RESEARCH ARTICLE

CACNA1S Variant Associated With a Myalgic Myopathy Phenotype

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Abstract

Background and Objectives

This study aimed to characterize the phenotype of a novel myalgic myopathy encountered in a Finnish family.

Methods

Four symptomatic and 3 asymptomatic individuals from 2 generations underwent clinical, neurophysiologic, imaging, and muscle biopsy examinations. Targeted sequencing of all known myopathy genes was performed.

Results

A very rare *CACNA1S* gene variant c.2893G>C (p.E965Q) was identified in the family. The symptomatic patients presented with exercise-induced myalgia, cramping, muscle stiffness, and fatigue and eventually developed muscle weakness. Examinations revealed mild ptosis and unusual muscle hypertrophy in the upper limbs. In the most advanced disease stage, muscle weakness and muscle atrophy of the limbs were evident. In some patients, muscle biopsy showed mild myopathic findings and creatine kinase levels were slightly elevated.

Discussion

Myalgia is a very common symptom affecting quality of life. Widespread myalgia may be confused with other myalgic syndromes such as fibromyalgia. In this study, we show that variants in *CACNA1S* gene may be one cause of severe exercise-induced myalgia.

Introduction

CACNA1S gene codes the $Ca_v1.1$ subunit of the dihydropyridine receptor (DHPR). DHPR couples depolarization of the muscle fiber membrane to muscle contraction by inducing ryanodine receptor to open for calcium influx during excitation-contraction coupling.[1] Pathogenic variants of genes encoding ion channels may result in skeletal muscle channelopathies by causing changes in cell membrane excitability and muscle contraction.[2] Specific gene variants encoding calcium channels, either type 1 ryanodine receptor (RyR1) or subunit $Ca_v1.1$ of the DHPR, have been associated with several myopathic conditions such as congenital myopathy,[3,4] periodic paralysis,[5,6] risk of statin-associated muscle symptoms,[7] susceptibility to malignant hyperthermia,[7-9] and exertional heat illness.[7,10] Pathogenic variants of *RYR1* are also a common cause of congenital myopathies. These include central core disease, multiminicore disease, centronuclear myopathy, congenital fiber type disproportion,[11] and core-rod myopathy.[3] Specific *RYR1* variants have been associated with axial myopathy[12,13] and benign calf distal myopathy.[14-16] Pathogenic variants of *CACNA1S* are mostly known to cause hypokalemic periodic paralysis (hypoPP).[17] Recently, a *CACNA1S* variant was reported to cause exertional heat stroke and rhabdomyolysis.[18]

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Glossary

ADM = abductor digiti minimi; **CMAP** = compound muscle action potential; **DHPR** = dihydropyridine receptor; **EDB** = extensor digitorum brevis; **hypoPP** = hypokalemic periodic paralysis; **NADH** = nicotinamide adenine dehydrogenase; **RyR1** = type 1 ryanodine receptor.

Some pathogenic *CACNA1S* variants have been associated with congenital myopathy with hypotonia, delayed motor development, and progressive muscle weakness.[4]

Musculoskeletal pain is a common complaint in the adult population.[19] When widespread and chronic, it can be diagnosed as fibromyalgia. Fibromyalgia shows a worldwide estimated average prevalence of 2.7%, with a greater prevalence of 4.2% in women[20] than in men. Several myopathies can also account for pain. Many patients with chloride channel myotonia or sodium channel myotonia experience myotonia and stiffness as painful.[21,22] One specific *SCN4A* variant (p.A1156T) has been reported to cause a myalgic syndrome with muscle stiffness and cramping without overt myotonia.[23] Myalgia can be the most disabling symptom also in myotonic dystrophy type 2.[24]

We have studied a Finnish family in which symptomatic members had exercise-induced myalgia, stiffness, cramping, muscle weakness, and fatigue. The phenotype included muscle hypertrophy and was similar in all symptomatic patients sharing a rare variant in *CACNA1S*.

Methods

Clinical Examinations

Seven family members from 2 generations of the Finnish family (Figure 1), the proband and his adult relatives, were examined. Their medical records and family history were reviewed. Clinical neuromuscular examination included detailed manual muscle strength assessment and myotonia testing by muscle percussion, handgrip, and eyelid closure. Features of fibromyalgia were screened for by using a Finnish questionnaire, which is based on previously published criteria for diagnosing fibromyalgia.[25,26]

Neurophysiology Studies

Standard neurography and needle EMG were conducted both in the proximal and distal parts of at least 1 upper and 1 lower limb. The reference values according to age and patient height were used in neurography. Compound muscle action potential (CMAP) exercise testing was performed, and the Fournier protocol was used consisting of short (10–12 seconds) and long (5 minutes) exercise tests.[27,28] Abductor digiti minimi (ADM) and extensor digitorum brevis (EDB) muscles were examined in the short exercise test, and ADM muscle in the long exercise test. Short exercise test was also conducted after cold exposure. ADM muscle was cooled by applying ice bag on the ADM muscle for 7 minutes. The temperature of the skin after cooling is usually 18–20°C. Neurophysiologists add a comment on temperature if it is outside this range. The target temperature was attained in all cooling tests of the study participants. Percentages regarding CMAP exercise test results refer to pre-exercise values.

Muscle Biopsy and Histology

Muscle biopsies were obtained from all examined family members, usually from tibialis anterior muscle. Standard histologic and histochemical stainings[29] were performed including hematoxylin and eosin, modified Gomori trichrome, periodic acid Schiff, nicotinamide adenine dehydrogenase (NADH), combined succinate dehydrogenase–cytochrome oxidase, and myosin heavy-chain double-staining.[30]

Muscle Imaging

Axial MRI sections of the upper and lower limb muscles included T1-weighted and T2-weighted sequences and short tau inversion recovery sequence. MRI of the upper limb covered muscles from the level of deltoid muscle to the level of wrist joint. MRI of the lower limb covered muscles from the level of gluteus maximus to the level of talocrural joint.

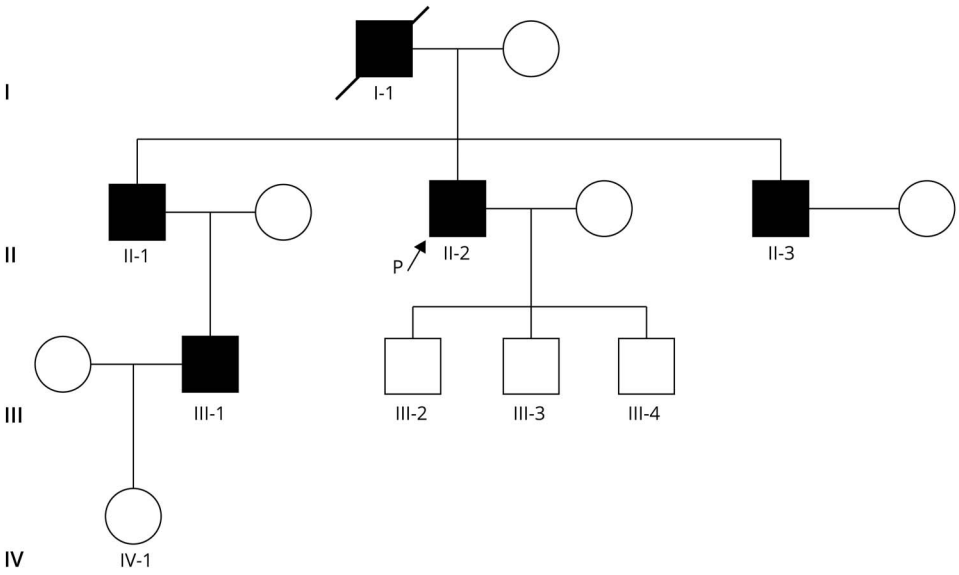
Molecular Genetics

Genomic DNA was isolated from peripheral blood samples by using standard methods. Genetic analysis was performed by using targeted next-generation sequencing with MYOcap gene panel.[31]

Western Blotting

Membrane fractions were isolated from snap-frozen muscle biopsies by using the ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem; Merck KGaA, Darmstadt, Germany), and the samples for Western blotting were prepared with a membrane protein compatible method, as described earlier.[32] SDS-PAGE and Western blotting were performed by using standard methods. Immunodetection: polyvinylidene difluoride filters were incubated in primary antibody solution overnight at +8°C and in secondary antibody 1 hour, at room temperature, with gentle agitation. Enhanced chemiluminescence detection was performed with ChemiDoc Reader and ImageLab software (Bio-Rad Laboratories, Hercules, CA). SERCA1 and SERCA2 were used as loading controls. The primary antibodies used (all mouse monoclonal antibodies) were CACNA1S/DHPR clone A1 (ab2862; Abcam, Cambridge, United Kingdom), SERCA1 clone VE121G9 (Research Diagnostics Inc., Flanders, NJ), and SERCA2 clone IID8 (ab2817; Abcam).

Figure 1 Pedigree of the Family



In addition to 4 living symptomatic patients, 3 sons of II:2 were examined. The deceased father I:1 had complained of symptoms similar to those of his sons but was never neurologically examined. There were no other family members than those in pedigree.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the Research Ethics Committee of the Northern Savo Hospital District (733/2019) and performed according to the Declaration of Helsinki. Written informed consent from all participants was obtained. Authorization was obtained for disclosure of photographs. The study was conducted together with Tampere Neuromuscular Center in Finland.

Data Availability

Data supporting the findings of this study are available from the corresponding author on reasonable request.

Results

Clinical Findings

Four family members had similar muscle symptoms with an age at onset ranging from 14 to 51 years. First symptoms were muscle fatigue and burning muscle aching in the limbs lasting for days after physical exercise. General fatigue was also an early symptom. After a few years, muscle stiffness and cramping began, both provoked by physical exertion. Myalgia became constant, and eventually, just normal walking was enough to aggravate myalgia. Symptoms fluctuated also without preceding exercise. Finally, the 3 older patients developed fixed muscle weakness after several years or up to 2 decades after symptom onset.

The disease progressed slowly, eventually leading to incapacity for work and causing disability. Aged patients required cane to assist walking. II:1 and II:2 also used a peroneal or an ankle brace. Except for III:1, the symptomatic patients had difficulty in climbing stairs, getting out of cars or up from chairs, lifting plates to shelves, and washing their hair. Opening jars and walking on uneven terrain were also challenging. Two patients complained of dysphagia, but formal swallowing tests had not been conducted.

At the initial stages, clinical examination did not reveal anything remarkable. Later, very mild ptosis, muscle hypertrophy in the upper limbs, and decreased muscle strength became evident over the years. Muscle weakness was more pronounced in the lower than in the upper limbs, and II:1 developed mild muscle atrophy. II:1 and II:2 reported allodynia and dysesthesia on sensory examination. The used questionnaire suggested fibromyalgia in II:1, II:2, and II:3. Creatine kinase levels were normal in others but slightly elevated in patient II:3. Clinical details are summarized in Tables 1 and 2. Clinical examination of the younger asymptomatic individuals III:2, III:3, and III:4 was unremarkable at ages 28–31 years.

Neurophysiologic Studies

EMG did not show myopathic or myotonic findings. Mild neurogenic findings suggestive of lumbar radiculopathy were obtained from II:1, II:2, and II:3. They all had a history of unilateral lower limb radiculopathy, were diagnosed with disk protrusion or disc rupture by lumbar MRI, and had undergone spine surgery. In III:2, the compound sensory nerve action potentials had low amplitudes in the lower limbs with preserved conduction velocity. CMAPs of the motor nerves in the lower limbs were normal, but the minimum latency of the F-wave in peroneal nerve on the left was prolonged, suggestive of very mild polyneuropathy in the lower limbs.

The short exercise tests with ADM and EDB muscles were unremarkable, the latter yielding slightly abnormal but

Table 1 Clinical Data of the Participants

Family member	II:1	II:2	II:3	III:1	III:2	III:3	III:4
Sex/age, y	M/66	M/61	M/57	M/33	M/30	M/28	M/31
Age at onset, y	14	30	51	27	—	—	—
Symptoms							
Exercise-induced myalgia	Yes, severe	Yes, severe	Yes, moderate	Yes, moderate	—	—	—
Myalgia at rest	Yes, severe	Yes, severe	Yes, mild	Yes, mild	—	—	—
Muscle stiffness	Yes	Yes	Yes	Yes	—	—	—
Muscle cramping	Yes	Yes	Yes	Yes	—	—	—
Proximal muscle weakness	Yes	Yes	Yes	Yes	—	—	—
Dysphagia	Yes	Yes	—	—	—	—	—
Fatigue	Yes	Yes	Yes	Yes	—	—	—
Clinical findings							
Ptosis	Yes	Yes	Yes	Yes	—	—	—
Weak eye closure	Yes	Yes	Yes	—	—	—	—
Facial weakness	Yes	—	—	—	—	—	—
Muscle atrophy	Shoulders, lower limbs	—	—	—	—	—	—
Upper limb muscle hypertrophy	Yes	Yes	Yes	Yes	—	—	—
Muscle weakness	Yes	Yes, LL > UL	Yes, LL > UL	—	—	—	—
Allodynia and dysesthesia	Yes	Yes	—	—	—	—	—
Questionnaire suggesting fibromyalgia	Yes	Yes	Yes	—	—	—	—

Abbreviations: LL = lower limbs; M = male; UL = upper limbs.

inconsistent results in 3 patients. In the long exercise test (Figure 2), immediate increase after exercise was registered in all others but II:2 and III:1. The increase ranged from +15% to +26%, occurring immediately or during the first 5 minutes after exercise. The overall mean for the greatest CMAP value observed was +16%, and SD was 9%. Unlike others, III:1 first showed immediate decrease (−26%), followed by early increase, which resulted in +19% increase above the pre-exercise value. Common for all, however, was the gradual decrease in amplitude starting after 3–5 minutes after exercise. The decrease in CMAP amplitude was greater than 40% from the maximum CMAP after exercise and greater than 20% compared with pre-exercise values in 3 cases. The amplitude, however, still continued to decrease when the last readings were obtained in 5 patients. The decrease ranged from −10% to −27%. The overall mean for smallest value was −16% with a SD of 10%. The short exercise test at cold yielded inconsistent results in 4 patients.

Muscle Histology

Biopsies from II:2 and III:1 showed myopathic findings (Figure 3), increased internalized nuclei, marked fiber size variation, and atrophic fibers. NADH staining showed mild irregularity of oxidative enzyme activity in both fiber types. Biopsy from II:3 showed fiber type grouping in addition to increased internal nuclei. His only biopsy was taken from left tibialis anterior muscle. His EMG, however, showed prolonged duration of motor unit potentials in the same muscle, which means that the histologic findings are probably at least partly caused by concomitant nerve root injury. The biopsy also showed some hybrid muscle fibers expressing more than 1 myosin heavy-chain isoform.[30] Biopsies of III:2 and III:3 had a few internalized nuclei with slightly larger type IIA muscle fibers compared with slow type I fibers.

Muscle Imaging

Muscle imaging showed no apparent muscle atrophy or hypertrophy in the muscles of the limbs. Only in patient II:2, MRI showed slightly increased generalized fatty degenerative changes (Figure 4).

Western Blotting

Western blotting analysis showed some variation in DHPR protein expression, which was not significantly different when compared with that in controls (not shown). Minor reduction of the total protein could not be excluded.

Table 2 Diagnostic Assessments of the Study Participants

Family member	II:1	II:2	II:3	III:1	III:2	III:3	III:4
CK level (U/L)	Normal (114–194)	Normal (49–229)	Normal or slightly elevated (202–413)	Normal (99–125)	Normal (126)	Normal (157)	Normal (107)
ENMG	Ulnar nerve entrapment l.a., mild L5 nerve root injury	Mild L5 nerve root injury	Median nerve entrapment l.a., L4/5 nerve root injury	Normal	Median nerve entrapment, very mild LL polyneuropathy	Normal	Normal
CMAP exercise test	Long: Immediate increase	Normal	Long: Immediate increase	Long: Immediate decrease, then early increase, late decrease	Long: Immediate increase, late decrease, compatible with Fournier pattern IV	Long: Immediate increase, late decrease, compatible with Fournier pattern IV	Long: Immediate increase
Muscle MRI	Normal	Slightly increased degenerative fatty replacement	Normal	Normal	Normal	Normal	Normal
Muscle biopsy	Normal	Myopathic	Muscle atrophy, increased internal nuclei, fiber-type grouping	Myopathic	Normal	Normal	Normal
***CACNA1S* variant**	c.2893G>C (p.E965Q)	c.2893G>C (p.E965Q)	c.2893G>C (p.E965Q)	c.2893G>C (p.E965Q)	c.2893G>C (p.E965Q)	c.2893G>C (p.E965Q)	c.2893G>C (p.E965Q)

Abbreviations: CK = creatine kinase; CMAP = compound muscle action potential; ENMG = electroneuromyography; l.a. = bilateral; LL = lower limbs. Biopsy findings of II:3 were partly explained by nerve root injury. EMG patterns are referring to patterns described by Fournier.[27,28] Normal range of CK for male participants aged 5 days–49 years was 50–400 U/L and for male participants aged older than 49 years, 40–280 U/L.

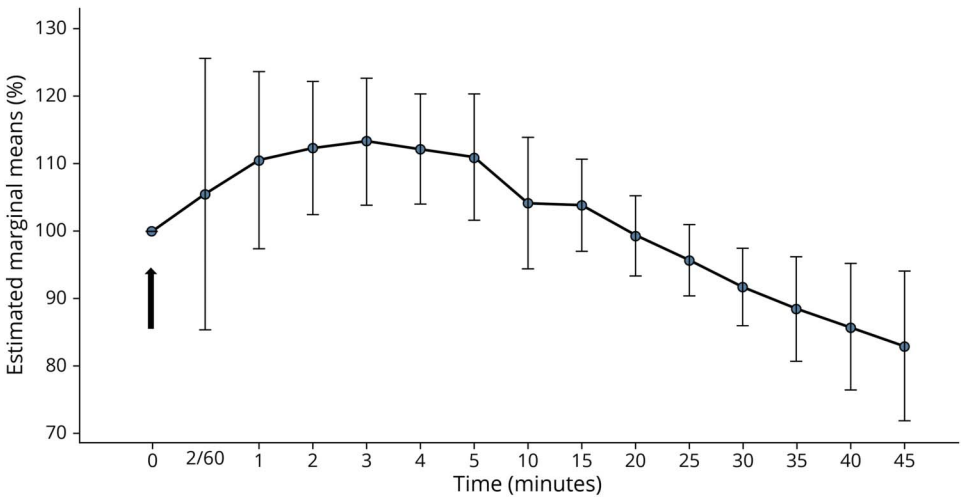
Genetics

All studied individuals carried a rare heterozygous *CACNA1S* variant c.2893G>C (p.E965Q) predicted to be pathogenic. This allele has a worldwide estimated prevalence of 3/100,000 according to Genome Aggregation Database.[33] No other potentially pathogenic variants were identified.

Discussion

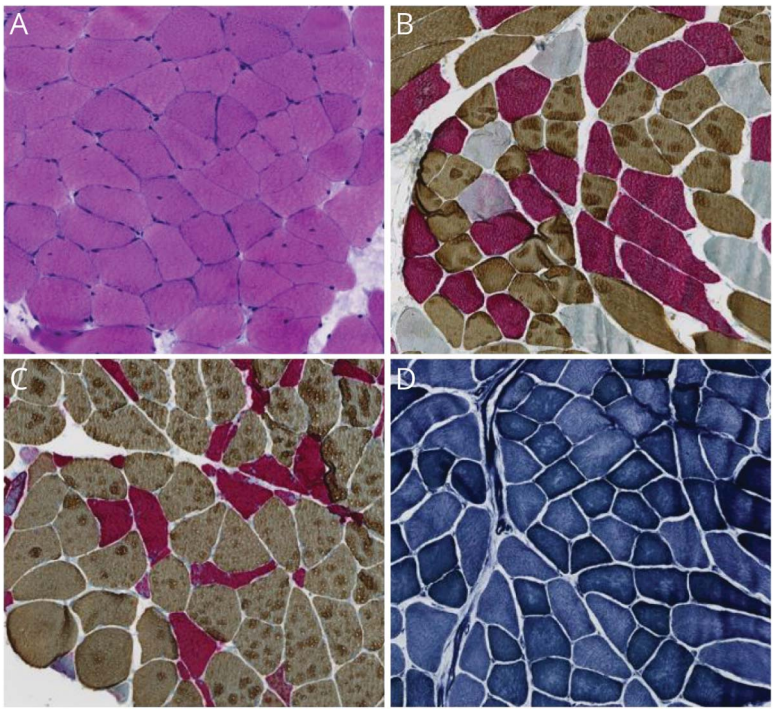
Functionally disabling myalgia is a common health problem in the population and a diagnostic challenge for the clinicians. Molecular genetics identified a rare *CACNA1S* variant c.2893G>C (p.E965Q) in all family members with exercise-induced myalgia, frequently combined with muscle stiffness,

Figure 2 Changes in CMAP Amplitude in ADM Muscle During the Long Exercise Test



Small circles represent the mean of CMAP amplitude compared with each participant's own pre-exercise value. CI is 95%. Arrow indicates exercise beginning after baseline values were obtained. The following time intervals indicate the time that had passed after cessation of 5-minute exercise. The first postexercise value was recorded 2 seconds after cessation of exercise. ADM = abductor digiti minimi; CMAP = compound muscle action potential.

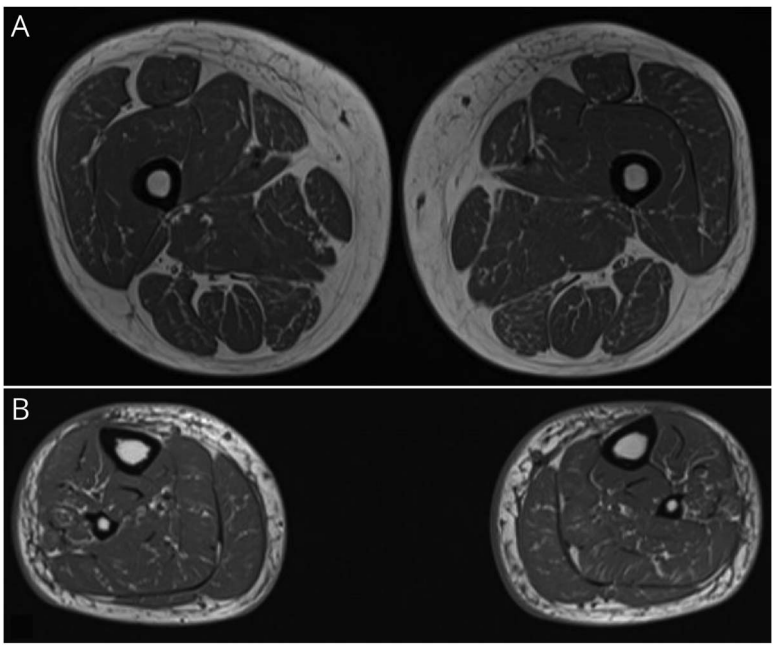
Figure 3 Muscle Biopsy



Muscle biopsy from the right deltoid muscle (cap. med.) from patient III:1. Light microscope images at 400× magnification. (A) Hematoxylin and eosin–stained biopsy section with slightly increased number of internalized nuclei. (B and C) Double myosin immunohistochemistry shows considerable fiber size variation being more pronounced in the fast fibers (red). (D) Mild central "moth-eaten" irregularity of oxidative enzyme activity in both fiber types by nicotinamide adenine dehydrogenase staining.[30]

cramping, fatigue, and, eventually, muscle weakness. In advanced stages, these symptoms resulted in disability and incapacity for work. Three members from the younger generation also carried the variant but have not yet reported any symptoms. However, the age at onset varied greatly ranging from adolescence to later adulthood, and therefore, these still asymptomatic variant carriers are followed up with consecutive studies.

Figure 4 MRI



Transverse T1-weighted MR images of II:2 showing slightly increased generalized and diffuse fatty replacement being more pronounced than would be expected for a person his age in (A) all thigh muscles and (B) lower legs.

Traditional neurophysiologic studies were unremarkable. The short CMAP exercise test was normal, and short exercise with cold provocation produced inconsistent results. In the long exercise test, immediate or early increase was observed in 6 participants and late decrease in 3 participants. Decrease in 4 others was similar but did not exceed 20% compared with pre-exercise values and 40% from the maximum CMAP value, which are considered abnormal.[21,27,34-36] The results of the long exercise test in 2 cases were compatible with the Fournier EMG pattern IV, common for patients with hyperkalemic periodic paralysis and pathogenic sodium channel variant (T704M).[27] This pattern means immediate increase and late decrease in CMAP amplitude in the long exercise test. The *CACNA1S* variant (R528H) results in hypoPP. These patients showed the same late decrease but without the immediate and transient increase in the long exercise test.[27] It is worth noting that not all patients with confirmed skeletal muscle channelopathy display changes in CMAP amplitude during exercise tests. An ion channel variant can also result in different EMG patterns despite the identical variant between patients.[27]

Pathogenic *CACNA1S* variants are known to result in hypoPP, accounting for 60%–80% of the cases.[17,37] Exertional myalgia and muscle cramps have been reported in *RYR1*-related myopathies[38,39] and most interestingly also together with muscle hypertrophy, muscle stiffness, and mild ptosis.[38] A hypothesis, but not experimentally proven, has been proposed that $Ca_v1.1$ variants whose interaction with RyR1 is altered may result in phenotypes similar to the ones produced by pathogenic RyR1 variants.[40] The downstream effects would be similar explaining the clinical overlap. This could explain possible analogies between phenotypes, which result from specific *CACNA1S* or *RYR1* variants. The features of our patients resemble greatly the phenotype previously reported in patients with specific *RYR1* variant[38] and have considerable overlap with fibromyalgia.

CACNA1S variant c.2893G>C (p.E965Q) affects the extracellular P-loop between S5 and S6 segments of the third domain in $Ca_v1.1$ protein.[40] S5 and S6 segments from all the 4 domains form the pore,[1] which is selective for Ca^{2+} ions.[41] P-loops contribute to the selectivity filter.[40,42] In our variant, glutamic acid with electrically charged side chain is replaced by glutamine with polar neutral side chain. At least 2 other variants have been described in which P-loops were affected, and they resulted in congenital myopathy. The first one was a compound heterozygous *CACNA1S* missense variant c.825C>A (p.F275L) and frameshift variant c.2371delC (p.L791Cfs*37).[4] The second one was a dominant c.4099C>G (p.L1367V) variant.[4] Moreover, malignant hyperthermia syndrome has been associated with the c.4060A>T (p.T1354S) variant, which locates in P-loop between the S5 and S6 segments of the fourth domain.[43] However, functional studies of DHPR variant channels are difficult to perform because the channel is located in the transverse tubule.

We describe a *CACNA1S* variant associated with a myalgic myopathy in a large Finnish family. We evaluated it to be likely pathogenic, thus opening the window for abnormal calcium handling as an additional mechanism contributing to the spectrum of myalgic conditions.

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Disclosure

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Anna Vihola, PhD	Tampere Neuromuscular Center, Tampere University Hospital; Folkhälsan Research Center, Helsinki; Medicum, University of Helsinki; Fimlab Laboratories, Tampere, Finland	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Tuomas Rauramaa, MD, PhD	Department of Pathology, Kuopio University Hospital, Finland; Unit of Pathology, Institute of Clinical Medicine, University of Eastern Finland, Kuopio	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data

Continued

Appendix *(continued)*

Name	Location	Contribution
Bjarne Udd, MD, PhD	Tampere Neuromuscular Center; Folkhälsan Research Center, Helsinki; Medicum, University of Helsinki, Finland	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

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