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Research paper

Hypokalemic periodic paralysis associated with the atypical CACNA1S c.2690G>A (p.Arg897Lys) variant: description of 14 affected individuals from five families

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ABSTRACT

This study describes five families (14 individuals) with hypokalemic periodic paralysis carrying a heterozygous pathogenic variant NM_000069.3:c.2690G>A (p.Arg897Lys) in the Calcium Voltage-Gated Channel Subunit Alpha1 S (*CACNA1S*) gene. The clinical exam showed pelvic weakness was common (10/14, with three being too young to exclude this age-dependent myopathy). Electromyography showed myogenic changes, and the long exercise test did not reveal a significant reduction of compound muscle action potential amplitude. Muscle MRI in three patients demonstrated involvement of axial musculature, the pelvic girdle, thighs (with relative sparing of sartorius and gracilis), and legs (especially the gastrocnemius muscles). A homozygosity haplotype analysis in three families revealed a shared segment of approximately 10 million base pairs, suggesting a common ancestor 2-8 generations ago.

1. Introduction

Periodic paralysis is a rare muscular disorder caused by dysfunction of an ion channel in muscle fibers, leading to episodes of painless muscle weakness triggered by physical exertion, stress, or carbohydrate intake [1].

Hypokalemic periodic paralysis (HypoPP) is characterized by weakness episodes associated with low serum potassium levels (<3 mmol/L). The condition affects approximately 1 in 100,000 individuals and is commonly caused by mutations in the Calcium Voltage-Gated Channel Subunit Alpha1 S (*CACNA1S*) gene or in the Sodium Voltage-Gated Channel Alpha Subunit 4 (*SCN4A*) gene. HypoPP is inherited in an autosomal dominant manner, with higher penetrance and attack frequency in males, leading to a male-to-female ratio of 3-4:1 [1,2].

Clinical symptoms usually manifest before the age of 20, with attacks occurring most frequently between ages 15 and 35. The episodes predominantly affect proximal limb muscles, especially the lower limbs, and are associated with hyporeflexia or areflexia and transient

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Table 1
Clinical and demographic characteristics of the patients. *Deceased in 2021 at the age of 86. Pt: Patient. W: Women. M: Men. DL: Dyslipidemia. HTN: Hypertension. AF: Atrial Fibrilation. NA: Not applicable.

Pt	Age	Age at symptom onset	Age at genetic diagnosis	Gender	Comorbidities	HypoPP	Myopathy	Tested treatment
1.II.2	61	50	52	W	DL, recurrent peripheral facial paralysis, actinic keratosis, blepharitis, fifth metacarpal fracture of the left hand (due to a fall), osteoporosis with two dorsal vertebral fractures, and chronic functional constipation.	NO	YES	Acetazolamide.
1.II.3	64	46	56	W	DL, proximal fibula fracture.	NO	YES	NA
1.II.4	54	Childhood	40	M	DL, chronic Achilles tendinitis, sacral insufficiency fracture, actinic keratosis, squamous cell carcinoma.	YES	NO	Oral potassium, spironolactone.
1.III.2	16	15	16	M	NA	YES	NO	Oral potassium.
2.III.5	84	Youth	64	W	Graves' disease, depression, right colon adenocarcinoma.	YES	YES	NA
2.III.6	80	Youth	67	W	HTN, insomnia.	YES	YES	NA
2.III.7	80	Youth	56	W	Lymphoma, breast cancer, and macular degeneration.	YES	YES	Oral potassium.
2.III.8	75	60	66	W	Multinodular goiter, appendectomy due to appendicitis, left knee prosthesis, cataracts.	NO	YES	NA
3.I.1	61	55	61	M	HTN, renal colic, diverticulitis.	NO	YES	NA
4.I.2	86*	42	72	W	HTN, multinodular goiter, osteoporosis.	YES	YES	NA
4.II.1	62	56	58	M	HTN, asthma, paroxysmal AF, left basal ganglia hypertensive hematoma.	NO	YES	NA
4.III.1	30	14	27	M	NA	YES	NO	Oral potassium, spironolactone.
4.III.2	27	17	26	W	Allergic asthma, eating behavior disorder.	YES	NO	Oral potassium.
5.II.2	70	57	62	W	DL, HTN, hypertrophic cardiomyopathy, adjustment disorder, osteoporosis.	NO	YES	NA

elevations in creatine kinase (CK) levels. Between attacks, neurological examination is typically normal, without the myotonia characteristic of hyperkalemic periodic paralysis. However, most patients develop progressive proximal myopathy over time, becoming clinically evident in most cases after the age of 50 [1,3].

Treatment during acute episodes consists of oral or intravenous potassium administration to abort paralysis attacks. Preventive strategies include avoiding vigorous exercise and high-carbohydrate meals, as well as pharmacological interventions such as carbonic anhydrase inhibitors and potassium-sparing diuretics [1]. A retrospective study of 74 patients found that only half responded to acetazolamide, with *CACNA1S* mutation carriers more likely to benefit compared to those with *SCN4A* mutations [4]. No effective treatment exists for late-onset myopathy [1].

The *CACNA1S* gene encodes the alpha-1S subunit of the voltage-gated L-type calcium channel, which is expressed in skeletal muscle plasma membranes. Two prevalent mutations, NM_000069.3: c.1583G>A (p.Arg528His) and NM_000069.3:c.3716G>A (p.Arg1239His), account for approximately 70 % of genetically known HypoPP cases. Most mutations involve missense substitutions of positively charged arginine residues within the S4 transmembrane segments of voltage-sensor domains II, III, or IV. These mutations lead to aberrant "gating pore currents," which are considered the primary pathogenic mechanism underlying susceptibility to anomalous depolarization and muscle inexcitability during paralytic attacks. Gating pore currents have been demonstrated for six of eight different HypoPP-associated *CACNA1S* mutations studied to date [5].

The NM_000069.3:c.2690G>A (p.Arg897Lys) variant was first reported in four males with HypoPP in Japan and France. This variant results in a charge-preserving substitution of arginine to lysine in S4 of domain III. Affected individuals experienced recurrent episodes of severe paresis with ictal hypokalemia (<3.0 mmol/L) from a young age, triggered by rest after exercise, alcohol consumption, or high-carbohydrate meals. They responded well to acetazolamide, potassium supplementation, or potassium-sparing diuretics. The long exercise test was consistent with HypoPP in one of the three tested patients, while the other two had borderline results (25-31 % decrement). Three individuals developed proximal myopathy at ages 46, 55, and 59 (two with pelvic girdle myopathy, one unspecified). Magnetic Resonance Imaging (MRI) from one of the patients revealed fatty infiltration in the paraspinal muscles, gluteus, soleus, and medial gastrocnemius muscles, while the quadriceps, gracilis, sartorius, semimembranosus, and semitendinosus muscles were grossly spared. A muscle biopsy showed vacuolar changes and T-tubular aggregates. Notably, gating pore currents were not detected in oocyte expression studies [6].

Failure to detect gating pore currents has been reported for only two HypoPP-associated *CACNA1S* mutations: p.Arg897Lys [6] and NM_000069.3:c.2699G>T (p.Arg900Ser) [7]. Interestingly, gating pore currents were observed for alternative missense mutations affecting the same residues: p.Arg897Ser and NM_000069.3:c.2698A>G (p.Arg900-Gly) [7]. These findings challenge the prevailing hypothesis that gating pore currents are the primary pathogenic mechanism in HypoPP, suggesting instead a more heterogeneous and complex pathophysiology.

In this study, we describe five families (14 individuals) carrying the pathogenic heterozygous c.2690G>A variant in *CACNA1S* gene, manifesting with HypoPP and pelvic girdle myopathy.

2. Materials and methods

2.1. Clinical evaluation

A total of 14 patients from five families in Mallorca, initially thought to be unrelated, were evaluated after being referred to neurology outpatient clinics for periodic paralysis or proximal lower limb weakness. A blood test was performed in all cases as part of the initial evaluation and subsequently for genetic testing. Additional examinations were conducted when necessary, including electrophysiological studies

in 10 patients, long exercise test in four patients, transthoracic echocardiography in two patients, and muscle MRI in four patients. Informed consent was obtained from all participants except for one deceased patient, whose son provided consent for inclusion in this retrospective study.

2.2. Electrophysiological studies

Studies were conducted using a Synergy electromyograph (Viasys Healthcare Ltd, UK). The number of muscles examined by conventional needle electromyography varied per patient at the examiner's discretion. Attention was given to the presence of denervation potentials, myotonic discharges, and interference pattern characteristics during maximum contraction. The long exercise test involved maximum contraction of the right abductor digiti minimi for five minutes, with rest periods of three to four seconds every 15 s. The maximum compound muscle action potential (CMAP) amplitude was recorded before exercise and then every two minutes for 30–50 min post-exercise.

2.3. Genetic analysis

Whole-exome sequencing was performed in index cases from each family using the "Illumina DNA Prep with Exome v2.0 PLUS Enrichment" protocol, covering 37.5Mb. The sample preparation followed the "Nextera Flex for Enrichment" (Illumina) protocol based on hybridization capture of targeted regions. Sequencing achieved an average depth of 100x, with 95 % of bases sequenced at ≥20x coverage. Library preparation and sequencing were performed on an Illumina NextSeq 500 platform. Annotation of bases, alignment to the human genome reference hg19 (GRCh37), quality filtering, and variant annotation were conducted using DRAGEN. Only variants sequenced at ≥20x coverage and meeting quality standards (>Q30, >20 % of reads and visual inspection) were considered. Variants meeting these quality criteria were not confirmed by Sanger sequencing. For the evaluation, relevant variants previously reported in databases such as dbSNP, GNOMAD, OMIM, Locus-Specific Mutation Databases, ClinVar, HGMD, Varsome, Franklin, LOVD, and UCSC were considered. Additionally, variants with a minor allele frequency (MAF) below 1 % in the 1000 Genomes Project, GNOMAD, and Global Minor Allele databases were included. The evaluation focused on variants located in exons and intronic regions flanking exons (±20 bp). Gene analysis included all genes related to HypoPP and/or pelvic girdle myopathy identified by the Geneyx analysis software.

In the remaining affected family members, once the p.Arg897Lys variant was identified in the index case, targeted sequencing of exon 21 of transcript NM_000069.3 was performed via PCR and Sanger sequencing.

To estimate the most recent common ancestor (MRCA) between families, exome sequencing data from four patients from three families (families 3, 4, and 5) were analyzed. Exomes from families 1 and 2 were sequenced by an external provider (raw data were unavailable). The analysis utilized the homozygosity haplotype approach, which identifies shared homozygous SNPs from variant call format files. Homologous regions around the variant between two samples were delimited when homozygous positions in the genome did not match [8]. The estimated number of generations to MRCA was calculated using the mutation age estimation method in R [9].

2.4. Muscle MRI

Whole-body MRI was performed in three patients from cranial vertex to tibial pilon using axial T2 or STIR sequences and coronal STIR sequences. In a fourth patient, only lower limbs were imaged from hip to tibial pilon using axial and coronal T1-weighted sequences, axial STIR, and coronal T2 DIXON (InPhase and water) sequences. MRI scans were performed using a 1.5T GE SIGNA Explorer high-field system.

3. Results

3.1. Clinical description

A summary of clinical features of the patients is provided in Table 1.

3.1.1. Family 1

Comprising six siblings: three affected (II.2, II.3, II.4) and three unaffected (II.6, II.7, II.8). Two affected nephews (III.1 and III.2) were also identified, though no additional data were available for III.1. Based on family history provided by the patients, affected relatives included their mother (I.2), an uncle (I.3) and a cousin (II.9); for whom no further information was obtainable (Fig. 1).

Patient 1.II.2 developed pelvic girdle myopathy at age 50, with

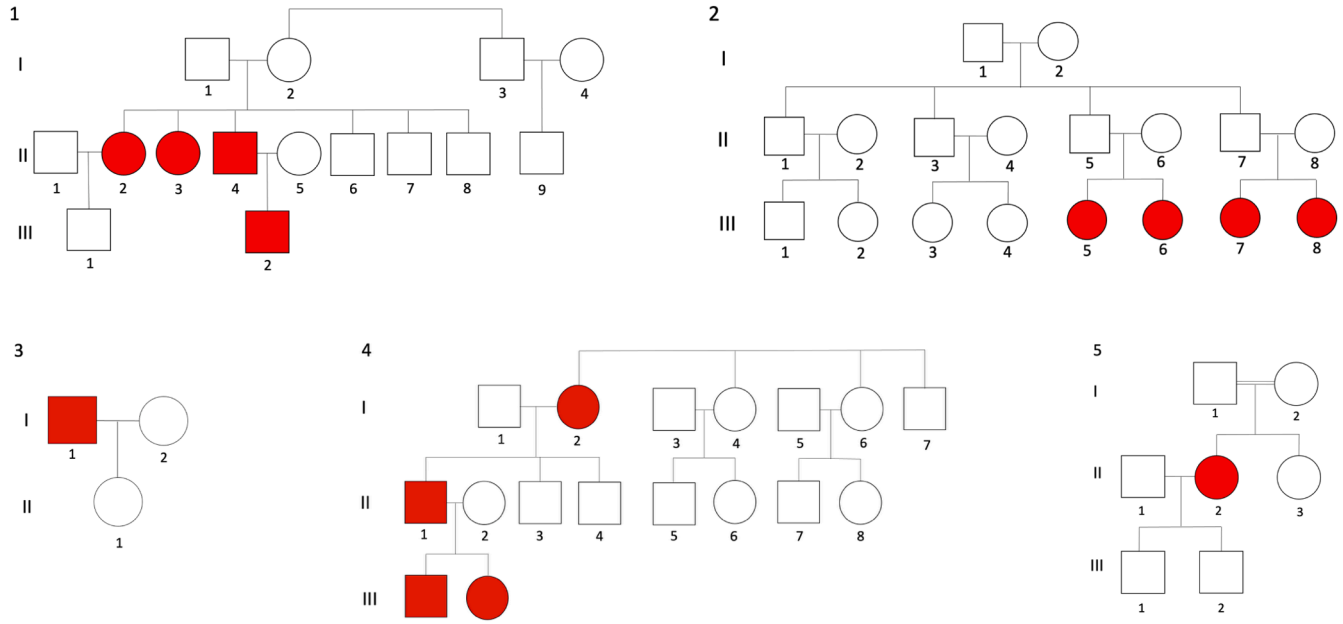

Fig. 1. Pedigree of the families. Two parallel lines denotes that patients 5.I.1 and 5.I.2 are cousins.

bilateral psoas and gluteal weakness graded as 4/5 on the Medical Research Council (MRC) scale but no periodic paralysis. No CK elevation was observed during follow-up. Acetazolamide was poorly tolerated. Patient 1.II.3 presented at the age of 46 with similar pelvic girdle myopathy without periodic paralysis and without CK elevation. Patient 1.II.4 had HypoPP episodes since childhood, resolving within hours following intravenous potassium administration. The patient experienced 2-3 mild episodes per week and 2-3 severe episodes annually. Triggers included carbohydrate intake, fasting, physical exertion, rest, stress, and cold. Preventive treatment with spironolactone and oral potassium was ineffective. No CK elevation was observed during follow-up. Patient 1.III.2 had occasional mild periodic paralysis episodes from age 15 without confirmed hypokalemia, resolving spontaneously without hospital care. No CK elevation was noted.

3.1.2. Family 2

Consisted of two affected sisters (III.5, III.6) and two affected cousins (III.7, III.8). Based on family history provided by the patients, additional affected relatives included another cousin (III.1), father (II.5), uncle (II.7) and grandmother (I.2); with no further details available (Fig. 1).

Patient 2.III.5 experienced HypoPP since youth and developed pelvic girdle myopathy after age 50. At age 84, severe HypoPP attacks persisted for several days. No CK elevation was observed during follow-up. Lumbosacral spine MRI showed paravertebral and iliopsoas muscle atrophy. Patient 2.III.6 experienced periodic paralysis episodes during youth, each lasting approximately two hours. These episodes were triggered by waking, rest, or postprandial periods and resolved after her first pregnancy. Pelvic girdle myopathy developed in her fifth decade. No CK elevation was observed. Patient 2.III.7 had three periodic paralysis episodes in youth, resolving with oral potassium. Severe progressive pelvic girdle weakness developed after age 50, requiring a wheelchair. The highest recorded CK level was 266 IU/L. Patient 2.III.8 had no periodic paralysis but severe pelvic girdle weakness (psoas 3/5, quadriceps and hamstrings 4/5 on the MRC scale), requiring support for standing. No CK elevation was observed. The patient had a concomitant diagnosis of atypical parkinsonism, presenting with a symmetric rigid-akinetic syndrome resistant to levodopa, cognitive decline, depression, REM sleep behavior disorder, constipation, and orthostatic hypotension. Cranial CT showed no abnormalities. During the last assessment at the movement disorders unit, the patient exhibited mild hypomimia, slight rigidity in the right upper limb, symmetric bradykinesia in both upper limbs, and slow gait with freezing episodes during turns.

3.1.3. Family 3

This family consisted of a 61-year-old male patient (I.1) with a healthy daughter (II.1) and no known family history (Fig. 1).

Patient 3.I.1 presented with progressive pelvic girdle myopathy starting at the age of 55, without periodic paralysis. Neurological examination revealed proximal lower limb weakness, with bilateral involvement of the psoas and gluteus muscles (4/5 on the MRC scale). The highest recorded CK level for this patient was 549 IU/L. A transthoracic echocardiogram was performed with no pathological findings.

3.1.4. Family 4

This family consisted of a 62-year-old male patient (II.1), his mother, who passed away at the age of 86 (I.2), and his two children (III.1 and III.2) (Fig. 1).

Patient 4.II.1 presented with pelvic girdle myopathy starting at the age of 56, without periodic paralysis. Neurological examination revealed abdominal and proximal lower limb weakness: psoas 4-/5, hamstrings 4/5, and quadriceps 4+/5 bilaterally on the MRC scale. The highest recorded CK level for this patient was 910 IU/L. Patient 4.I.2 experienced two self-limited episodes of lower limb weakness at the age of 42, which were not diagnosed at the time but were compatible with periodic paralysis (potassium levels were not recorded). In her sixth decade, she developed pelvic girdle myopathy and first sought medical

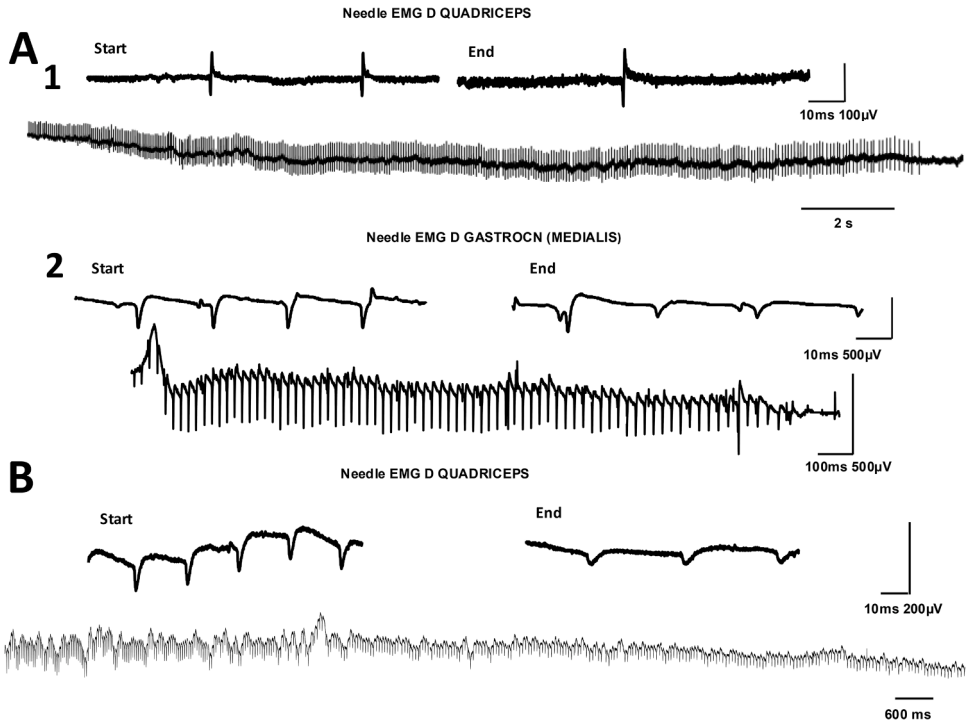

Fig. 2. Myotonic discharges A Patient 2.III.7. 1 A discharge lasting approximately 20 s, consisting of potentials with fibrillation-like morphology, an initial frequency of around 20 Hz, and no amplitude changes, although with a slight decrease in frequency throughout the discharge. 2 A discharge lasting approximately 4 s, consisting of potentials with positive wave morphology and an initial frequency of around 40 Hz, with a progressive reduction in both amplitude and frequency over the course of the discharge. B Patient 2.III.6. A discharge lasting approximately 10 s, consisting of potentials with positive wave morphology and an initial frequency of around 50 Hz, with a progressive reduction in both amplitude and frequency over the course of the discharge.

attention at the age of 72, when she already exhibited bilateral iliopsoas weakness (4/5 on the MRC scale) and gluteal weakness (2/5 on the MRC scale). Her condition progressively worsened, leading to loss of ambulation at 78 years old. The highest recorded CK level for this patient was 245 IU/L. Patient 4.III.1 experienced HypoPP episodes starting at the age of 14, most of which were mild and improved with oral potassium administration. However, at the ages of 14 and 19, he had severe episodes requiring ICU admission due to profound weakness associated with severe hypokalemia and electrocardiographic abnormalities. He is on preventive spironolactone therapy, which has reduced the frequency of episodes. The highest recorded CK level for this patient was 2,276 IU/L. Patient 4.III.2 experienced HypoPP episodes starting at the age of 17, with improvement after oral potassium administration. Most episodes were mild, but she had three severe episodes requiring hospitalization. The highest recorded CK level for this patient was 323 IU/L.

3.1.5. Family 5

This family consisted of a 70-year-old woman (II.2) whose parents were cousins and had no known family history (Fig. 1).

Patient 5.II.2 presented with progressive pelvic girdle myopathy starting at the age of 57. At the beginning of her follow-up in 2016, she exhibited only proximal weakness in the gluteus and psoas muscles (4/5 on the MRC scale), which progressively worsened until she developed severe paraparesis requiring a wheelchair. The highest recorded CK level for this patient was 530 IU/L. A transthoracic echocardiogram showed left ventricular hypertrophy, particularly in the basal septum. She was diagnosed with concomitant atypical parkinsonism, characterized by an asymmetric rigid-akinetic syndrome predominantly affecting the left side, dysphagia, and camptocormia, with no response to levodopa. Cranial MRI showed mild bilateral cerebellar atrophy, and DaTSCAN revealed degenerative dopaminergic degeneration.

3.2. Electrophysiological studies

The findings from needle electromyography performed in 10 patients varied depending on the severity of myopathy in affected individuals. Greater involvement of the quadriceps compared to the tibialis anterior or gastrocnemius was observed when these muscles were included in the examination. Additionally, fibrillation potentials and positive waves were noted. In patients with less severe involvement, a dense interference pattern was observed despite clinical weakness. In the most severely affected patients (such as 1.II.3, 2.III.5, 2.III.6, and 2.III.8), increased muscle consistency of the quadriceps was evident, with numerous silent zones and a reduced interference pattern, reflecting significant replacement of muscle tissue by connective tissue.

Notably, scarce fibrillation potentials or positive waves with electrophysiological and acoustic characteristics similar to myotonic discharges were detected in patients 2.III.6 and 2.III.7 (Fig. 2).

The long exercise test was performed in three patients from Family 1. In patient 1.II.2, a 20 % decrease in CMAP amplitude was observed between 10 and 15 min post-exercise. In patient 1.II.4, the decrease was also approximately 20 %, but it occurred progressively from 5 min onwards. In patient 1.II.3, no significant reduction in CMAP amplitude was observed.

3.3. Genetic analysis

The heterozygous c.2690G>A variant in *CACNA1S* gene was found to be absent from population databases (gnomAD 4.10/1,612,000). This variant alters the p.Arg897 amino acid residue in *CACNA1S*, and other variants affecting this residue have been determined to be pathogenic, associated with a severe HypoPP phenotype [10,11]. This suggests that this residue is located in a functionally critical domain of the protein and is clinically significant. Additionally, it has been reported in ClinVar in an individual with HypoPP, where it was classified as pathogenic [12] and a clinical description of four patients with HypoPP has been published [6]. Based on these and other considerations, and according to the criteria of the American College of Medical Genetics: PS3, PS4, PM1, PM2, PM5, PP3, PP5, this variant has been classified as pathogenic [12].

All families carrying the heterozygous c.2690G>A variant in *CACNA1S* gene share a common geographic origin despite not having shared surnames. This suggests a possible founder effect in the past, implying a common ancestor. To explore this hypothesis, the shared homologous region among the different families was determined (patients 3.I.2, 4.I.2, 4.II.1 and 5.II.2). The results indicate that individuals from different families share between 9.8 and 46.1 million base pairs flanking the *CACNA1S* gene (Fig. 3). Overall, families 3, 4, and 5 share a common genomic fragment of at least 9,803,437 base pairs (Fig. 3). Based on this

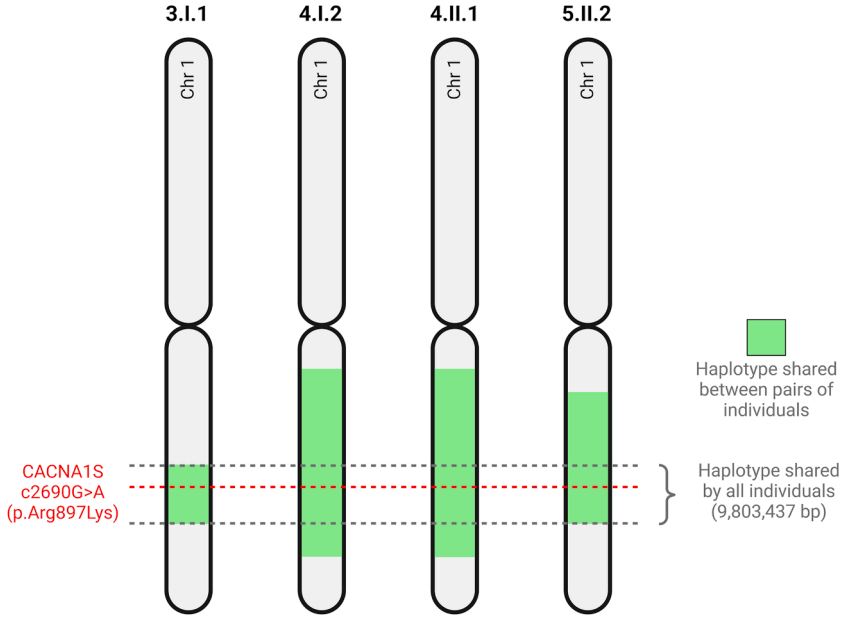

Fig. 3. Shared haplotype at the *CACNA1S* gene locus in patients from three families with the c.2690G>A (p.Arg897Lys) mutation. The image depicts the shared haplotype among four patients from three different families affected by the c.2690G>A (p.Arg897Lys) variant (red dashed line). Colored segments indicate genomic regions shared between two or more samples. Dashed lines mark the boundaries of the minimally shared region across all samples.

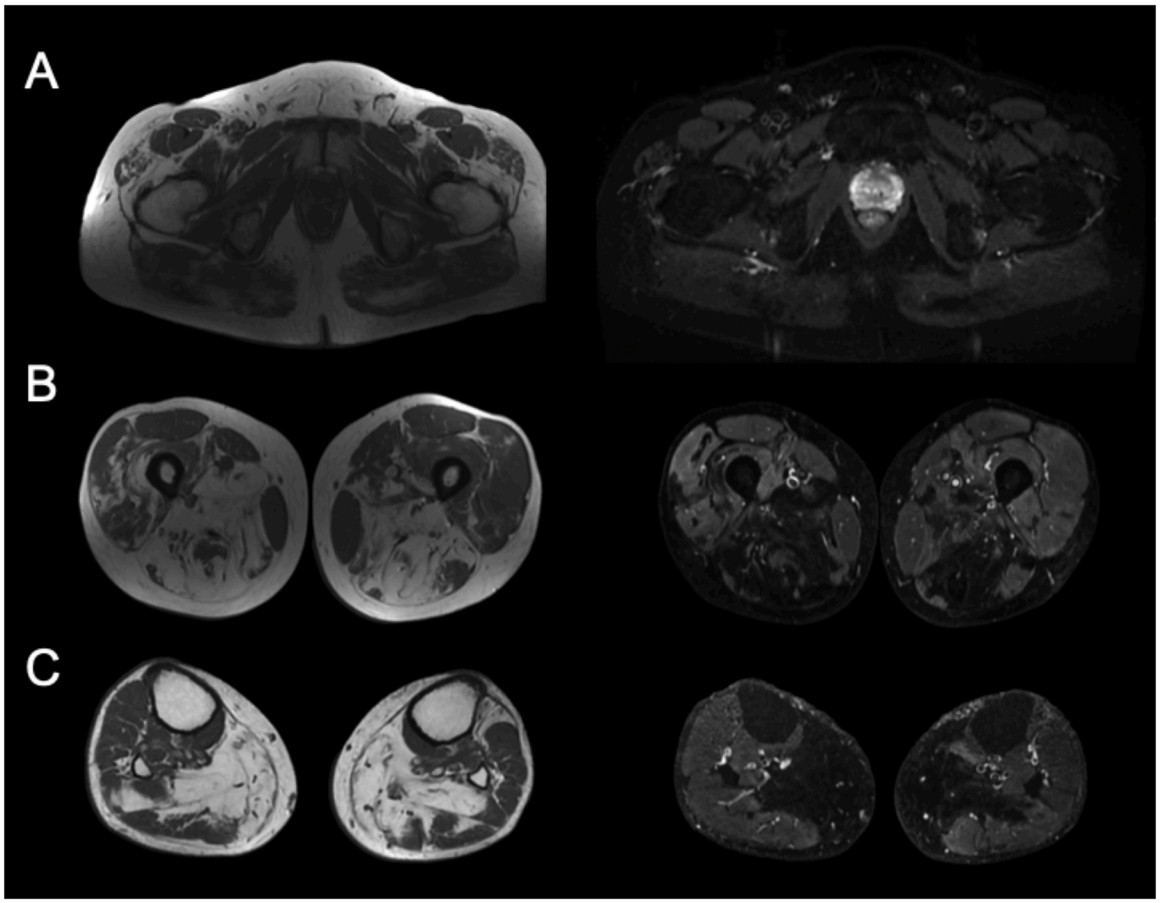

Fig. 4. Lower limb muscle MRI of patient 3.I.1. Comparative axial slices are shown at the hip level (A), mid-thigh (B), and leg (C). The left column displays T1-weighted sequences, while the right column shows STIR sequences, except for the hip slice, which corresponds to a DIXON T2 water sequence.

data, it is estimated that these families share a common ancestor approximately 4.5 generations ago (95 % CI: 2.6-8.1 generations), which corresponds to just over 100 years ago (95 % CI: 65-203 years).

3.4. Muscle MRI

A muscle MRI of the lower limbs was performed in patient 3.I.1, revealing diffuse symmetric fatty degeneration in both extremities. At the hip level, bilateral fatty infiltration was observed in the gluteus maximus. In the thigh, the posterior and medial compartments were particularly affected, involving the semitendinosus, semimembranosus, biceps femoris, and adductor muscles. The sartorius, gracilis, and rectus femoris muscles were bilaterally preserved. In the legs, fatty infiltration was notable in the left tibialis anterior and peroneal muscles, with severe involvement of the superficial posterior compartment, affecting the soleus and both the medial and lateral gastrocnemius. The rest of the leg musculature was preserved (Fig. 4). In patient 4.II.1, a whole-body MRI showed significant atrophy and fatty degeneration of the bilateral pectoralis major, anterior abdominal wall musculature (rectus abdominis and obliques), bilateral lumbar paravertebral muscles, bilateral iliopsoas, and bilateral gluteus maximus. In the thighs, there was severe bilateral fatty infiltration in all muscle except for the gracilis and sartorius, which remained intact, with relatively less involvement of the right rectus femoris, left vastus intermedius, and bilateral adductors. In the legs, there was marked atrophy and fatty degeneration of the medial and lateral gastrocnemius muscle bilaterally (Fig. 5). Patient 4.III.1, who did not exhibit clinical signs of myopathy, underwent a whole-body MRI that showed no abnormalities. In patient 5.II.2, a whole-body MRI revealed a predominance of fatty tissue over muscle tissue, suggestive of severe atrophy, localized bilaterally in the iliopsoas muscles, bilateral lumbar paravertebral muscles, gluteus maximus, minor abductor, semimembranosus, semitendinosus, vastus lateralis, right vastus medialis, and medial gastrocnemius bilaterally and symmetrically. In the thighs, the gracilis and sartorius muscles remained unaffected, with relative preservation of the right rectus femoris and the left vastus medialis and vastus intermedius (Fig. 5). None of the MRIs images showed significant muscular edema in the examined muscles (Fig. 4).

4. Discussion

In this study, we described 14 patients from five families, representing the largest reported cohort to date with the p.Arg897Lys variant.

The three families for which genomic data were available shared approximately 10 million base pairs and a common ancestor within an estimated 2 to 8 generations. It is important to note that homozygosity haplotype calculations tend to overestimate the shared region, as they only consider homozygous regions [8]. Additionally, exome sequencing data leave some genomic regions uncovered, making it difficult to delineate shared segments precisely. As a result, the actual shared region might be smaller than predicted by this method, leading to an increased estimated number of generations and a longer time to the most recent common ancestor.

Most affected individuals in this cohort were female (9/14, 64 %). Even when considering affected but unstudied relatives, the proportion remains high (12/23, 52 %). This differs from previous descriptions of the disease [1,2] and even from reports on the same variant [6].

Notably, the majority of patients (10/14, 71 %) exhibited myopathy exclusively symptomatic at the pelvic girdle. In six of these patients, myopathy was the only symptom, with an average onset age of 54 years. Among the patients with periodic paralysis (8/14, 57 %), disease onset

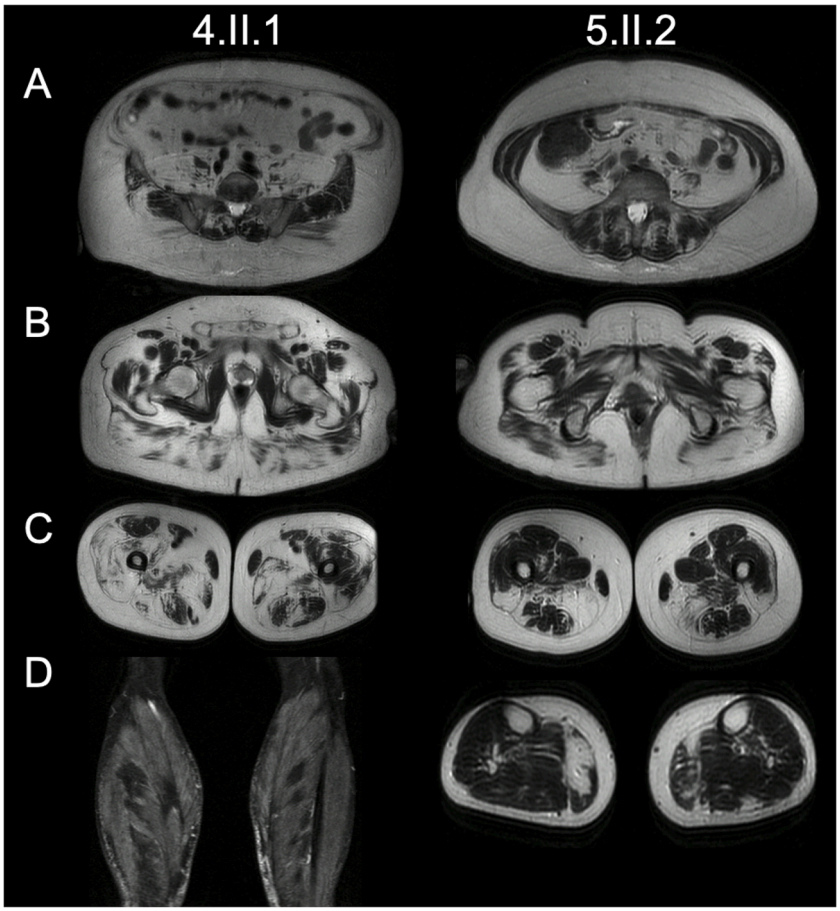

Fig. 5. Whole-body MRI of patients 4.II.1 and 5.II.2. Comparative axial T2-weighted sequences are shown at the lumbosacral level (A), hip (B), and mid-thigh (C). At the leg level (D), a coronal STIR sequence comparison is presented for patient 4.II.1, while for patient 5.II.2, an axial T2-weighted sequence comparison is shown.

generally occurred in childhood or adolescence, consistent with previous descriptions [1], with four cases subsequently developing pelvic girdle myopathy. CK elevations were recorded in 7/14 cases, with an average value of 728 IU/L. Severe periodic paralysis episodes occurred in four patients (4/8, 50 %), while the remaining cases were mild. Regarding myopathy, three patients (3/10, 30 %) developed progressive weakness leading to wheelchair dependence in old age. Remarkably, patients without myopathy were the youngest (mean age: 32 years), raising the possibility that myopathy might develop later as the disease progresses. The clinical presentation in our cohort aligns with previous descriptions of this variant [6], particularly its late-onset asymmetric myopathy with exclusive pelvic girdle involvement.

A study of 55 patients with HypoPP due to the most common *CACNA1S* mutation, p.Arg528His, found that MRI revealed fatty muscle infiltration even in individuals without detectable muscle weakness. No correlation was observed between the periodic paralysis and the development of permanent weakness or MRI fatty muscle infiltration severity. Additionally, three patients (5 %) had no periodic paralysis episodes but exhibited weakness comparable to the rest of the cohort. The most affected muscles were the paraspinal, abdominal, iliopsoas major, and proximal lower limb muscles. The authors concluded that this mutation causes myopathy beyond the damage inflicted by periodic paralysis attacks alone [13].

Similarly, in another series of 11 patients with HypoPP due to the p.Arg528His mutation, muscle MRI consistently showed atrophy and fatty infiltration, primarily affecting the posterior thigh compartment, with initial involvement of the adductor magnus and semimembranosus, while the biceps femoris, sartorius, and gracilis were spared. This pattern was observed in both symptomatic and asymptomatic carriers, with a linear relationship between fatty infiltration and age [14]. Muscle MRI findings were consistent across the three patients with pelvic girdle myopathy in our study. The imaging revealed involvement of the axial musculature, including the pectoralis major, abdominal wall, and lumbar paravertebral muscles. Additionally, the pelvic girdle muscles, particularly the iliopsoas and gluteus maximus, were affected. In the thighs, there was no clear compartmental predominance; however, a characteristic sparing of the sartorius and gracilis muscles was observed, with relative preservation of the rectus femoris. In the lower legs, the gastrocnemius muscles were predominantly involved. These findings align with previous reports on this variant [6] and other *CACNA1S* mutations [13,14], differing from previously described hereditary myopathy patterns [15], making them useful for diagnostic purposes.

From an electrophysiological perspective, EMG during paralysis can show reduced CMAP amplitude with decreased motor unit recruitment or electrical silence, depending on the severity of weakness. Other variable findings include increased insertional activity, a higher proportion of polyphasic MUPs, and reduced muscle fiber conduction velocity [16]. The presence of myotonic discharges in EMG strongly suggests hyperkalemic periodic paralysis (observed in up to 80 % of patients) but is rarely seen in patients with sodium channel variants or those without an identified mutation [17]. In our study, two related patients exhibited prolonged fibrillation potentials or positive waves with acoustic characteristics resembling myotonic discharges, though lacking the characteristic fluctuating amplitude and frequency of myotonic dystrophy [18]. Instead, both amplitude and, to a lesser extent, frequency decreased progressively without oscillations. While not typical, this discharge pattern has been observed in myotonic dystrophy type II [19] and is considered a myotonia subtype [18]. Recent reviews have stated

that the presence of typical myotonic discharges is incompatible with a HypoPP diagnosis [5]. However, both myotonic discharges and even clinical myotonia have been reported in patients with sodium channel-related HypoPP [20,21], although their electrophysiological characteristics were not precisely described. These findings suggest that atypical myotonic discharges may occur in HypoPP, regardless of whether the causative mutation affects the sodium or calcium channel.

In the long exercise test, a gradual >40 % CMAP amplitude reduction at 30–40 min post-exercise is considered suggestive of periodic paralysis [14,22–25]. In our cohort, no significant CMAP amplitude reduction was observed in the three patients who underwent the test (two of whom had never experienced a symptomatic attacks of transient weakness). While this finding is consistent with previous reports on this variant [6], the small sample size limits its interpretation, especially considering the test's low sensitivity (59.3 %) [26].

Preventive treatment for periodic paralysis episodes was attempted in three patients. One patient could not tolerate acetazolamide, and spironolactone was only effective in one of the two patients who received it.

HypoPP is a rare and diagnostically challenging disease, and the patients described herein exhibit significant phenotypic variability, even among members of the same family. This probably contributes to considerable diagnostic delays, with some patients in our cohort experiencing delays exceeding 40 years from symptom onset to genetic diagnosis.

5. Conclusion

The cohort of 14 affected individuals reported herein adds confidence to the assignment of *CACNA1S* p.Arg897Lys as a pathogenic mutation for susceptibility to HypoPP with permanent myopathy. Our findings confirm previous descriptions in *CACNA1S* variants and may be useful for diagnosis, such as the muscle MRI pattern (characterized by sparing of the sartorius and gracilis muscles).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT to translate the manuscript and improve readability and language. After using this tool, the authors have reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Data availability

The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

CRediT authorship contribution statement

Oriol Barrachina-Esteve: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Marc Ventayol-Guirado:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis. **Victor J Asensio:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis. **Damià Heine-Suñer:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Ricardo Corrales:** Visualization, Resources. **Noemí Vidal:** Writing – review & editing, Visualization, Resources. **Trajche Ivanovski:** Writing – review & editing, Resources. **Clara Arbós:** Resources. **Maite Agirre:** Resources. **Carles Montalà:** Resources. **Jordi Ballabriga:** Writing – review & editing, Resources. **Ana Valero:** Writing – review & editing. **M Magdalena Rosselló:** Writing – review & editing. **Pablo Dávila:** Writing – review & editing. **Margalida Mestre:** Writing – review & editing. **Ana Sánchez:** Writing – review & editing. **Elena Deyá:** Writing – review & editing. **Inés Legarda:** Writing – review & editing, Resources. **Ana Espino:** Writing – review & editing, Resources. **Montse Olivé:** Writing – review & editing, Resources. **Francesc Miralles:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

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References

[1] Fialho D, Griggs RC, Matthews E. Periodic paralysis. Handbook of clinical neurology, 148. Elsevier; 2018. p. 505–20. https://doi.org/10.1016/B978-0-444-64076-5.00032-6.
[2] Ke Q, Luo B, Qi M, Du Y, Wu W. Gender differences in penetrance and phenotype in hypokalemic periodic paralysis. Muscle Nerve 2013;47:41–5. https://doi.org/10.1002/mus.23460.
[3] Links TP, Zwarts MJ, Wilmink JT, Molenaar WM, Oosterhuis H. Permanent muscle weakness in familial hypokalaemic periodic paralysis: clinical, radiological and pathological aspects. Brain 1990;113:1873–89. https://doi.org/10.1093/brain/113.6.1873.
[4] Matthews E, Portaro S, Ke Q, Sud R, Haworth A, Davis MB, et al. Acetazolamide efficacy in hypokalemic periodic paralysis and the predictive role of genotype. Neurology 2011;77:1960–4. https://doi.org/10.1212/WNL.0b013e31823a0cb6.
[5] Cannon SC. Periodic paralysis. Handbook of clinical neurology, 203. Elsevier; 2024. p. 39–58. https://doi.org/10.1016/B978-0-323-90820-7.00002-1.
[6] Kubota T, Wu F, Vicart S, Nakaza M, Sternberg D, Watanabe D, et al. Hypokalaemic periodic paralysis with a charge-retaining substitution in the voltage sensor. Brain Commun 2020;2:fcaa103. https://doi.org/10.1093/braincomms/fcaa103.
[7] Wu F, Quinonez M, Cannon SC. Gating pore currents occur in CaV1.1 domain III mutants associated with HypoPP. J Gen Physiol 2021;153:e202112946. https://doi.org/10.1085/jgp.202112946.
[8] Miyazawa H, Kato M, Awata T, Kohda M, Iwasa H, Koyama N, et al. Homozygosity haplotype allows a genomewide search for the autosomal segments shared among patients. Am J Hum Genet 2007;80:1090–102. https://doi.org/10.1086/518176.
[9] Gandolfo LC, Bahlo M, Speed TP. Dating rare mutations from small samples with dense marker data. Genetics 2014;197:1315–27. https://doi.org/10.1534/genetics.114.164616.
[10] Chabrier S, Monnier N, Lunardi J. Early onset of hypokalaemic periodic paralysis caused by a novel mutation of the CACNA1S gene. J Med Genet 2008;45:686–8. https://doi.org/10.1136/jmg.2008.059766.
[11] Hanchard NA, Murdock DR, Magoulas PL, Bainbridge M, Muzny D, Wu Y, et al. Exploring the utility of whole-exome sequencing as a diagnostic tool in a child with atypical episodic muscle weakness. Clin Genet 2013;83:457–61. https://doi.org/10.1111/j.1399-0004.2012.01951.x.
[12] National Center for Biotechnology Information. ClinVar; [VCV000473979.7], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000473979.7 (accessed Feb. 19, 2025). n.d.
[13] Holm-Yildiz S, Witting N, Dahlqvist J, De Stricker Borch J, Solheim T, Fornander F, et al. Permanent muscle weakness in hypokalemic periodic paralysis. Neurology 2020;95. https://doi.org/10.1212/WNL.0000000000009828.
[14] Carbonell-Corvillo P, Rivas E, Cabrera M, García-Redondo A, Fernández A, Paradas C. Redefining periodic paralysis with CACNA1S mutation in a Spanish cohort. Neurología 2024. https://doi.org/10.1016/j.nrl.2023.02.009. S0213485324001282.
[15] Aivazoglou LU, Guimarães JB, Link TM, Costa MAF, Cardoso FN, De Mattos Lombardi Badia B, et al. MR imaging of inherited myopathies: a review and proposal of imaging algorithms. Eur Radiol 2021;31:8498–512. https://doi.org/10.1007/s00330-021-07931-9.
[16] Sharma C, Nath K, Parekh J. Reversible electrophysiological abnormalities in hypokalemic paralysis: case report of two cases. Ann Indian Acad Neurol 2014;17:100. https://doi.org/10.4103/0972-2327.128566.

[17] Miller TM, Dias Da Silva MR, Miller HA, Kwiecinski H, Mendell JR, Tawil R, et al. Correlating phenotype and genotype in the periodic paralyses. Neurology 2004;63:1647–55. https://doi.org/10.1212/01.WNL.0000143383.91137.00.
[18] Miller TM. Differential diagnosis of myotonic disorders. Muscle Nerve 2008;37:293–9. https://doi.org/10.1002/mus.20923.
[19] Logigian EL, Ciafaloni E, Quinn LC, Dilek N, Pandya S, Moxley RT, et al. Severity, type, and distribution of myotonic discharges are different in type 1 and type 2 myotonic dystrophy. Muscle Nerve 2007;35:479–85. https://doi.org/10.1002/mus.20722.
[20] Sun J, Luo S, Song J, Huang J, Cai S, Zhu W, et al. Electromyographic features in a Chinese cohort with hereditary skeletal muscle channelopathies. J Clin Neurophysiol 2020;37:231–8. https://doi.org/10.1097/WNP.0000000000000635.
[21] Luo S, Sampedro Castañeda M, Matthews E, Sud R, Hanna MG, Sun J, et al. Hypokalaemic periodic paralysis and myotonia in a patient with homozygous mutation p.R1451L in NaV1.4. Sci Rep 2018;8:9714. https://doi.org/10.1038/s41598-018-27822-2.
[22] Simmons DB, Lanning J, Cleland JC, Puwanant A, Twydell PT, Griggs RC, et al. Long exercise test in periodic paralysis: a Bayesian analysis. Muscle Nerve 2019;59:47–54. https://doi.org/10.1002/mus.26157.
[23] Ribeiro A, Suetterlin KJ, Skorupinska I, Tan SV, Morrow JM, Matthews E, et al. The long exercise test as a functional marker of periodic paralysis. Muscle Nerve 2022;65:581–5. https://doi.org/10.1002/mus.27465.
[24] Tengan CH. The exercise test as a monitor of disease status in hypokalaemic periodic paralysis. J Neurol Neurosurg Psychiatry 2004;75:497–9. https://doi.org/10.1136/jnnp.2003.013870.
[25] Kuntzer T, Flocard F, Vial C, Kohler A, Magistris M, Labarre-Vila A, et al. Exercise test in muscle channelopathies and other muscle disorders. Muscle Nerve 2000;23:1089–94. https://doi.org/10.1002/1097-4598(200007)23:7<1089::AID-MUS12>3.0.CO;2-Q.
[26] Periviita V, Jokela M, Palmio J, Udd B. A retrospective study of accuracy and usefulness of electrophysiological exercise tests. J Neurol 2024;271:1802–12. https://doi.org/10.1007/s00415-023-12110-5.