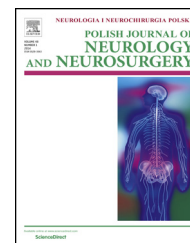


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Case report

CAV3 mutation in a patient with transient hyperCKemia and myalgia



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ABSTRACT

Mutations in caveolin-3 (CAV3) can lead to different clinical phenotypes affecting skeletal or cardiac muscles. Here, we describe a patient with Klinefelter syndrome, ulcerative colitis and Sjögren syndrome, who developed transient hyperCKemia, myalgia and mild muscular weakness. Using whole exome sequencing (WES), a missense mutation G169A was found in the CAV3 gene. In addition, we identified a homozygous frameshift deletion in MS4A12 that may contribute to inflammatory bowel disease, further demonstrating usefulness of WES in dual molecular diagnoses.

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1. Introduction

Caveolin-3 is a muscle-specific membrane protein. It is a principal component of caveolae, the specialized lipid rafts of the sarcolemmal membrane [1]. Mutations in the caveolin 3 gene (CAV3) lead to several different clinical phenotypes:

autosomal dominant limb-girdle muscular dystrophy type 1C (LGMD1C) [2], rippling muscle disease [3], distal myopathy [4], isolated hyperCKemia [5] and cardiomyopathy [6]; other phenotypes e.g., long QT syndrome have been also reported [7]. Patients may show an overlap of these symptoms and in some cases the same mutation can lead to different phenotypes [8–10,17]. Such abundance of phenotypes suggests

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a modifying role of other genes or environmental factors. Here, we present a case of a patient with a previously described mutation in the *CAV3* gene, accompanied by another genetic condition (Klinefelter syndrome) and autoimmune diseases, either of which could modify the phenotype of myopathy.

2. Case report

2.1. Patient's history

The patient was a 48-year-old male born of non-consanguineous parents. His medical history included gonadal dysgenesis, which led to diagnosis of Klinefelter syndrome made at the age of 31 on the basis of testicle biopsy, increased LH and FSH levels, and decreased testosterone, and confirmed later by karyotype analysis. The patient suffered also from ulcerative colitis, with onset at the age of 46 years. Patient's deceased mother had been diagnosed with extrapyramidal syndrome (no detailed medical documentation was available); none of the other family members had any neuromuscular symptoms.

First muscular symptoms started acutely at the age of 46 years in form of myalgia during an upper respiratory tract infection. Other symptoms during the infection included shortness of breath and macular rash on the cheeks and upper torso. At that time, an increased creatine kinase (CK) level of 1910 U/L was noted, as well as increased hs-troponin and the NT-proBNP values. Inflammatory biomarkers were also elevated: ESR 67 mm/h and C-reactive protein 30.4 ng/ml (reference range <10). Antinuclear antibodies were present in 1:2560 titre, and anti-Ro and anti-La antibodies were detected. Echocardiography did not reveal abnormalities of the heart, the ejection fraction was 60%. Electromyography was generally concluded as showing signs of generalized sensory-motor polyneuropathy and myopathic changes in tibialis anterior and fist dorsal interosseous muscle (original recordings were not available to us). On the basis of the clinical findings and laboratory test results, the patient was diagnosed with

polymyositis and received intravenous methylprednisolone followed by oral prednisone 40 mg/d, with slow tapering to a dose of 5 mg/over 16 months. After 2 weeks of treatment, normalization of CK levels was observed. 3 months later, the patient was hospitalized again. Creatine kinase level was normal, rheumatoid factor was present (296 IU/ml, reference range <34 IU/ml), and antinuclear (1:1280), anti-Ro (+++), and anti-Ku (+) antibodies were positive. ESR was 24 mm. Ophthalmologic Schirmer test was positive. A muscle biopsy was fixed in formaldehyde and only poor quality paraffin sections were available, which revealed no signs of myositis or vasculitis. Sparse atrophic muscle fibers with multiple nuclei and single hypertrophic fibers were found (results not shown). Electroneurography showed normal conduction velocities and electromyography was normal. MR of the lumbosacral spine demonstrated L4/L5 and L5/S1 disc bulging with mild intervertebral foramen restriction. The patient was diagnosed with Sjögren syndrome. Additionally, osteopenia, and vitamin D deficiency were found.

Because of non-specific neuromuscular symptoms and sporadic occurrence, WES analysis was performed to establish possible genetic background. The results of the genetic analysis (described below), pointed to a *CAV3* mutation as the cause of the myopathy. To evaluate the patient's phenotype, he was admitted to our neurology department. The patient's major complaints at that time were myalgias and generalized weakness; he also reported problems with climbing stairs and getting up from a sitting position.

2.2. Clinical examination

On admission into our department (16 months after first symptoms), the patient was alert and fully oriented in the place and time. Scoliosis and varus deformity of the knees and elbows were noticeable (Fig. 1). Mild atrophy of the calf muscles was present. Signs of postthrombotic syndrome were visible in the lower legs. On neurological examination, the cranial nerves function was normal. Muscle strength in the

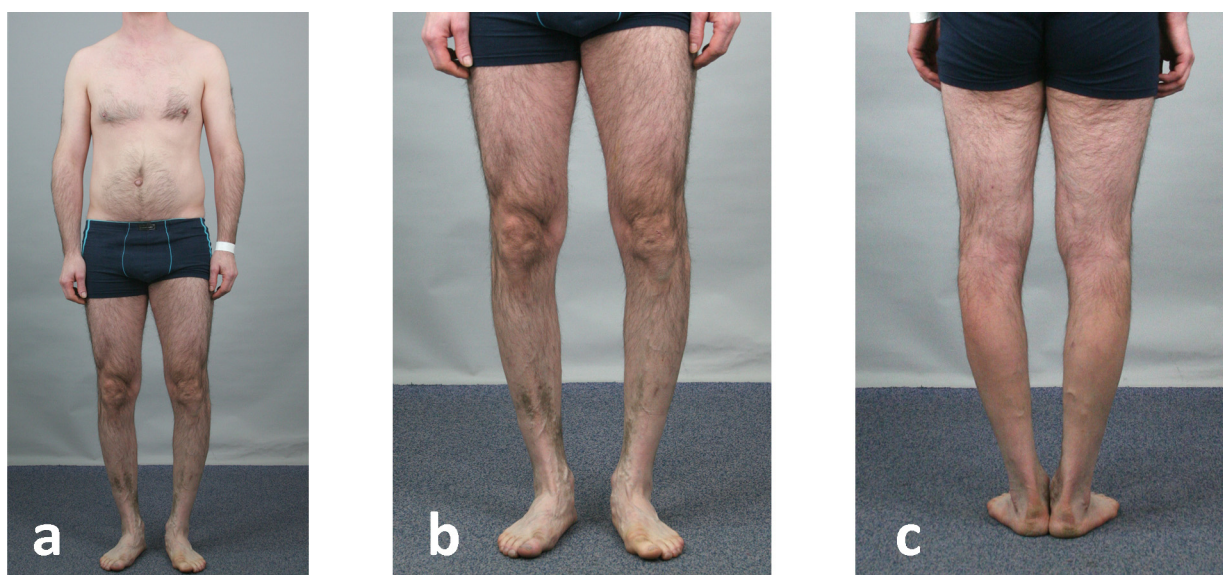


Fig. 1 – The patient's appearance (a). The elbows and knees show varus deformity. Note mild calf muscle atrophy (b and c).

upper and lower limbs was mildly decreased proximally (4/5 in Lovett scale), and more prominent weakness of the left lower limb was present, with degree difficult to assess due to pain of the left knee. There was also mild global sensory impairment in the left lower limb. Deep tendon reflexes in the upper limb were normal, while knee and ankle reflexes were markedly decreased. No pathological pyramidal signs were present.

2.3. Laboratory tests and diagnostic procedures

CK, blood count, urine, electrolytes, renal and hepatic parameters, lipid profile, thyroid hormones, vitamin B12, and folic acid level were all in the normal range. Troponin I and NT-proBNP levels were not elevated. Laboratory test abnormalities included slightly elevated ESR (15 mm/h), increased follicle-stimulating and luteinizing hormone levels (respectively: 24.81 mIU/ml, normal range 1.5–12.4 and 10.72 mIU/ml, normal range 1.7–8.6) and increased level of gamma-globulins.

Electroneurography showed normal conduction parameters in the peripheral nerves. Electromyographic parameters of proximal and distal muscles of the limbs were normal. Degenerative changes of the left hip joint were found in the radiogram, while the radiogram of the left knee was normal. Brain CT scan demonstrated no abnormalities. Ophthalmologic examination with fundoscopy confirmed dry eye syndrome and revealed a small retinal lesion of nondescript character. Spirometry showed normal respiratory parameters. MR of the thigh muscles showed no abnormalities (Fig. 2).

2.4. Molecular analyses

Patient's genomic DNA extracted from a peripheral blood was analyzed using whole exome sequencing (WES) at Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC) through the Baylor Hopkins Center for Mendelian Genomics as previously described [11–13].

From the initial set of all variants, we filtered out synonymous single nucleotide variants (SNVs) and variants with minor allele frequency > 1% in 1000 Genomes [14], Exome Variant Server [15], and in-house exome databases [16], which left 705 potentially deleterious mutations (Supp Table 1). A query for previously reported pathogenic variants revealed nine variants annotated in Human Gene Mutation Database (HGMD) as disease causing mutations (DMs) and three variants

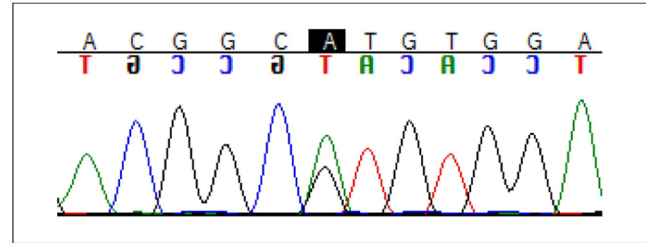


Fig. 3 – DNA sequencing chromatogram of exon 2 of the CAV-3 gene showing a heterozygous missense mutation c. G169A in the proband.

annotated as likely DMs. We found that all but one of these variants was observed in at least one of the above-mentioned reference databases and thus they are less likely to contribute to the patient's phenotype. The single HGMD-DM variant not reported in external and internal exome datasets was a missense mutation c.G169A; p.V57M in exon 2 of CAV3 (NM_001234; chr3:8787266 G > C [hg19]). The predictions from MutationTaster, RadialSVM, Polyphen2, LRT, and PhyloP consistently classified this variant as conserved and deleterious. Sanger sequencing confirmed the mutation (Fig. 3) and showed that it was absent in the patient's father and brother, and half-sister. Unfortunately, the proband's mother is deceased and no DNA sample is available for testing.

Besides the HGMD mutations, we investigated other potentially pathogenic variants, including 68 novel, 113 putative compound heterozygous, and 54 homozygous variants (Supp. Table 1). Among them, we identified an additional variant that may contribute to the patient phenotype, i.e. the homozygous frameshift deletion in MS4A12 (Membrane-spanning 4-domains subfamily A member 12) (NM_001164470; chr11:60264977:CA > C). The allele frequency of this variant in control databases vary between 0.22% and 0.36%.

2.5. Incidental confirmation of Klinefelter syndrome

We performed the analysis of B-allele frequency data obtained from WES to identify the regions with Absence of Heterozygosity (AOH) which could potentially reveal the presence of larger copy-number variants (CNVs) or suggest that parents

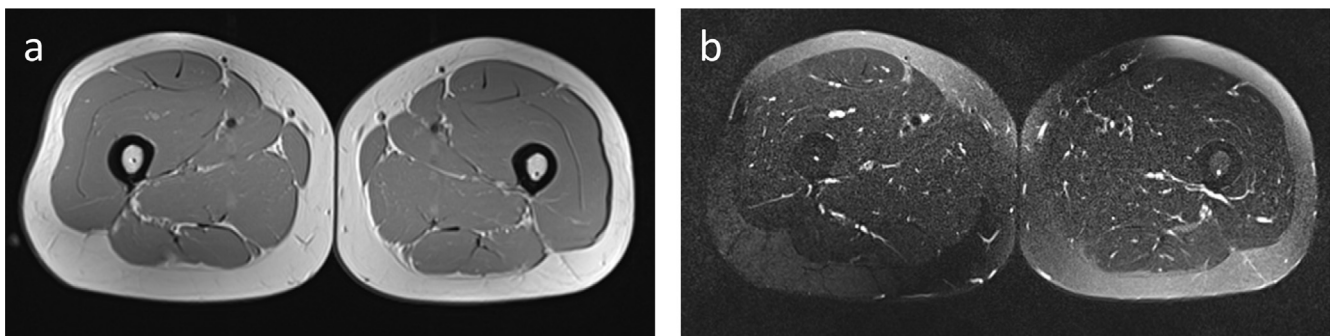


Fig. 2 – T1-weighted (a) and spectral fat-suppressed T2-weighted (b) transverse MR image of the thighs of the patient. The signal from thigh muscles is normal in both sequences, without signs of fatty degeneration or inflammatory changes.

are consanguineous. Although no larger AOH was detected, we noticed the excess of heterozygous SNPs on X chromosome in this male patient. Analysis of 100 WES cases (47 males and 53 females) randomly selected from BH-CMG cohort revealed that the fraction of heterozygous SNPs on X chromosome vary from 0.13 to 0.22 (mean = 0.17) in males and between 0.41 and 0.63 (mean = 0.56) in females. In our patient, we found that the percentage of heterozygous SNPs on X chromosome was 0.59, indicating the presence of two X chromosomes, and further confirming the previous cytogenetic diagnosis of 46,XXY (Klinefelter syndrome) in this patient.

3. Discussion

CAV3 mutations can lead to different phenotypes: limb-girdle muscular dystrophy type 1C (LGMD1C), rippling muscle disease, distal myopathy, isolated hyperCKemia, and cardiomyopathy. Moreover, the same mutation can cause different phenotypes; variable as well as overlapping phenotypes have been observed within families [8–10,17].

The described patient presents several symptoms found in caveolinopathies, constellation of which cannot be classified as one of the classic caveolinopathy phenotypes. Myalgia, muscle weakness, and high CK levels are frequent symptoms in patients with caveolinopathies. Despite the mild proximal muscle weakness, however, the course of the disease in our patient is different from LGMD1C, which is characterized by an earlier onset (varying from 5 years to adulthood), mild-to-moderate proximal muscle weakness, calf hypertrophy, and progression of symptoms [18]. Our patient, in contrast to most cases of LGMD1C, presented with slight atrophy of the calves, which was more suggestive of distal myopathy [19]. Myalgia, which was our patient's main complaint, is characteristic both for LGMD1C and rippling muscle disease, however, we did not find clinical signs of rippling muscle disease in this case. Interestingly, most patients with caveolinopathy, with the exception of familial hypertrophic cardiomyopathy, have persistently increased level of CK [9,20], whereas in our patient hyper-CK-mia was transient.

Muscle biopsy of the presented patient showed very mild myopathic changes (not shown), consistent with other literature reports of muscle biopsy in caveolinopathies [20]. In contrast, MR imaging demonstrated normal muscle tissue (Fig. 3), while in both cases of rippling muscle disease and LGMD caused by the CAV3 mutations, MR of the muscles demonstrated fatty infiltration and atrophy of muscles of the thigh [10,21,22].

The G169A mutation found in the described case had been previously reported in families with asymptomatic hyperCKemia [23], autosomal dominant RMD [24] as well as in one sporadic case of myotonia [25]. In contrast to those observations, our patient had clinical symptoms of myopathy, but experienced only transient, infection-related, increased CK levels; no signs of rippling muscles or myotonia were present either clinically or on EMG studies. This is an interesting phenomenon of the same mutation leading to different phenotype that could be explained by the modifying effects of other genes.

The patient's Klinefelter syndrome could be a factor contributing to neuromuscular symptoms, as muscle weakness is often observed in Klinefelter syndrome [26]. Therefore, the described case can constitute an example of genetic “double trouble” phenomenon. Recent genomic approaches to disease, such as WES of a large cohort of subjects have revealed that two or more pathogenic variants can be found in as many as 5–7% of patients when compared with unrelated control individuals [27]. These studies also facilitated dual molecular diagnoses of “blended Mendelian phenotypes”, which should be taken into account in atypical presentation of neuromuscular disorders, and have been described also in caveolinopathy [28].

Moreover, the patient's muscular symptoms could be attributed to the accompanying acquired diseases as well as to the primary muscle disease. Increased troponin and NT-proBNP levels accompanying transient hyper-CKemia could suggest infective myocarditis despite the normal echocardiography result. On the other hand, infection is often a precipitating factor of rhabdomyolysis in patients with hereditary myopathy. Our patient also has Sjögren syndrome, in which diffuse myalgia and muscle weakness are commonly found [29]. Of note, association of Klinefelter syndrome and autoimmune disease, especially female-biased ones like systemic lupus erythematosus or Sjögren syndrome, has been described in several reports [30,31]. Asymmetrical left lower limb weakness with accompanying pain could be also explained by degenerative hip joint disease, however, asymmetry has been found in caveolinopathies [8,19]. Therefore, the patient's muscular symptoms could be a composite result of several coexisting conditions. However, the fact that the CAV3 mutation was not found in the patient's relatives potentially implicates its pathogenic role in the described case.

Detection of Klinefelter syndrome by WES can be of practical importance in the future, as this relatively common chromosomal disorder is currently grossly underdiagnosed [32].

Notably, WES revealed also a homozygous frameshift deletion in MS4A12 that may contribute to another part of patient's phenotype, inflammatory bowel disease. MS4A12 is a cell surface protein found primarily in the apical membrane of colonocytes and it was found that silencing of this gene in colon cancer inhibits the proliferation, cell motility and chemotactic invasion of cells [33]. Strict restriction of expression to colonocytes as well as involvement of MS4A12 in modulation of EGFR signaling indicate an important role of this protein in control of epithelial cell properties, such as growth, healing, adhesion and cell migration. The involvement of MS4A12 in inflammatory bowel disease has not been studied, however, we hypothesize that homozygous loss of function variant in MS4A12 gene could be at least a modifying factor in this condition.

4. Conclusion

The CAV3 mutation phenotype spectrum can be extended to transient hyperCKemia with mild muscular weakness and myalgia, without overt dystrophic features in the muscle biopsy and MR imaging. Co-existing Klinefelter syndrome as

well as accompanying autoimmune disorders can be the phenotype-modifying factors in the described case. Hereditary myopathy should be taken into consideration even in the presence of other – genetic and acquired – diseases which could account for muscular symptoms.

Conflict of Interest

None declared.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pjnns.2016.06.008.

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