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Genetic background effects on age-related hearing loss associated with *Cdh23* variants in mice

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ABSTRACT

Inbred strain variants of the *Cdh23* gene have been shown to influence the onset and progression of age-related hearing loss (AHL) in mice. In linkage backcrosses, the recessive *Cdh23* allele (*ahl*) of the C57BL/6J strain, when homozygous, confers increased susceptibility to AHL, while the dominant allele (*Ahl*+) of the CBA/CaJ strain confers resistance. To determine the isolated effects of these alleles on different strain backgrounds, we produced the reciprocal congenic strains B6.CBACa-*Cdh23*$^{Ahl+}$ and CBACa.B6-*Cdh23*^{ahl} and tested 15-30 mice from each for hearing loss progression. ABR thresholds for 8 kHz, 16 kHz, and 32 kHz pure-tone stimuli were measured at 3, 6, 9, 12, 15 and 18 months of age and compared with age-matched mice of the C57BL/6J and CBA/CaJ parental strains. Mice of the C57BL/6N strain, which is the source of embryonic stem cells for the large International Knockout Mouse Consortium, were also tested for comparisons with C57BL/6J mice. Mice of the C57BL/6J and C57BL/6N strains exhibited identical hearing loss profiles: their 32 kHz ABR thresholds were significantly higher than those of CBA/CaJ and congenic strain mice by 6 months of age, and their 16 kHz thresholds were significantly higher by 12 months. Thresholds of the CBA/CaJ, the B6.CBACa-*Cdh23*$^{Ahl+}$, and the CBACa.B6-*Cdh23*^{ahl} strain mice differed little from one another and only slightly increased throughout the 18-month test period. Hearing loss, which corresponded well with cochlear hair cell loss, was most profound in the C57BL/6J and C57BL/6NJ strains. These results indicate that the CBA/CaJ-derived *Cdh23*$^{Ahl+}$ allele dramatically lessens hearing loss and hair cell death in an otherwise C57BL/6J genetic background, but that the C57BL/6J-derived *Cdh23*^{ahl} allele has little effect on hearing loss in an otherwise CBA/CaJ background. We conclude that although *Cdh23*^{ahl} homozygosity is necessary, it is not by itself sufficient to account for the accelerated hearing loss of C57BL/6J mice.

1. Introduction

Age-related hearing loss (AHL), or presbycusis, poses a serious human health concern as it negatively affects the life of many elderly individuals (Dalton et al., 2003). In a 2003 CDC study, presbycusis was found to be second only to arthritis as a handicapping condition within the elderly segment of our society (Bielefeld et al., 2010). Susceptibility to AHL has a significant genetic component although AHL presents with a complex pathophysiology (DeStefano et al., 2003). A range of environmental factors, including exposure to loud noises or ototoxic drugs, can exacerbate and accelerate AHL. The high degree of genetic complexity in human populations coupled with highly variable environmental factors make it difficult to identify the genetic components underlying AHL in the human population, although some limited success has been achieved (Bared et al., 2010; DeStefano et al., 2003; Friedman et al., 2009; Garringer et al., 2006; Rodriguez-Paris et al., 2008; Unal et al., 2005).

Laboratory mice are more amenable to studies of AHL predisposition because of their well-defined genetics, short life span and the ability of researchers to control for environmental factors. Like humans, many inbred mouse strains express variable degrees of AHL, and at least ten quantitative trait loci associated with AHL have been identified in mice (Latoche et al., 2011; Noben-Trauth and Johnson, 2009). C57BL/6J (B6J) and CBA/CaJ (CBA) are the two most widely used inbred strains in hearing research. The B6J strain

Abbreviations: ABR, auditory brainstem response; AHL, age-related hearing loss; IHC, inner hair cell; OHC, outer hair cell; ANOVA, analysis of variance; st dev, standard deviation; SEM, standard error of the mean.

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has been used extensively as a model of early onset AHL (Henry and Chole, 1980; Hequembourg and Liberman, 2001; Mikaelian, 1979; Wang et al., 2008; Willott, 1986). B6J mice exhibit a high frequency hearing loss by 3–6 months of age that progresses to a profound impairment by 15 months. In contrast, CBA mice remain resistant to AHL until 15 months of age or older and are often used as good hearing controls (Henry and Chole, 1980; Li and Borg, 1991; Zheng et al., 1999). B6J and CBA mice also exhibit dramatically different rates of cochlear hair cell loss (Spongr et al., 1997).

One of the major genetic factors contributing to hearing loss in B6J mice is the *ahl* locus on Chromosome 10 (Johnson et al., 1997), which has been shown to be a splice variant of the cadherin 23 gene (*Cdh23*) (Noben-Trauth et al., 2003). The CBA strain does not have this predisposing *Cdh23* splice variant, consistent with its much slower rate of hearing loss. Cadherin 23 has been shown to be a component of the tip links of hair cell stereocilia, along with protocadherin 15 (Kazmierczak et al., 2007). Mutations in the genes encoding these two proteins affect tip link formation and stability, which is necessary for normal hair cell mechanotransduction (Alagramam et al., 2011). Mice with an ENU-induced missense mutation in the ectodomain of Cdh23 show a loss of tip links that correlates with a progressive hearing loss that is similar to, but more severe than the hearing loss associated with the *ahl* splice variant of *Cdh23* (Schwander et al., 2009).

Here we report on the characterization of two reciprocal congenic strains that we developed to evaluate the isolated effects of *Cdh23* allelic variants when on different strain backgrounds. The CBACa.B6-*Cdh23*^{ahl} congenic stain (CBA.B6-*ahl*) is homozygous for the recessive AHL susceptibility allele (*ahl*) of *Cdh23*, which we transferred from the B6J strain onto the CBA background. The reciprocal B6.CBACa-*Cdh23*$^{Ahl+}$ congenic strain (B6.CBA-*Ahl*+) is homozygous for the dominant AHL resistance allele (*Ahl*+) of *Cdh23,* which we transferred from the CBA strain onto the B6J background. To follow the progression of hearing loss in the congenic strain mice and mice of the B6J and CBA parental strains, auditory brainstem response (ABR) thresholds were measured at 3, 6, 9, 12, 15 and 18 months of age. After the final ABR test, inner ears of mice from each strain were examined for cochlear hair cell loss to determine its correspondence with hearing loss.

We also report on hearing loss in mice of the C57BL/6N strain (B6N), an NIH subline of C57BL/6 separated from B6J in 1951. A complete description of the B6N strain at The Jackson Laboratory (C57BL/6NJ, Stock #005304) and its development is given in the JAX mice database (http://jaxmice.jax.org/strain/005304.html). Like B6J, the B6N strain carries the *Cdh23*^{ahl} hearing loss susceptibility allele, but it has not previously been assessed for AHL. Because the B6N strain is the source of embryonic stem cells for the International Knockout Mouse Consortium (http://www.knockoutmouse.org/), establishment of a hearing profile for this control strain is important for assessing hearing-related phenotypes of the many knockout mice being generated by this program.

2. Materials and methods

2.1. Mice and congenic strain development

All mice examined in this study originated from The Jackson Laboratory (http://www.jax.org/), and all procedures involving their use were approved by the Institutional Animal Care and Use Committee. The Jackson Laboratory is accredited by the American Association for the Accreditation of Laboratory Animal Care.

We use the following abbreviated strain designations throughout the paper, which are followed in parentheses by their corresponding full inbred strain names and Jackson Laboratory Stock Numbers: CBA (CBA/CaJ, Stock #000654), B6J (C57BL/6J, Stock #000664), B6N (C57BL/6NJ, Stock #005304), B6.CBA-*Ahl*+ (B6.CBACa-*Cdh23*$^{CBA/CaJ}$, Stock #10615) and CBA.B6-*ahl* (CBACa.B6-*Cdh23*^{ahl}, Stock #10614). The region of the *Cdh23* gene that is associated with AHL in inbred mouse strains (Noben-Trauth et al., 2003) was amplified by PCR from the genomic DNA of each of these strains, and *Cdh23*^{ahl} and *Cdh23*$^{Ahl+}$ genotypes were confirmed by DNA sequence analysis (Supplementary Fig. 1).

The B6.CBA-*Ahl*+ and CBA.B6-*ahl* reciprocal congenic strains were developed by genetic introgression of B6J- and CBA-derived segments of Chromosome 10 (containing the *Cdh23* gene) into the genomes of CBA and B6J, respectively. To accomplish this introgression, mice from the B6J and CBA parental strains were first intercrossed to generate F1 hybrids. The F1 hybrids were then backcrossed to B6J mice to produce N2 generation mice for development of the B6.CBA-*Ahl*+ strain and to CBA mice to produce N2 mice for development of the CBA.B6-*ahl* strain. Repeated backcrossing of hybrid progeny (selected on the basis of Chromosome 10 marker genotypes) to the parental (B6J or CBA) strain continued until 10 backcross generations (N10) were completed. Finally, the N10 generation mice of each line were interbred to produce the two homozygous congenic strains. To define the extent of the congenic regions in each strain, single nucleotide polymorphisms (SNPs) along the length of Chromosome 10 were genotyped. The CBA-derived congenic interval of B6.CBA-*Ahl*+ extends from 53.46 Mb (SNP *rs3696307*) to 105.44 Mb (SNP *rs13480749*), and the B6J-derived congenic interval of CBA.B6-*ahl* extends from 38.69 Mb (SNP *rs13480581*) to 74.61 Mb (SNP *rs13480654*).

2.2. Hearing assessment: auditory-evoked brainstem response

Hearing in mice was assessed by ABR threshold analysis, essentially as previously described (Zheng et al., 1999). Mice were anesthetized with an intraperitoneal injection of tribromoethanol (0.2 ml of 12.5 mg/ml stock per 10 g of body weight), and then placed onto a 37 °C heating pad in a sound-attenuating chamber. The evoked brainstem responses to 8, 16 and 32 kHz tone-bursts stimuli were amplified and averaged. By varying the sound pressure level (SPL) in 5 dB increments, ABR thresholds were defined as the lowest dB SPL level at which an ABR pattern could be clearly recognized with peaks 1–2 being the most prominent at high levels and still recognizable at intensities near threshold. Stimulus presentation, data acquisition and analysis were performed using computerized equipment from Intelligent Hearing Systems (IHS; Miami, Florida). 100 dB was the maximum SPL presented for all stimuli. With our testing system, average ABR thresholds (in dB SPL) for normal hearing mice are about 40 dB for 8 kHz, 20 dB for 16 kHz, and 45 dB for 32 kHz stimuli.

2.3. Cochlear histology

Cochleae were prepared and evaluated as described in detail in earlier publications (Ding et al., 2001; Johnson et al., 2010; Zheng et al., 2009). Mice were euthanized by CO_2 asphyxiation, and decapitated. The temporal bones were removed, immersed in 4% paraformaldehyde, and shipped to the University of Buffalo for analysis. Cochleae were stained with Ehrlich's hematoxylin solution, the organ of Corti carefully microdissected out into two or three segments and mounted as a flat surface preparation in glycerin on glass slides. One person, blind to the ABR results, dissected the cochleas and prepared the surface preparation. A second person blind to the experimental conditions made the hair cell counts and had no prior knowledge of the ABR results. Surface preparations were examined with a light microscope (Zeiss Standard, 400× magnification). With careful inspection, the hair cells can be readily distinguished from support cells in hematoxylin-stained