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Research Report

Evaluation of HCN2 abnormalities as a cause of juvenile audiogenic seizures in Black Swiss mice

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jams1, juvenile audiogenic monogenic seizures
HCN2, hyperpolarization-activated cyclic nucleotide-gated channel subunit 2
Bsg, basigin
MCOI, multi-copper oxidase
cM, centimorgan

ABSTRACT

Epilepsy is an often-debilitating disease with many etiologies. Genetic predisposition is common for many of the generalized epilepsy syndromes, and mutations in genes encoding neuronal ion channels are causative in many cases. We previously identified a locus for juvenile audiogenic monogenic seizures (*jams1*) in the Black Swiss mouse strain, delimited by the gene basigin (*Bsg*) and the marker *D10Mit140*. This region includes *Hcn2*, the gene encoding the hyperpolarization-activated cyclic nucleotide-gated channel subunit 2 (HCN2), an ion channel implicated in epilepsy. By sequencing genomic DNA, we found that Black Swiss mice have a single polymorphism in exon 2 within the *Hcn2* gene. This single G/C to A/T base change alters the third position of a codon specifying alanine residue 293, without changing the predicted amino acid sequence. Furthermore, we found no detectable differences in HCN2 protein expression in the brains of Black Swiss mice, compared to control mice. We therefore reason that juvenile audiogenic seizures in Black Swiss mice are unlikely to be due to abnormalities of HCN2 channel function.

1. Introduction

Epilepsy, the condition in which affected individuals suffer recurrent seizures, affects up to 2% of the population. Whereas environmental factors such as traumatic head injury and brain ischemia can be causative in some cases, many familial epilepsy syndromes have been characterized, pointing to genetic factors that may predispose toward or underlie many cases of epilepsy. Recent studies have identified numerous genes responsible for monogenic epilepsy syndromes, which are largely generalized epilepsy syndromes as opposed to partial epilepsy syndromes seen with many environmental causes. Notably, the majority of identified gene defects underlying familial human epilepsy syndromes have involved

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voltage- or ligand-gated ion channels (Scheffer and Berkovic, 2003), illuminating the importance of neuronal membrane and network excitability in the pathophysiology of epilepsy (Mccormick and Contreras, 2001). Furthermore, consistent with abnormal excitability, human patients with familial and sporadic generalized epilepsy syndromes may suffer seizures in response to photic stimulation or somatosensory or audiogenic stimuli (Ferlazzo et al., 2005). This phenomenon is known as "reflex seizures" and can cause significant disability in patients who must avoid inciting stimuli.

In addition to the use of genetic approaches to identify genes involved in human epilepsy syndromes, mouse models of seizure susceptibility have further illuminated the role of ion channels in epilepsy, and have demonstrated overlap between human and mouse epilepsy genes (Steinlein and Noebels, 2000; Noebels, 2003). Besides the benefits of large numbers of affected animals to facilitate genetic analysis, spontaneous heritable epilepsy in mice provides a model for detailed biochemical and electrophysiological analysis not possible in human patients.

One of the best-studied models of generalized epilepsy in mice is susceptibility to audiogenic seizures, wherein affected mice develop generalized seizures in response to noxious auditory stimuli (Brennan et al., 1997; Ross and Coleman, 1999). Whereas mouse audiogenic seizure susceptibility loci have been mapped to chromosomes 4, 7, and 12 in the mouse DBA/2J strain (Neumann and Seyfried, 1990), others have identified a monogenic locus on chromosome 13 in the Frings mouse, which was cloned to reveal a single gene encoding a novel protein, Mass1, that shares homology with the Na^+/Ca^{2+} exchanger and contains a multi-copper oxidase (MCOI) domain (Skradski et al., 2001). The role of Mass1, also known as the very large G-protein-coupled receptor-1 (VLGR1), in epilepsy has been further strengthened by a report of a family with febrile and nonfebrile convulsions with a mutation of the *MASS1* gene (Nakayama et al., 2002), as well as studies demonstrating that mice with targeted disruption of the transmembrane and cytoplasmic domains of Mass1/VLGR1 or the entire gene are susceptible to audiogenic seizures (Mcmillan and White, 2004; Yagi et al., 2005). Although Mass1 mutations may result in abnormal brain excitability, mice with audiogenic seizures often have hearing loss, complicating attribution of pathogenesis to abnormal brain excitability. Indeed, Mass1/VLGR1 animals have profound cochlear abnormalities and hearing loss (Johnson et al., 2005), and certain mutations of the same gene in humans result in Usher syndrome type IIC (USH2C), characterized by congenital hearing loss and progressive retinitis pigmentosa but no seizures (Weston et al., 2004). Thus, whereas Frings mice have audiogenic seizures due to loss of Mass1/VLGR1, the primary underlying pathophysiology remains unclear.

We previously identified a unique monogenic locus for juvenile audiogenic seizures in the Black Swiss mouse strain (*jams1*), whose seizures are unrelated to hearing loss and may better model reflex epilepsy in humans (Misawa et al., 2002). The *jams1* locus maps to a 1.6 centimorgan (cM) region on mouse chromosome 10, delimited by the marker D10Mit140 and the gene *Bsg* (Basigin). Interestingly, this region is largely syntenic to a region on human chromosome 19p13.3 that has been implicated in familial juvenile febrile convulsions (Johnson et al., 1998). The *jams1* locus contains 128 known or predicted genes, of which one, *Hcn2*, encodes a voltage-gated ion channel subunit, the hyperpolarization-activated cyclic nucleotide-gated ion channel subunit 2 (HCN2). HCN channels are critical for cellular excitability, and the HCN2 subunit has been implicated in mechanisms of generalized epilepsy as well as febrile convulsions in rodents (Ludwig et al., 2003; Brewster et al., 2002). Furthermore, HCN2 channels are enriched in the inferior colliculus, a brainstem nucleus critical for auditory processing (Koch et al., 2004) Thus, we reasoned that the HCN2 channel is an excellent candidate protein to consider underlying juvenile audiogenic seizures in Black Swiss mice.

To address whether mutations in the *Hcn2* gene within the *jams1* locus are responsible for audiogenic seizures in Black Swiss mice, we analyzed the *Hcn2* gene and protein in juvenile and adult Black Swiss mice. By sequencing Black Swiss genomic DNA, we found that Black Swiss mice harbor a single point mutation within the coding region of Exon 2 of the *Hcn2* gene that does not change the primary amino acid sequence of HCN2. Furthermore, we found no detectable differences in HCN2 protein expression in the brains of juvenile and adult Black Swiss mice, compared to control C57/B6 mice. We therefore reason that juvenile audiogenic seizures in Black Swiss mice are not due to abnormalities of the *Hcn2* gene.

2. Results

2.1. *The Hcn2 coding region lacks significant mutations in Black Swiss mice*

The monogenic locus for juvenile audiogenic seizures in the Black Swiss mouse strain (*jams1*) maps to a 1.6 cM region on mouse chromosome 10, delimited by the marker D10Mit140 and the gene *Bsg* (Basigin) (Misawa et al., 2002). This region contains 128 known or predicted genes, of which one *Hcn2*, encodes a voltage-gated ion channel subunit, HCN2. Because HCN ion channels have been implicated in epilepsy, we undertook sequencing of the *Hcn2* gene exons and intron/exon boundaries in Black Swiss mice. The *Hcn2* gene coding region includes 8 exons that generate an 863 AA protein consisting of 6 transmembrane domains, a cyclic nucleotide binding domain and a predicted C-terminal PSD-95-Discs Large-Zona Occludens (PDZ) ligand (Fig. 1A). We performed PCR-based cloning of the exons of *HCN2* to determine if there were sequence differences between Black Swiss and control mice that do not exhibit audiogenic seizures. In each case, primer pairs were chosen to include 50–100 nucleotides of adjacent intron sequence and intron/exon boundaries for each exon (Table 1). To reduce the possibility of PCR-generated mutations, we used high-fidelity *Pfx* polymerase, and by varying magnesium concentration and using 10% DMSO in PCR reactions (Table 1), we were able to generate PCR fragments of all *Hcn2* exons (Fig. 1B) and subcloned these fragments into pcDNA3 (Fig. 1C). Sequence analysis of the cloned PCR fragments revealed a single difference between Black Swiss and that published for the C57BL/6J strain. This polymorphism occurs within the coding region of exon 2, and