Case Report

De novo Mutation in *CACNA1S* Gene in a 20-Year-Old Man Diagnosed with Metabolic Myopathy

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Abstract

The calcium channel, voltage-dependent, L-type, alpha 1S subunit *(CACNA1S)* gene encodes a skeletal Ca^{2+} channel which is involved in calcium-dependent processes such as muscle contraction and neurotransmitter release. Mutations in this gene have been accompanied by hypo- and normokalemic periodic paralysis, thyrotoxic periodic paralysis, and susceptibility to malignant hyperthermia. We report the clinical and genetic findings in a patient diagnosed with metabolic myopathy who had episodic attacks of muscle pain and weakness but with no family background of the disease. Next-generation sequencing (NGS) using a panel targeting metabolic myopathy and myotonia genes identified a *de novo* heterozygous pathogenic variant c.3724A>G, p.Arg1242Gly, in exon 30 of *CACNA1S*. As the second report of this variant, this case may broaden the *CACNA1S*-related disease spectrum to include normokalemic periodic paralysis.

Keywords: *CACNA1S*, channelopathies, next-generation sequencing, normokalemic, periodic paralysis

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Introduction

The muscle channelopathies consist of a rare inherited group of disorders which cause either muscle hyper- or hypoexcitability.[1] Most of the muscle channelopathies are caused by mutations in ion channel encoding genes such as *CNCL1*, *SCN4A*, *CACNA1S*, *KCNJ2*, and *RYR1*. A subset of channelopathies, hypokalemic periodic paralysis (HOKPP), is characterized by episodic attacks of muscle weakness and pain with decreased levels of serum potassium during paralytic episodes. While hyperkalemic periodic paralysis (HYPP) is clinically similar to HOKPP, it is distinguished by elevated serum potassium levels during muscle weakness attacks. Both types of periodic paralyses are dominantly inherited and categorized as either familial or sporadic forms. Depending on which gene is mutated and which position and domain of the protein are affected, the age of onset of HOKPP may range from 1 to 26 years.[2,3] To date, mutations in *CACNA1S* have mainly been reported as a genetic cause of HYPP in OMIM (Online Mendelian Inheritance in Man). However, for most patients with HYPP, heterozygous mutations in the sodium channel gene *SCN4A* have been reported. Using Next-generation sequencing (NGS), we present a patient diagnosed with metabolic myopathy who was found to have a *de novo* mutation in a gene causing muscle channelopathies.

Case Report

A 20-year-old man with non-consanguineous parents was referred for evaluation of episodic muscle weakness with a primary diagnosis of metabolic myopathy. Before the study, we obtained informed consent from the proband, siblings and his parents. The proband (IV-1) (Figure 1) was born to a non-consanguineous family from Isfahan, central Iran, following a normal pregnancy and pre-term delivery. Shortly after birth, he presented with hypotonia followed by delayed neck holding and sitting. However, at the age of 14 months, he was able to walk normally. He was hospitalized for 20 days after an episode of generalized muscle weakness at the age of 2 years with complete recovery. Further attacks occurred at approximately 1-year intervals and lasted for 3 – 14 days. The episodes were characterized by muscle weakness, swelling and pain, usually triggered by febrile illness. Since the age of 7 years, he developed muscle pain and spasm following exercise. Subsequently, constant muscle weakness in the lower limbs appeared 3 years ago. He also complained of periodic upper limb muscle weakness following exercise. Cognitive development was normal. Between the episodes, he had mild proximal muscle weakness (4/5) in the upper and lower extremities. He also had generalized hyporeflexia. A sensory exam was normal and cranial muscles were intact. During the most severe attacks, he had plasma creatine kinase (CK) levels up to 33000 U/L. Potassium levels were usually normal but in

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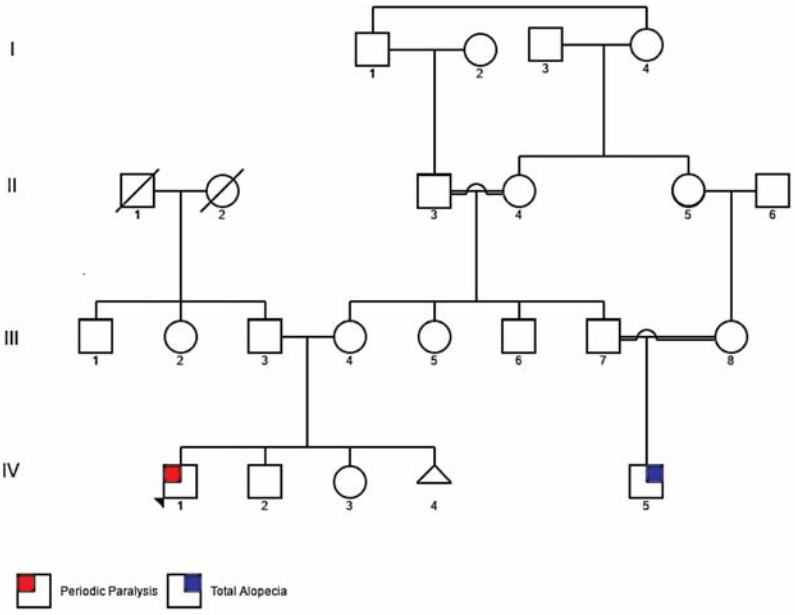

Figure 1. Pedigree of the family in this study. The proband is IV-1

one episode, it reached 5.3 mEq/L, reflecting mild hyperkalemia. Echocardiography was normal. Muscle biopsy showed mild nonspecific myopathic changes with normal glycogen and lipid content and no mitochondrial abnormality. The proband has two normal siblings (IV:2, IV:3) and his mother (III:4) had an abortion for an unexplained reason. None of his extended family members had a history of myopathy.

We obtained 10 mL of peripheral blood from the patient, his parents and normal siblings and only the sample from the patient was subjected to whole exome sequencing (WES). Genomic DNA was extracted using an established salting out protocol.[4] The sequence target regions were captured with a SureSelect custom enrichment kit (Agilent Technologies Inc., Santa Clara, CA, USA) and paired-end sequencing was performed on a NextSeq Sequencer (Illumina Inc., San Diego, CA, USA). The sequence reads were aligned to the hg19 human reference sequence using a Burrows-Wheeler Aligner[5] and coverage of > 20x for 97.5% of the targeted regions was achieved. Variant calling was performed using GATK HaplotypeCaller[6] and the annotation process was carried out using VarSeq software (GoldenHelix Inc, Bozeman, MT, USA). The variants within the 52 known genes responsible for metabolic myopathies and myotonia were then subjected to filtering and prioritization considering the allele frequency in the general population and dbNSFP function prediction scores.[7]

Our filtering led to identification of a heterozygous pathogenic variant, c.3724A>G (p.Arg1242Gly) in exon 30 of *CACNA1S* gene (NM_000069.2), most compatible with the patient's phenotype. The affected individual and his family members were screened for the detected variant using Sanger sequencing on a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). As expected, the detected pathogenic heterozygous variant was not identified in the family members apart from the affected individual; thus, the substitution site is considered to be a potential *de novo* arisen variant. Maternity and paternity were confirmed using QF-PCR.

Discussion

Myopathies are a heterogeneous group of muscle diseases which arise from various biological processes such as metabolic, inflammatory, or collagen-related processes. They are mainly classified as inherited or acquired forms.[8] In the group of inherited myopathies, metabolic myopathy, a deficiency in enzymes resulting from a defective gene, involves metabolic pathways which eventually affect the muscles. Metabolic myopathy manifests with muscle dysfunction such as chronic or episodic muscle weakness, hypotonia, cramp, or stiffness.[9] As metabolic myopathies have symptoms similar to most neuromuscular disorders, it is sometimes difficult to make an accurate clinical diagnosis. Thus, genetic testing can be helpful in detecting disease-causing variants and determining the type of neuromuscular disorder, particularly in conditions such as those manifested by our patient.

Periodic paralyses (PP) are uncommon genetic disorders which are divided into hypokalemic PP (HOKPP), normokalemic PP and hyperkalemic PP (HYPP) based on the level of serum potassium during attacks.[10] Heterozygous mutations in the *SCN4A* gene can cause both HOKPP and HYPP; the latter is a potentially life threatening disease because of possible sudden respiratory paralysis and arrhythmia;[11,12] However, heterozygous substitutions in the *CACNA1S* gene give rise to approximately 70% of cases of HOKPP.[13] In this study, we report a *de novo* mutation, c.3724A>G (R1242G), in exon 30 of the *CACNA1S* gene. This gene encodes the α_1 subunit of the L-type skeletal muscle voltage-gated calcium channel which plays a key role in muscle contraction.[14] The α_1 subunit consists of four similar domains I–IV, each of which is made up of six transmembrane helical segments S1–S6 (Figure 2A).[15] There are four positively charged R residues in the voltage-sensitive S4 segments of each domain. During membrane depolarization, by shifting the more deeply situated R residues to the membrane surface into an almost extracellular location, the

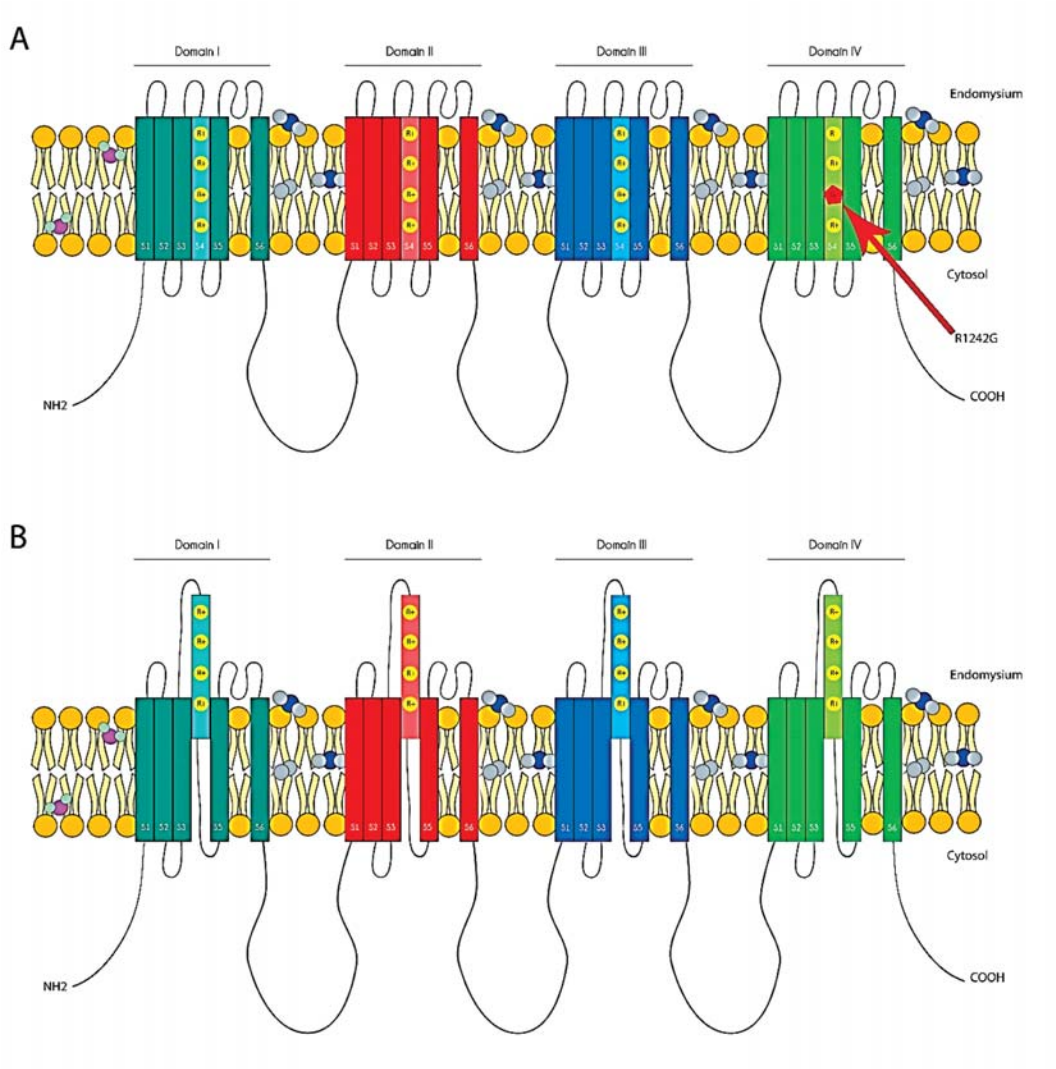

Figure 2. **A)** Shows the four repeated domains in the alpha subunit of the L-type voltage-gated calcium channel and the location of the R1242G substitution. There are four positively charged R residues in each S4 segment which play a critical role in the outward movement of the S4 segments. R1242G is located on the S4 segment of domain IV, shown by the red arrow; **B)** Shows the outward movement of the S4 segments which cause a conformational change in the whole protein structure leading to the opening of the alpha pore.

Table 1. Features of probands harboring c.3724A>G in *CACNA1S*

Phenotype	IV-1 (proband in this study)	Fan, et al.[10]		
		Patient 1 (I-1)	Patient 2 (II-2)	Patient 3 (II-4)
Gender	Male	Male	Female	Female
Age of onset	2-year-old	Infancy	Early teen	Early teen
Hypotonia at birth	Yes	N/A	N/A	Yes
Delayed motor development	No	Yes	N/A	Yes
Painful muscle cramping	Yes	Yes	Yes	Yes
Flaccid weakness	Yes	Yes	Yes	Yes
Leg edema	Yes	N/A	Yes	Yes
Respiratory failure	No	Yes (cause of death)	No	No
Foot drop	No	N/A	Yes	Yes
Triggers	Cold, hard exercise, and febrile illness	Cold and rest after exercise	Walking and febrile infection	Cold and walking
Total serum potassium level	Normal	Normal	Normal	Normal
CPK level	Elevated	N/A	N/A	Elevated
CPK: creatine phosphokinase; N/A: not available.				

outward movement of each S4 segment causes a conformational change in the whole protein structure leading to opening of the alpha pore (Figure 2B). In the majority of the previously reported mutations in the *CACNA1S* gene, there were substitutions of arginine residue, such as R900S and R528G, which neutralized the positively charged R residues in the S4 segments of each domain.[13,16] The R residue substitutions in the S4 segments cause alterations in the alpha pore current which conducts ions into the cytosol. In addition, these mutations facilitate an aberrant so-called omega current which contributes to the associated pathogenic phenotype. Our detected *de novo* pathogenic variant, R1242G, is a glycine substitution at the third charged R residue in the voltage sensor S4 segment of domain IV (Figure 2A). In a study conducted by Fan, et al. in 2013 on a two-generation family with progressive muscle weakness and myopathy defined as normokalemic periodic paralysis, they reported a novel mutation, R1242G, in the voltage-gated calcium channel gene *CACNA1S*.[10] After a functional study, a significant reduction in central alpha pore inward currents was reported together with the presence of outward omega currents in the cell lines that expressed R1242G.[10]

In line with the previous report by Fan, et al.[10] which provides additional evidence for the variant of c.3724A>G in *CACNA1S* resulting in normokalemic periodic paralysis, our report adds another family harboring this variant (Table 1).

These findings indicate that genome analysis of suspected cases may reveal additional symptoms and signs, help define the spectrum of the phenotype and provide greater understanding of the role of this variant in potassium-related paralysis. Finally, our study emphasizes the importance of WES in detecting the genetic causes underlying such a heterogeneous neuromuscular disorder.

Conflict of interest

The authors declare no conflicts of interest.

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