

An Arg1239His mutation of the *CACNL1A3* gene in a Korean family with hypokalemic periodic paralysis

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= Abstract =

Familial hypokalemic periodic paralysis (hypoPP) is a rare inherited channelopathy that often presents with episodic weakness accompanied by hypokalemia. Thus far, mutations in the gene encoding two ion channels (*CACNL1A3*, L-type calcium channel alpha-1 subunit and *SCN4A*, a sodium channel type IV alpha subunit) have been identified. Several cases of familial hypoPP in children have been reported in Koreans, but there are only a few cases with identified mutations. We report a 12-year-old boy and his affected mother with hypoPP who has a heterozygous G to A substitution at codon 1239 in exon 30 of the *CACNL1A3* gene that causes a change from arginine to histidine (Arg1239His, *CACNL1A3*). This mutation is common among Caucasians; however, it has not yet been reported in Koreans. The patients were treated with oral acetazolamide and potassium replacement and were instructed to avoid precipitating factors. After the medication and lifestyle modification, the paralytic attacks significantly decreased. (**Korean J Pediatr 2008;51:771-774**)

Key Words : Hypokalemic periodic paralysis, *CACNL1A3*, Arg1239His mutation, Korean

Introduction

Familial hypokalemic periodic paralysis (hypoPP) is an autosomal dominant disorder of ion channel dysfunction characterized by episodic flaccid weakness with concomitant fall in blood potassium levels¹. The onset of hypoPP usually occurs within the first and the second decade of life and the frequency of attacks often decreases after 40¹. Attacks can occur spontaneously or be provoked by prolonged rest after vigorous exercise or a carbohydrate-rich meal on the day prior to the attack². Attacks can also be triggered by stress including intercurrent viral illness, lack of sleep or menstruation².

Linkage analysis has been used to map the candidate locus responsible for HypoPP. A variety of skeletal muscle ion-channel-related genes has been identified such as *CACNL1A3*, L-type calcium channel alpha-1 subunit, and *SCN4A*, a sodium channel type IV alpha subunit³. Each mutation has

some differences in gender penetrance and clinical characteristics such as age of onset and the duration of episode⁴.

Several Korean cases of familial Hypo PP in children have been reported, but cases with identified gene mutations are rare³. In the current report of hypoPP, we describe a 12-year-old boy and his affected mother with a heterozygous G to A substitution at codon 1239 in exon 30 of the *CACNL1A3* gene that resulted in a change from arginine to histidine (*CACNL1A3* Arg1239His). Even though this mutation has been reported in other racial groups⁵⁻⁷, especially Caucasians, it has not been reported in a Korean case previously.

Case report

A boy 12 years of age presented to our hospital due to episodic paralysis, which began about 7 years ago. The symptoms had usually appeared in the early morning when he was awakened. In addition, overeating or rest after vigorous exercise induced paralysis at any time. Weakness was focal or generalized and lasted several minutes to several hours. The patient recovered from the weakness in all four limbs and paralytic attack in the afternoon. The frequency of the attacks was approximately 1-3 times a week.

Received : 8 March 2008, Accepted : 26 May 2008

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The family history was positive for other affected member, his mother. She, 36 years old, was also suffering from similar attacks of weakness since she was three. However, her parents and all of her five siblings were without symptoms (Fig. 1). Her parents took her to primary care physicians to be evaluated and treated; however, no specific diagnosis was ever made. She presented to a tertiary care hospital when she was 20 years old. She recalled that the doctor told her that her serum potassium level was low and that the paralysis might be due to hypokalemia. The doctor recommended further evaluations such as electromyography (EMG) or muscle biopsy. However, she did not follow up with these recommendations for additional testing. She was then lost to follow up.

On admission, the boy showed non-specific findings on the physical, radiological and laboratory examinations including electrolytes, muscle enzymes and thyroid hormone testing. The patient could cause an attack by eating large amounts of food especially sweet food. During two paralytic episodes, serum electrolytes and EMG were evaluated. He had low serum potassium levels (2.4 mEq/L and 2.5 mEq/L) and electrocardiography findings consistent with hypokalemia including U-wave changes. Electromyography performed in the right lower extremity, where the paralytic attacks were most severe, showed decreased compound motor action potentials in the tibial and sural nerves.

Mutation analysis of the candidate locus responsible for HypoPP (e.g. *CACNLIA3*, L-type calcium channel alpha-1 subunit, and *SCN4A*, a sodium channel type IV alpha subunit) was performed in the proband, his affected mother and unaffected younger sister with his parental consent. Genomic DNA was extracted from the peripheral blood using standard methods, after informed consent was obtained from the mother. Each index site (e.g. exon 11 and 30 of *CACNLIA3*

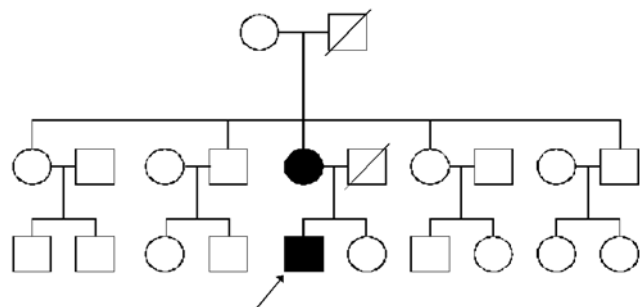


Fig. 1. Pedigree of the Korean family with hypokalemic periodic paralysis. The dark symbols are affected individuals. The proband is indicated by an arrow.

and exon 12 of *SCN4A*) was amplified by the polymerase chain reaction (PCR) using the following appropriate primers: exon 11 of *CACNLIA3*, forward 5'-GGGAGTCAGGAGAA GGGAAAG-3' and reverse 5'-TTTCAAGGAGGGAGGGA AGT-3'; exon 30 of *CACNLIA3*, forward 5'-ACATCTCCC CAAAACACACA-3' and reverse 5'-GAGCGCCAGTCAGT GTCTTA-3'; exon 12 of *SCN4A*, forward 5'-ATGCATGCC ACTCCTGCTCCTCTCA-3' and reverse 5'-CCTCGTTTT CAGCCCTCTAGCTTCC-3'. The PCR conditions were an initial denaturing cycle at 95°C for 7 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 63°C for the exon of *SCN4A* and at 60°C for the exons of *CACNLIA3*, and then an extension at 72°C for 1 min. A final extension step of 72°C for 5 min was also used. The PCR products were electrophoresed on a 1.2% agarose gel; the amplified genomic DNA fragments were extracted from the gel and purified according to the manufacturer's protocol (QIAquick gel extraction kit; Quiagen, Germany). Genomic DNA was extracted from the peripheral blood using standard methods, after informed consent was obtained from the mother. Direct sequencing on both strands with BigDye terminator chemistry (PE Biosystems, USA) identified a heterozygous G to A substitution at codon 1239 in exon 30 of *CACNLIA3* in the proband and his affected mother (Fig. 2A) but no mutation

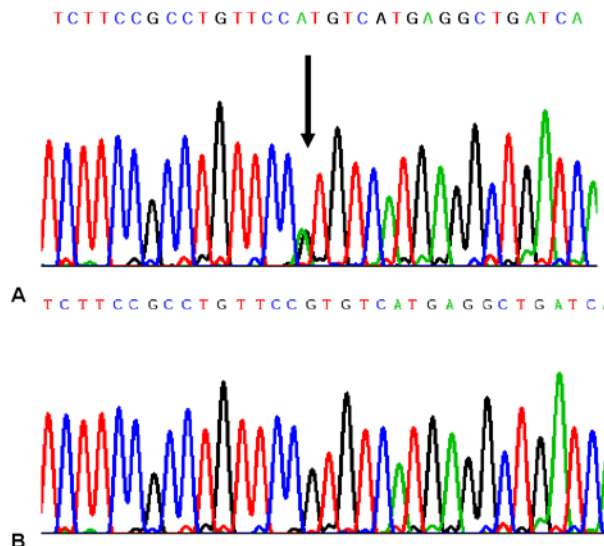


Fig. 2. Electropherogram from the proband, his affected mother and his unaffected sister. Direct sequence analysis of *CACNLIA3* shows an identical heterozygous G to A substitution at codon 1239 resulting in an Arg1239His mutation in the proband and his affected mother (A, a filled arrow indicating the nucleotide substitution); however, no mutation was observed in his unaffected sister (B).

in his unaffected sister (Fig. 2B). This mutation causes a change from arginine to histidine at the amino acid position 1239 of the L-type calcium channel alpha-1 subunit.

The boy and his mother were treated with acetazolamide and oral potassium supplements and were instructed to avoid precipitating triggers through lifestyle and dietary modification such as eating small frequent meals. After the medication and lifestyle modification, the frequency and severity of the attacks gradually decreased in both mother and son.

Discussion

Familial HypoPP presents as either an autosomal dominant condition (in two-thirds of cases) or a sporadic (one-third of cases) event¹⁾. This disorder is caused by mutations in the alpha subunit of either the skeletal muscle L-type calcium channel gene *CACNLIA3* (Arg528His and Arg528Gly in exon 11; Arg1239His and Arg1239Gly in exon 30)⁸⁾ or the sodium channel gene *SCN4A* (Arg669His, Arg672His, Arg672Gly, Arg672Ser and Arg672Cys in exon 12)⁸⁾. In a large-scale study, the majority of families identified were found to have missense mutations in *CACNLIA3*, whereas missense mutations in *SCN4A* were reported in 9% of families; in 22% of cases, no mutation was identified⁹⁾. Sternberg et al.⁹⁾ screened for mutations in a population of 58 hypoPP probands; one of the largest studies to date. This screening resulted in the following distribution: 26 cases (45%) carrying *CACNLIA3* Arg528His, 14 cases (24%) carrying *CACNLIA3* Arg1239His, 3 cases (5%) with *SCN4A* Arg672His, one case (2%) with *SCN4A* Arg672Gly and one (2%) with *SCN4A* Arg672Ser⁹⁾. *CACNLIA3* mutation at 528 and 1239 codons may occur more frequently compared with other mutations, because these two codons have CpG dinucleotide and G to A transition that are the most common mutations within the gene coding regions that cause human genetic disease¹⁰⁾.

There are several studies on genotype/phenotype correlations^{5, 8, 9)}. For example, the mean age at onset of hypoPP in *CACNLIA3* Arg528His carriers had been reported to be significantly later than in *CACNLIA3* Arg1239His carriers⁸⁾. Furthermore, the treatment response to acetazolamide, with regard to the frequency and severity of attacks, increased in patients with *SCN4A* Arg672His but decreased in *SCN4A* Arg672Gly carriers⁵⁾. The treatment with acetazolamide, a carbonic anhydrase inhibitor, has been very effective in most hypoPP patients. However, several patients with the *SCN4A* Arg669His mutation have an exacerbation of symp-

oms after acetazolamide treatment³⁾. Therefore, genetic diagnosis of familial hypoPP, with identification of the specific mutation in a given family, is useful for the prediction of response to acetazolamide treatment.

The mechanism of action of the carbonic anhydrase inhibitors is unclear; however, it is independent of carbonic anhydrase inhibition¹⁾. In vitro studies show that the carbonic anhydrase inhibitors relieve weakness in potassium-deficient rats through activation of calcium-activated potassium channels rather than by direct inhibition of carbonic anhydrase¹¹⁾. The observation that prophylactic treatment improves fixed weakness is currently under investigation in a randomized controlled trial¹⁾.

With the use of PCR amplification, the proband and his affected mother in the current report were found to have the Arg1239His mutation in *CACNLIA3* encoding the muscle dihydropyridine (DHP)-sensitive calcium channel alpha1 subunit. The alpha1 subunit is composed of four transmembrane domains (I to IV), each of which consists of six segments (S1-S6)³⁾. This mutation, *CACNLIA3* Arg1239His, caused the replacement of a positively charged arginine at position 1239, in segment S4 of domain II, with a weakly positive histidine³⁾.

The mutation, *CACNLIA3* Arg1239His, is one of two common mutations (the other, Arg528His) in the Caucasian population¹⁾. In Asia, Kusumi et al.¹²⁾ reported four patients, in one Japanese family, with the *CACNLIA3* Arg1239His mutation and Ke et al.¹³⁾ reported two patients in one Chinese family. Kusumi et al.¹²⁾ reported that three of four patients treated with acetazolamide and potassium chloride medication showed decrease or disappearance of paralytic attacks although difficulty in running continued to be a problem. The other patient, who did not have much improvement, was not taking medication on a constant basis¹²⁾. In Koreans, only three types of mutations (*CACNLIA3* Arg528His in four families, Arg1239Gly mutation in one family and *SCN4A* Arg672Cys mutation in one family) have been reported to date^{3, 8)}. The current case is the first report of a *CACNLIA3* Arg1239His mutation in the Korean population. The index cases in our study were treated with acetazolamide and oral potassium replacement in addition to instructions for avoiding precipitating triggers through lifestyle and dietary modification such as eating small and frequent meals. With medication, the patients showed gradual improvement in the frequency and severity of the paralytic attacks.

한글 요약

가족성 저칼륨성 주기성 마비 1예

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가족성 저칼륨성 주기성 마비는 골격근에 존재하는 ion channel의 장애로 인해 저칼륨혈증과 연관되어 나타나는 주기성 이완성 마비를 보이는 드문 유전 질환이다. 이 질환의 발생에 관여하는 유전자로는 골격근육의 1q31-32 염색체에 위치하는 칼슘 채널과 나트륨 채널의 alpha subunit을 encoding하는 CACNA1S gene과 SCN4A gene이 밝혀져 있다. 국내에서도 소아의 가족성 저칼륨성 주기성 마비가 수 예 보고된 바 있지만, 유전자 분석을 통해 변이가 확인된 예는 드물다. 이에 우리는 CACNA1S의 Arg1239His 변이에 의한 저칼륨성 주기성 마비로 진단된 12세 환자의 증례를 보고하는 바이다. 이 변이는 현재까지 알려진 CACNA1S의 변이 중 비교적 흔한 것으로 알려져 있으나, 국내에서는 보고된 바가 없다. 저자들은 경구 acetazolamide와 칼륨 복용, 유발인자를 회피할 것을 교육함으로써 이 환아를 치료했으며, 현재 환아는 주기성 마비의 빈도와 증증도의 개선을 보이며 삶의 질 역시 향상되었다

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