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# Linkage data suggesting allelic heterogeneity for paramyotonia congenita and hyperkalemic periodic paralysis on chromosome 17*

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**Summary.** Paramyotonia congenita (PC), an autosomal dominant non-progressive muscle disorder, is characterised by cold-induced stiffness followed by muscle weakness. The weakness is caused by a dysfunction of the sodium channel in muscle fibre. Parts of the gene coding for the α-subunit of the sodium channel of the adult human skeletal muscle (SCN4A) have been localised on chromosome 17. To investigate the role of this gene in the etiology of PC, a linkage analysis in 17 well-defined families was carried out. The results ($z = 20.61$, $\Theta = 0.001$) show that the mutant gene responsible for the disorder is indeed tightly linked to the SCN4A gene. The mutation causing hyperkalemic periodic paralysis (HyperPP) with myotonia has previously been mapped to this gene locus by the same candidate gene approach. Thus, our data suggest that PC and HyperPP are caused by allelic mutations at a single locus on chromosome 17.

## Introduction

Paramyotonia congenita (PC) was first described by Eulenburg (1886) and Rich (1894). Symptoms are usually noticed within the first weeks of life. Following exposure to cold, patients develop stiffness of the face or fingers (cold-induced myotonia). Exercise of the exposed muscles increases the stiffness and is followed by localised muscle weakness. After rewarming, the muscle strength gradually returns within hours. Some patients occasionally experience attacks of weakness without exposure to cold (periodic paralysis). During paralytic attacks, the legs are mainly affected, although muscle weakness can be generalized; paramyotonic stiffness is lacking (Becker 1970, 1977). All patients show myotonia on electromyographic investigation. Electrophysiological studies on muscle fibres from patients with PC show an abnormal temperature dependence of the sodium channel in the muscle fibre. Cold-induced weakness is caused by a disturbance in the gating mechanism of the tetrodotoxin-sensitive sodium channel. This abnormality results in depolarisation and subsequent inexcitability of the muscle fibre (Rüdel and Lehmann-Horn 1985; Lehmann-Horn et al. 1981, 1987a).

In 1956, hyperkalemic periodic paralysis (HyperPP), an autosomal dominant muscle disorder, was recognized and subsequently shown to occur without myotonia, with myotonia or with paramyotonia (Gamstorp 1956, 1963; Engel 1986). Attacks of muscle weakness in these patients may start in the first or second decade of life, and usually occurs while resting following exercise. During an attack of generalised weakness, the patient may be unable to move and serum potassium is usually increased. The weakness may last for hours or even days. In contrast to PC, these patients do not show stiffness of the face or fingers during exposure to cold. HyperPP has been primarily associated with an abnormal function of the muscle sodium channel (Lehmann-Horn et al. 1983, 1987b, 1991). The gene for parts of the α-subunit of the sodium channel of the adult human skeletal muscle (SCN4A) has been localised to the long arm of chromosome 17 and shows close linkage to the disease locus in members of one American and six European families with myotonic HyperPP (Fontaine et al. 1990; Koch et al. 1991).

Since PC and HyperPP are both thought to be caused by altered functions of the muscle sodium channel and a

*Dedicated to Professor P. E. Becker on the occasion of his 83rd birthday

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number of investigators have debated their nosological entities on clinical grounds, it was considered reasonable to test the hypothesis that a mutation in this gene (a "candidate gene") is responsible for the hereditary disorder PC (Engel 1986; Serratrice and Desnuelle 1982; De Silva et al. 1990). We therefore carried out an investigation in families with PC to study possible linkage to the SCN4A gene.

## Patients and methods

### *Patients*

A total of 23 families with suitable pedigree structures for a linkage study were ascertained. Of the German families, 6 were found to be non-informative for the analysis. Following informed consent, all families were personally seen by one of the authors (MCK). Five German families (Ravensberg families) originated from a distinct area in Westphalia and are thought to derive from a common ancestor. These families were first visited by P. E. Becker, 20 years ago (Becker 1970). Ten families came from other parts of West Germany and reports about all but four have been previously published (Becker 1970, 1977; Lehmann-Horn et al. 1981, 1987a; Ricker et al. 1986, 1990). Data from two British families were also included in the study; investigations of both families have been published (Trush et al. 1972; Harper 1989). A total of 114 affected cases (aged 3–86 years), 45 non-affected individuals and 34 spouses were typed for the linkage study.

All affected patients had a history of cold-induced muscle stiffness and localized weakness. All family members were studied clinically for the presence of minimal myotonic signs. In doubtful cases, a needle elelctromyographic study was performed. A standardised fore-arm cooling test was carried out in 21 patients from 10 families (Ricker et al. 1990). In the remaining 7 families, the diagnosis was beyond doubt. Four families had a so-called "pure" form of PC with no history of attacks of weakness in the absence of exposure to cold. In all other families, at least one member reported occasional or frequent attacks of periodic paralysis.

### *DNA analysis and probes*

Total human DNA was extracted from leukocytes from peripheral blood by a modification of the salting out procedure (Miller et al. 1988). DNA (5–8 μg) was digested to completion with the appropriate restriction enzyme under conditions recommended by the manufacturer, fractionated by electrophoresis in 0.8% agarose gels, transferred to nylon membranes and hybridised to the radiolabelled cDNA insert of the two probes used throughout the study. The first probe, hNa2, was a 0.66-kb *Hin*dIII/*Bam*HI fragment corresponding to parts of the SCN4A gene with a chromosomal location of 17q23.1–25.3 (Fontaine et al. 1990; George et al. 1991). The second probe, hGH, was a 0.8-kb *Hin*dIII cDNA fragment encoding the human growth hormone gene (GH1) on 17q22–24 (Chakravarti et al. 1984). The restriction fragment length polymorphisms (RFLPs) of these two cDNA probes were shown to be linked with no recombinants in a reference pedigree (Fontaine et al. 1990). Therefore, it was considered appropriate to generate haplotypes with the hNa2 and hGH gene probes. Each probe detects a two-allele polymorphism with the restriction enzymes *Bgl*II, and *Hin*dII.

### *Linkage analysis*

The lod (logarithm of the odds) score was calculated with the LINKAGE package of computer programs, version 5.03, up-dated by J. Ott (Lathrop et al. 1984). A lod score above +3.00 at a given recombination fraction indicates statistical evidence of linkage between two randomly chosen genetic loci. Approximate confidence limits are obtained by the support interval (= maximum lod score minus 1).

Co-segregation of the PC phenotype and a specific allele for the candidate gene (SCN4A) would suggest that the gene and the disease are associated. Conversely, any evidence of recombination between the PC locus and the gene probe would eliminate the SCN4A gene as the possible site of the molecular defect in the tested PC family. Autosomal dominant inheritance with 100% penetrance of the PC gene was assumed in the analysis; all unaffected individuals were beyond the age of risk.

## Results

The lod scores at a recombination fraction of 0.001–0.40 between PC and either the SCN4A or the GH1 polymorphisms (separately and their combined haplotype) for the different subsets of families are shown in Table 1.

**Table 1.** Lod scores for PC, according to two-point linkage analysis with SCN4A and GH1 gene probes on chromosome 17

| Linkage comparison | Recombination fraction $\Theta$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.001 | 0.01 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 |
| A All families ($n = 17$) | | | | | | | |
| PC – SCN4A | 20.61 | 20.20 | 18.51 | 16.35 | 11.90 | 7.43 | 3.22 |
| PC – GH1 | 9.33 | 9.06 | 7.96 | 6.61 | 4.10 | 2.04 | 0.62 |
| PC – SCN4A/GH1 | 26.90 | 26.36 | 24.11 | 21.21 | 15.23 | 9.38 | 4.06 |
| B Ravensberg families ($n = 5$) | | | | | | | |
| PC – SCN4A/GH1 | 11.82 | 11.57 | 10.57 | 9.25 | 6.53 | 3.89 | 1.60 |
| C Other German families ($n = 10$) | | | | | | | |
| PC – SCN4A/GH1 | 13.96 | 13.68 | 12.54 | 10.07 | 7.04 | 5.05 | 2.23 |
| D British families ($n = 2$) | | | | | | | |
| PC – SCN4A/GH1 | 1.13 | 1.10 | 1.00 | 0.89 | 0.66 | 0.45 | 0.24 |
| E Families without periodic paralysis ($n = 4$) | | | | | | | |
| PC – SNC4A/GH1 | 4.76 | 4.66 | 4.28 | 3.79 | 2.78 | 1.78 | 0.84 |

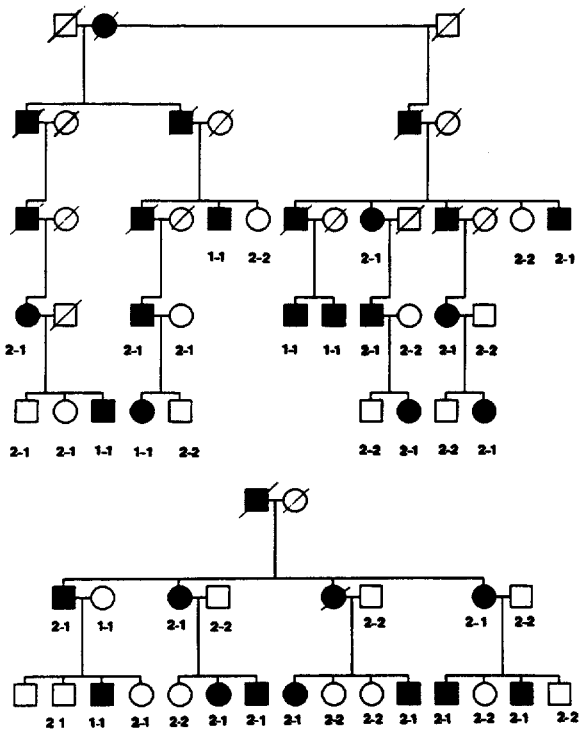

**Fig. 1.** Pedigrees of two German families with autosomal dominant PC, showing linkage to the 25-kb fragment of the *Bgl*II polymorphism of the SCN4A gene on chromosome 17, corresponding to allele 1: *1-1* indicates homozygosity for allele 1, *2-2* homozygosity for allele 2, *2-1* heterozygosity for the alleles 1 and 2. The *Hin*dII polymorphism of the GH1 gene was also informative in these two families, but the results did not significantly affect the lod score. SCN4A RFLP: *Bgl*II A1 (25 kb), A2 (15 kb), allele frequencies 0.25 and 0.75; GH1 RFLP: *Hin*dII A1 (6.7 kb), A2 (4.5 kb), allele frequencies 0.62 and 0.38

All key family members were typed with both gene probes; the informative polymorphism was used to type the remaining family members. There were 70 scorable meioses. No recombination event between the markers and PC was observed. Each gene probe separately, and the combined haplotype of the two, were tightly linked to PC. The maximum multipoint lod score for all families was $z = 26.90$ at a recombination fraction $\Theta = 0.001$ (support interval 0.001–0.02). The pedigrees of two representative German PC families are shown in Fig. 1.

## Discussion

This study conclusively shows that PC is tightly linked to the genes for SCN4A and GH1 on the long arm of chromosome 17. Since there is considerable heterogeneity in symptoms of PC, different subsets of families were studied. There was no evidence for a recombination event between the disease and the candidate gene probe in the 5 Ravensberg families. The disease co-segregated in all affected family members with the 25-kb fragment of the *Bgl*II polymorphism, corresponding to allele 1. This supports the hypothesis that the mutation has a single origin in this population (founder effect). The possibility of a homozygous individual for the PC gene was examined by investigating the family history and the pedigree structure. There was no single proven case.

After having shown linkage to the SCN4A gene in the Ravensberg families, further linkage studies were carried out to ascertain whether mutations in the same genetic locus are responsible for PC in families not related to this geographical region. Data in additional German families support the linkage model. In these families, the PC gene was linked to either of the *Bgl*II polymorphisms.

Data from two British families, which were not conclusive on their own, support the locus being on chromosome 17, since recombination was not observed. Finally, data from 4 families with "pure" PC, but with no history of periodic paralysis, were calculated separately. Again, linkage without evidence for recombination could be demonstrated. Thus, there is no evidence for genetic heterogeneity in PC in German families. Further linkage studies are required to ascertain whether mutations at the same genetic locus are responsible for PC in other populations.

The data in this study confirm the earlier proposal that the electrophysiological basis of PC is a malfunction of the muscle sodium channel (Rüdel and Lehmann-Horn 1985; Lehmann-Horn et al. 1981, 1987a). In addition, the data indicate that, in the families studied to date, both PC and HyperPP with myotonia are diseases of the same muscle sodium channel (Fontaine et al. 1990; Koch et al. 1991). Studies in well-defined families with HyperPP but without myotonia are needed to clarify whether this disorder is linked to the same gene locus on chromosome 17.

The positive lod scores of the present linkage analysis end the long-standing discussion as to whether PC and myotonic HyperPP are distinct entities (Engel 1986; De Silva et al. 1990). In nosological terms, these two disorders should be considered as one entity with the following clinical classification: "pure" PC, HyperPP with paramyotonia (= paralysis periodica paramyotonica), HyperPP with myotonia (Becker 1977, Engel 1986). The clinical and electrophysiological differences between these disorders remain to be explained. Electrophysiological in vitro studies have revealed different mechanisms causing the cold-induced weakness and the hyperkalemic weakness (periodic paralysis) (Lehmann-Horn et al. 1987a, b; Ricker et al. 1989; Moxley et al. 1989). These facts are supported by the observations that effective drug treatment to prevent cold-induced weakness or hyperkalemic weakness is different (Engel 1986; Ricker et al. 1986).

Thus, further studies at the DNA level are required to explain the genotype-phenotype correlation. The clinical heterogeneity observed might be explained by the nature and location of the mutations in the sodium channel gene and by the biological effects of these mutations on the structurally abnormal protein. Such studies might also provide an insight into the normal physiological function of membrane channels.

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