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A novel in-frame mutation at the boundary between exon 21 and intron 21 of *SCN4A* in a family with paramyotonia congenita

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Abstract

Nondystrophic myotonias and periodic paralyses are an important group of genetic skeletal muscle disorders characterized by dysfunction of ion channels that regulate cell membrane excitability. Mutations in the *Sodium Voltage-Gated Channel Alpha Subunit 4* (*SCN4A*) gene are associated with a spectrum of a heterogeneous group of skeletal muscle such as sodium channel myotonia, paramyotonia congenita, hyperkalemic periodic paralysis, congenital myasthenia, and congenital myopathy. Gain of function mutations in *SCN4A* cause three muscle disorders with overlapping clinical phenotypes: myotonia, paramyotonia congenita, and hyperkalemic periodic paralysis. Here, we describe the clinical and genetic features of a new family with paramyotonia. The proband, an eight-year-old girl, began to experience muscle stiffness in her hands and limbs on exposure to exercise exercise at the age of four and presented with myotonia. She was initially misdiagnosed with myotonic dystrophy because of worsening weakness with significant elevation of serum creatinine kinase levels. Two other affected family members had paradoxical myotonia in the face and hands on exposure to cold muscle stiffness in her face, hands, and limbs on exposure to cold and showed grip myotonia on physical examination. A novel heterozygous in-frame insertion, c.3911_3912+1dupAGA, at the boundary between exon 21 and intron 21 of *SCN4A* was identified using whole exome sequencing. Our finding enhances our understanding of the genotype and phenotype of patients with paramyotonia congenita, caused by mutations in the *SCN4A* gene.

Keywords: Channelopathy, nondystrophic myotonia, paramyotonia congenital, *SCN4A* gene, next generation sequencing

INTRODUCTION

Nondystrophic skeletal muscle channelopathies are a rare heterogeneous group of disorders caused by genetic mutations in different ion channels.[1,2] Nondystrophic myotonia and periodic paralysis are two important groups of genetic nondystrophic skeletal muscle channelopathies.[1,2] Voltage-gated sodium channels are expressed in skeletal muscle plasma membranes; the mainly expressed sodium channel is a heterodimer of the pore-forming voltage-gated sodium channel protein type 4 alpha-subunit (NaV1.4) and the non-covalently associated beta1-subunit.[3] The NaV1.4 is encoded by the *SCN4A* gene (MIM# 603967) located on chromosome 17q23. Nav1.4 is essential for the generation and propagation of muscle action potential, which is crucial for skeletal muscle contraction. Mutations in NaV1.4 produce six allelic skeletal muscle disorders, which include myotonia (#608390), paramyotonia congenita (PMC, MIM#168300), hyperkalemic periodic paralysis (MIM#170500), hypokalemic periodic paralysis (MIM#613345), congenital myasthenia (MIM#614198), and congenital myopathy.[1,4] Currently, more than 80 mutations in *SCN4A* have been identified in patients with skeletal muscle disorders.[3,5-10] Three allelic disorders of myotonia congenital, PMC, and hyperkalemic periodic paralysis constitute the main forms and have overlapping clinical phenotypes and genotypes.[2,3,10] The three allelic disorders are caused by heterozygous mutations, the majority of which are missense mutations with high penetrance.[2,3] These mutations have been established as gain-of-function resulting in increased sodium channel current.[1,3]

Here, we report three affected individuals of a new family showing cold- and/or exercise-induced

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paradoxical myotonia, grip myotonia, and episodic weakness. This family was found to harbor a rare, novel, heterozygous, in-frame insertion of c.3911_3912+1dupAGA located in the boundary of the consensus splice site of exon 21 and intron 21 of *SCN4A* by whole exome sequencing (WES).

CASE REPORT

A small, new family was identified at the Nowon Eulji Medical Center (Seoul, Republic of Korea). The pedigree chart is illustrated in Figure 1A. This family comprised of three identified patients (II-1, II-2, and III-1). The proband's (III-1) grandfather (I-1) had died a long time ago; therefore, his clinical features could not be checked.

Patient 1 (proband, III-1)

The proband, an eight-year-old girl, was born at term with a birth weight of 2 900 g (10-25th percentile) as the first child of non-consanguineous Korean parents. She had complained of repetitive tingling sensation or muscle contractions in limbs from age four, the frequency of which increased after exercise (for example, when riding a bicycle). At five years of age, she had first visited our emergency room for sustained stiffness, pain, and severe weakness of both legs after riding a bicycle. On laboratory testing during episodes of weakness at the emergency room, significant elevation of serum creatine kinase (CK) levels (537 IU/L; normal range: 26-140 IU/L) and elevation of serum potassium (K^+) levels (5.4 mmol/L; normal range: 3.5-5.3 mmol/L) were observed. Other laboratory tests, including a complete blood count, chemical test panel, blood gas analysis, and urinalysis, were normal. On examination, she showed hand grip myotonia. Initially, she was clinically considered in the differential diagnosis of myotonic dystrophy. Molecular diagnostic tests using Southern blot were used to check the *DMPK* (MIM#605377) and *CNBP* (MIM# 116955) genes for myotonic dystrophy and normal results were obtained. The patient's abnormal CK and K^+ levels returned to normal levels on follow-up check-up after 1 month. At 6 years of age, nerve conduction studies were done on the right upper and bilateral lower extremities, the results of which were normal. We attempted to perform electromyography; however, we were unsuccessful due to non-cooperation.

Patient 2 (father, II-2)

The patient's 37-year-old father presented symptoms of paramyotonia, especially on the upper limbs and eyelid, 1-2 times per month since childhood, the intensity and frequency of which did not increase with age. The father had an episodic prolonged stiffness with weakness at childhood after eating oriental melon, a potassium-rich fruit. His stiffness and episodic weakness did not cause significant disability and functional limitations, but he felt uncomfortable with eyelid closing paramyotonia, which worsened in winter after washing in the morning. On examination, he showed fluctuating hand grip myotonia. Percussion myotonia was not prominent. Electromyography performed on the right upper and lower extremities revealed abnormal features of systemic spontaneous myotonia-like discharges, myopathic motor unit potentials, and abnormal early recruitment, predominantly in proximal limb muscles.

Patient 3 (uncle, II-1)

The patient's uncle was also afflicted with paramyotonia on the eyelid and limbs. However, his daily life and general athletic ability were not limited by his condition. Episodes of stiffness did not worsen with age.

Genetic results

Genomic DNA was extracted from peripheral blood leukocytes of four family members (patients: II-2 and III-1; controls: I-2 and II-3). WES was performed for the proband (III-1) and her father (II-2). SureSelect Human All Exon V5 (Agilent Technologies) was used for library preparation, and sequencing was performed on the Illumina NextSeq500 platform (Illumina Inc., San Diego, USA) generating 2 × 150 bp paired-end reads at GC Genome (Yongin, Republic of Korea). Alignment of sequence reads and indexing of the reference genome (hg19), and variant calling were conducted with a pipeline based on GATK Best Practice. The heterozygous in-frame insertion c.3911_3912+1dupAGA at the boundary between exon 21 and intron 21 of *SCN4A* was identified based on the reference sequence NM_000334.4. This variant was classified as a variant of uncertain significance (VUS) with PM4 (in-frame insertion), PP1 (cosegregation in the family), and PP4 (highly specific phenotype), according to the guidelines of the American College of Medical Genetics and Genomics.[11] The identity and heterozygosity of this mutation were confirmed by PCR amplification and direct Sanger sequencing of the affected (II-2 and III-1)

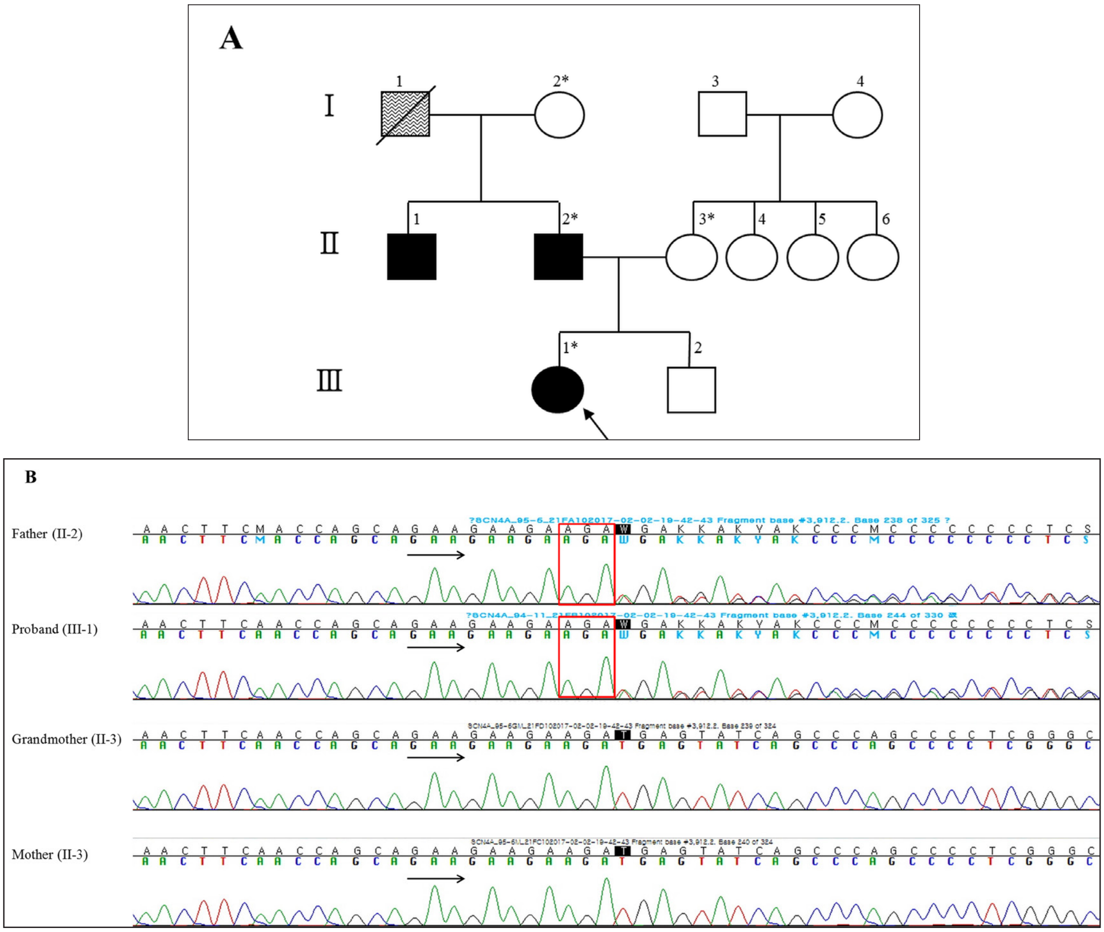

Figure 1. (A) Pedigree of the family with paramyotonia congenita. The black arrow indicates the proband patient III-1. Darkened symbols represent the affected members. The wave symbol denotes a putative patient who could not be checked clinically. Asterisks indicate sampled individuals. (B) Sanger sequencing confirmed the mutation in patients (II-2 and III-1) and the wild type genotype in unaffected family members (I-2

and unaffected family member samples (I-2 and II-3) (Fig. 1B). No pathological mutations in any other known genes associated with nondystrophic myotonia or periodic paralysis, including *CLCN1*, *CACNA1S*, *KCNJ2*, and *KCNJ18*, were detected.[4]

DISCUSSION

Skeletal muscle channelopathies, consisting of sodium, calcium chloride, and potassium channel disorders, are a rare heterogeneous spectrum of diseases with significant clinical overlap that can be challenging to diagnose.[1] Nav1.4 is a large protein composed of four homologous domains (DI–DIV), each consisting of six transmembrane segments (S1–S6). Segments S5 and S6 of each domain form a single ion-conducting pore, while S1–S4 form the voltage sensing domains. Of the six clinical phenotypes associated with NaV1.4 mutations (myotonia, PMC, hyperkalemic periodic paralysis, hypokalemic periodic paralysis, congenital myasthenia, and congenital myopathy), the myotonia, PMC, and hyperkalemic periodic paralysis are caused by NaV1.4 gain-of-function defects with an autosomal inheritance pattern. Hypokalemic periodic paralysis arise from moderate loss-of-function changes due to a *SCN4A* missense mutation, especially in S4 segments of the voltage sensor domains.[3] The very rare syndrome of congenital myasthenia and congenital myopathy was attributed to a loss-of-function mechanism with recessive inheritance.[3] The heterozygous in-frame insertion c.3911_3912+1dupAGA in our family was classified as a VUS with a dominant cosegregation and a definite PMC phenotype. In addition, based on WES data, other possible genetic disorders were excluded. Interestingly, the intron 21 of *SCN4A* is a very rare class of introns, known as the AT-AC type II, which are self-splicing ribozymes, and the splicing defects caused by the *SCN4A* intron 21 mutation have been confirmed.[12-14] Kubota et al. reported a patient with myotonia caused by a deletion/insertion located in intron 21 of *SCN4A* showing a gain-of-function defect.[14] AT-AC type II introns are extremely rare, and most of them are found in members of the NaV gene family.[14] Thus, mutations in these introns are expected to be associated with channelopathies.[14] In the present study, the in-frame insertion was located at the boundary intron 21 of *SCN4A*, suggesting that it may alter the production of gain-of-function changes. We suggest that the heterozygous in-frame insertion c.3911_3912+1dupAGA, classified as VUS in *SCN4A*, is a pathogenic variant responsible for the phenotype observed in our family with PMC.

Patients with PMC/hyperkalemic periodic paralysis typically complain of muscle stiffness, which can present as focal weakness from infancy or early childhood. However, pediatric patients who cannot explain their exact symptoms are especially more difficult to diagnose. Furthermore, most patients, even those without muscle disease, feel that their muscles do not function well when they are cold. Especially, cases of pediatric patients with an initial chief complain of weakness with significant elevation of CK levels and myotonia may be confused with myotonic dystrophy at the first visit, as in the case of our proband. Despite having nondystrophic myotonia, several patients with PMC show transient but mild elevation in CK levels during the attack of weakness.[1,6] Careful consideration of all channelopathies is important for accurate early diagnosis when evaluating pediatric patients, even those with high CK levels.

In summary, we have identified with WES a family with a rare PMC caused by a novel heterozygous in-frame insertion at the boundary of AT-AC type II intron (intron 21) of *SCN4A*. Our study provides additional information that expands our understanding and delineation of skeletal sodium channelopathies.

DISCLOSURE

Ethics: The Institutional Review Board of the Nowon Eulji Medical Center (IRB #2014-06-007-001) approved the use of human clinical materials and blood in this study. Written informed consent for genetic testing was obtained from patients and family members before participation.

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Conflict of interest: None

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