Voltage sensor charge loss accounts for most cases of hypokalemic periodic paralysis

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ABSTRACT

Background: Several missense mutations of *CACNA1S* and *SCN4A* genes occur in hypokalemic periodic paralysis. These mutations affect arginine residues in the S4 voltage sensors of the channel. Approximately 20% of cases remain genetically undefined.

Methods: We undertook direct automated DNA sequencing of the S4 regions of *CACNA1S* and *SCN4A* in 83 cases of hypokalemic periodic paralysis.

Results: We identified reported *CACNA1S* mutations in 64 cases. In the remaining 19 cases, mutations in *SCN4A* or other *CACNA1S* S4 segments were found in 10, including three novel changes and the first mutations in channel domains I (*SCN4A*) and III (*CACNA1S*).

Conclusions: All mutations affected arginine residues, consistent with the gating pore cation leak hypothesis of hypokalemic periodic paralysis. Arginine mutations in S4 segments underlie 90% of hypokalemic periodic paralysis cases. ***Neurology*® 2009;72:1544–1547**

GLOSSARY

HypoPP = hypokalemic periodic paralysis.

Hypokalemic periodic paralysis (HypoPP) is an important autosomal dominant muscle channelopathy with onset in the first or second decade. It is characterized by episodes of flaccid paralysis in association with low serum potassium. Typically, attacks occur during the night or in the early morning and can last from several hours to days. Point mutations in *CACNA1S* or *SCN4A*, which encode the skeletal muscle voltage-gated calcium and sodium channels, associate with HypoPP.[1-10] However, in most studies at least 20% of cases remain genetically undefined.[8,11,12]

Sodium and calcium channels have homologous pore-forming α subunits, each containing four domains containing six transmembrane segments. Two common mutations in *CACNA1S* and several additional mutations in *SCN4A* have been reported in HypoPP. All of these mutations affect arginine residues in S4 segments that contribute to voltage sensing. Functional characterization of known mutations suggests HypoPP is associated with loss of normal channel function. However, evidence also exists that at least some of these mutations allow an abnormal cation flux to pass through the aqueous omega pore that lines the voltage sensor.[13,14] The full spectrum of HypoPP mutations has not been defined. In particular, it remains to be determined whether the gating pore cation leak hypothesis accounts for all cases. We therefore undertook an extensive analysis of all voltage sensors in *SCN4A* and *CACNA1S* in 83 clinically definite HypoPP cases.

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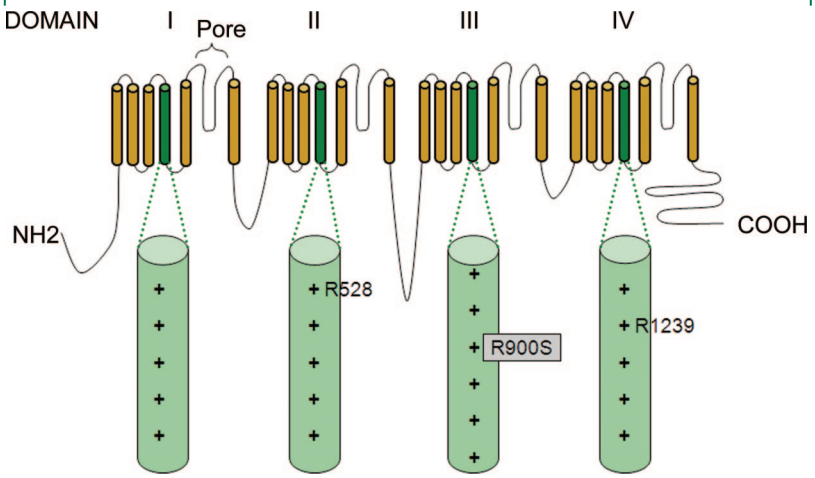
Figure 1 **Diagrammatic representation of the alpha subunit of *CACNA1S* with S4 segments and arginine residues known to be mutated in hypokalemic periodic paralysis highlighted**



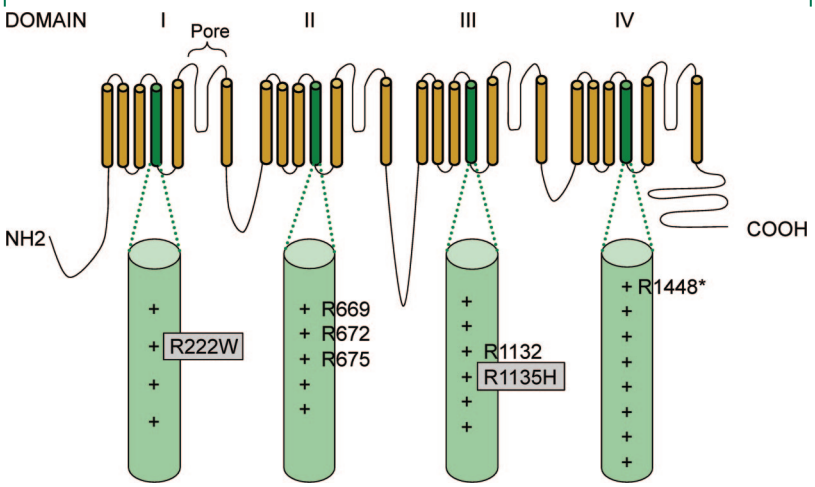
The novel arginine substitution reported here is boxed in gray. + indicates the number of positively charged residues in each S4 segment.

METHODS We examined 83 cases referred to our national patient referral center for skeletal muscle channelopathies with a firm clinical diagnosis of HypoPP. All patients gave written informed consent for the DNA analysis in this study, which had ethics committee approval from the National Hospital for Neurology and Neurosurgery & Institute of Neurology Joint Research Ethics Committee. We first undertook direct automated sequencing of *CACNA1S* for the common mutations described at amino acid positions R528 and R1239. For cases who were negative for these mutations we performed direct automated DNA sequencing of all regions of *CACNA1S* and *SCN4A* coding for the remaining S4 segments (exons 4 and 21 of *CACNA1S* and exons 5, 12, 13, 18, and 24 of *SCN4A*).

PCR reactions to amplify each exon of *CACNA1S* using the following reagents and conditions were performed: a 25 μL reaction contained 200 ng genomic DNA, 5 μL of 10 x PCR buffer without $MgCl_2$ (Applied Biosystems), 4 μL of 25 mM $MgCl_2$, 5 μL of 2 mM dNTPs, 15 pmol of each primer (forward and reverse), and 2.5 units of AmpliTaq Gold polymerase (Applied Biosystems). PCR reactions to amplify each exon of *SCN4A* using the following reagents and conditions were additionally performed: a 50 μL reaction contained 200 ng genomic DNA, 10 μL of 5 x PCR buffer (Promega), 5 μL of 2 mM dNTPs, 10 pmol of each primer (forward and reverse), and 1.0 unit of GoTaq polymerase (Promega).

Cycling conditions consisted of an initial denaturing step of 95°C for 10 minutes followed by 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension step of 72°C for 7 minutes. Samples were sequenced (bidirectionally) using the ABI Big Dye Terminator Sequencing Kit version 1.1, M13 universal primers, and an ABI Model 3730xl Automated DNA Sequencer. Data were analyzed using version 2.5 SeqScape Analysis software (ABI). See supplemental data on the *Neurology*® Web site at www.neurology.org for oligonucleotide primer sequences.

Figure 2 **Diagrammatic representation of the alpha subunit of *SCN4A* with S4 segments and arginine residues known to be mutated highlighted**



Novel arginine mutations reported here are boxed in gray. + indicates the number of positively charged residues in each S4 segment. *Note substitutions of R1448 cause a phenotype of paramyotonia congenita.

RESULTS In 64 out of 83 cases of HypoPP, we identified previously reported mutations: either R528G/H (25 cases) or R1239G/H (39 cases) in *CACNA1S*. Of the remaining 19 cases, 10 harbored additional mutations in other S4 segments of *CACNA1S* or *SCN4A*. Six of these were positive for mutations that have been reported previously, namely the substitutions R672C/H/S and R1132Q in *SCN4A*. In the other four cases, we found three novel mutations, all of which neutralized arginine residues in S4 segments. These mutations were absent from 240 control chromosomes. One mutation was in the S4 segment of domain III (DIII/S4) of *CACNA1S*: c.2700G>T; p.R900S (figure 1). The other two mutations neutralized arginine residues in S4 segments of *SCN4A*; the first in DI c.664C>T; p.R222W and the second in DIII c.3404G>A; p.R1135H (see figure 2 and the table for details and figure e-1 for electropherograms). R222W was found in two apparently unrelated kindreds.

All four individuals with the new mutations had typical HypoPP phenotype. Age at onset of attacks of muscle paralysis was in the second decade, with attacks usually occurring at night or in the early morning, and associated with low serum potassium levels or with provocative factors that would induce low serum potassium. The frequency and severity of attacks were not different from those in individuals with previously reported mutations. To our knowledge, these individuals received no pharmacologic therapy or potassium supplementation only.

DISCUSSION Our results expand the spectrum of S4 segment arginine mutations which cause HypoPP. These data add new genetic evidence to support the hypothesis that loss of positive charge in S4 voltage sensors is important in the molecular pathogenesis of this muscle channelopathy. We iden-

Table Details of all reported S4 segment mutations in *CACNA1S* and *SCN4A*

Gene	S4 segment mutation	Loss of positive charge	Residue position in the S4 segment	Domain	Phenotype
CACNA1S	R528G/H†	Yes	R1	DII	Hypokalemic periodic parlaysis
CACNA1S	R900S*	Yes	R3	DIII	Hypokalemic periodic paralysis
CACNA1S	R1239G/H†	Yes	R2	DIV	Hypokalemic periodic paralysis
SCN4A	R222W*	Yes	R2	DI	Hypokalemic periodic parlaysis
SCN4A	R669H	Yes	R1	DII	Hypokalemic periodic parlaysis
SCN4A	R672C/G/H/S†	Yes	R2	DII	Hypokalemic periodic parlayis
SCN4A	R675G/Q/W	Yes	R3	DII	Potassium sensitive normokalemic periodic paralysis
SCN4A	R1132Q†	Yes	R3	DIII	Hypokalemic periodic paralysis
SCN4A	R1135H*	Yes	R4	DIII	Hypokalemic periodic paralysis
SCN4A	R1448C/H/L/P/S	Yes	R1	DIV	Paramyotonia congenita
SCN4A	G1456E	No		DIV	Paramyotonia congenita

*Novel mutations identified in this study.
†Other mutations identified in our cohort.
R1 represents the outermost arginine and R4 the innermost. Each arginine residue is separated from the next by two other amino acids. All hypokalemic periodic paralysis mutations cause loss of positive charge.

tified new point mutations in domain III of *CACNA1S* and in domains I and III of *SCN4A*. There are no previous reports of mutations in domain III of *CACNA1S* or in domain I of *SCN4A* in HypoPP. The present findings indicate that loss of positively charged residues in S4 segments in these domains can also cause HypoPP. Overall, the 74 genetically characterized HypoPP cases reported here argue that arginine mutations in the voltage sensors of both channels are the overwhelmingly most important cause of HypoPP. The present results, furthermore, suggest that screening for arginine mutations in S4 segments should yield a genetic diagnosis in approximately 90% of cases of HypoPP. We propose that analysis for S4 substitutions in domains II and IV of *CACNA1S* should be followed by analysis of the S4 regions in domains I, II, and III of *SCN4A* and in domain III of *CACNA1S*.

It remains unclear how S4 mutations lead to the HypoPP phenotype. Previous functional studies of the effect of the *CACNA1S/SCN4A* S4 mutations on the gating properties of the main pore all pointed to a loss of function defect. However, such a loss of function mechanism does not readily explain the prolonged depolarization of the sarcolemmal membrane associated with attacks of paralysis[15] or the episodic occurrence of hypokalemia. The individual contribution of each S4 segment to channel gating is not yet fully understood. Current evidence suggests that the S4 segments of DIII and IV of Nav1.4 play a more significant role in fast inactivation.[16] It is therefore of interest to note that replacement of the outermost arginine in DIV/S4 of *SCN4A* does not produce a HypoPP phenotype but rather one of paramyotonia congenita.[17] In contrast, replacement of the third outermost arginine in DII/S4 of *SCN4A* produces a potassium-sensitive periodic paralysis.[18] Only one non-arginine substitution has been described in an S4 segment of either channel, G1456E[19] [*SCN4A*] also in domain IV, which resulted in a phenotype of paramyotonia congenita (table). Our genetic evidence indicates that loss of positive charge in the voltage sensor in domains I, II, and III of *SCN4A* and in domains II, III, and IV in *CACNA1S* is important in the pathogenesis of HypoPP.

Two recent studies have suggested that a gating pore current may be important in the pathogenesis of HypoPP,[13,14] and our data are fully consistent with this hypothesis. In response to depolarizing voltages, S4 segments undergo a conformational change that moves these segments outwards through a gating pore (omega pore) which leads to the opening of the central pore of the channel (alpha pore). The outermost arginines in S4 occupy and occlude the narrowest part of the gating pore at the resting membrane potential while internal arginine residues occlude it at depolarized potentials. Recently, it was shown that in addition to disrupting the gating of the main pore (alpha pore), mutations that neutralize the two outermost arginines (R669/R672) in DII/S4 of Nav1.4 also generate a monovalent cation leak through the gating pore at hyperpolarized potentials.[13] A histidine substitution at R672 produces a proton specific pore leak whereas other amino acid substitutions at this position cause a nonselective cation leak. The leak current is thought to be mainly mediated by protons and could contribute to the pathophysiology of HypoPP, possibly by an accumulation of intracellular protons and disruption of the intracellular homeostasis of pH.[14] Consistent with the role of arginines in occluding the gating pore, a 10-fold larger leak current occurs with a glycine substitution compared with the histidine substitution at R663 in the rat isoform of NaV1.4 (comparable to R669 in the human isoform).[14] Our finding of additional mutations that affect S4 arginines adds to the possibility that these arginines play a central role in HypoPP. Furthermore, our findings expand the number of channel domains and the range of arginines affected, notably including arginines buried more deeply in the channel. Interestingly, in the Shaker potassium channel, replacing more intracellular arginine residues with histidine residues has been shown to produce proton leak currents at depolarized potentials in contrast to substitutions of outer arginines that generate leak currents at hyperpolarized potentials.[20] If the intracellular arginines that we have found lead to

similar changes in *SCN4A*, then patients with mutations affecting these residues may have leak currents at depolarized potentials rather than at rest. Functional characterization of our new mutations will be important for determining the pathophysiologic effect of these more intracellular arginine residues.

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