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Research paper

A new spontaneous mutation in the mouse protocadherin 15 gene

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Abstract

We have characterized a new allele of the protocadherin 15 gene (designated *Pcdh15*$^{av\text{-}6J}$) that arose as a spontaneous, recessive mutation in the C57BL/6J inbred strain at Jackson Laboratory. Analysis revealed an inframe deletion in *Pcdh15*, which is predicted to result in partial deletion of cadherin domain (domain 9) in Pcdh15. Morphologic study revealed normal to moderately defective cochlear hair cell stereocilia in *Pcdh15*$^{av\text{-}6J}$ mutants at postnatal day 2 (P2). Stereocilia abnormalities were consistently present at P5 and P10. Degenerative changes including loss of inner and outer hair cells were seen at P20, with severe sensory cell loss in all cochlear turns occurring by P40. The hair cell phenotype observed in the 6J allele between P0 and P20 is the least severe phenotype yet observed in *Pcdh15* alleles. However, young *Pcdh15*$^{av\text{-}6J}$ mice are unresponsive to auditory stimulation and show circling behavior indicative of vestibular dysfunction. Since these animals show severe functional deficits but have relatively mild stereocilia defects at a young age they may provide an appropriate model to test for a direct role of Pcdh15 in mechanotransduction.
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Keywords: Deafness; Cochlear hair cells; Pcdh15; Mouse

1. Introduction

The mouse Ames waltzer (*av*) is a recessive mutation, which causes deafness and vestibular dysfunction associated with degeneration of the inner ear neuroepithelia. The gene that harbors the *av* mutation is Protocadherin 15, *Pcdh15* (Alagramam et al., 2001a). Mutation in *PCDH15* causes Usher syndrome type 1F (Ahmed et al., 2001; Alagramam et al., 2001b) and non-syndromic deafness DFNB23 (Ahmed et al., 2003). Recently, the R245X mutation of *PCDH15* was reported to account for 58% of USH1 cases in the Ashkenazi Jewish population (Ben-Yosef et al., 2003). The importance of studying the *av* mouse as a model for inner ear dysfunction in these patients has grown since the identification of the Pcdh15 gene in 2001 (Alagramam et al., 2001a).

Documenting the salient features of cochlear pathology in different alleles of *av* will help us understand (a) the function of *Pcdh15* in hair cell development and (b) the cause of inner ear disorders in USH1F and DFNB23 patients. Reports on *av* mutants in the literature show that mutation in *Pcdh15* affects hair bundle morphogenesis and polarity (Hampton et al., 2003; Pawlowski et al., 2006; Raphael et al., 2001; Washington et al., 2005) and mechanotransduction (Alagramam et al., 2005). More recently, a detailed study on the localization and function of Pcdh15 in hair cells by Senften et al. (2006) strongly supports the role of Pcdh15 in bundle morphogenesis and polarity. Specifically, the localization of Pcdh15 to the base of the stereocilia in young mice further strengthens the view that Pcdh15 plays a role in bundle polarization during early stages of development.

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Little is known about the function of protocadherins. It has been suggested that Pcdh15 is involved in hair bundle cohesion via its cadherin domains. Cadherins are known to link cell membranes together (Gumbiner, 2005; Patel et al., 2003). Pcdh15 has 11 cadherin domains that are thought to adhere to similar domains of a second molecule on an adjacent stereocillium in a homophylic manner, forming lateral links between stereocilia early in development. However, they can also act as signaling molecules through homophylic or heterophylic binding at the extracellular domains, resulting in interactions with other molecules at the cell membrane or with the intracellular cytoskeleton (Gumbiner, 2005; Patel et al., 2003). Cadherin domain 1, which is affected in the *Pcdh15*$^{av\text{-}2J}$ mutation, is thought to be key for adhesion, yet the effect of the mutation in this allele varies between subtle and severe hair cell pathology, with occasional animals showing some preservation of auditory function. The affected cadherin domain in the *Pcdh15*$^{av\text{-}6J}$ allele reported here is cadherin domain 9 and animals having this mutation are consistently unresponsive to auditory stimulation by young adulthood. How do mutations in the cadherin chain produce such functional deficits? Close analysis of the more subtle Pcdh15 mutations, such as the one described here, will be important for better understanding the function(s) of this class of cadherin.

2. Materials and methods

2.1. Mice

The new allele described in this report arose as a spontaneous mutation at The Jackson Laboratory (TJL) and was maintained in a C57BL/6J (B6) background. The Animal Care and Use Committee at TJL approved the care and use of the mice included in part of the investigation. A total of 200 mice of both sexes were used at TJL. The Animal Care and Use Committee at Case Western Reserve University (CWRU) approved the care and use of the mice included in the remaining part of the investigation. A total of 50 mice of both sexes were used (25 deaf-circlers; 25 controls) at CWRU.

2.2. Auditory-evoked brain stem response (ABR)

Auditory brainstem responses (ABRs) were conducted as previously described (Zheng et al., 1999). Briefly, mice were anesthetized and their body temperature was maintained at 37–38 °C by placing them on a heating pad in a soundproof chamber during testing. Intelligent Hearing System (Miami, FL) was used to generate acoustic stimuli and ABR recording. Platinum subdermal needle electrodes were inserted at the vertex (active), ventrolaterally to the right ear (reference) and the left ear (ground). Alternating click stimuli of 50 ms duration and tone bursts with 3 ms duration (1.5 ms rise-fall time with no plateau) of 8, 16, and 32 kHz were presented to both ears of the animals through plastic tubes (a closed system). ABR threshold was obtained for each animal by reducing the stimulus intensity from 100 dB SPL in 10 dB steps and finally in 5 dB steps until the lowest intensity that could evoke a reproducible ABR pattern was detected on the computer screen.

2.3. Mutation screening

2.3.1. Reverse transcriptase polymerase chain reaction (RT-PCR)

RT-PCR was used to screen for mutations in the *Pcdh15* coding sequence. RNA was isolated from affected mice and reverse transcribed to complementary DNA (cDNA). RNA isolated from brain was used for initial screening. RNA isolated from cochlear tissues was used later to confirm results of preliminary screening from brain RNA. RNA was extracted from five av6J homozygous mutants and five unaffected siblings as described previously (Washington et al., 2005). Standard PCR technique was used to amplify specific fragments of cDNA that were resolved on a standard agarose gel to verify size of the PCR product. These products were cloned followed by DNA sequence analysis. Results from the affected mice were compared to results from the unaffected siblings. Multiple clones were sequenced ($n = 3$) to ensure that results were not errors introduced by PCR. RT reactions were performed with ~2 μg of total RNA. The Superscript First-Strand Synthesis System for RT-PCR (Invitrogen, CA) was used to generate and amplify *Pcdh15* cDNA. Primers designed to amplify 0.5 kb or 1 kb overlapping fragments of the coding region were synthesized. Conditions used for amplification were similar to those used previously to amplify *Pcdh15* (Washington et al., 2005). PCR products were analyzed on 2% agarose gels for large fragment products (~1 kb) or 3% agarose gels for smaller fragments (~0.5 kb). The following PCR conditions were used: 94 °C for 2 min followed by 34 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min (or 72 °C for 30 s with 0.5 kb fragment). Temperature for annealing was adjusted according to T_m of the set of primers used. RT-PCR product was cloned into pCR2.1-TOPO vector (Invitrogen, CA) prior to sequence analysis. The sequences of RT-PCR products were determined using BigDye Terminator Cycle sequencing reagents and protocols (Applied Biosystems, CA). The ABI Prism 377 DNA sequencer (Applied Biosystem, CA) was used to analyze and display the resulting sequence data. The primer pairs used to confirm abnormal splicing as a result of mutation and the PCR conditions used are described here: KA100 (5′TTT TTG CAC TGC ATC CAT TC 3′) and KA113 (5′GTG GGA TCT CTC CAG GAT GT 3′) were used to amplify a 510 bp fragment spanning the region from exon 20 to exon 23. KA684 (5′ACA TGA ATG ACT ACC CTC CA 3′) and KA685 (5′CTG CTG GAC ATC ACA GGT 3′) were used to amplify a smaller 304 bp fragment spanning the region from exon 21 to exon 23. cDNA obtained from either

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