The Genotype and Clinical Phenotype of Korean Patients with Familial Hypokalemic Periodic Paralysis

Familial hypokalemic periodic paralysis (HOPP) is a rare autosomal-dominant disease characterized by reversible attacks of muscle weakness occurring with episodic hypokalemia. Mutations in the skeletal muscle calcium (CACNA1S) and sodium channel (SCN4A) genes have been reported to be responsible for familial HOPP. Fifty-one HOPP patients from 20 Korean families were studied to determine the relative frequency of the known mutations and to specify the clinical features associated with the identified mutations. DNA analysis identified known mutations in 12 families: 9 (75%) were linked to the CACNA1S gene and 3 (25%) to the SCN4A gene. The Arg528His mutation in the CACNA1S gene was found to be predominant in these 12 families. Additionally, we have detected one novel silent exonic mutation (1950C>T) in the SCN4A gene. As for a SCN4A Arg669His mutation, incomplete penetrance in a woman was observed. Characteristic clinical features were observed both in patients with and without mutations. This study presents comprehensive data on the genotype and phenotype of Korean families with HOPP.

Key Words: Hypokalemic Periodic Paralysis; Calcium Channels; Sodium Channels; Mutation

INTRODUCTION

Familial hypokalemic periodic paralysis (HOPP) is an autosomal dominant skeletal muscle ion channelopathy resulting in episodic attacks of muscle weakness with concomitant hypokalemia. Familial HOPP is the most prevalent form of periodic paralysis, but it is still a rare disorder (the prevalence is estimated at about 1/100,000) (1). The onset of this disease is usually in the first or second decade of life. Acute crises most frequently occur at night and in the early morning, and last for hours, sometimes days. Although the frequency of attacks is variable, ranging from a few in a lifetime to daily attacks, it is maximal between ages 15 and 35 yr and then decreases with age (2, 3). The precipitating factors consist mainly of carbohydrate- or sodium-rich meals, emotional stress, exposure to cold, and strenuous exercise.

Molecular genetic analysis has shown that familial HOPP is caused by mutations in a calcium channel gene (CACNA1S) or a sodium channel gene (SCN4A) (4). The majority (70%) of familial HOPP patients have missense mutations in the CACNA1S gene located on chromosome 1q32 (4, 5). The CACNA1S gene encodes the alpha 1-subunit of a skeletal muscle calcium channel protein (6). To date, three causative HOPP mutations have been identified in the highly conserved putative membrane spanning domains (Arg528His, Arg1239His, and Arg1239Gly) of the calcium channel protein (6, 7). Five missense mutations in SCN4A gene have been implicated in familial HOPP. These include Arg669His, Arg672Ser, Arg672His, Arg672Gly, and Arg672Cys (8-10). In a previous large-scale study, missense mutations in the sodium channel gene were found in about 9% of HOPP families (11).

The phenotypes of each mutation have some differences in gender penetrance and the clinical characteristics, including the response to medications (12-14). Comparison between the males and females with disease-causing mutations has shown that the symptoms are less frequent in females than in males (12). Acetazolamide is a carbonic anhydrase inhibitor, and it has been considered to be highly effective for treating most HOPP patients. However, several patients with the Arg672Gly SCN4A mutation and a patient with the Arg672Ser SCN4A mutation showed an exacerbation of their symptoms after acetazolamide treatment (4, 14). In light of this information, which is possibly based on the genetic or allelic heterogeneity found in familial HOPP patients, further understanding of the genotype and characteristic phenotype of this disorder has been suggested to be useful for facilitating the diagnosis and providing appropriate treatment (15, 16). The number of Asian patients with familial HOPP is presumed to be much smaller than that of patients with HOPP.
in Western countries (17, 18). Relatively little work on direct comparisons of patients with and without known mutations has been done to date. In the present study, each index case and some relatives from 20 Korean families with HOPP were screened for the eight known causative mutations in the CACNA1S and SCN4A genes. The purpose of this study was to determine the relative frequency of the known mutations and to ascertain the clinical features of those with or without these mutations.

MATERIALS AND METHODS

Subjects

We identified 51 patients who were diagnosed with HOPP from January 2000 to June 2006 at the clinics of the Catholic University of Korea, the Seoul National University, and the Samsung Medical Center. All of the clinical and mutation data, except those of the patients in five families, were obtained prospectively from the Department of Pediatrics at the Catholic University of Korea. The clinical details of four families have been previously reported (19, 20). The search for a causal mutation was undertaken in 20 independent probands having the diagnosis of HOPP and also in some of their relatives. All the patients fulfilled the diagnostic criteria for HOPP: 1) each patient had episodic paralysis; 2) one or more members of each kindred had shown a low serum potassium level (<3.5 mEq/L) during attacks of weakness; and 3) myotonia was neither clinically present nor detectable by electromyography. We confirmed the absence of secondary causes of hypokalemia, such as thyrotoxicosis, hyperaldosteronism, and renal tubular acidosis. Blood samples were obtained with given informed consent from all participants in compliance with the institutional review board of the Catholic University of Korea, College of Medicine.

Mutation analysis

Each proband was initially screened for the known causative mutations in the CACNA1S gene (Arg528His, Arg1239His, and Arg1239Gly). The Arg528His, Arg1239His, and Arg1239Gly mutations disrupt the BstE I, Sau 96 I, and Bsp LU III restriction sites, respectively. The restriction analysis products of the patients were compared with those of the three known mutations (controls). For those patients in whom a mutation was not identified in the CACNA1S gene, direct sequence analysis of exon 12 of the SCN4A gene was performed. Molecular genetic screening for known mutations was available as a commercial clinical service at the Neurogenetics DNA Diagnostic Lab, Massachusetts General Hospital (Boston, MA, U.S.A.). In all the affected members of one family with HOPP, sequencing of exon 12 of the SCN4A gene did not identify any of the 5 previously reported mutations. However, a heterozygous silent mutation (1950C>T) was found in the exon 12. We searched for the same change in 50 unrelated and unaffected Koreans by performing a polymerase chain reaction (PCR) and analyzing the PCR products by means of restriction digestion analysis and sequencing. From each control individual, a 273-bp fragment containing the transition site was amplified by PCR. Primers used were 5′-GTTGGAGTGGGTGGAGAC-3′ (F) and 5′-CCCTGGCGCTTTCGTTG-3′ (R). PCR was performed on 50 μL of standard PCR buffer containing 2 mM MgCl2, 250 μM of each dNTP, 10 pmole of each primer, 1 unit of i-MAX II Taq polymerase (Intron biotechnology, Seoul, Korea), and 100 ng genomic DNA. The PCR products were electrophoresed on 1.8% agarose gel, and the amplified genomic DNA fragments were extracted from the gel and then purified using the method described by the manufacturer’s recommended protocol (QIAquick® gel extraction kit; Quiagen, Hilden, Germany). The C-to-T alteration at nucleotide position 1950 in exon 12 of the SCN4A gene destroys a Hpa I restriction site, thus permitting the change to be screened by restriction analysis with Hpa I. A 25-μL aliquot of each 273-bp PCR product was mixed with 1 IU of Hpa I (New England BioLabs Inc; Ipswich, MA, U.S.A.), 5 μL of appropriate 10X buffer and 19.8 μL of H2O. The mixtures were left at 37°C for 16 hr. The resulting digestion products were electrophoresed on 1.8% agarose gel. Direct sequencing was performed according to the manufacturer’s protocol (ABI 3700; Applied-Biosystems, Foster city, CA, U.S.A.).

Statistical analysis

Statistical analysis was performed using SPSS for windows 13.0 (SPSS Inc., Chicago, IL, U.S.A.). Data were analysed with nonparametric tests (Mann-Whitney). Statistical data were expressed as mean, unless indicated otherwise. p<0.05 was considered statistically significant.

RESULTS

Mutation analysis

The screening for known HOPP mutations in 20 independent index cases showed 12 families (60%) with mutations in either the calcium channel gene or the sodium channel gene (Table 1). Calcium channel mutations were found in 9 families, and sodium channel mutations in 3 families. The following distribution was found amongst those with calcium channel mutations: 6 cases with Arg528His (4 familial, 2 sporadic), 2 cases with Arg1239His (familial), and one case carrying Arg1239Gly (familial). The Arg528His mutation was the most common causal mutation of familial HOPP in the present study.
Incomplete penetrance was observed in a woman with Arg669His SCN4A mutation: she was the mother of a male patient who carried the same Arg669His mutation, but she had never experienced any paralytic attacks in her lifetime (Fig. 1). In one proband, a heterozygous C-to-T transition was identified at nucleotide 1950 in exon 12 of the SCN4A gene. This alteration does not predict an amino acid change at codon 650. The same change was confirmed in the DNA of the proband’s mother, who had similar attacks of paralysis. Other members of the family were not affected, and the samples from them were not available. This change has not been reported previously. We confirmed its absence in 50 unaffected and unrelated Korean individuals, i.e., 100 chromosomes.

Genotypes and phenotypes in HOPP patients

Table 2 describes the clinical characteristics for Korean patients with HOPP. The mean age at onset of the disease was younger in those with mutations (average, 11 yr; range, 1-19 yr) than in those without (average, 17 yr; range, 1-34 yr) (p<0.05). Patients with the Arg1239Gly CACNA1S mutation presented at the earliest age (average, 2.6 yr) (p<0.05). The patient with the Arg669His SCN4A mutation had much shorter attacks that were on average 1 hr-long, compared with those with the Arg1239Gly mutation, for whom the average length of the attacks was 16 hr (p<0.05). Patients with the Arg528His CACNA1S mutation had attacks, on average, once per month. In contrast, patients with the Arg1239Gly CACNA1S and Arg672Gly SCN4A mutations had daily attacks even after the age of 30. Postcritic myalgias were reported in 18 patients with Arg528His and one patient with Arg669His in whom the myalgias could last from several minutes to hours. Both percritic and postcritic myalgias were reported in one patient with Arg672Cys and in one index case without mutations. All the affected members of the family with the Arg1239Gly mutation displayed bilateral ankle equinus. This deformity seemed to be slowly progressive. One affected member of the family underwent muscle biopsy and electrodagnostic study, including nerve conduction study and needle electromyogram. No definite abnormality was found on either studies. Strenuous exercise was the most common triggering factor for an attack both in those with and without mutations. Carbohydrate- or sodium-rich meals were another common precipitant. Compared with those with other mutations, the patients with Arg528His were less sensitive to cold exposure (p<0.05). Although alcohol intake was frequently reported as a provocative factor in many cases, all patients with Arg1239His and most of patients with Arg528His patients did not complain of paralytic attacks after alcohol consumption. Prophylactic acetazolamide treatment was administered to most cases. One patient with the Arg1239Gly mutation, one patient with the Arg672Gly mutation, and two patients in different kindreds without mutations reported worsening of their symptoms with acetazolamide treatment. These patients were successfully treated with a combination of oral spironolactone, amiloride, and potassium supplementation. The severe paralytic attacks in patients with the Arg1239Gly, Arg528His, Arg672Gly, and Arg672Cys mutations were often accompanied by respiratory distress.

Table 1. Results of molecular genetic testing in Korean families with HOPP

| Type          | Families | Patients |
|---------------|----------|----------|
| All HOPP      | 20       | 51       |
| HOPP without mutations | 8 (40%) | 14 (27%) |
| HOPP with mutations | 12 (60%) | 37 (73%) |
| CACNA1S gene  | 9 (75%, 9/12) | 32 (86%, 32/37) |
| Arg528His     | 6        | 22       |
| Arg1239His    | 2        | 4        |
| Arg1239Gly    | 1        | 6        |
| SCN4A gene    | 3 (25%, 3/12) | 5 (14%, 5/37) |
| Arg669His     | 1        | 1        |
| Arg672Gly     | 1        | 3        |
| Arg672Cys     | 1        | 1        |

HOPP, hypokalemic periodic paralysis.
All except one patient with Arg672Cys and two patients with Arg528His were familial cases. Two patient with Arg528His were de novo (20).

![Fig. 1. Pedigree of a family with the SCN4A Arg669His mutation. Dark symbols represent the affected individuals. The symbol with a dot designates an asymptomatic carrier. Slash marks represent deceased individuals. The proband is indicated by an arrow. The age of the family members is designated by the number.](image-url)
DISCUSSION

We investigated the molecular genetic basis of 20 Korean families suffering from HOPP. Twelve families (60%) had known mutations in either the calcium channel or the sodium channel. When considering the previous notion of the low prevalence of Asian HOPP patients with mutations (17, 18), this figure is high and analogous to those reported in the previous Western studies (4, 11). Eight probands remained without the known mutations, and three of them had other affected members in their families. Compared to the patients with known mutations, those without mutations showed an older age at onset of their disease. Although they did not have electrodiagnostic studies or muscle biopsies performed, overall, they had the typical clinical features, including dietary triggers and their response to medications. Given that the allelic or genetic heterogeneity of HOPP has been consistently suggested, undescribed mutations are likely to be discovered in these kindreds. We have to admit the limitation in the level of the genetic analysis at this stage, but we have already been performing sequencing for novel mutations in the CACNA1S gene for the mutation-negative cases and linkage studies to exclude the two loci for the familial cases without mutations. In comparison with the previous study (11) that showed the majority were secondary to both Arg528His and Arg1239His CACNA1S mutations, the Arg528His mutation alone was predominant in the present study. The observation of the identical mutation in unrelated families and de novo mutations as already described (20) argues in favor of the recurrent mutation in the CACNA1S gene, which may contribute to the predominance of this mutation in the Korean population.

Some genotype-phenotype correlations in HOPP patients are emerging. As shown by the clinical details in Table 2, some of the clinical phenotypes displayed by our series of families and the previously reported families were similar. One asymptomatic female case with the Arg669His SCN4A mutation was documented, which is consistent with the general observation that the phenotype of HOPP is often less pronounced in females. There were certain phenotypic differences, however, between the previous studies and our results. Patients with the Arg672Gly SCN4A mutation in the present study showed an earlier age of onset of the disease than that in the previous report (4), and they did not display postictal myalgias and cramps. In contrast to the previous report (11), the patient with Arg672Cys did not complain of worsening after potassium intake. Potassium administration restored the patients’ strength during attacks. Emotional stress-induced weakness was present in 32% of those with the Arg528His CACNA1S mutation. This result stands in contrast
to the previous finding (11), that weakness is rare in the patients with Arg528His following exposure to acute stress. In addition, compared with the attack frequency reported by Miller et al. (11) for their patients with Arg528His, a low frequency of attacks was observed for the Korean patients with Arg528His. However, the prevalence of each of the different precipitating factors was higher in the Korean patients with Arg528His than in Miller’s patients with Arg528His. There may have been a different way in assessing precipitating factors or a difference in exposition to precipitating factors. Otherwise, these differences, overall, may support the earlier suggestion of the modifying influence of other unidentified genetic factors (21).

We observed one novel silent mutation (1950C>T) in exon 12 of the SCN4A gene. Samples from unaffected siblings were not available. We confirmed its absence in 50 unaffected and unrelated Korean individuals. There have been reports showing that silent mutations can also be pathogenic by creating a new cryptic splicing site or by interrupting other functional processes in the nucleus (22, 23). Given that C1950 is located near the middle of exon 12, however, we are not considering conducting further studies to exclude this single-base substitution as a rare sequence variant. Instead, we have already been performing an exhaustive screening of other regions of the CACNA1S and SCN4A in this and other HOPP families without known mutations. Additional studies such as RT-PCR analysis to test for aberrant pre-mRNA splicing will be required to establish a possible pathogenic role of 1950C>T.

All the known mutations causing HOPP change positively charged arginines to weakly charged amino acid residues in the voltage-gated calcium (CACNA1S) and sodium (SCN4A) channels. The functional effects of the mutations have been investigated by electrophysiologic studies of in vitro expression systems. Altogether, those mutations reduce the respective ion current density and result in a slow activation of each ion channel. However, the linkage between the electrophysiologic defects of the mutations and hypokalemia remains to be elucidated.

Acetazolamide has been reported to increase the frequency and the severity of attacks in some HOPP patients with either a specific calcium channel mutation or sodium channel mutation (4, 11, 14). Before performing genetic testing, we preferred to use spironolactone and its derivatives, which we preferred to use spironolactone and its derivatives, which are already been performing an exhaustive screening of other regions of the CACNA1S and SCN4A in this and other HOPP families without known mutations. Additional studies such as RT-PCR analysis to test for aberrant pre-mRNA splicing will be required to establish a possible pathogenic role of 1950C>T. In the present study, none of the patients who were prescribed a combination of oral spironolactone, amiloride, and potassium supplementation reported exacerbation of their symptoms or other side effects, including gynecomastia.

In conclusion, we identified the known genetic defects underlying the HOPP in 60% of the individuals we tested. These included the Arg528His, Arg1239His, and Arg1239-Gly mutations in the CACNA1S gene and the Arg669His, Arg672Gly, and Arg672Cys mutations in the SCN4A gene. The Arg528His mutation was the most common cause of familial HOPP in the present study. An asymptomatic case of a woman with an Arg669His mutation was observed. We also found a novel silent mutation, which is most likely a single nucleotide polymorphism, in the SCN4A gene. Some characteristic clinical features were observed in the patients with and without the previously reported mutations. Although a strict correlation may be difficult to establish for some genotypes due to the small number of families, further collection of this type of data will help clarify the clinical phenotypes, and this will lead to a better understanding of the disease and help to provide appropriate treatment.

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