been associated with primary caveolin-3 deficiency include limb girdle muscular dystrophy, rippling muscle disease, distal myopathy and hyperCKaemia. This is the first report describing the clinical, pathological and genetic features of patients with caveolinopathy from the UK. Ten patients (six families) were identified via the National Commissioning Group (NCG) service for patients with limb girdle muscle dystrophy in Newcastle. Myalgia was the most prominent symptom in our cohort of patients and for 50% it was the reason for referral. Muscle weakness was only found in 60% of the patients, whereas rippling muscle movement was present in 80%. One of the patients reported episodes of myoglobinuria and another one episodes of hypoglycaemia. Five different mutations were identified, two of which were novel and three that had previously been described. Caveolinopathy needs to be considered as a differential diagnosis in a range of clinical situations, including in patients who do not have any weakness. Indeed, rippling muscles are a more frequent symptom than weakness, and can be detected in childhood. Presentation with myalgia is common and management of it as well as of myoglobinuria and hypoglycaemia may have a major impact on the patients' quality of life.

Keywords: Caveolin-3; Rippling muscle disease; Limb girdle muscular dystrophy; Myalgia; Myoglobinuria; Hypoglycaemia

1. Introduction

Caveolae ('little caves') are 50–100 nm small membrane invaginations on the surface of cells, which represent appendages or subcompartments of plasma membranes [1]. They participate in membrane trafficking, sorting, transport and signal transduction, including endocytosis and potocytosis. Caveolin is believed to play a role in the formation of the caveolae membranes, acting as a scaffolding protein that organizes and concentrates caveolin-interacting proteins and lipids in caveolae microdomains [2]. The mammalian caveolin gene family consists of three caveolins, caveolin-1, -2 and -3. Caveolins-1 and -2 are co expressed in adipocytes, whereas the expression of caveolin-3 is muscle specific [3].

Caveolin-3 is a 21–24 kDa integral membrane protein formed of 151 amino acids (aa). Three separate segments can be identified in this protein, an N-terminal region (aa 1–73), a central hydrophobic transmembrane domain (aa 75–106) and a C-terminal (aa 107–151) domain. The transmembrane domain is believed to form a hairpin loop struc-

☆ *Disclosure:* The authors have no conflicts of interest.

* Corresponding author. Tel.: +44 191 241 8762/8655; fax: +44 191 241 8770.
E-mail address: volker.straub@ncl.ac.uk (V. Straub).

0960-8966/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2008.05.003

ture in the cell membrane, allowing both the N- and C-terminal ends to face the cytoplasm. The N-terminal domain contains a caveolin signature sequence (aa 41–48, FEDVIAEP present in all caveolins) and a scaffolding domain (aa 55–74) known to bind various signalling proteins. It is also responsible for the homo-oligomerization and the interaction with caveolin-associated signalling molecules such as eNOS, β-adrenergic receptors, protein kinase C isoforms, G proteins, Src-family kinases, and multiple components of the dystrophin-glycoprotein complex [4,5].

Mutations in the CAV3 gene have been described in different domains of the protein. They are usually inherited in an autosomal dominant pattern and are associated with a broad spectrum of clinical phenotypes including LGMD1C, distal myopathy, rippling muscle disease (RMD) and hyperCKaemia. Recently, an autosomal recessive inheritance of some CAV3 mutations was considered [6–9].

Different pathogenic mechanisms have been postulated to explain muscle pathology in caveolin-3 deficient muscles. First, it was found that mutated caveolin-3 behaved in a dominant-negative fashion, causing the retention of wild type protein at the level of the Golgi apparatus [10]. Second, altered caveolin-3 expression changed the outcome of phosphoinositol-3-kinase activation from cell survival to cell death [11]. Third, caveolin-3 null mice showed T-tubule abnormalities that could lead to dysregulation of muscular calcium homeostasis initiating a dystrophic process and possibly RMD [3].

Here, we describe the clinical details of the first group of patients with caveolinopathy from the UK representing six families with five different mutations. Our cohort of patients showed various phenotypes that extend the clinical spectrum of patients with caveolin-3 deficiency. In addition, we found two novel mutations in the caveolin gene in our patients.

2. Patients and methods

The Institute of Human Genetics in Newcastle Upon Tyne, UK serves as the national referral centre for patients with a possible diagnosis of limb girdle muscular dystrophy. The service is commissioned by the National Commissioning Group (NCG), covers residents from England, Wales and Scotland and provides clinical assessment, biopsy analysis and genetic testing.

We describe 10 patients (3 male and 7 female) from six families with genetically confirmed mutations in the CAV3 gene. All patients underwent a thorough neurological assessment and six of them (one patient from each family) had a muscle biopsy. The manifestations of mechanical irritability of the muscles (percussion induced rapid contractions/PIRCs, mounding and rippling muscle movements) were assessed as previously described [12]. Serum creatine kinase levels were measured in all patients. Six patients had electrophysiological investigations.

2.1. Muscle biopsy studies

Muscle biopsies were assessed by routine histochemistry and immunoanalysis. Optimised immunohistochemical and multiplex Western blot protocols were used as previously described [13,14].

Commercial antibodies to the laminin α5 chain (mAb 1924), laminin β1 chain (mAb 1921), laminin γ1 chain (mAb 1920) and Collagen VI (mab 1944) were obtained from Chemicon International (Temecula, CA). Antibodies against α-dystroglycan (clone IIH6) was a gift from Kevin Campbell, β-spectrin (RBC2/3D5), β-dystroglycan (43DAG/8D5), C-terminal dystrophin (Dy8/6C5) and the N-terminal dystrophin (DY10/12B2), α-sarcoglycan (Ad1/20A6), β-sarcoglycan (1/5B1), γ-sarcoglycan (35DAG/21B5), δ-sarcoglycan (3/12C1), neonatal Myosin (MHCn), laminin α2 chain (NCL-Merosin) and dysferlin NCL-Hamlet/NCL-Hamlet were all from Novocastra. Caveolin-3 C38320 was supplied by Becton–Dickinson.

Briefly, sections were washed for 15 min in PBS, pH 7.3, containing 0.1% Triton X to permeabilise the membranes. Excess buffer was removed and sections incubated overnight at 4 °C in optimally diluted primary antibody diluted in 40% foetal calf serum containing 0.1 M lysine. Following 2× 10 min washes in PBS/Triton sections were incubated for 90 min at room temperature in HRP rabbit anti mouse IgG (Dako P260) diluted 1:100 in 0.1 M lysine in 40% foetal calf serum. Sections were washed for 2× 10 min as above, visualised with 3,3′-diaminobenzidene (DAB) and counterstained with Carazzi's haematoxylin prior to dehydration and mounting. Control sections were labelled without primary antibodies, and all sections were compared with control samples from other neuromuscular disorders, and with normal muscle.

Multiplex Western blot analysis was performed using biphasic polyacrylamide gradient gels 4–12% gels and antibodies against dysferlin (NCL-hamlet), calpain 3 (Calp3 d/2C4 and Calp3c/12A2), dystrophin (Dy8/6C5 C-terminus and Dy4/6D3 rod domain), α-sarcoglycan (Ad1/20A6), β-dystroglycan (43DAG/8D5), γ-sarcoglycan (35DAG/21B5), the laminin α2 chain (Chemicon MAB 1922) and Caveolin-3 C38320. Myosin heavy chain staining on the post blotted gel was used to indicate how much actual muscle protein (as opposed to fat and fibrous connective tissue) was loaded in each lane [13].

2.2. Mutation analysis

Genomic DNA (50–100 ng) extracted from peripheral blood lymphocytes was used as a template for PCR amplification. Reactions with exon-specific primers were performed as previously described [6]. Thirty-five cycles of amplifications were performed as described above with an annealing temperature of 55 °C [6]. PCR products were digested with BclI (LifeTechnologies, Karlsruhe, Germany) and products were analyzed by electrophoresis on a 2%