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***Ahl2*, a Second Locus Affecting Age-Related Hearing Loss in Mice**

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Abstract

Inbred mouse strains with age-related hearing loss (AHL) provide valuable models for studying the genetic basis of human presbycusis. Here we report the genetic mapping of a second AHL locus in mice (designated *Ahl2*) that is a major contributor to the 8- to 10-month difference in hearing loss onset times between NOD/LtJ and C57BL/6J mice. A whole-genome linkage scan of 110 progeny from a (C57BL/6J × NOD/LtJ) × NOD/LtJ backcross revealed statistically significant associations of ABR thresholds with markers on chromosome 5, with a peak lod score of 5.5 for *D5Mit309*. At 6 months of age, backcross progeny that inherited two copies of the recessive NOD/LtJ-derived allele at this locus (genotype *ahl2/ahl2*) exhibited ABR thresholds that were on average 26 decibels above those of heterozygous mice. Analysis of a (CAST/Ei × NOD/LtJ) × NOD/LtJ backcross, which segregates strain-specific alleles at both *Ahl2* and the *Ahl* locus on chromosome 10, showed that the hearing loss attributable to *Ahl2* is dependent on a predisposing *Ahl* genotype. The statistically significant effect of *Ahl2* observed in crosses with NOD/LtJ was not seen in crosses involving three other strains with early onset AHL: A/J, BUB/BnJ, and SKH2/J.

Age-related hearing loss (or presbycusis) is the most common form of sensory impairment in human populations [1,2] and adversely affects the quality of life of many elderly individuals [3]. The genetic basis of presbycusis is poorly understood because of the extreme difficulty in studying such a late-onset, complex disease. The cumulative effects of non-genetic factors such as noise trauma, disease, or ototoxic drugs vary greatly among individuals over the course of their lifetimes, confounding estimates of genetic components. One approach for unraveling the complex genetic basis of human presbycusis is to use inbred strains of the laboratory mouse as models. Mice offer a promising approach because of their short lifespan and ease of experimental manipulation, and because of the anatomical and functional similarities between the human and mouse cochlea [4,5].

Several inbred strains of mice, such as C57BL/6J, DBA/2J, and BALB/cByJ, exhibit age-related hearing loss (AHL) and provide valuable models for human presbycusis [6–10]. By backcross linkage analysis of F1 hybrids between CAST/Ei and strains that exhibit AHL, we previously mapped a locus (symbol *Ahl*) on mouse chromosome (Chr) 10 and showed that it is a major contributor to AHL susceptibility in all 10 inbred strains examined [11,12]. Although all 10 strains exhibit AHL, severity and onset times vary greatly. For example, C57BL/6J mice do not exhibit hearing loss until 10 months of age or older, whereas NOD/LtJ mice exhibit a much earlier onset—before 3 months. Analysis of progeny from crosses between C57BL/6J and NOD/LtJ mice showed that variation in AHL onset time was not associated with the Chr 10 *Ahl* locus [12], indicating that other genes must be responsible.

Here we report the genetic mapping of a second AHL locus (*Ahl2*) on Chr 5 that is a major contributor to the variation in hearing loss onset times between NOD/LtJ (NOD) and C57BL/6J (B6) mice. (To alleviate husbandry difficulties encountered with diabetic mice, we used the

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resistant NOD.NON-*H2^{nb1}* congenic strain, rather than its diabetic NOD/LtJ progenitor, but for brevity have designated these mice as NOD.) Hearing in mice was assessed by auditory brainstem response (ABR) threshold analysis, as described [10]. Briefly, the evoked brainstem responses of anesthetized mice were amplified and averaged and their wave patterns displayed on a computer screen. Auditory thresholds were obtained for each specific auditory stimulus by varying the sound pressure level (SPL) to identify the lowest level at which an ABR pattern could be recognized. With our testing system, average ABR thresholds (in decibels (dB) SPL) for mice with normal hearing are about 40 for click, 30 for 8 kHz, 20 for 16 kHz, and 45 for 32 kHz stimuli. We considered 20–40 dB SPL above normal to be a mild impairment, 41–60 dB above normal to be intermediate, and greater than 60 dB above normal to be a profound impairment.

At 6 months of age, B6 mice and F1 hybrids between B6 and NOD mice (B6NODF1) have normal ABR thresholds, whereas NOD mice exhibit a profound hearing loss [12,13]. Therefore, to maximize linkage information, we backcrossed B6NODF1 hybrids to NOD mice rather than intercrossing. The frequency distribution of ABR thresholds among the 6-month-old N2 backcross mice was roughly bimodal rather than bell shaped (Fig. 1A), indicating the likelihood of a large contribution from one or a small number of loci.

DNA genotyping of 110 backcross progeny for 90 marker loci distributed throughout the genome was performed by the Center for Inherited Disease Research (CIDR). ABR thresholds for click, 8 kHz, 16 kHz, and 32 kHz stimuli were used as quantitative traits and quantitative trait loci (QTL) linkage analyses were performed using the computer program Map Manager QTX [14]. This program uses a fast regression method to detect and localize QTL within intervals defined by genetic markers and can perform pair-wise locus analysis to search for QTL interactive effects. Analysis of the B6NODF1 $\times$ NOD backcross data revealed a strong association of ABR thresholds with loci on Chr 5 (Fig. 1B). A peak lod score of 5.5 was obtained for the linkage association with *D5Mit309* (Fig. 1C). Segregation at this locus, designated *Ahl2*, could account for about 20% of the total ABR threshold variation observed among the backcross mice. ABR thresholds of mice that were homozygous for the NOD-derived allele at this locus (genotype *ahl2/ahl2*) were at least 20 dB higher than in heterozygous mice (genotype *ahl2/+*, where the “+” allele derives from B6), for all four test stimuli (Fig. 2A). No other locus associations with lod scores above 2.0 were detected (Fig. 1B). Tests of all locus-pair combinations with ABR thresholds revealed no statistically significant interactive effects in this backcross.

To examine possible effects of *Ahl2* in another backcross involving NOD mice, we analyzed mice from a previously described backcross of (CAST/Ei $\times$ NOD) F1 hybrids (CASNODF1) with NOD mice used to refine the map position of *Ahl* on Chr 10 [12]. QTL linkage analysis of the 290 backcross progeny from this cross revealed a highly significant linkage association of ABR thresholds with Chr 5 markers, with a peak at *D5Mit235*. The lod score for the association of *D5Mit235* with click ABR thresholds in the CASNODF1 $\times$ NOD backcross was 4.8 at 3 months of age and 4.2 at 6 months of age. As in the B6NODF1 $\times$ NOD backcross, the most likely QTL location was in the mid-region of Chr 5 and thus probably represents the same *Ahl2* locus. The Chr 5 map positions (Mouse Genome Database) for the markers with peak lod scores are 44.0 for *D5Mit309* (B6NODF1 $\times$ NOD backcross) and 42.0 for *D5Mit235* (CASNODF1 $\times$ NOD backcross). Detection of the same locus on Chr 5 in two independent linkage crosses involving NOD/LtJ confirms the important contribution of *Ahl2* to the early onset of AHL in this strain.

We detected a pronounced interactive effect of *Ahl2* with *Ahl* in the CASNODF1 $\times$ NOD backcross, which segregates strain-specific alleles for both loci (Fig. 2B). All backcross mice that were heterozygous at the Chr 10 *Ahl* locus (genotype *ahl/+*; where the recessive “*ahl*”

susceptibility allele derives from the NOD strain and the dominant “+” resistance allele derives from CAS) had normal hearing thresholds, regardless of *Ahl2* genotypes. Only *ahl/ahl* mice exhibited hearing impairment that was strongly influenced by genotypes at the *Ahl2* locus. The *Ahl2* locus accounts for about 12% of the ABR threshold variation among the 143 N2 mice with *ahl/ahl* genotypes from the CASNODF1 $\times$ NOD backcross. The average ABR thresholds for mice that were homozygous for the recessive NOD-derived allele at *Ahl2* (genotype *ahl2/ahl2*) were about 20 dB above those of heterozygotes (genotype *ahl2/+*, where the dominant “+” resistance allele derives from CAS). Lod scores were highly significant (3.4–4.3) for thresholds obtained with all four test stimuli. We have observed a similar epistatic interaction between the *Ahl* gene and A/J-derived mitochondrial DNA (mtDNA), wherein homozygosity for the predisposing *Ahl* allele is a prerequisite for manifestation of the mtDNA effect on hearing loss [15].

To test for *Ahl2* effects in inbred strains other than NOD/LtJ, we examined progeny from three other previously described backcrosses: (A/J $\times$ CAST/Ei) $\times$ A/J, (SKH2/J $\times$ CAST/Ei) $\times$ SKH2/J, and (BUB/BnJ $\times$ CAST/Ei) $\times$ BUB/BnJ [12]. To control for the strong epistatic effect of the *Ahl* locus in mice with a CAS-derived allele, only those N2 mice that were homozygous for the non-CAS predisposing allele (genotype *ahl/ahl*) were analyzed (Fig. 2B). No statistically significant associations of ABR thresholds with *Ahl2*-linked markers on Chr 5 were found in any of the three backcrosses. Thus, the influence of *Ahl2* on hearing loss seems to be more strain specific than that of *Ahl*, and may be restricted to NOD and related strains.

QTL mapping has proven to be a valuable method for identifying genes underlying complex traits in mammals [16]. High-resolution genetic mapping of QTLs and the eventual molecular identification of AHL-contributing genes such as *Ahl* and *Ahl2* in experimentally amenable inbred mouse strains will help to identify similar genes in human populations. These findings will lead to a better understanding of the pathophysiology of AHL and may contribute to the development of diagnostics, preventive interventions, and therapies for human presbycusis.

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FIG. 1.

Linkage analysis of AHL in B6NODF1 $\times$ NOD backcross mice. All backcross mice were tested at 6 months of age. ABR thresholds (in dB SPL) for the click stimulus are shown; similar results were obtained with the 8 kHz, 16 kHz, and 32 kHz pure-tone test stimuli. (A) Frequency distribution of ABR thresholds among backcross progeny. For comparison, ABR thresholds of progenitor B6 mice and B6NODF1 hybrids are less than 50 dB SPL at this age, and those of NOD mice are greater than 90 dB SPL. (B) Genome-wide tests for associations of the genotypes of 90 marker loci with ABR thresholds of 110 backcross mice. The dotted line represents the threshold value (LOD = 3.3) for significant linkage in a backcross population [17]. (C) Most likely position of *Ahl2* on mouse Chr 5. The peak lod score is at *DSMit309*, as determined from regression analysis of multiple Chr 5 markers with ABR thresholds.

FIG. 2.

Effects of the *Ahl2* locus on ABR thresholds of backcross mice. All mice were tested at 6 months of age. (A) The (NOD/LtJ × C57BL/6J) × NOD/LtJ backcross. Light gray bars indicate means and standard errors of ABR thresholds for 59 backcross mice that were heterozygous at the *Ahl2*-linked marker *D5Mit309* (genotype NB; N, NOD/LtJ-derived alleles; B, C57BL/6J-derived alleles), and dark bars indicate threshold means and standard errors of 50 homozygotes (genotype NN). The lod scores for associations of *D5Mit309* with ABR thresholds are shown below each test stimulus. (B) Backcrosses of F1 hybrids with CAST/Ei. Light gray bars indicate means and standard errors of ABR thresholds for backcross mice that were heterozygous at the *Ahl2*-linked marker *D5Mit235*, and dark bars indicate threshold means and standard errors of homozygotes. The numbers of mice tested for each genotype are shown below the appropriate bars. An interactive effect of *Ahl2* with *Ahl* genotypes (*ahl/+* and *ahl/ahl*) is apparent in the NOD/LtJ backcross. No statistically significant *Ahl2* effects were detected in the backcrosses involving the A/J, SKH2/J, or BUB/BnJ strains. Results shown are for the click stimulus, but ABR thresholds for the 8, 16, and 32 kHz test stimuli gave similar results.