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A Korean Family with Arg1448Cys Mutation of SCN4A Channel Causing Paramyotonia Congenita: Electrophysiologic, Histopathologic, and Molecular Genetic Studies

A family with paramyotonia congenita (PC) is presented. At least 10 family members were affected in an autosomal dominant inheritance pattern. The proband had cold-sensitive muscle stiffness, paradoxical myotonia, and intermittent muscle weakness since childhood. The serum level of creatine kinase was mildly elevated and short exercise test with cooling revealed a drastic reduction of compound muscle action potentials with repetitive discharges. Muscle biopsy revealed marked variation in the fiber size and increased internal nuclei. The molecular biological study revealed a common missense mutation (Arg1448Cys) at the voltage-gated sodium channel gene *(SCN4A)*. The repetitive CMAP discharges during short exercise test with cooling observed in the proband has not been reported previously. This observation needs to be confirmed among PC patients with different mutations. This is the first report on a PC family confirmed by the molecular biological technique in Korea.

Key Words : *Myotonic Disorders; Sodium Channels*

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INTRODUCTION

The familial periodic paralysis syndrome used to be classified into hypokalemic, normokalemic, hyperkalemic, and paramyotonic forms. Recently, however, a combination of electrophysiologic and molecular biologic studies has led to a reclassification of the disease. In this scheme, the periodic paralyses are divided into two major subtypes, hypokalemic and hyperkalemic forms, according to the genes affected (1). Because the paramyotonic form is caused by a mutation of the voltage-gated sodium channel gene *(SCN4A)* (2), it is now considered allelleic to hyperkalemic periodic paralysis (HyperPP).

Paramyotonia congenita (PC) is a disease with autosomal dominant inheritance which is clinically characterized by paradoxical myotonia, cold-sensitive myotonia, and inter-attack periodic paralysis. We have recently identified a common *(SCN4A)* gene mutation in a Korean family with PC and describe here its clinical, electromyographic, histopathologic, and molecular genetic findings in detail.

CASE REPORT

The proband was a 33-yr-old man with a history of intermittent muscle weakness and stiffness since childhood. He had experienced a transient generalized or focal muscle weakness on awakening, which most frequently affected shoulder, back, and lower leg muscles, lasting several hours to 1-2 days. He also had a hand or facial muscle stiffness provoked by cold exposure, which worsened by repetitive short, strong exercise. Eating icecream consistently produced a stiffness of mouth and tongue. However, ingestion of potassium-rich fruits (watermelon, pears, orange juice, and bananas) (5) did not produce attacks of weakness or stiffness. The frequency of the muscle weakness was variable ranging 2-15 times a year, and the cold-sensitive muscle stiffness clustered during winter, up to 20-30 times a day. Family history revealed that at least 10 family members were affected, suggesting the autosomal dominant inheritance pattern (Fig. 1). On examination, the muscle power was normal in all groups without atrophy, hypertrophy, or fasciculation. Neither percussion myotonia nor grip myotonia was observed. The serum level of creatine kinase was mildly elevated (245 IU/L, upper normal limit, 190 IU/L) and other routine laboratory tests including chest radiography, EKG, complete blood count, liver and renal function tests, erythrocyte sedimentation rate, electrolytes, and blood glucose were normal.

The needle electromyography, performed on right biceps and quadriceps muscles, showed spontaneous activities and myotonic discharges with typical dive-bomber sound. The motor unit potentials were normal in both size and shape.

In order to evaluate the effects of cold temperature and exer-

cise on the size of the compound muscle action potentials (CMAPs), short exercise test with cooling was performed with some modification (Fig. 2) (3). The test was first performed on the abductor digiti minimi muscle (ADM) without cooling (skin temperature, 32℃) and then after immersing hand into the ice-cold water until the skin temperature decreased to 20℃. Without cooling, no change was observed in the CMAP throughout the test. With cooling, however, a drastic reduction of the CMAP amplitude and repetitive CMAP discharges (Fig. 2) were noted immediately after exercise. Such a change was maximally seen 30 sec after exercise (2.8 mV, reduction in 80.4%) and gradually recovered thereafter. He also showed difficulty in relaxing hand following forceful grip after cold exposure.

The muscle biopsy revealed moderate to marked variations in muscle fiber size and an increase in internal nuclei (about 25% of the fibers). Muscle fiber necrosis, regenerating fibers, vacuolar changes, or abnormalities in the fiber type proportion was not observed (Fig. 3).

The proband, his wife, and his affected son (III-3, 4, and IV-2 in Fig. 1) were available for the genetic testing and gave us informed consent for the genetic analysis using their blood cells. The genomic DNA was extracted from whole blood as described previously (4). The primer pairs for the polymerase chain reaction (PCR), 5′-AGTGGCATTTGCAAACAGC-CTTGGGAATGGG-3′ (forward) and 3′-AGTGAGGG-GCAGAGATTCGAATGTTC-5′ (reverse), were designed to include the common sites of mutation in exon 24 of the *SCN4A* gene. The condition of the thermal cycling reaction was as follows: an initial denaturation at 94℃ for 4 min and 35 cycles of 94℃ for 1 sec, 62℃ for 1 min, and 72℃ for 2 min. A final extension at 72℃ for 10 min was added at the end of the reaction. The PCR products were then cleaned using QIAGEN PCR purification kits (QIAGEN, U.S.A.) and bi-directional nucleotide sequence analysis was performed by automated sequencer (Perkin Elmer 377, PE Applied Biosystems, U.S.A.) using BigDye™ Terminator Cycle sequencing kit (PE Applied Biosystems, U.S.A.). The results of the DNA sequencing of exon 24 revealed a transition of cytosine to thymidine at nucleotide 4343, which would substitute the amino acid cysteine for arginine at codon 1448 in the proband (III-4) and his son (IV-2) (Fig. 4).

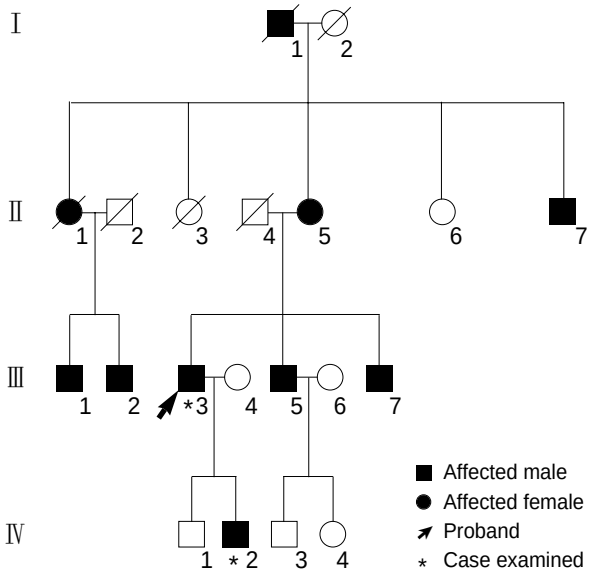

Fig. 1. Pedigree of the family.

DISCUSSION

The phenotypic profile of the presented family was consis-

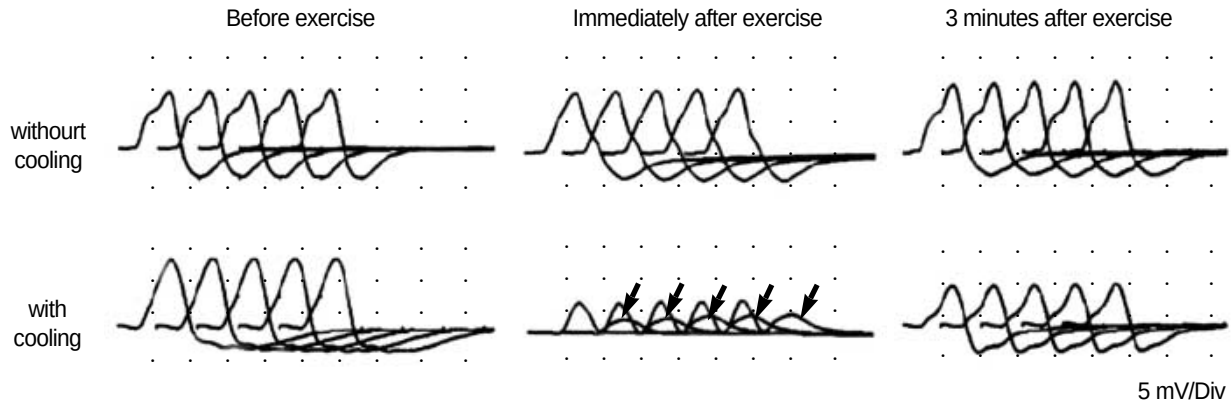

Fig. 2. The results of the short exercise test with (lower row) and without (upper row) cooling. The recording electrodes were attached on the abductor digiti minini muscle and five consecutive supramaximal stimulations at a frequency of 2 Hz were applied to the ulnar nerve at the elbow. The stimulation was performed before exercise, immediately after 30 sec of exercise (finger fanning), and every 10 sec for 3 min. After exercise with cooling (lower row), compound muscle action potential amplitude is markedly reduced (14.3 to 5.6 mV, reduction in 60.8%) along with the repetitive discharges. At the end of the observation (3 min after exercise), the CMAP amplitude is nearly recovered to the baseline level.

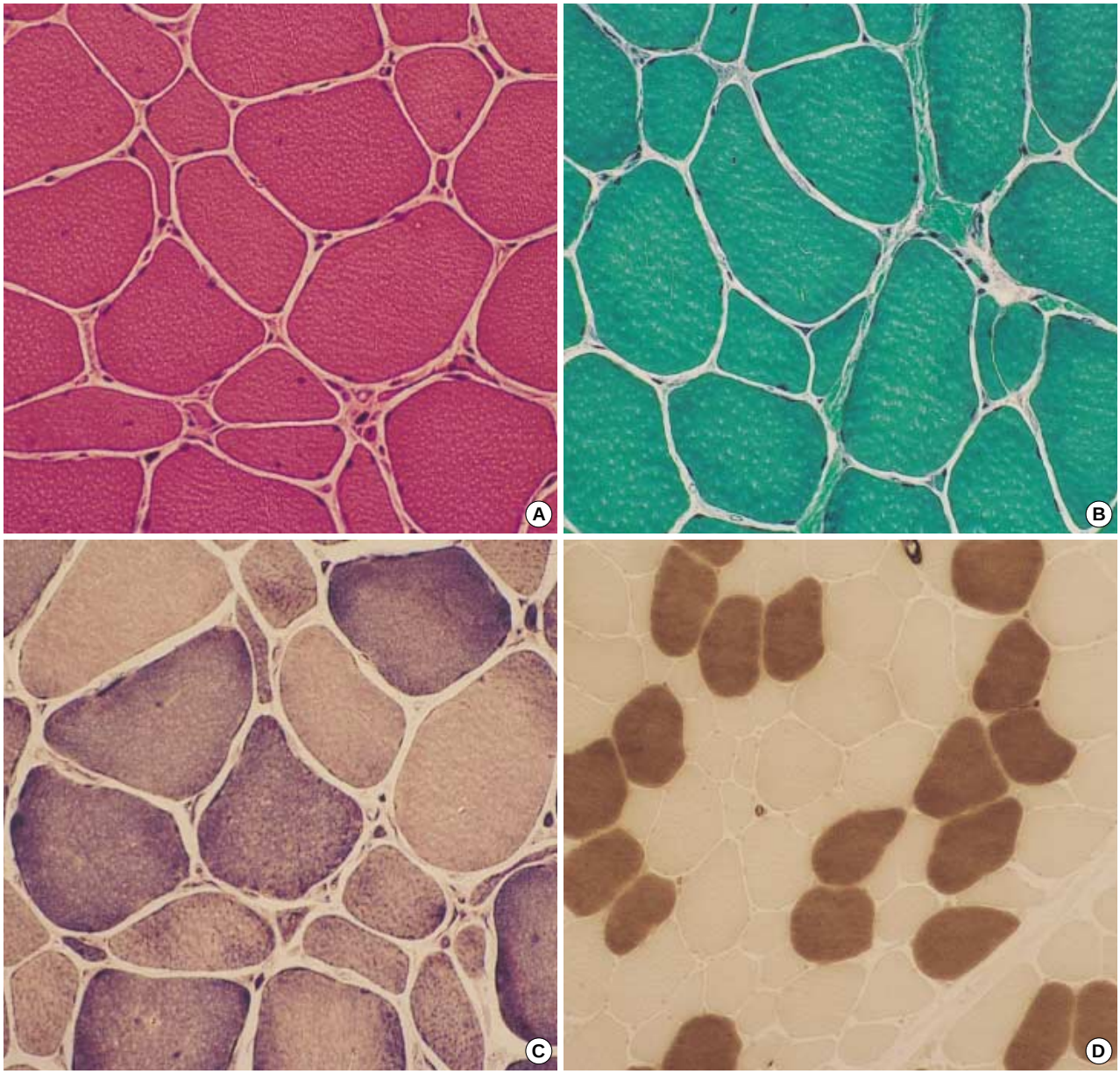

Fig. 3. Muscle biopsy finding. (A) H&E stain, ×200. (B) modified Gomori-trichrome stain, ×200. (C) NADH stain, ×200. (D) ATPase stain at pH 4.3, ×100. Moderate to severe fiber size variation and increase in internal nuclei are seen. There is only minimal infiltration of the connective tissue. There is no abnormality in the distribution of muscle fiber type.

tent with an autosomal dominant disease with cold-sensitive stiffness, paradoxical myotonia, and frequent interattack weakness, all of which are characteristic features of PC. While most patients with typical PC are hypokalemic during attacks of weakness (1), some families with PC superimposed by HyperPP showed hyperkalemia during the attacks (6). Unfortunately, we did not have a chance to measure the potassium level during the episodes of weakness and the potassium loading test could not be done. However, lack of history of provocation by potassium-rich fruits suggests potassium loading would not produce paralytic episodes in this family. In addition, the lack of decrement in CMAP amplitude after short exercise at room temperature is not consistent with HyperPP (6). Thus, overall clinical findings suggest the affected members of the family have pure PC not association with HyperPP.

The hallmark of the electrophysiologic study in the present family was the drastic reduction of CMAP amplitudes after short exercise with cooling. Although post-exercise decrement in CMAP amplitude at room temperature is a common finding in both short and prolonged exercise test in various myotonic syndromes (7), it does not differentiate specific subtypes (8). In addition, exercise test at room temperature often produce

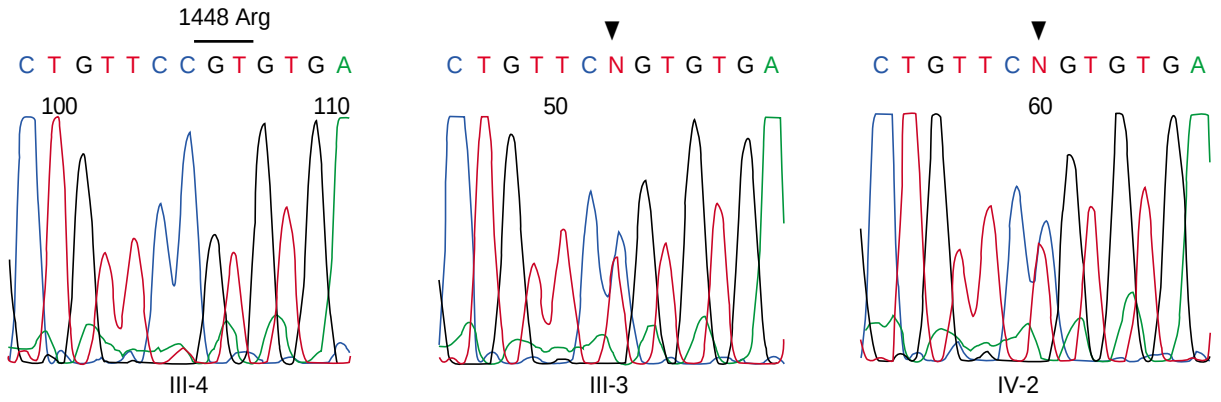

Fig. 4. The results of automated sequencing. In III-3 (proband) and IV-2 (son of the proband), there is a C to T single base change, predicting substitution of arginine to cysteine at α-position 1448 of the sodium channel subunit. The sequence of III-4 (spouse of the proband) is normal.

a normal response in patients with PC, while it is consistently abnormal when performed after cooling (3). Thus, the short exercise with cooling seems to be the single most important electrodiagnostic test for PC. The findings that no change was observed in the CMAP during the short exercise test without cooling, and cooling alone did not produce any change in the size of the CMAP were indicative of cold-sensitive, exercise-induced myotonia. Also, a greater reduction of CMAP amplitudes up to 30 sec suggested that the myotonia was transiently worsened by the repetitive muscle contraction (paradoxical myotonia). The occurrence of the repetitive CMAP discharges is also noteworthy. The repetitive CMAP discharges have been well-documented in some patients with acute organophosphate poisoning (9), myasthenia gravis with pyridostigmine toxicity (10), and occasionally in patients with myotonic disorders (11). However, with the Medline-based literature searches, we could not find any report documenting the repetitive CMAP discharges during short exercise test with cooling in patients with PC. Because PC also belongs to a group of myotonic syndrome, and bears a defect in fast inactivation of the sodium channel, which leads to a prolonged muscle action potential generation, it can be speculated that the repetitive CMAP discharge can be found in patients with PC especially at cold atmosphere. Thus, we think this is mainly attributable to the relatively recent introduction of the short exercise test with cooling for PC (3), although the rarity of the cases and lack of careful observation of the electrophysiologic findings may also have contributed.

The increase in central nuclei and variation in muscle fiber size, as in our case, are the most common pathological abnormalities in PC (12), although occasional necrotic fibers and a few vacuolated fibers have been described in some patients with PC superimposed by HyperKPP (13). The selective type 2B fiber deficiency, which had been described in a patient with PC (14), was not observed in our case.

In PC, the amino acid arginine at position 1448 in segment four (S4) of the fourth repeated domain of the SCN4A is known to be the most common site of mutation, and four different mutations affecting this residue (R1448H (2), R1448C (2), R1448P (15), R1448S (16)) have been reported. Because the S4 segment contains a repeating motif with positively charged amino acids (arginine or lysine), it is believed to serve as a voltage-sensor of the channel, which shifts mechanically in response to membrane depolarization and opens sodium channel (17). Therefore, the replacement of the positively charged amino acid arginine by neutral cysteine will cause a defect in inactivation and deactivation of the channel, which eventually will lead to clinical myotonia (15). Interestingly, patients with four different mutations affecting codon 1448 have different phenotypes (16). Among them, patients with R1448P mutation have the most severe form of myotonia (18), and those with R1448C mutation have some disabilities from stiffness in association with frequent paralytic attacks (2) as in our case.

In brief, our case showed typical clinical, electrophysiological and histopathologic features of PC, and was confirmed to have R1448C mutation in the SCN4A gene. This is the first case of PC confirmed by molecular biological technique in Korea and it is suggested that this mutation may be also common among families with PC in Korea. The repetitive CMAP discharges in short exercise test with cooling, shown in the proband seems to be an important finding not reported elsewhere before, and needs to be confirmed among families with different mutations of PC.

REFERENCES

1. Ptáček LJ. *The familial periodic paralyses and nondystrophic myotonias. Am J Med 1998; 105: 58-70.*
2. Ptáček LJ, George AL Jr, Barchi RL, Griggs RC, Riggs JE, Robertson M, Leppert MF. *Mutations in an S4 segment of the adult skeletal muscle sodium channel cause paramyotonia congenita. Neuron 1992; 8: 891-7.*

3. Jackson CE, Barohn RJ, Ptáček LJ. *Paramyotonia congenita: abnormal short exercise test, and improvement after mexiletine therapy. Muscle Nerve 1994; 17: 763-8.*
4. Miller SA, Dykes DD, Polesky HF. *A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.*
5. National Rural Living Science Institute. *Food composition table, 5th ed. Korea: Rural Development Administration, 1996.*
6. Kim J, Hahn Y, Sohn EH, Lee YJ, Yun JH, Kim JM, Chung JH. *Phenotypic variation of a Thr704Met mutation in skeletal sodium channel gene in a family with paralysis periodica paramyotonia. J Neurol Neurosurg Psychiatry 2001; 70: 618-23.*
7. Streib EW, *AAEE minimonograph #27: Differential diagnosis of myotonic syndromes. Muscle Nerve 1987; 10: 603-15.*
8. Kuntzer T, Flocard F, Vial C, Kohler A, Magistris MR, Labarre-Vila A, Gonnaud PM, Ochsner F, Soichot P, Chan V, Monnier G. *Exercise test in muscle channelopathies and other muscle disorders. Muscle Nerve 2000; 23: 1089-94.*
9. Jager KW, Roberts DV, Wilson A. *Neuromuscular function in pesticide workers. Br J Ind Med 1970; 27: 273-8.*
10. Park KH, Kim DE, Arnold TW, Oh SJ, Bradley R. *Pyridostigmine toxicity. Electrophysiological study. Electromyogr Clin Neurophysiol 1993; 33: 323-8.*
11. Kirby JF Jr, Kraft GH. *Electromyographic studies in myotonia congenita. Arch Phys Med Rehabil 1973; 54: 47-50.*
12. Lehmann-Horn F, Engel AG, Ricker K, Rüdel R. *The periodic paralysis and paramyotonia congenita. In Engel AG, Franzine-Armstrong C, eds. Myology. 2nd ed. New York: McGraw-Hill, 1994: 1291-334.*
13. Thrush DC, Morris CJ, Salmon MV. *Paramyotonia congenita: a clinical, histochemical and pathological study. Brain 1972; 95: 537-52.*
14. Heene R, Gabriel RR, Manz F, Schimrigk K. *Type 2B muscle fibre deficiency in myotonia and paramyotonia congenita. A genetically determined histochemical fibre type pattern? J Neurol Sci 1986; 73: 23-30.*
15. Lehmann-Horn F, Jurkat-Rott K. *Voltage-gated ion channels and hereditary disease. Physiol Rev 1999; 79: 1317-72.*
16. Lerche H, Mitrovic N, Dubowitz V, Lehmann-Horn F. *Paramyotonia congenita: the R1448P Na^+ channel mutation in adult human skeletal muscle. Ann Neurol 1996; 39: 599-608.*
17. Ptáček LJ, Johnson KJ, Griggs RC. *Genetics and physiology of the myotonic muscle disorders. N Engl J Med 1993; 328: 482-9.*
18. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. *SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 1995; 80: 805-11.*