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Hyperkalemic Periodic Paralysis and the Adult Muscle Sodium Channel α-Subunit Gene

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Hyperkalemic periodic paralysis (HYPP) is an autosomal dominant disorder characterized by episodes of muscle weakness due to depolarization of the muscle cell membrane associated with elevated serum potassium. Electrophysiological studies have implicated the adult muscle sodium channel. Here, portions of the adult muscle sodium channel α-subunit gene were cloned and mapped near the human growth hormone locus (*GH1*) on chromosome 17. In a large pedigree displaying HYPP with myotonia, these two loci showed tight linkage to the genetic defect with no recombinants detected. Thus, it is likely that the sodium channel α-subunit gene contains the HYPP mutation.

THE PRIMARY PERIODIC PARALYSES are autosomal dominant disorders displaying episodes of muscle weakness accompanied by slight abnormalities in the concentration of blood potassium: elevated in the hyperkalemic form and reduced in the hypokalemic form (*1–3*). Recurrent paralysis attacks are associated with the eventual development of an irreversible vacuolar myopathy, which may be debilitating. The ultimate pathogenetic step in each form of the disorder is muscle membrane inexcitability due to depolarization (*4*), leading to paralysis.

Hyperkalemic periodic paralysis (HYPP; also known as Gamstorp's disease or adynamia episodica hereditaria) typically begins in the first decade with attacks of muscle weakness precipitated by cold, rest after exercise, fasting, or ingestion of potassium (*5*). The three clinical variants of HYPP (*1–3, 6*) breed true and include HYPP with myotonia (muscle stiffness following contraction), which includes the majority of cases, HYPP without myotonia, and HYPP with paramyotonia (cold-induced myotonia). In vitro studies have demonstrated that in HYPP with myotonia, a tetrodotoxin-sensitive sodium current is triggered in affected muscle by a slight increase in extracellular potassium that would normally not be sufficient to open the voltage-gated sodium channel (*6, 7*). The α subunit of the sodium channel can itself form a voltage-gated sodium channel that can be blocked by tetrodotoxin in vitro (*8, 9*), albeit with abnormal kinetics involving slow inactivation in the absence of an additional low molecular weight factor (*10–14*). We have therefore tested for genetic linkage between HYPP and the locus for the α subunit of the adult muscle sodium channel.

Recently, rat cDNA clones encoding the α subunits of the adult muscle sodium channel (*12*) and of the fetal muscle sodium channel (*15*) were isolated. We took advantage of strong evolutionary conservation to clone two intracellular domains of the human adult muscle sodium channel α subunit (Fig. 1A). Na2 included ~600 bp of the intracellular domain between repeats II and III (nucleotides 2434 to 3099 of the rat coding sequence) and was expected to be highly homologous only to the adult human isoform. Na3 spanned ~300 bp of the carboxyl terminal end (nucleotides 4834 to 5166) and was expected to be homologous to both adult and fetal human α subunits. These regions were amplified by the polymerase chain reaction (PCR) with normal adult human skeletal muscle single-stranded cDNA as the template, and the PCR products of appropriate size were cloned (plasmids h-Na2 and h-Na3) and sequenced (Fig. 1B) (*16*).

DNA blot analysis of human genomic DNA digested with 11 restriction enzymes and probed with h-Na2 showed only a single band for each, consistent with the existence of a single-copy gene. H-Na2 and h-Na3 were localized by somatic cell hybrids

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Table 1. Lod scores for linkage of HYPP to the sodium channel α-subunit gene.

Lod score ($\hat{z}$)	Recombination fraction ($\hat{\theta}$)					
	0.00	0.05	0.10	0.20	0.30	0.40
Two-point lod score: H-Na2 versus *GH1*	9.89	8.96	7.96	5.83	3.52	1.23
Two-point lod score: HYPP versus h-Na2	4.00	3.63	3.23	2.39	1.47	0.53
Two-point lod score: HYPP versus *GH1*	2.40	2.15	1.88	1.32	0.74	0.23
Multipoint lod score: HYPP versus h-Na2/*GH1*	7.02	6.42	5.79	4.43	2.92	1.15

to the long arm of chromosome 17 (Fig. 2). H-Na3 also hybridized to a lesser extent to chromosome 3, probably indicating cross-hybridization with the fetal gene.

To identify a restriction fragment length polymorphism (RFLP), we used h-Na2 and h-Na3 to probe human genomic DNA from five unrelated individuals digested with 30 restriction enzymes. A single Bgl II RFLP was revealed. The two allelic fragments, of 25 and 15 kb, appeared at frequencies of 0.27 and 0.73 (n = 48) respectively, in 24 unrelated individuals of Western European ancestry. The Bgl II RFLP was placed on the genetic linkage map of chromosome 17 with the use of the Venezuela reference pedigree (*17*). H-Na2 was linked with no recombinants to the human growth hormone gene (*GH1*) on 17q (Table 1; $\hat{z}$ = 9.89 at $\hat{\theta}$ = 0.00) (*18*). With the program MAPMAKER (*19*), h-Na2 was located with odds greater than 10^7:1 between *NGFR* and *TK1*, coincident with *GH1* (*17*).

We identified a large family affected by HYPP with myotonia (Fig. 3) in which the mean age of onset was 6.5 ± 5.3 years (SD, n = 23, range = 2 to 15 years). The h-Na2 RFLP was tightly linked to the disorder (Table 1; $\hat{z}$ = 4.00 at $\hat{\theta}$ =0.00) which segregated with the 25-kb Bgl II fragment (*18*). To extract information from matings uninformative for the h-Na2 RFLP (see Fig. 3: matings involving individuals III-5 and III-7), we used the tightly linked *GH1* marker. Analysis of linkage between the Hinc II RFLP at *GH1* (*20*) and HYPP resulted in a logarithm of the likelihood ratio for linkage (lod score) ($\hat{z}$) of 2.40 at $\hat{\theta}$ = 0.00 (Table 1). Analysis for linkage of HYPP with both h-Na2 and *GH1* yielded a multipoint lod score of $\hat{z}$ = 7.02 at $\hat{\theta}$ = 0.00 (Table 1) (*21*). The most likely interpretation of our data is that the α subunit of the adult muscle sodium channel is the site of the primary defect in this family. Interestingly, in 1887 G. Couzot [quoted in (*1*)] reported a periodic paralysis pedigree with cosegregating short stature, raising the possibility of a linked *GH1* mutation in that family.

Linkage analysis with additional families should clarify whether the other HYPP variants are the result of mutation of different genes or to allelic mutations of the α subunit of the adult muscle sodium channel. They should also establish whether hypokalemic periodic paralysis shares the same molecular mechanism as HYPP. In one family with

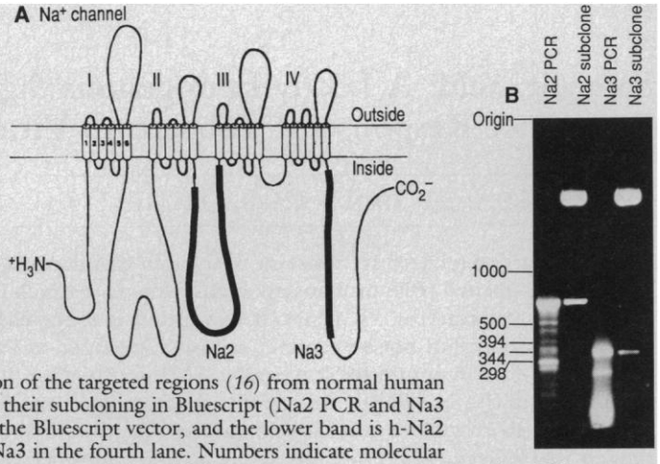

Fig. 1. Cross-species PCR-mediated cloning of portions of the α subunit of the adult muscle sodium channel. (**A**) Model of the rat sodium channel (*8, 9, 23*). The two regions of the adult rat skeletal muscle sodium channel (*12*) that were targeted for cross-species PCR cloning are shown in bold lines and are labeled Na2 and Na3. (**B**) Ethidium bromide–stained agarose gel illustrating the amplification of the targeted regions (*16*) from normal human skeletal muscle cDNA and their subcloning in Bluescript (Na2 PCR and Na3 PCR). The upper band is the Bluescript vector, and the lower band is h-Na2 in the second lane and h-Na3 in the fourth lane. Numbers indicate molecular size markers (base pairs). Sequence analysis verified that each clone showed greater than 90% sequence homology with the α subunit of the adult muscle sodium channel from the rat.

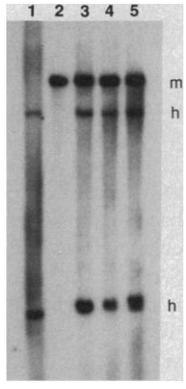

Fig. 2. Bgl II RFLP and chromosomal localization of the α subunit of the adult muscle sodium channel (h-Na2). Physical localization of h-Na2 by means of a regional somatic cell hybrid mapping panel for chromosome 17. Preliminary physical mapping was carried out with nine human × hamster hybrids for h-Na2 and h-Na3 (*24*). For h-Na2, the segregation of the hybridization signal was completely concordant with that of chromosome 17. The discordant fractions for the sex chromosomes and for all autosomes except chromosome 7 ranged from 0.29 to 0.78. The discordant fraction for chromosome 7 was 0.11. In addition to the chromosome 17 component, h-Na3 identified a locus likely to be on chromosome 3, although the discordant fraction was only 0.11 for several autosomes. In view of the chromosome 17 localization of the α-subunit gene, we carried out mapping of h-Na2 using the regional panel shown here digested with Hind III (*25–27*). Mouse-specific and human-specific fragments are designated "m" and "h", respectively. The human specific bands (lane 1) are absent from the mouse cell line LTK$^-$ (lane 2), and present in the human × mouse hybrids NF-13 (17q11.2-17qter) (lane 3); DCR-1 (17q11.2-17qter) (lane 4); and Sp3 (17q11.2-17qter) (lane 5), suggesting a 17q localization (*28*).

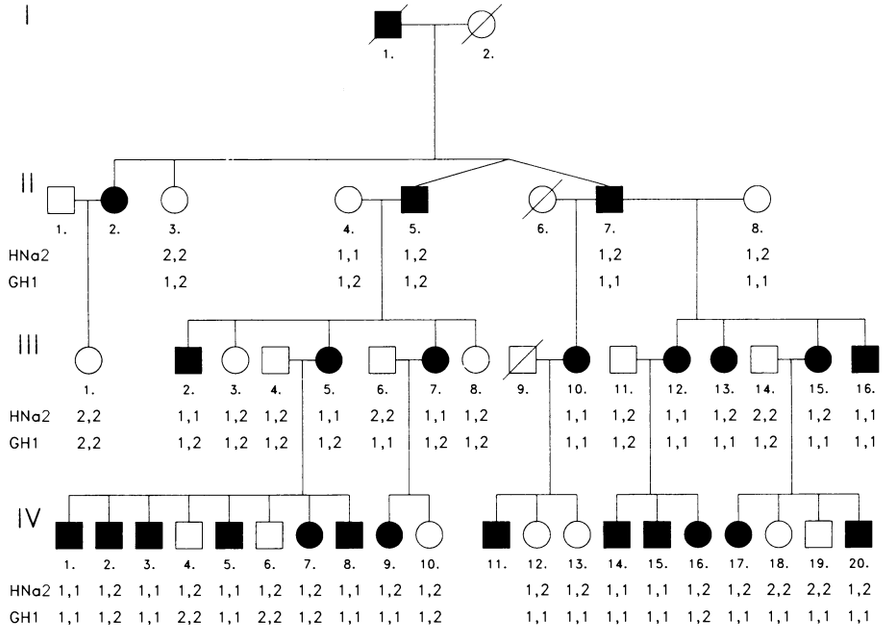

Fig. 3. Typing for the sodium channel and its closely linked marker *GH1* in the hyperkalemic periodic paralysis (HYPP) with myotonia pedigree. Blood was collected from each cooperative member of this family for initiation of lymphoblastoid cell lines. Criteria used for the diagnosis of HYPP were those described in (*1–3*). Alleles indicated for the Bgl II RFLP detected by h-Na2 are 25 kb (*1*) and 15 kb (*2*) and for the Hinc II RFLP at *GH1* are 6.7 kb (*1*) and 4.5 kb (*2*).

hypokalemic periodic paralysis, we have found a recombinant between this disorder and the sodium channel gene (*22*), suggesting that separate loci may exist.

The ultimate proof of a defect in the sodium channel gene in HYPP will require definition of the molecular lesion. This is the next step toward understanding of the structure and function of the sodium channel, establishment of molecular diagnostic procedures in HYPP, and realization of an animal model of the disorder.

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Salicylic Acid: A Likely Endogenous Signal in the Resistance Response of Tobacco to Viral Infection

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Some cultivars of tobacco are resistant to tobacco mosaic virus (TMV) and synthesize pathogenesis-related (PR) proteins upon infection. In a search for the signal or signals that induce resistance or PR genes, it was found that the endogenous salicylic acid levels in resistant, but not susceptible, cultivars increased at least 20-fold in infected leaves and 5-fold in uninfected leaves after TMV inoculation. Induction of PR1 genes paralleled the rise in salicylic acid levels. Since earlier work has demonstrated that treatment with exogenous salicylic acid induces PR genes and resistance, these findings suggest that salicylic acid functions as the natural transduction signal.

LIVING ORGANISMS HAVE EVOLVED A complex array of biochemical pathways that enable them to recognize and respond to signals from the environment. These pathways include receptor organs, hormones, second messengers, and enzymatic modifications. At present, little is known about the signal transduction pathways that are activated during a plant's response to attack by a pathogen, although this knowledge is central to our understanding of disease susceptibility and resistance. A common form of plant resistance is the restriction of pathogen proliferation to a small zone surrounding the site of infection. In many cases, this restriction is accompanied by localized death (necrosis) of host tissues. Together, pathogen restriction and local tissue necrosis characterize the hypersensitive response (HR). In addition to local defense responses, many plants respond to infection by activating defenses in uninfected parts of the plant. As a result, the entire plant is more resistant to a secondary infection. This systemic acquired resistance (SAR) can persist for several weeks or more (*1*) and often confers cross-resistance to unrelated pathogens (*2*).

The interaction between tobacco (*Nicotiana tabacum* Linn.) and TMV has been used extensively as a model for the study of plant disease and resistance. In general, infection of tobacco leaves with TMV results in one of two distinct responses. TMV-infected tobacco cultivars that have the dominant N gene are resistant and display both HR and SAR (*1*). In contrast, tobacco cultivars that lack the N gene (nn genotype) are susceptible to TMV. The virus replicates and spreads rapidly throughout these plants, causing stunting and the appearance of mosaic patterns of chlorosis on the youngest leaves.

Resistant (NN genotype), but not susceptible (nn), cultivars produce several new proteins in response to TMV infection, including five distinct families of proteins referred to as pathogenesis-related (PR) proteins (PR1 through PR5) (*3, 4*). PR proteins are found in many plants, and different subsets can be induced by various viral, viroid, fungal, and bacterial pathogens and by certain environmental and chemical stresses (*5*). The defense-related enzymatic activities of some of these PR proteins (*6, 7*) and the correlation between PR gene expression and resistance suggest that these

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