Mutations of SCN4A gene cause different diseases: 2 case reports and literature review

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SCN4A encodes the Nav1.4 channel and mutations in SCN4A lead to different ionic channelopathies. In this study, one sporadic individual of periodic paralysis, one paramyotonia family and 200 normal healthy controls are enrolled. Genomic DNA was extracted from peripheral blood leukocytes, followed by polymerase chain reaction and DNA sequencing of candidate genes, including SCN4A and CACNA1S. As a result, heterozygous mutations c.2024G>A (R675Q) and c.1333G>A (V445M) of gene SCN4A were identified in the hypokalemic periodic paralysis patient and the paramyotonia congenita family respectively. Both mutations were not detected in healthy controls. Compared with reported cases, patients with mutation R675Q usually do not present hypokalemic periodic paralysis but hyperkalemic or normokalemic periodic paralysis. The mutation V445M was first reported in Chinese patients with nondystrophic myotonias. In addition, we carried out literature review by summarizing clinical features of the 2 mutations and establish the genotype–phenotype correlations to provide guidance for diagnosis.

Introduction

The skeletal muscle sodium channel gene SCN4A is located on chromosome 17q23–25 and encodes the pore-forming α-subunit of skeletal muscle sodium channel.¹ Mutations in SCN4A are associated with various neuromuscular disorders that are labeled collectively as skeletal muscle sodium channelopathy. These disorders include hyperkalemic periodic paralysis (hyperPP, OMIM: 170500), hypokalemic periodic paralysis type 2 (hypoPP2, OMIM:613345), paramyotonia congenita (PMC, OMIM:168300), sodium channel myotonias (SCM, OMIM:608390) and congenital myasthenic syndrome (OMIM:614198).² SCN4A mutations leading to periodic paralysis or nondystrophic myotonia have been found located throughout every domain and segment of this channel and may interfere with muscle hyperexcitability or inexcitability by changing channel kinetics or functions, thereby producing changes in the micro- or macroscopic electrophysiological properties. HypoPP2 is a common autosomal dominant muscle channelopathy with onsets in the first or second decade. It is characterized by episodes of flaccid paralysis in association with low serum potassium.³ Interestingly, all the documented mutations for hypoPP2 are at arginine residues of the voltage sensor S4 of domains in SCN4A, especially in domain II.⁴ PMC is inherited autosomal-dominantly and is characterized by muscle stiffness exacerbated by cold and exertion. This disease is also caused by mutations in the sodium-channel gene SCN4A. Similar or even same mutations in the same gene (SCN4A) can cause distinct clinical disorders.⁵

In this study, we reported one sporadic individual of periodic paralysis and one autosomal-dominant paramyotonia family, both of whom were caused by mutations of SCN4A gene but having completely different phenotypes. In addition, we carried out literature review by summarizing reported clinical features of the SCN4A mutations and established the genotype–phenotype correlations to provide guidance for diagnosis.

Result

Case 1

A 25-year-old male had a history of transient quadripareisis since the age of 15 y He was unable to move his limbs on an empty stomach when he got up from a nap in the morning at school and fell to the ground after running a few steps. Physical examination showed weakness of limbs with absent or reduced reflexes. No sensory impairment was present. Laboratory investigation suggested hypokalemia (2.78mmol/L) at the time and muscle weakness relieved remarkably after one-week oral potassium supplement. Afterwards, such attack repeated and always relieved with one week’s potassium supplement. The patient experienced the most serious episode after he drank a beer in the...
evening and couldn’t walk in the next morning. The serum potassium was 3.22 mmol/L (ref., 3.5–5.1 mmol/L) during the attack of weakness. The creatine kinase was 276 IU/L, mildly higher than the normal range (ref., 22–269 IU/L). The symptoms relieved with 2 days’ oral potassium treatment. Attacks subsequently appeared about 3 to 4 times per month, and there was a tendency of spontaneous remission by rest or taking food at times. All the attacks occurred in the morning. Predisposing factors that increased the frequencies of episodes include poor sleep, emotional excitement or irritability, rest after strenuous exercise. There was no myotonia. Results of routine hematological and biochemical tests including complete blood cell count, serum thyroxin concentration test, and urinalysis were all within normal ranges. A long exercise test indicated that the compound muscle action potential (CMAP) amplitude was progressively decreased by 16%.

Case 2

The proband, a 20-year-old girl, complained of paroxysmal muscle stiffness that lasted for about 20 seconds since she was 7–8 y old. The symptoms were precipitated by movement, emotional stress and fatigue. The attacks often involved both legs without involuntary limb trembling. The myotonia symptom was more apparent and frequent in winter than summer. The frequency of episodes was usually less than 10 times per day. Her father, aunt and grandma also had similar symptoms when they were young, but the frequency and severity of the attacks relieved in their forties. And the myotonia symptoms rarely occurred in recent years. The creatine kinase level as well as other routine blood parameters was normal. There is no overt history of muscle symptoms aggravated by ingestion of potassium-rich foods. The neurological examination was normal. EMG revealed prominent myotonic discharges almost in both distal and proximal muscles of upper and lower limbs (Fig. 1B, Fig. 1C). To our knowledge, these individuals received no pharmacologic therapy so far.

Genetic analysis of SCN4A and CACNA1S genes in the proband was negative except SCN4A. A heterozygous mutation c.2024 G > A (p.R675Q) was identified in Case 1 (Fig. 1D). All the patients in the PMC family harbored mutation c.1333 G > A (V445M) whereas their normal relatives did not (Fig. 1D). Two mutations mentioned above were not detected in 200 healthy persons.

Discussion

The Nav1.4 channel is composed of a principal pore-forming and voltage-sensing subunit (α-subunit) that is associated with an accessory β1 subunit in the muscle. The α-subunit consists of 4 homologous domains (DI–DIV), and each domain contains 6 transmembrane segments designated S1–S6.6 Mutations that decrease Na+ channel function generally lead to periodic paralysis phenotypes, while increased channel activity, or non-inactivatable channels, results in non-dystrophic myotonia phenotypes. Mutations in SCN4A gene have been shown to be responsible for approximately 10% of cases of hypokalemic periodic paralysis.4 In this study, we identified one sporadic hypoPP case and a PMC family by screening SCN4A.

Recently, a number of studies7–9 have revealed that mutation R675Q in SCN4A mainly caused 2 kinds of disease: hyperPP and normoKPP (normokalemic periodic paralysis). In addition, more and more findings supported the notion that normoKPP was not a distinct disease and normokalemia could occur during attacks in patients with hyperPP.9 However, low blood potassium level was observed in our patient who carries a mutation at codon 675 of the gene SCN4A. The patient exhibited the characteristic clinical and laboratory features of hypoPP, including hypokalemia during attacks and effective remission of weakness after supplying potassium. Among the reported cases, no patients with mutation R675Q of gene SCN4A presented as hypoPP. Our study widened the phenotype spectrum of SCN4A. A summary of clinical features in patients carrying R675Q mutation of gene SCN4A was shown in Table 1.

As with hypoPP, the age of onset in R675Q mutation carriers is usually in the first or second decade of life, attacks may last from minutes to days. Weakness is precipitated not only by a carbohydrate load or rest after exercise but also by cortisone, coldness season alteration and potassium supplements.7–9 The latter was relatively rare predisposition in hypokalemic periodic paralysis. All patients had periodic paralysis and never exhibit myotonia in their lifetime. Among them, only one patient developed a persistent weakness but not his brother. With the exception of our patient, potassium could exacerbate weakness in 2 patients and sodium relief attacks in one patient. Interestingly, muscle pain and stiffness were presented in one family, but typical myotonic discharges were not observed on EMG. Patients with EMG evaluations were normal or a decrease in amplitude after short or long excise. In periodic paralysis, more extensive but often non-specific changes may be seen including vacuolar myopathy, tubular aggregates and myopathic changes. Muscle biopsy findings were nonspecific in the single patient. Acetazolamide is effective in all the patients that were treated. One patient was sensitive to potassium and thiazide diuretics simultaneously.8

The R675Q mutation was localized in the membrane spanning segment S4 of domain II, which is known to be involved in the voltage sensing of the channel. It is of interest that all documented mutations at codon 675 may result in loss of positive charge in the S4 segment of the sodium channel Nav1.4, thus making patients harboring the mutations susceptible to hypoPP due to abnormal gating pore currents.4 Another study also demonstrated that hypoPP mutations of arginine residues within the domain II create a cation leakage, which is not caused by a defect of the central pore but rather by the gating pore formed by the S4 segment.10 The R675Q mutation of SCN4A gene shifted activation in the depolarizing direction but had little effect on fast inactivation and its recovery. It dramatically impaired slow inactivation, prolonged slow inactivation and impeded recovery from slow inactivation, which suggested that R675Q mutation may be involved in fast activation and slow inactivation converge.11

PMC is an autosomal dominant disorder characterized by cold or exercise induced myotonia and caused by mutations in

Table 1.
Unlike the majority of myotonic conditions, the stiffness associated with PMC worsens rather than improves with repeated muscle contractions. The symptom of our patient was precipitated by movement and the attacks occurred more frequently and severe in winter. Thus we conducted the genetic test for SCN4A and identified a heterozygous mutation c.1333G >A (V445M), which is co-segregated with the disease in the family. Moreover, the valine residue at codon 445, which is located in the sixth transmembrane segment of domain I (DIS6), is completely conserved in voltage-dependent sodium channels of different species. Functional consequences by Masanori et al demonstrated that fast gating behavior was altered by V445M in a manner predicted to increase excitability: an impairment of fast inactivation increased the persistent Na current at 10 ms and activation had a hyperpolarized shift (4 mV). In contrast, slow inactivation was enhanced by V445M due to both a slower recovery and an accelerated entry rate (1.fold6). DIS6 is crucial for slow inactivation and enhanced slow inactivation cannot prevent myotonia. The above evidence all strongly implicated the mutation as the basis of this disorder.

Mutations in SCN4A gene have been reported to cause 3 clinically distinct myotonia diseases: PMC, hyperPP and SCM. Our proband never complained of exacerbation by potassium-rich foods or drugs and only performed transient periodic paralysis following stiffness triggered by cold or movement stimulus. Although phenotype of the proband was characterized by a mild myotonia with cold sensitivity and without common performance of paramyotonia, we tend to consider the probable diagnosis as paramyotonia. The diagnosis of PMC was further supported by EMG exploration. Besides typical muscle stiffness without weakness, in the first family carrying the V445M mutation reported by Rosenfeld et al. in 1997, all affected members were characterized by debilitating pain, which is especially severe in the intercostal muscles. Similarly, patients with V445M showed severe generalized painful myotonia with severe muscle hypertrophy in French-Canadians. Our patient and her affected relatives manifested no pain clinically, which is different from previous report. In 2009, Bradley reported extraocular muscle hypertrophy and myotonia, in addition to eyelid myotonia, in a father and son with a mutation in SCN4A gene (V445M). To sum up, phenotype of V445M mutation in SCN4A may have region and race specificity and shows genetic heterogeneity. A summary of clinical features in patients carrying V445M of gene SCN4A was shown in Table 1, too.

Myotonia usually improves after further exertion, known as the ‘warm-up’ phenomenon. The warm-up phenomenon was observed only in one family from Canada, suggesting it is not a
| Year | Present Family | [8] Family B | [7] Family 5 | [9] | Present Family | [15] | [17, 18] | [16] |
|------|---------------|--------------|--------------|-----|---------------|-----|----------|-----|
| Ethnicity | Chinese-Han | French | Chinese-Han | Korea | Ethnicity | Chinese-Han | American-Atlanta | Canadians | French-Canadian |
| SCN4A mutation | c.2024 G > A | c.2024 G > A | c.2024 G > A | c.2024 G > A | SCN4A mutation | c.1333 G > A | c.1333 G > A | pV445M | pV445M |
| Protein alteration | p.R675Q | p.R675Q | p.R675Q | p.R675Q | Protein alteration | p.V445M | p.V445M | p.V445M | p.V445M |
| Demographic data | | | | | Demographic data | | | | |
| Family history | | | | | Family history | | | | |
| Affected individuals | 1 | 5 | 2 | 3 | Affected individuals | 4 | 8 | 2 | NA |
| Patient | Proband | BIII2; BIII3 | 2 | 3 | Patient | 4 | Proband | Son; father | Proband |
| Gender | male | male; male | female(2); male | | Gender | female | male; male | | |
| Age at onset, year | 15 | 18; 16 | 13–14 | <10y | Age at onset, year | 7–8 | 16 | NA | NA |
| Age, year | 25 | 40; 38 | 16–40 | ND | Age, year | 20 | 54 | 28; 58 | NA |
| Phentotype | | | | | | | | | |
| Permanent weakness | No | Yes | No | No | Weakness | Yes | No | No | No |
| Myotonia | No | No | No | No | Myotonia | Yes | Yes | Yes | Yes |
| Periodic paralysis | Yes | Yes | Yes | Yes | Precipitants | RAE, cold emotion, fatigue | | | |
| Precipitants | RAE, poor sleep, emotion | RAE, fasting; RAE, cortisol | Coldness, season alteration, violent exercise | Potassium, coldness | Warm up | | | | |
| Duration | 7d | 24-48h; <24h | 7–15d | NA | Alleviating factors | None | Coldness | NA | NA |
| Frequency | 3-4/month | 5/week; 3/week | 34/year; 1-2/year | NA | Extremities | Lower | All | All | All |
| Extremities | All | Lower; All | All | NA | Others | None | Intercostal muscle, face | NA | |
| Ictal K +/- | Low | NA; Normal/Low Exacerbation/NA | Normal | High Exacerbation/NA | Hypertrophy | Yes | NA | Yes; Yes | Yes |
| Response to potassium/sodium | Remission/NA | | | | Muscle atrophy | No | No | No; No | No |
| Muscle pain | No | Yes; Yes | No | No | Muscle pain | No | Yes | No; No | Yes |
| Muscle stiffness | No | No; Yes | No | No | Muscle stiffness | Yes | Yes | Yes; Yes | Yes |
| EMG | CMAP↓ | CMAP↓ | Normal | NA | EMG | myotonic | myotonic | myotonic | myotonic |
| Muscle biopsy | NA | NA; NSP | NA | NA | Muscle biopsy | NA | PC | NA | NA |
| Diagnosis | HypoPP | NormoPP | NormoPP | HyperPP | Diagnosis | PC | PCM | NDM# | PCM |
| Treatment | Potassium | No; ACZ, potassium, thiazide | ACZ | ACZ | Treatment | None | Dantrolene sodium, flecainide | NA | Ethanol or mexiletine |

**Abbreviations:** SD: standard deviation; K+: potassium; PP: periodic paralysis; RAE: rest after exercise; NA: Not available; NSP: Nonspecific changes; ACZ: acetazolamide; #: Extraocular muscle hypertrophy in a patient with myotonia and a mutation in the SCN4A gene (V445M); PMC: paramyotonia congenital. PCM: Painful congenital myotonia; NDM: Nondystrophic myotonia.
reliable distinguishing factor from other sites in SCN4A. In contrast to classic paramyotonia, partial affected individuals report subjective improvement of muscle symptoms in cold temperatures. Typical myotonic discharges were observed on EMG in all patients, which is in accordance with myotonia. Most anecdotal evidences were based on clinical experience and had various effects to antiarrhythmic agent. Ethanol, mexiletine, flecainide and dantrolene sodium provided dramatic relief of symptoms in certain patients. Among of them, flecainide, as a potent sodium channel blocker, acting by effectively inhibiting the abnormal sodium current associated with expression of the mutant (V445M) channel. Mexiletine is a class 1b antiarrhythmic medication with a high affinity for muscle sodium channels. Mexiletine resulted in greater improvement in stiffness both in human and rat model. However, the reason why they are effective only for individual patients and different drugs are all effective for disease caused by the same mutation. For a more precise explanation of mechanism such as ethanol or dantrolene sodium and so on, indeed, a relevant functional study will be needed.

Phenotypic variability observed in individuals harboring the same SCN4A mutation strongly demonstrated that the genetic background and other epigenetic factors perhaps influenced the clinical expression of particular mutations. In addition, individual Nav channel mutations can have more than one physiological phenotype can also be found in the predominant heart sodium channel isoform, Nav1.5, which might be the result of additional modifiers of channel behavior, such as other genetic variation and alterations in transcription, RNA processing, translation, post-translational modifications, and protein degradation. Above-mentioned mechanisms in connection with Nav1.5 may one be of contributing interpretations for phenotypic variability in Nav1.4. In addition, the exact pathophysiological mechanisms how these mutations in SCN4A gene result in 2 different diseases such as paramyotonia congenita and hypokalemic periodic paralysis are not identified.

Our research expanded the phenotypic spectrum of S4 segment arginine mutations, which also supported the hypothesis that loss of positive charge in S4 voltage sensors was important in the molecular pathogenesis of muscle channelopathies. Moreover, the mutation V445M in SCN4A was first reported in Chinese patients with nondystrophic myotonias.

**Material and Methods**

**Subjects**

One hypoPP patient and a 3-generation Chinese paramyotonia congenita family (Fig. 1A) were enrolled in the study. Blood samples were collected from all affected subjects and consanguineous members of the paramyotonia congenita family. Two hundred genetically unrelated healthy controls were recruited from the medical examination center (set in outpatient clinic in our hospital) with the same Han Chinese ethnic background. All participants signed written informed consent, and the protocol was conducted in compliance with the Ruijin Hospital Ethics Committee, Shanghai Jiao Tong University School of Medicine.

**Methods**

Genomic DNA was extracted from peripheral blood through the standardized phenol/chloroform extraction method. We conducted the genetic test for CACNA1S (RefSeq NM_000069) and SCN4A (RefSeq NM_000334). The primers flanked the entire coding exons and intron-exon boundaries of the above-mentioned genes were designed using the web based Primer 3.0 program. Polymerase chain reaction was carried out at appropriate annealing temperature. The purified PCR products were sequenced on an ABI 3730 XL sequencer (Applied Biosystems, USA).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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