A Novel Mutation in the Gene for the Adult Skeletal Muscle Sodium Channel α-Subunit (SCN4A) That Causes Paramyotonia Congenita of von Eulenburg

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Background: Paramyotonia congenita (PMC) of von Eulenburg is an autosomal dominant muscular disease characterized by exercise- and cold-induced myotonia and weakness. To date, 18 missense mutations in the adult skeletal muscle sodium channel α-subunit (SCN4A) gene have been identified to cause a spectrum of muscular diseases, including PMC of von Eulenburg, PMC without cold paralysis, PMC without cold paralysis (MIM 168350). Hyperkalemic periodic paralysis (MIM 170500) is also a dominantly inherited disease in which periodic paralysis is associated with hyperkalemia. Because hyperkalemic periodic paralysis can occur in some families with PMC, it has been suggested that the 2 diseases share a common pathologic basis. In fact, PMC and hyperkalemic periodic paralysis have been genetically mapped to the same locus on chromosome 17q23-25, and various missense mutations in the gene for the subunit of the skeletal muscle sodium channel (SCN4A) at this locus have been identified as causative mutations for both diseases. Furthermore, other autosomal dominant myotonias characterized by potassium sensitivity (potassium-aggravated myotonia) have also been demonstrated to be caused by mutations in the SCN4A gene.5-18

Although 18 missense mutations in the SCN4A gene have been identified in various forms of sodium channelopathies,4-18 no obvious correlations have been demonstrated between the location or nature of amino acid substitutions in SCN4A and the clinical phenotypes. Herein, we describe a novel mutation in the SCN4A gene in a Japanese family with PMC of von Eulenburg. This observation provides an important insight into the genotype-phenotype correlation of sodium channelopathies.

RESULTS

CLINICAL FEATURES

The proband (IV-2) was a 16-year-old girl who began to experience muscle stiffness that affected her face and limbs in a cold environment, such as being outdoors in the winter (approximately 5°C-10°C), at the age of 4 years. She also experienced stiffness and concomitant weakness in her hands after immersing them in ice-cold water. She had not experienced episodes of...
SUBJECTS, MATERIALS, AND METHODS

SUBJECTS AND SAMPLES

A 4-generation Japanese pedigree (P1043) with cold-induced myotonia was investigated (Figure 1, A). Clinical history was ascertained for 10 individuals (II-1, III-1, III-2, III-4, III-5, III-6, IV-1, IV-2, IV-3, and IV-4), including the 6 affected individuals (III-1, III-4, III-6, IV-2, IV-3, and IV-4), through interview. Four individuals (III-6, IV-1, IV-2, and IV-4), including the 3 affected individuals (III-6, IV-2, and IV-4), were examined by neurologists (K.K., K.K., and A.H.). High-molecular-weight genomic DNA was extracted from peripheral blood leukocytes of the family members interviewed and 90 unrelated Japanese individuals under informed consent, according to the standard procedure.

LINKAGE AND MUTATIONAL ANALYSIS

Linkage analysis of the SCN4A gene locus was made using 2 dinucleotide repeat markers, including (dGdA)n, in intron 22 and (dGdT)n, in intron 23 of the SCN4A gene.10 Pairwise lod scores were calculated using the MLINK subprogram of the LINKAGE package version 5.1 using the autosomal dominant inheritance model.11 Because previously reported causative mutations for PMC are frequently located in exons 22 and 24 of the SCN4A gene,6,7 we started single strand conformation polymorphism analysis12 with these exons. Exon 22 was amplified by the polymerase chain reaction (PCR) using primers (5’-TGGAGGCGAGGAGGGGAAC-3’ and 5’-GGACGACACACAGGACAGG-3’) in a total volume of 10 µL containing dNTPs, 200 µmol/L each; [α-32P]dCTP, 185 kBq; Tris hydrochloride (pH 8.0), 10 mmol/L; potassium chloride, 50 mmol/L; magnesium chloride, 1.5 mmol/L; genomic DNA, 100 ng; and Taq polymerase (Takara, Tokyo, Japan), 0.1 U. The PCRs were performed with an initial denaturation at 96°C for 2 minutes and 30 cycles (1 minute each at 96°C, 58°C, and 72°C). For exon 24, we first amplified the entire exon 24 using primers (5’-AGTGGCATTTGCAAAACGCGTTGGGAAAGT-3’ and 5’-AGTGAGGGCAGAGATTCTGTTCTGAC-3’) with a polymerase mix (Klen Taq; Clontech, Palo Alto, Calif), according to the manufacturer’s instructions. Using 1 µL of the 1:200 diluted PCR products as the template, 8 overlapping DNA fragments covering the exon 24 were amplified by nested PCR (Figure 1) under the same conditions as those used for the amplification of exon 22. The PCR products were diluted 6-fold with a buffer containing 98% formamide; EDTA (pH 8.0), 10 mmol/L; 0.025% xylene cyanol; and 0.025% bromophenol blue and electrophoresed through a 0.5% agarose gel with a 0.5× mutation detection enhancement gel (MDE; Toyobo, Tokyo, Japan) containing 10% glycerol at 10 W for 18 hours at 4°C. The gel was transferred to Whatman 3MM paper (Whatman International, Maidstone, UK) and autoradiographed to Fuji RX film (Fuji Photo Film, Tokyo, Japan) at −80°C using an intensifying screen. The PCR products, which showed aberrant conformers in the single-strand conformation polymorphism analysis, were subcloned into a pT7Blue T-vector (Novagen, Madison, Wis), followed by bidirectional nucleotide sequence analysis by the dideoxynucleotide chain termination method using an automated DNA sequencer (Pharmacia, Uppsala, Sweden). Nucleotide and amino acid residues were numbered as described.10

Only 3 (III-1, III-6, and IV-4) of the affected members reported episodes of severe generalized paralysis—always muscle stiffness after exposure to cold such as air conditioning in summer—and never while resting after exercising or eating large meals. Serum potassium levels during generalized paralyses were not measured.

LINKAGE ANALYSIS TO THE SCN4A LOCUS AND MUTATIONAL ANALYSIS OF THE SCN4A GENE

Results of the analysis of the (dGdA)n and (dGdT)n dinucleotide markers are shown in Figure 1, A. The highest pairwise lod scores of 1.84 (θ = 0.0) and 1.81 (θ = 0.0) were obtained for the (dGdA)n and (dGdT)n markers, respectively. Single strand conformation polymorphism analysis of the SCN4A gene of the proband and other members of this family revealed aberrantly moving bands in 2 regions, one of which represented a G to A polymorphism at nucleotide position 4869 in exon 24 of SCN4A complementary DNA.20 Haplotype analysis [(dGdA)9-(dGdT)9-G-A] revealed perfect cosegregation of a haplotype of GA4-GT9-G with the disease in this family (Figure 1, A). The other aberrant conformer also segregated with the disease in all affected members of this family (Figure 1, B), but not in 90 unrelated Japanese individuals. Nucleotide sequence analysis revealed that this generalized flaccid weakness. On examination, she showed grip and percussion myotonia in her hands in a warm environment (approximately 20°C), which was further exacerbated by exercise. Serum sodium, potassium, and chloride levels were normal, and serum creatine kinase levels were normal, and serum creatine kinase level was 83 U/L (normal range, 7-160 U/L). Needle electromyographic studies revealed myotonic discharges at rest but otherwise normal findings. The amplitude of compound muscle action potentials of the thenar muscles that were elicited by electric stimulation of the left median nerve was 26.6 mV, which decreased to 1.0 mV after immersing her left hand in ice-cold water for 10 minutes.

In addition to the proband (IV-2), 5 members of her family (III-1, III-4, III-6, IV-3, and IV-4) interviewed experienced cold-induced muscle stiffness in their faces and hands with onsets in infancy, and 3 other members (I-2, II-2, and II-3) were thought to have cold-induced muscle stiffness based on information obtained from the interviewed family members (II-1 and IV-2). These members (I-2, II-2, II-3, III-1, III-4, III-6, IV-3, and IV-4) were also judged to be affected (Figure 1, A). Two members (III-4 and IV-3) experienced stiffness in their throats when eating ice cream. Percussion and grip myotonia were observed in all the affected members examined (III-6, IV-2, and IV-4); none was aware of any food that exacerbated muscle stiffness, except ice cream.
entire exon 24 as a template, the nested polymerase chain reaction was performed with primers (5'-ATCGATGCCCGACTCCTTCTTG-3' and 5'-ATGATGCGGACTCTTCTTG-3'), which amplified the fragment encompassing the adult skeletal muscle sodium channel α-subunit complementary DNA 4315 to 4542 (see “Subjects, Materials, and Methods” section).

**Figure 1.** A, Japanese pedigree with paramyotonia congenita (PMC) of von Eulenburg. Filled squares and circles indicate affected male and female individuals, respectively; open symbols, unaffected individuals. Genotypes of the (dGdA) and (dGdT) dinucleotide markers and the G·A polymorphism at nucleotide position 4869 are shown. Haplotype segregating with PMC of von Eulenburg is boxed. Inferred haplotypes are indicated by brackets. B, Autoradiogram of single strand conformation polymorphism analysis. The aberrant conformer segregated with affected family members. Using the first polymerase chain reaction product of the entire exon 24 as a template, the nested polymerase chain reaction product of the clinical features of the affected individuals in this family were associated with mutations in the SCN4A gene can vary, the clinical features of the affected individuals in this family are close to that described by von Eulenburg.

Linkage and haplotype analyses suggested that the disease was linked to the SCN4A gene. The mutation that segregated with the disease in this family substituted a glutamic acid for a glycine at position 1456, which is highly conserved among voltage-gated sodium channels. This mutation was not found in 90 healthy Japanese individuals. These observations strongly support this mutation being the pathogenic mutation, although we analyzed only exons 22 and 24 in the SCN4A gene.

**SCN4A** has 4 similar domains (D1-D4) connected by cytoplasmic loops (Figure 2), each domain consisting of 6 transmembrane segments (S1-S6). Their function is not fully understood. The S4 segments, which have a positive charge due to repeats containing positively charged amino acids (X-X-R-K), are thought to act as voltage sensors for channel activation and to be involved in activation-inactivation coupling. The present mutation (G1456E) is located in the D4/S4 and predicted to alter the charge of the D4/S4 by substituting negatively charged glutamic acid for glycine. Interestingly, 3 mutations (R1448C, R1448H, and R1448P) affecting the same arginine at position 1448 in the D4/S4 are reported to be associated with the phenotype of PMC of von Eule-

This family exhibited various forms of myotonia, including cold-induced myotonia, grip and percussion myotonia, and paradoxical myotonia. The mode of inheritance is consistent with autosomal dominant inheritance. The proband experienced cold-induced muscle stiffness and weakness only in her hands, and the 3 other affected members experienced attacks of cold-induced muscle stiffness followed by generalized weakness. Interestingly, muscle weakness was always associated with exposure to cold temperature and never with rest after exercising or eating large meals. Although the clinical presentations associated with mutations in the SCN4A gene can vary, the clinical features of the affected individuals in this family are close to that described by von Eulenburg.
These mutations, including the G1456E identified in the present study, are all predicted to alter the charge of the D4/S4 (Figure 2). These observations suggest that mutations causing a decreased positive charge in the S4/D4 are associated with the phenotype of PMC of von Eulenburg. Other mutations not located in the D4/S4 have also been described to cause cold-sensitive myotonia. However, some of them, including S804F and G1306V, are reported to be associated with potassium-aggravated myotonia without cold sensitivity in some families. Our observation that mutations identified in the D4/S4 are associated with PMC of von Eulenburg provides an important genotype-phenotype correlation in sodium channelopathies.

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