

## Clinical Diversity of *SCN4A*-Mutation-Associated Skeletal Muscle Sodium Channelopathy

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**Background and Purpose** Mutations of the skeletal muscle sodium channel gene *SCN4A*, which is located on chromosome 17q23-25, are associated with various neuromuscular disorders that are labeled collectively as skeletal muscle sodium channelopathy. These disorders include hyperkalemic periodic paralysis (HYPP), hypokalemic periodic paralysis, paramyotonia congenita (PMC), potassium-aggravated myotonia, and congenital myasthenic syndrome. This study analyzed the clinical and mutational spectra of skeletal muscle sodium channelopathy in Korean subjects.

**Methods** Six unrelated Korean patients with periodic paralysis or nondystrophic myotonia associated with *SCN4A* mutations were included in the study. For the mutational analysis of *SCN4A*, we performed a full sequence analysis of the gene using the patients' DNA. We also analyzed the patients' clinical history, physical findings, laboratory tests, and responses to treatment.

**Results** We identified four different mutations (one of which was novel) in all of the patients examined. The novel heterozygous missense mutation, p.R225W, was found in one patient with mild nonpainful myotonia. Our patients exhibited various clinical phenotypes: pure myotonia in four, and PMC in one, and HYPP in one. The four patients with pure myotonia were initially diagnosed as having myotonia congenita (MC), but a previous analysis revealed no *CLCN1* mutation.

**Conclusions** Clinical differentiating between sodium-channel myotonia (SCM) and MC is not easy, and it is suggested that a mutational analysis of both *SCN4A* and *CLCN1* is essential for the differential diagnosis of SCM and MC.

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**Key Words** myotonic disorders, familial periodic paralyses, *SCN4A*.

## Introduction

The skeletal muscle sodium channel comprises a principal pore-forming and voltage-sensing subunit (the alpha subunit), which is associated with an accessory beta-1 subunit. Its alpha subunit is encoded by the gene *SCN4A*, which is located on chromosome 17q23-25,<sup>1</sup> comprises 24 exons with a 5.5-kb open reading frame,<sup>2</sup> and is associated with various neuromuscular disorders. The beta-1 subunit has not been reported to be linked to any human disease.

More than 40 different types of *SCN4A* mutation have been identified, most of which are single-base substitutions producing missense mutations.<sup>3</sup> In contrast to the chloride and

calcium channelopathies, which produce relatively uniform phenotypes, *SCN4A* mutations produce several clinically distinct skeletal muscle disorders including hyperkalemic periodic paralysis (HYPP), paramyotonia congenita (PMC), potassium-aggravated myotonia (PAM), hypokalemic periodic paralysis, and congenital myasthenic syndrome.<sup>4-8</sup> Previous studies have also shown that certain *SCN4A* mutations are associated with a specific phenotype, and it has been suggested that the mutation-specific differences in aberrant channel gating behavior underlies the genotype-phenotype correlation in skeletal muscle sodium channelopathy.<sup>9</sup>

In this context, we studied six Korean patients with *SCN4A* mutations who showed various clinical types of nondystro-

phic myotonia and periodic paralysis syndromes. We reasoned that this would help to clarify the genotype-phenotype correlations in Korean patients with this group of disorders. We therefore performed a mutational analysis of *SCN4A* in these patients, and analyzed their clinical features in detail.

## Methods

### Subjects

Six unrelated Korean patients with nondystrophic myotonia and periodic paralysis syndrome were included in the study. The patients comprised one patient with HYPP, one with PMC, and four with pure myotonia. Mutation of *CLCN1* had previously been excluded in all four patients with the pure myotonia phenotype, by full sequence analysis of its coding regions.<sup>10</sup> The control group comprised 100 healthy Koreans. All of the patients and controls provided written informed consent to participate in this study, which was reviewed and approved by the Pusan National University Hospital Institutional Review Board.

### Clinical evaluation

A detailed clinical history was taken from all patients, and all underwent a standard neurological examination. History-taking revealed several clinical data, including age of onset, first clinical symptom, the most disabling symptom at the time of presentation, and family history. On neurological examination, special notice was taken of the presence of muscle atrophy or hypertrophy, distribution of muscle weakness (if any), status of the deep-tendon reflexes, and types of maneuver that provoke myotonia. All patients submitted to electrodiagnostic studies, including routine nerve conduction studies and needle electromyography (EMG) from at least two different muscles in each extremity. Routine laboratory tests were performed, including complete blood count, liver and renal function tests, thyroid function tests, blood glucose, electrolytes, serum creatine kinase levels, chest X-ray, and electrocardiogram. Muscle biopsy procedures were performed in three pa-

tients. The biopsied muscle samples were flash frozen in isopentane prechilled with liquid nitrogen and then processed for routine histochemical reactions including hematoxylin-eosin, modified Gomori trichrome, NADH, and ATPase stains.

### DNA analysis

The *SCN4A* mutations were identified by direct sequence analysis of whole coding regions of the gene. DNA was extracted from the patients' anticoagulated whole blood, and 21 sequence specific primer pairs covering the entire coding region of *SCN4A* were subjected to PCR amplification. Primer sequence data and information on the PCR conditions are available upon request.

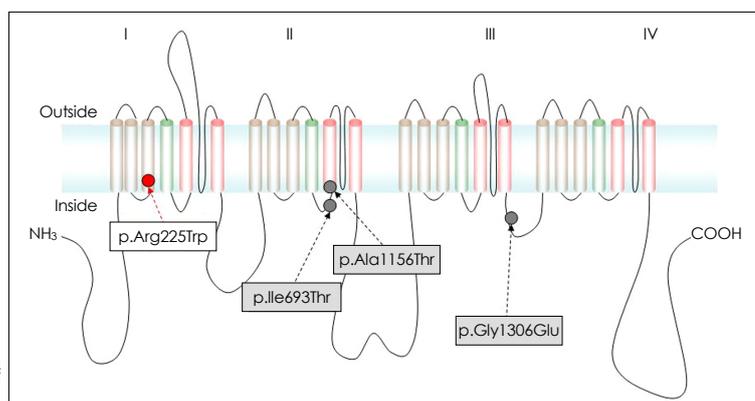
The amplified PCR products were separated on 2% agarose gels, purified, cycle-sequenced with PCR primers using the BigDyeTerminator Sequencing Kit (PE Applied Biosystems, Foster, CA, USA), and electrophoresed using an ABI PRISM 3730XL DNA analyzer (PE Applied Biosystems).

PCR-restriction fragment length polymorphism (RFLP) was conducted on patient 3 and the 100 normal controls to confirm the presence of the novel c.673C>T (p.R225W) mutation found in this patient and to establish that it is not a benign polymorphism. The mutation was identified using the restriction enzyme *Aci* I to discriminate the mutated allele from normal alleles. The primer pair 5'-TGCACTGTCCT TCCCAACCC-3' (forward) and 5'-GCCTCTCAAACGCC CATCCT-3' (reverse) was used for the PCR reaction. Since the mutated allele will lose the normal *Aci* I restriction site, a heterozygous mutation of c.673C>T will produce three DNA bands at 472, 245, and 227 bp, while normal alleles will produce bands at only 245 and 227 bp. All enzyme digestion reactions were conducted at 37°C for 2 hours, and the electrophoresis was performed on 2% agarose gel.

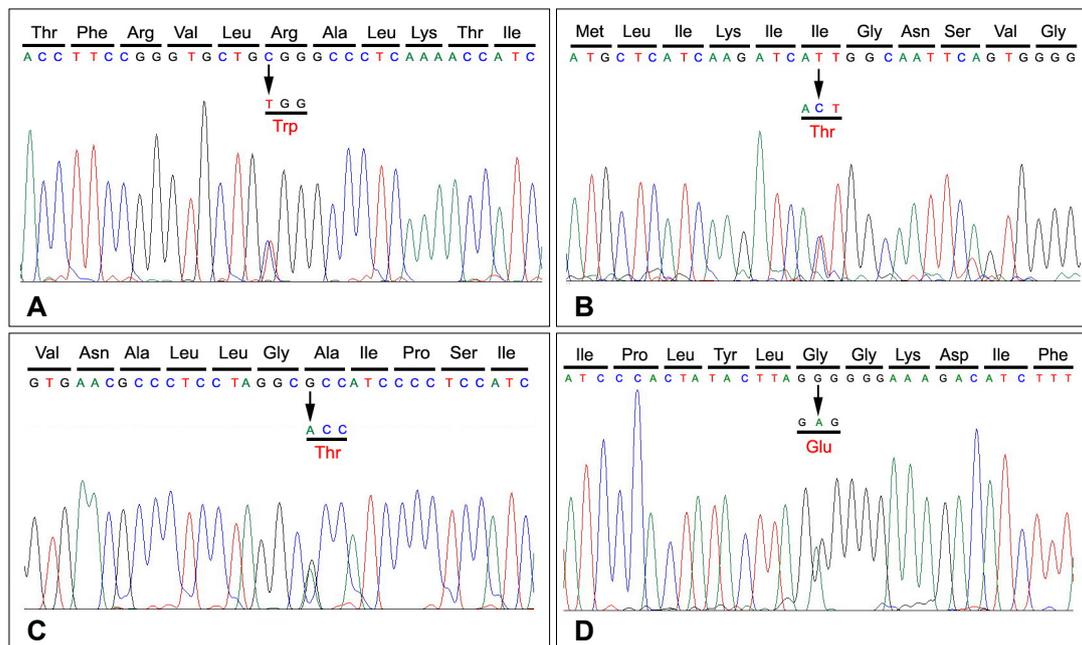
## Results

### *SCN4A* mutations

The sequence analysis revealed four different mutations in-



**Fig. 1.** Membrane-folding model of the sodium channel alpha subunit and locations of the missense mutations identified in the present study. A novel mutation, p.Arg 225Trp, is located at the transmembrane S3 segment of domain I.



**Fig. 2.** Chromatograms of the patients. A: A novel mutation in exon 5 was identified in patient 3. A substitution of cytosine to thymidine at position 673 changes the codon for arginine at position 225 into tryptophan. B: A substitution of thymidine to cytosine at position 2078 in patient 4 changes the codon for isoleucine at position 693 into threonine. C: A substitution of guanine to adenine at position 3466 in patients 2 and 5 changes the codon for alanine at position 1156 into threonine. D: A substitution of guanine to adenine at position 3917 in patients 1 and 6 changes the codon for glycine at position 1306 into glutamate.

**Table 1.** *SCN4A* mutations identified in the study

Change in nucleotide	Change in codon	Change in amino acid	Site	Patient numbers
c.673C>T	CGG>TGG	p.R225W	Exon 5	3
c.2078T>C	ATT>ACT	p.I693T	Exon 13	4
c.3466G>A	GCC>ACC	p.A1156T	Exon 22	2, 5
c.3917G>A	GGG>GAG	p.G1306E	Exon 21	1, 6

cluding one that has not been described previously. The types and locations of the identified mutations are summarized in Figs. 1 and 2, and Table 1. A novel missense mutation, c.673C>T, was identified in exon 5 from patient 3, which is expected to change a codon for arginine into tryptophan at position 225 (p.R225W). This is considered a pathogenic mutation because 1) we were unable to observe the same alteration in any of the 100 normal controls using PCR-RFLP analysis (Fig. 3A), and 2) the mutated amino acid arginine at position 225 is highly conserved between different species in multiple alignment analysis of the amino acid sequence (Fig. 3B).

Two patients (patients 2 and 5) shared a c.3466G>A (p.A1156T) mutation in exon 19, and another mutation in exon 22 (c.3917G>A, p.G1306E) was shared by two other patients (patients 1 and 6). In patients 4, the mutation c.2078T>C (p.I693T) was identified in exon 13 (Table 1, Figs. 1 and 2).

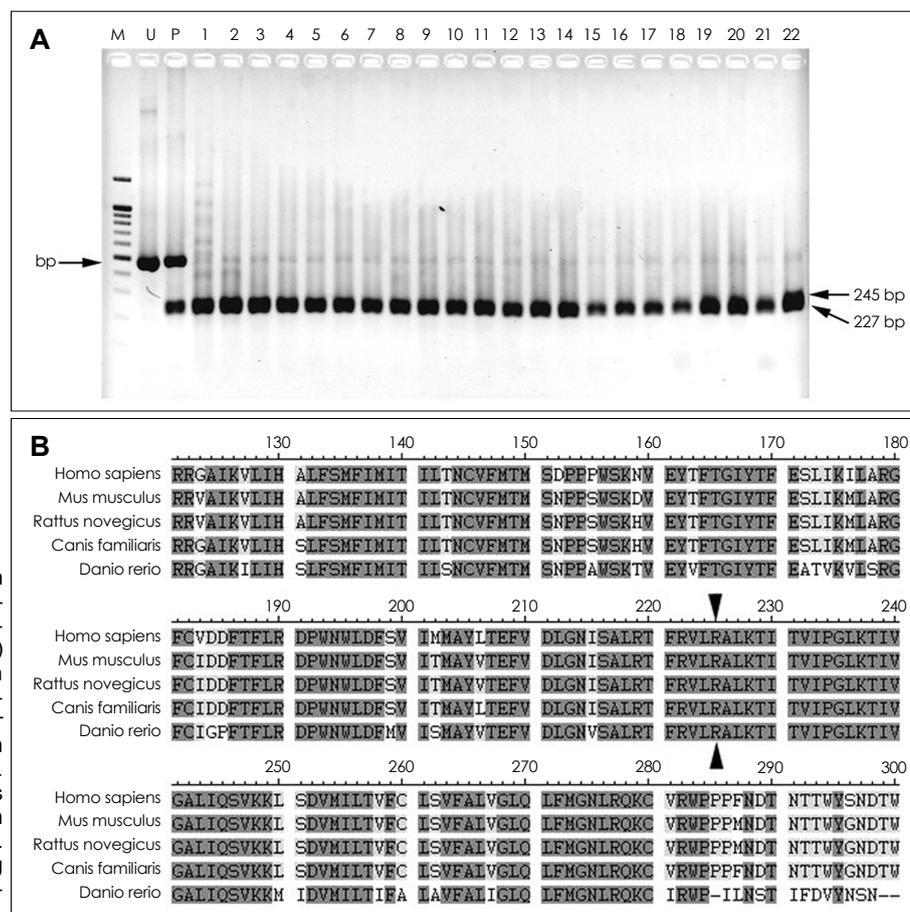
### Clinical features

The clinical features of the six patients are summarized in

Table 2. They comprised six men with ages of onset from birth to 30 years. The four patients with the pure myotonia phenotype (patients 1, 2, 3, and 6) had myotonia with the warm-up phenomenon, which initially affected predominantly the lower extremities. In this group of patients, muscle stiffness was provoked by voluntary contraction of the skeletal muscles after a period of rest, and typical myotonic discharges were observed on EMG. Patient 4 presented with myotonia that worsened by repetitive exercise and cold exposure, and was classified as having the PMC phenotype. EMG in this patient also revealed prominent myotonic discharges. Periodic weakness was the main symptom in patient 5, together with elevated serum potassium levels. Needle EMG also revealed myotonic discharges.

## Discussion

We have identified four different *SCN4A* mutations (one of which has not been reported previously) in six unrelated Korean patients with nondystrophic myotonia and periodic paralysis syndrome. A novel heterozygous missense mutation c.673C>T (p.R225W) was found in patient 3, who presented with mild nonpainful pure myotonia (Table 1 and 2). This mutation is located at the cytoplasmic side of transmembrane S3 segment of domain I (DI/S3; Figs. 1 and 2), and is noteworthy because most of the previously reported *SCN4A*



**Fig. 3. A:** The results of PCR-restriction fragment length polymorphism using restriction enzyme *Aci I* for the identification of the novel c.673C>T (p.Arg225Trp) mutation. No similar digestion pattern was observed among 100 normal control chromosomes. M, 100-bp molecular marker; U, undigested PCR product from patient 3; P, patient 3; numbers, control. **B:** Multiple alignment of the homologous sodium channel alpha subunit protein between different species of eukaryotes. The mutated protein in patient 3 (p.Arg 225Trp, arrowheads) is highly conserved between species.

**Table 2.** Summary of patients' clinical findings

Patient number	Gender	Age (years)	Age at first symptom (years)	Affected family	Clinical features	Phenotype	SCN4A mutation
1	Male	21	Birth	(-)	Painful myotonia with warm-up phenomenon involving the face and extremities; excessive sweating in the hands and soles; intermittent mild subjective generalized weakness; generalized muscular hypertrophy; no cold sensitivity	Pure Myotonia	p.G1306E
2	Male	33	30	(-)	Relatively mild, nonpainful myotonia with warm-up phenomenon involving the posterior legs; no muscle hypertrophy; no cold sensitivity	Pure myotonia	p.A1156T
3	Male	21	11	(-)	Nonpainful myotonia with warm-up phenomenon involving the lower extremities; myotonia is provoked by cold exposure and associated with transient weakness; no muscle hypertrophy	Pure myotonia	p.R225W
4	Male	7	5	(-)	Myotonia worsened by repetitive exercise; myotonic stiffness in the extremities; generalized muscular hypertrophy; no transient weakness	PMC	p.I693T
5	Male	28	27	(-)	Periodic profound generalized muscle weakness lasting a few days associated with hyperkalemia; intermittent short-lasting muscle weakness after strenuous exercise; no clinical myotonia; generalized weakness was induced by potassium loading	HYPP	p.A1156T
6	Male	32	Childhood	(+)	Nonpainful myotonia with warm-up phenomenon; affects the face and extremities; generalized muscular hypertrophy; no transient weakness; no cold sensitivity; a son and a daughter are also affected	Pure myotonia	p.G1306E

PMC: paramyotonia congenita, HYPP: hyperkalemic periodic paralysis.

mutations are clustered in domains III or IV of the protein. Furthermore, it is the most proximal mutation ever reported in *SCN4A*. Thus far only two mutations affecting domain I have been reported.<sup>11,12</sup> This mutation is also remarkable because there is only one previous report of an *SCN4A* mutation affecting the S3 segment (p.L1433R).<sup>13</sup>

Another mutation, c.2078T>C (p.I693T), was identified in patient 4, who exhibited a typical PMC phenotype. This mutation is located at the cytoplasmic link between S4 and S5 of DII, and has been reported previously in patients with PMC (Table 1, Figs. 1 and 2).<sup>14</sup>

The c.3466G>A (p.A1156T) mutation was shared by two of our patients who exhibited completely different phenotypes (Table 1, Figs. 1 and 2): while patient 2 had pure myotonia affecting the lower extremities, with warm-up phenomenon, patient 5 manifested as HYPP without clinical history or signs of myotonia. Although the mutation p.A1156T has been described previously both in patients with HYPP and PMC,<sup>15</sup> it has never been associated with the pure myotonia phenotype, as in our patient 2. This phenotypic variability observed in individuals harboring the same *SCN4A* mutation strongly suggests that the genetic background-and perhaps other epigenetic factors-influences the clinical expression of particular mutations.

The c.3917G>A (p.G1306E) mutation, located at the cytoplasmic linker between domains III and IV (ID3-4), was also found in two patients, in both of whom it manifested as pure myotonia (patients 1 and 6). This mutation had been reported in patients with PAM and has been functionally characterized (Table 1, Figs. 1 and 2).<sup>16,17</sup>

Our study shows that *SCN4A* mutations produce different phenotypes of nondystrophic myotonia and periodic paralysis syndrome. We have also shown that certain *SCN4A* mutations may not be associated with specific clinical phenotypes, suggesting poor genotype-phenotype correlations in skeletal muscle sodium channelopathy.

In sodium channelopathy, there is a group of patients who manifest with pure myotonia. These patients exhibit one of several clinical phenotypes including myotonia fluctuans, myotonia permanens, and acetazolamide-responsive myotonia.<sup>18</sup> They are distinct from those with PMC by the absence of paradoxical myotonia and cold sensitivity. Since many such patients have myotonia that becomes much more pronounced after the ingestion of potassium-rich food,<sup>21</sup> the condition has been collectively called PAM. However, some patients with *SCN4A*-related pure myotonia do not exhibit such potassium sensitivity,<sup>11,19</sup> and thus the condition may be more appropriately labeled sodium-channel myotonia. The myotonia in this group of patients is very similar to that observed in those with myotonia congenita (MC), and clinical differenti-

ation may not be possible. The four patients with pure myotonia in our study had also initially been considered as having MC, but they were finally shown to have *SCN4A* mutations. A recent study involving the screening of *SCN4A* and *CLCN1* mutations in large numbers of patients with nondystrophic myotonia found that 20% of patients who had been clinically diagnosed as MC had *SCN4A* mutations.<sup>20</sup>

Our study also showed that sodium channelopathy comprises highly variable clinical phenotypes, and that the genotype-phenotype correlation is not unequivocal. Although careful clinical evaluation is of great help, we suggest that a definite molecular diagnosis using sequence analysis of both *SCN4A* and *CLCN1* is essential for the differential diagnosis of sodium channelopathy from other conditions, especially when a patient presents with pure myotonia.

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