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Paramyotonia Congenita and Hyperkalemic Periodic Paralysis Map to the Same Sodium-Channel Gene Locus

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Summary

Paramyotonia congenita (PC), an autosomal dominant muscle disease, shares some clinical and electrophysiological similarities with another myotonic muscle disorder, hyperkalemic periodic paralysis (HYPP). However, clinical and electrophysiologic differences allow differentiation of the two disorders. The HYPP locus was recently shown to be linked to a skeletal muscle sodium-channel gene probe. We now report that PC maps to the same locus (LOD score 4.4, $\theta = 0$ at assumed penetrance of .95). These linkage results, coupled with physiological data demonstrating abnormal sodium-channel function in patients with PC, implicate a sodium-channel gene as an important candidate for the site of mutation responsible for PC. Furthermore, this is strong evidence for the hypothesis that PC and HYPP are allelic disorders.

Introduction

Paramyotonia congenita (PC), a muscle disease that is manifest in infancy or early childhood by either transient episodes of paralysis and myotonia or abnormal relaxation of muscle, was first reported by Eulenburg (1886) and then by Rich (1894). These authors noted the very prominent myotonia of face and tongue muscles in affected individuals, especially after muscle cooling and rest after exercise. Stiffness and weakness may also occur when muscles in the extremities are involved. Attacks are typically frequent and of short duration. Myotonia is apparent on clinical or electromyographic examination (Barchi 1988; Riggs 1988). The myotonia and the periodic episodes of paralysis are features similar to those associated with another genetic muscle disease, hyperkalemic periodic paralysis (HYPP).

There are several features that set PC and HYPP apart as clinical entities. Experiments have shown that increasing the extracellular potassium concentration causes depolarization of muscle from patients with HYPP, during in vitro electrophysiologic recording (Lehmann-Horn et al. 1983, 1987*a*). These potassium-induced effects were not seen when similar studies were performed on muscle from a PC patient (Lehmann-Horn et al. 1987*b*). In vitro studies of muscle from PC patients show increased sodium conductance and depolarization on muscle cooling, a change that is not seen in muscle of patients with HYPP (Lehmann-Horn et al., 1981, 1987*b*, Moxley et al. 1989). In vivo, dramatic decrease in the frequency and amplitude of electrical activity is noted in the muscles of patients with PC when the muscles are cooled to 15°C. Muscle cooling does not have this effect in HYPP muscle (Subramony et al. 1986; Streib 1987). Moreover, though myotonia is present in both disorders, the predominant distribution of this abnormal muscle activity is different in the two diseases. The facial muscles are more prominently affected in PC, while muscles of the extremities and tongue are the predominant site of affection in HYPP. Some of the similar and distinguishing clinical and electrophysiological features of PC and HYPP are outlined in table 1.

Electrophysiologic investigation has implicated ab-

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Table 1

Clinical Features of PC and HYPP

Clinical Feature	PC	HYPP
Age at onset	Infancy	Infancy
Primary distribution of myotonia	Face	Extremities and tongue
Myopathic changes	Never	Sometimes present
Attacks exacerbated by rest after exercise	Yes	Yes
Excessive muscle depolarization elicited by increased K^+	No	Yes
Excessive muscle depolarization elicited by muscle cooling	Yes	No

normal sodium-channel (NaCh) function as the defect in both diseases (Lehmann-Horn et al. 1981, 1983, 1987*a*, 1987*b*; Rüdel et al. 1989). The abnormal sodium current in both disorders can be reversibly blocked by tetrodotoxin, an NaCh-blocking agent. These similarities, along with the report of a family whose affected members show clinical and electrophysiologic features of both diseases (DeSilva et al. 1990), have led to the genetic hypothesis that PC and HYPP are allelic disorders. Since the HYPP locus recently has been shown to be linked to a skeletal muscle NaCh locus (Fontaine et al. 1990; Ptacek et al. 1991), we tested this hypothesis by using pM8, a rat probe that recognizes an allele known to cosegregate with the HYPP disease allele (Ptacek et al. 1991).

Material and Methods

Family Studies and Genomic DNA Collection

Descendants in a branch of the kindred originally reported by Rich (1894) form the basis of the linkage study reported here (fig. 1). A diagnosis of PC was made when clinical symptoms of myotonia occurred and when one or both of the following findings were present: percussion myotonia (of the tongue or thenar muscles) or action myotonia of facial muscles. Of 28 family members evaluated, a diagnosis of PC was confirmed clinically ln 18. Some have very mild and infrequent attacks, while others have numerous attacks daily that limit their physical activity. All individuals considered to be affected had myotonia clinically, and none demonstrated dystrophic muscle changes. Patients III-3, IV-1, and IV-2 underwent electromyography (by J.H.P.; data not shown) and were noted to have findings typical of PC (Streib 1987).

DNA Preparation, Isolation of cDNA Probe, and Hybridization

Isolation of human DNA samples, enzyme digestion, and southern gel transfer were performed by routine techniques (Ptacek et al. 1991). The 2,064-bp cDNA clone pM8, containing nucleotides 3162–5225 (J. S. Trimmer, unpublished data) of the μI NaCh (GenBank accession number M26643) was isolated from a rat skeletal muscle cDNA library (Trimmer et al. 1989). This cDNA encodes amino acids 1052–1740 of the μI NaCh, from segment S2 of domain III to the middle of the carboxyl-terminal tail, and thus contains many of the regions that are the most highly conserved across the NaChs of different species. DNA samples digested with *Bgl*II reveal a polymorphism within the human locus homologous to pM8 (Ptacek et al. 1991). Two alleles are observed; the larger is a 25-kb band, and the second consists of 18-kb and 7-kb bands.

Perparation of the probe and hybridization to nylon filters were carried out as described elsewhere (Ptacek et al. 1991). Genotyes were scored without knowledge of affection status.

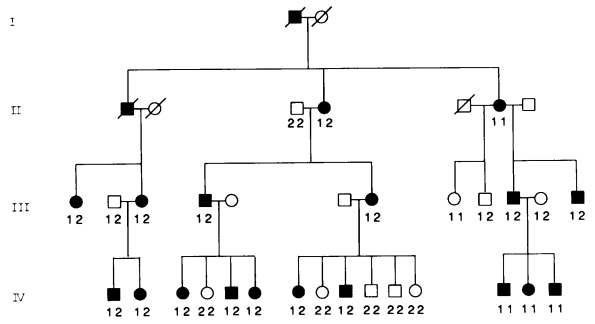

Figure 1 Pedigree of kindred 1637, with shaded figures representing affected individuals. Genotypes of *Bgl*II-digested DNA with the pM8 probe are listed below each individual. One of the authors (L.P.) examined each of the 28 family members from whom DNA samples were obtained. The pedigree structure has been altered by changing birth order and sex of select individuals to protect patient confidentiality. The disease allele cosegregates with allele 1. Paternity was evaluated in the pedigree by two highly polymorphic VNTRs, TBQ7 and YNH24. No misinheritance of alleles was found.

Linkage Analysis

We performed pairwise analysis with LINKAGE programs (Lathrop et al. 1985) by using maximum-likelihood methods. Allele frequencies at the marker locus pM8 (larger allele, .25; smaller allele, .75) were calculated from genotypes of 24 unrelated individuals and were used in the analysis. Two-point analysis of the NaCh probe to the disease locus was performed using assumed penetrances ranging from .70 to 1.00. A disease allele frequency of .001 and a normal allele frequency of .999 were used in the analysis.

Results

Complete linkage was demonstrated between the marker pM8 and the PC disease allele. The Appendix lists the pairwise lod scores for the pM8 and PC loci, at various recombination fractions (θ's) at an assumed penetrance of .95. A maximum LOD score of 4.4 was calculated at $\theta = 0$. When calculated at assumed penetrances varying from .7 to 1.0, the LOD scores varied from 3.96 to 4.54 and were always maximal at $\theta = 0$. The 25-kb allele cosegregated with the disease allele in this family. No obligate recombinants were observed, as would be expected for a candidate gene locus. In addition, there were no unaffected individuals who were obligate disease gene carriers.

Eighteen of the 28 individuals examined met disease criteria. All patients with symptoms had objective clinical signs of the disease. There were no individuals with objective signs in the absence of symptoms. Phenotypically normal individuals were coded as unaffected for the purpose of the linkage analysis, since all such individuals in this pedigree were older than 10 years of age. This disorder is typically expressed in infancy (Streib 1987).

Discussion

This study demonstrates that the PC disease allele is linked to the human homologue of a rat gene encoding a skeletal muscle NaCh. Because the electrophysiologic features of PC suggest NaCh dysfunction, the conserved region identified by pM8 is a strong candidate locus for the site of a molecular defect in PC. Moreover, linkage of pM8 to both PC and HYPP loci supports the hypothesis that these disorders, though clinically distinct, represent different mutations in the same gene. An alternate explanation is that the defects for PC and HYPP reside in distinct members of a very closely linked and functionally related gene family.

Specific mutations in the DNA of patients with PC and HYPP must now be identified in order to prove that this NaCh gene is the site of molecular alterations leading to these two diseases. Knowledge of such mutations may assist in the design of more effective medical therapies for patients with these and other myotonic muscle diseases. The differences in the distribution of muscle dysfunction in PC and HYPP raise important questions regarding differential expression of gene products that modulate excitable membranes.

A third myotonic muscle disease, myotonia congenita, is classically considered as a distinct clinical entity but shares some clinical similarities with PC and HYPP. Myotonia congenita may represent a third distinct allelic form of myotonic muscle disease. Linkage analysis using NaCh probes in myotonia congenita families will allow resolution of this question.

Delineation of molecular defects in these disorders will lead to clarification of these important human models of ion-channel dysfunction. It will provide another approach to better understanding both of the physiology of normal muscle and of differential expression of ion channels in excitable tissues.

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Appendix

LOD Scores for Linkage of pM8 and PC

LOD scores at various recombination fractions were as follows:

4.43 at $\theta = 0$
4.04 at $\theta = .05$
3.64 at $\theta = .10$
2.79 at $\theta = .20$
1.90 at $\theta = .30$
0.97 at $\theta = .40$

LOD scores were calculated on the basis of an assumed penetrance of .95. This high penetrance was based on an estimated segregation ratio in excess of 50% in this family. Because of the relatively small number of

individuals from whom the segregation was estimated, a conservative assumption (.95) was used in the analysis. Frequencies of .25 for the larger allele and .75 for the smaller allele were calculated on the basis of the genotypes of 24 unrelated individuals.

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