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CAV3 mutations causing exercise intolerance, myalgia and rhabdomyolysis: Expanding the phenotypic spectrum of caveolinopathies

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Abstract

Rhabdomyolysis is often due to a combination of environmental trigger(s) and genetic predisposition; however, the underlying genetic cause remains elusive in many cases. Mutations in *CAV3* lead to various neuromuscular phenotypes with partial overlap, including limb girdle muscular dystrophy type 1C (LGMD1C), rippling muscle disease, distal myopathy and isolated hyperCKemia. Here we present a series of eight patients from seven families presenting with exercise intolerance and rhabdomyolysis caused by mutations in *CAV3* diagnosed by next generation sequencing (NGS) (n = 6). Symptoms included myalgia (n = 7), exercise intolerance (n = 7) and episodes of rhabdomyolysis (n = 2). Percussion-induced rapid muscle contractions (PIRCs) were seen in five out of six patients examined. A previously reported heterozygous mutation in *CAV3* (p.T78M) and three novel variants (p.V14I, p.F41S, p.F54V) were identified. Caveolin-3 immunolabeling in muscle was normal in 3/4 patients; however, immunoblotting showed more than 50% reduction of caveolin-3 in five patients compared with controls. This case series demonstrates that exercise intolerance, myalgia and rhabdomyolysis may be caused by *CAV3* mutations and broadens the phenotypic spectrum of caveolinopathies. In our series, immunoblotting was a more sensitive method to detect reduced caveolin-3 levels than immunohistochemistry in skeletal muscle. Patients presenting with muscle pain, exercise intolerance and rhabdomyolysis should be routinely tested for PIRCs as this may be an important clinical clue for caveolinopathies, even in the absence of other "typical" features. The use of NGS may expand current knowledge concerning inherited diseases, and unexpected/atypical phenotypes may be attributed to well-known human disease genes.

Keywords: *CAV3*; Rhabdomyolysis; Myoglobinuria; Caveolinopathy; Exercise Intolerance; Myalgia

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1. Introduction

Acute rhabdomyolysis (RM) is a serious event often requiring critical care management. Precipitating causes include a range of environmental trigger(s) with and without a known genetic predisposition [1]. In many cases no cause is found. Here we report eight patients who on next-generation sequencing (NGS) were found to carry four heterozygous missense *CAV3* mutations after extensive earlier investigations had been negative. Our findings expand the *CAV3*-related phenotypical spectrum, so far comprising limb girdle muscular dystrophy type 1C (LGMD1C), rippling muscle disease, distal myopathy, isolated hyperCKemia and familial hypertrophic cardiomyopathy [2].

2. Materials and methods

Eight patients presenting with exercise intolerance, myalgia and/or recurrent RM who remained genetically unresolved despite extensive previous investigations are reported. Six patients (patients 1–5 and 8) were identified from a larger cohort of 225 patients with exercise intolerance, myalgia and/or recurrent RM. DNA from Patient 6 (the father of Patient 5) was assessed following a genetic diagnosis in his son. Patient 7 was genetically investigated following muscle biopsy analysis. Approval was obtained from the regional ethics committee, and informed consent was obtained from all subjects for genetic studies. Medical notes were reviewed retrospectively and patients were reassessed following the genetic diagnosis, except patients 1, 3 and 4 who failed to attend follow up visits, and Patient 6, who is deceased. Clinical findings are summarised in Table 1, including: age of onset, age at assessment, presenting symptom, recurrent RM and its triggers, reported rippling muscle contraction, percussion-induced rapid muscle contractions (PIRCs) assessed during physical exam (by a reflex hammer (percussion of a muscle)), muscle pain, muscle weakness assessed during physical exam, reported exercise intolerance (defined as pain and/or a cramp-like sensation during exercise), fatigue and baseline serum creatine kinase (CK) levels. Histopathological studies were performed as described in the Supplementary Material. Polyacrylamide gel electrophoresis and western blotting were performed as previously described [3]. Blots were incubated with 43DAG/8D5 (β-dystroglycan, Leica Biosystems, NCL-b-DG, dil. 1/350) and caveolin-3 (BD Biosciences BD610421, dil. 1/350). Myosin heavy chain staining with Coomassie blue on the post-blotted gel was used as a control for protein loading and quality of the transfer. Bands were visualised with SuperSignal West Pico Chemiluminescent Substrate detection (Life Technology) using AlphaInnotech FluorChemR Q platform and AlphaViewR software v3.0. Densitometric analysis was undertaken using ImageJ v1.47 software with data normalised to the density of the myosin heavy chain band on the Coomassie blue stained post-blotted gel and expressed as a percentage of the control sample.

DNA from six patients was sequenced by a NGS Illumina 'Trusight One' enrichment panel; designed to screen for 60 relevant genes, previously associated or putatively linked with RM (for review [1]). Mutations identified on NGS were confirmed by Sanger sequencing. Patient 7 was evaluated by bi-directional sequence analysis for mutations in *CAV3* and Patient 8 by whole exome sequencing (SOLiD™). Whole exome sequencing was performed as outlined previously [4]. Three μg of DNA was fragmented by sonication and ligated to SOLiD™ system sequencing adaptors. The resulting library was enriched for exomic sequences using the SeqCap EZ Human Exome Library v2.0 exome capture system (Nimblegen, Roche Diagnostics) and sequenced using a 5500XL Genetic Analyser (Life Technologies). After sequencing and alignment, average coverage was 56-fold with 73% of the exome covered to 20-fold or greater. Variant calling was performed using LifeScope™ 2.5 (Life Technologies) and the resulting variants were filtered using ANNOVAR. The *CAV3* mutations were confirmation by bi-directional Sanger sequencing. Mutations were described using the single letter nomenclature to describe non-synonymous variants.

3. Results

The clinical history from each patient is outlined below and key findings are summarised in Table 1. Patient 1 presented with fatigue, muscle pain, and recurrent episodes of myoglobinuria (highest CK: 28,000 IU/L) without apparent trigger. Inflammatory markers (CRP and ESR), HIV testing and auto-antibodies for auto-immune myositis were negative. Plasma acylcarnitine profile, urine organic acids, fatty acid oxidation flux and CPT2 activity in skin fibroblasts were all normal. Patient 2 presented with a longstanding history of muscle pain and tenderness exacerbated by mild physical activities and exercise that interfered with normal daily activities. Examination was unremarkable except for muscle pain evoked by muscle palpation. Routine biochemistry was normal, apart from raised CK. Inflammatory markers (CRP and ESR) and autoantibodies including ANA, GAD, Anti-DNA, Rheumatoid Factor, Anti-Hu, Anti-Yo, Anti-Ri, were negative. Patient 3 had exercise intolerance throughout adult life. RM occurred at age 37 following a few hours of moderate intensity swimming. At the time he was also taking antibiotics for an infection. Severe pain and acute muscle weakness were accompanied by myoglobinuria. A second episode was associated with exercise (swimming) in conjunction with fever. A third episode occurred spontaneously with no apparent precipitant. Patient 4 presented with exercise-related muscle cramps and stiffness since childhood. Examination was unremarkable. He had hypoglycaemic seizures in the neonatal period. Genetic testing for *GLUT1*, *HADH* and *LPIN1* were normal. Patient 5 had muscle symptoms from childhood. He had mild muscle weakness, and could not perform endurance activities. Paroxysmal weakness lasting 2–3 hours occurred after strenuous exercise. Post exercise muscle pain was also a feature. Hypertrophic cardiomyopathy was diagnosed in his 40s during a routine health check. Extensive genetic investigations were negative (including full mutation screening of *VCP*, *DES*, *MYOT*, *CRYAB*, *ZASP*, *TTN*, targeted sequencing of *POLG1* and *PEO1*, plus testing for large scale rearrangements and full sequencing of muscle-extracted mtDNA). Muscle biopsy slides were not available for review. Patient 6, the father of Patient 5,