Familial Periodic Paralysis and Charcot-Marie-Tooth Disease in a 7-Generation Family

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**Background:** A family with a complicated constellation of neurologic findings, including neuropathy, myotonia, and periodic paralysis, has been described in 4 studies in the medical literature since 1934. The underlying cause of their disease has been the subject of considerable speculation and has never been identified until now.

**Objective:** To identify the molecular basis of this family’s neurologic disease.

**Design:** The coding regions of 6 genes that cause peripheral neuropathy and regions of the muscle sodium channel gene (SCN4A) were sequenced.

**Results:** A novel missense mutation (Arg67Pro) in the myelin protein zero gene was identified in 2 patients with Charcot-Marie-Tooth disease, and a common missense mutation (Thr704Met) was identified in the SCN4A gene in 4 family members. We discuss the difficulties of genotype-phenotype correlation in this family.

**Conclusions:** These findings indicate that 2 independent mutations segregating in this family are responsible for the puzzling clinical picture.


**METHODS**

**FAMILY HISTORY**

The family history was obtained from 6 individuals, available medical records were reviewed, and 4 individuals were personally examined. Eventually, I found a 1954 study in the AMA Archives of Neurology and Psychiatry on 17 affected family members that included electromyographic and muscle biopsy results. This study cited generations I through III, published in 1934 and 1943. Clinical features in 7 generations are summarized in Figure 1. Participants gave written, informed consent under a protocol approved by the Yale University (New Haven, Conn) School of Medicine Human Investigations Committee. DNA samples were isolated from blood or buccal swabs using standard methods.

**MOLECULAR ANALYSIS OF THE SCN4A AND CMT GENES**

Seven common mutations (Thr704Met, Gly1306Val, Thr1313Met, Leu1433Arg, Arg1448His, Met1592Val, and Val1589Met) in the SCN4A gene were screened using direct sequencing. Thr704Met was amplified using primers 5’-GTCTTCAAGCTGGCCAAGTC-3’ and 5’-AGACGATGAGGAAGGAGTGG-3’ and the conditions described in the following paragraph for exon 2 of myelin protein zero (MP2). Mutations were verified by sequencing both DNA strands.

Peripheral myelin protein 22 (PMP22) gene duplication or deletion was negative, and 6 CMT genes (PMP22, MPZ, connexin 32 [CX32], early growth response factor 2 [EGR2], neurofilament...
light [NFL], and periaxin [PRX]) were analyzed by sequencing the entire coding region (Athena Diagnostics Inc, Worcester, Mass). A missense mutation in MPZ was detected. Exon 2 of MPZ was amplified using primers P0ex2-P21 (5'-CTCTCTGCTTATGACCTTACTG-3') and P0ex2-M6 (5'-CTCCTTAGCCATGTTATCT-3'). Polymerase chain reaction (PCR) was performed in a 25-µL reaction containing 50 ng of genomic DNA, 50 ng of each primer, 1.5 mM magnesium chloride, 200 µM deoxynucleotide triphosphates, and 1/100 PCR Buffer II and 2.5 U AmpliTaq DNA polymerase (Roche Molecular Biochemicals, Indianapolis, Ind) using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif). A modified touchdown protocol was used: 94°C for 5 minutes, 94°C for 45 seconds, annealing temperature (3 cycles each at 65°C, 62°C, 59°C, 56°C, and 53°C) for 45 seconds, and extension at 72°C for 1 minute (15 cycles total), followed by 25 cycles at 94°C for 45 seconds, 50°C for 45 seconds, 72°C for 1 minute, 72°C for 10 minutes, and a 4°C hold. The PCR products were purified (ExoSAP-IT; USB, Cleveland, Ohio) and then were sequenced using an automated sequencer (ABI 377; Applied Biosystems).

RESTRICTION DIGESTION

Restriction digests of MPZ PCR products were performed at 37°C with 10 U of HaeIII (New England Biolabs, Beverly, Mass) for 2 hours and separated by agarose gel electrophoresis. An affected patient's sample served as a positive control.

RESULTS

A 51-year-old man (patient V:8) was referred for familial neuropathy (Figure 1). At age 35 years, he began having nocturnal leg cramps and diaphragm cramping while singing opera. He had a history of paroxysmal atrial fibrillation. At age 47 years, he developed gait difficulty and distal leg numbness. Examination results showed distal muscular atrophy, myotonia, occasional fasciculations, reduced sensation below the middle of the calf, absent reflexes throughout, steppage gait, and pes planus. The median motor NCV was 28 m/s, and electromyography showed fibrillations and myotonia. His creatine kinase level was 822 U/L (reference range, 24-195 U/L). DNA test results for myotonic dystrophy, PMP22 duplication, and CX32 were negative. Based on his sister's periodic paralysis and his myotonia, acetazolamide therapy was started, with relief of the muscle cramping.

Patient IV:9, the father of patients V:8 and V:9, had episodes of periodic paralysis since infancy. His calves were enlarged in childhood, with foot deformities by age 10 years and multiple orthopedic procedures. Progressive leg weakness and muscle wasting began during his teens. Examination results disclosed absent deep tendon reflexes in the legs, footdrop, pes cavus, myotonic response to muscular percussion, absent position and vibration sense, steppage gait, and the Romberg sign. The median motor NCV was 31.4 m/s, and electromyography showed myotonia. His creatine kinase level was 822 U/L (reference range, 24-195 U/L). DNA test results for myotonic dystrophy, PMP22 duplication, and CX32 were negative. Based on his sister's periodic paralysis and his myotonia, acetazolamide therapy was started, with relief of the muscle cramping.

Patient V:10 was a 16-year-old boy with weekly episodes of periodic paralysis that lasted 10 to 30 minutes, beginning in the first year of life. Triggers included hot weather, rest after exertion, and certain foods. Myotonia was elicited on examination, and his reflexes were normal.

Figure 1. Pedigree of a family with Charcot-Marie-Tooth disease (CMT) and hypokalemic periodic paralysis.
mal. His feet showed pes planus. The median motor NCV was 62 m/s, and electromyography showed myotonia.

Patient IV:12 was a 51-year-old man with difficulty running in childhood who began to experience tripping, gait difficulty, and leg weakness in his 40s. A magnetic resonance image of the lumbar spine showed prominent cauda equina and thickened nerve roots. He had supraventricular tachycardia and type 1 diabetes mellitus. Examination showed distal weakness, atrophy and sensory changes, areflexia, steppage gait, pes cavus, and hammer toe deformity. His creatine kinase level was 867 U/L (reference range, 24-195 U/L). The median motor NCV was 26 m/s, and electromyography showed spontaneous fibrillations and positive waves.

Sequencing DNA from individuals V:8, V:9, VI:7, IV:12, IV:13, and V:10 showed a C→T point mutation resulting in the Thr704Met mutation of SCN4A. B, Control (top) and patient IV:12 (bottom) heterozygous for a G→C substitution (arrow) resulting in the Arg67Pro mutation of MPZ. C, The Arg67Pro mutation in MPZ results in the gain of an HaeIII restriction site and a new band at 195 base pair (arrow). D, The MPZ protein with the location of the Arg67Pro mutation and the amino (NH) and carboxyl (COOH) termini indicated.

Figure 2. Mutations in the α subunit of the muscle sodium channel (SCN4A) gene and the myelin protein zero (MPZ) gene. A, Control (top) and patient V:10 (bottom) heterozygous for a C→T substitution (arrow) resulting in the Thr704Met mutation of SCN4A. B, Control (top) and patient IV:12 (bottom) heterozygous for a G→C substitution (arrow) resulting in the Arg67Pro mutation of MPZ. C, The Arg67Pro mutation in MPZ results in the gain of an HaeIII restriction site and a new band at 195 base pair (arrow). D, The MPZ protein with the location of the Arg67Pro mutation and the amino (NH) and carboxyl (COOH) termini indicated.

A growing number of paroxysmal disorders, including epilepsy, hemiplegic migraine, hypokalemic periodic paralysis, myotonia congenita, episodic ataxia, Andersen syndrome, long QT syndrome, and malignant hyperther-
nia, have been found to result from mutations in a variety of ligand-gated or voltage-gated ion channels.18-22

The evaluation of patient V:8 with CMT showed a father with CMT and a sister with HYPP. Occam’s razor (a medieval principle of parsimony that remains a pearl of clinical medicine: among multiple explanations for a phenomenon, the simplest version should be chosen) would predict that neuromuscular disease in a parent and 2 of his children results from variable expression of a single, mutated gene. This was considered because SCN4A mutations cause not only the prototypical HYPP but also normokalemic periodic paralysis, myotonia without paralysis, paramyotonia congenita, and myotonia congenita.1,5 Patient V:8’s history of cardiac arrhythmia, myotonia, and response to acetazolamide therapy are consistent with a channelopathy. However, he does not carry the Thr704Met mutation. Furthermore, peripheral neuropathy has not been reported in any other family with HYPP, and the full pedigree showed that some members had only CMT, whereas others had only HYPP. Thus, I considered the alternative hypothesis of 2 separate genetic conditions. Despite the proband’s earlier “negative CMT genetic testing,” 4 additional CMT genes were tested and showed a private mutation in MPZ.

The occurrence of 2 genetically linked disorders in a patient is rare, but studies include descriptions of a patient with Duchenne muscular dystrophy and myotonic dystrophy,23 a family with mutations in the breast cancer genes BRCA1 and BRCA2, and a patient who inherited Leri-Weill dyschondrosteosis from his mother and neurofibromatosis type 1 from his father.24 This is more likely if 1 of the disorders is common (such as neurofibromatosis type 1, with an incidence of 1 in 3500, or Duchenne muscular dystrophy, with a similar incidence in boys).

In an era of rapidly increasing availability of genetic testing, its appropriate use and interpretation can be challenging. This family illustrates principles of molecular neurology, including variable expression and difficulties in assigning genotype-phenotype correlations. Despite having the same MPZ mutation, patient V:8 had pes planus and late-onset CMT, and his father had severe pes cavus with early orthopedic deformities. Because of the sister’s HYPP, it would be reasonable to attribute patient V:8’s muscle cramping, myotonia, response to acetazolamide therapy, and arrhythmia to an SCN4A mutation. The molecular studies ruled this out. Therefore, his myotonia and cardiac arrhythmia could be related to CMT or could be incidental. In I series,25 cardiac arrhythmias were observed in 21% of patients with uncomplicated CMT, and myotonia occurred in 5% of atypical patients. However, another series26 found a low frequency of cardiac conduction abnormalities (7%). Although the family pedigree was key to recognizing the possibility of 2 genetic conditions, modern molecular analysis allowed precise identification of the contributing mutations, answering a question posed 50 years ago.

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