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A Major Gene Affecting Age-Related Hearing Loss Is Common to at Least Ten Inbred Strains of Mice

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Inbred strains of mice offer promising models for understanding the genetic basis of human presbycusis or age-related hearing loss (AHL). We previously mapped a major gene affecting AHL in C57BL/6J mice. Here, we show that the same Chromosome 10 gene (*Ahl*) is a major contributor to AHL in nine other inbred mouse strains—129P1/ReJ, A/J, BALB/cByJ, BUB/BnJ, C57BR/cdJ, DBA/2J, NOD/LtJ, SKH2/J, and STOCK760. F1 hybrids between each of these inbred strains and the normal-hearing inbred strain CAST/Ei retain good hearing, indicating that inheritance of AHL is recessive. To follow segregation of hearing loss, F1 hybrids were backcrossed to the parental strains with AHL. Auditory-evoked brain-stem response thresholds were used to assess hearing in more than 1500 N2 mice and analyzed as quantitative traits for linkage associations with Chromosome 10 markers. Highly significant linkage was found in all nine strain backcrosses, with the highest probability (LOD > 70) near the marker *D10Mit112*. This map position for *Ahl* is near the waltzer mutation (*v*) and the modifier of deaf waddler locus (*mdfw*), suggesting the possibility of allelism. Results from an intercross of C57BL/6J and NOD/LtJ mice indicate that the 6- to 10-month difference in AHL onset between these two strains is not due to allelic heterogeneity of the *Ahl* gene.

INTRODUCTION

Age-related hearing loss (presbycusis) is the most common type of human hearing impairment, affecting about half the population by age 80 (Gorlin *et al.*, 1995; Morton, 1991). It is one of the most common chronic health problems of elderly individuals and adversely affects the quality of their lives (Mulrow *et al.*, 1990). Little is known about the genetic causes of human presbycusis because of confounding nongenetic factors such as noise trauma, disease, or ototoxic drugs (Gorlin *et al.*, 1995; Willott, 1991). Acquired mitochondrial mutations may also contribute to presbycusis (Fischel-Ghodsian, 1999). Comparisons of hearing level aggregations between genetically related and unrelated people provide evidence for a significant genetic effect on age-related hearing loss (Gates *et al.*, 1999). The interaction of predisposing genetic factors with various environmental factors is likely responsible for most forms of presbycusis, but because of the difficulties of dissecting complex traits in human populations, no contributing loci have been identified. It has been proposed that genes underlying monogenically inherited, late-onset hearing impairment, such as *COCH* (de Kok *et al.*, 1999), may also affect presbycusis susceptibility, but such associations have yet to be proven.

One approach for unraveling the genetic basis of human presbycusis is to use inbred strains and mutations of the laboratory mouse as models. In both humans and mice, high-frequency losses occur earliest, a consequence of cochlear hair cell loss progressing from base to apex. Molecular mechanisms involved in the hair cell degeneration associated with presbycusis are unknown. Because of the similarities between the human and the mouse auditory systems, identification of the genes causing hearing impairment or deafness in mice may also identify homologous human genes and genetic diseases (Brown and Steel, 1994; Steel, 1991, 1995). The genetic analysis of mouse deafness mutations already has proven valuable for the identification of genes causing human nonsyndromic deafness, such as *MYO7A* (Gibson *et al.*, 1995; Liu *et al.*, 1997), *MYO15* (Probst *et al.*, 1998; Wang *et al.*, 1998), and *POU4F3* (Erkman *et al.*, 1996; Vahava *et al.*, 1998).

The deafness caused by most mouse mutations is congenital and usually associated with other phenotypic effects. In contrast, certain inbred strains of mice exhibit a progressive, nonsyndromic hearing loss, with onset at more advanced ages. These strains have provided useful models for human presbycusis (Henry, 1982; Hunter and Willott, 1987; Li and Borg, 1991; Willott *et al.*, 1995; Zheng *et al.*, 1999). The genetic nature of age-related hearing loss (AHL) in the few inbred strains examined so far has been attributed to one to three major genes per strain (Erway *et al.*, 1993);

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however, it is not known how many different AHL genes are present collectively in existing mouse strains. A large-scale screening program to identify mouse strains with hearing impairment is being undertaken at The Jackson Laboratory. Hearing in more than 80 inbred strains of mice has been evaluated by auditory-evoked brain-stem response (ABR) threshold analyses (Zheng *et al.*, 1999). In this survey, 19 inbred strains were identified with significantly elevated ABR thresholds before the age of 3 months and 16 inbred strains were identified with later onset impairment. The age-related hearing loss in these strains is recessive: F1 hybrids with inbred strains that do not exhibit AHL (such as CAST/Ei or CBA/CaJ) retain good hearing beyond 12 months of age.

Functional complementation was assessed in F1 hybrids between some of the inbred strains with AHL and suggested that some of the hearing loss genes are common to some of the strains (Zheng *et al.*, 1999). Complementation test results for hearing loss, however, are difficult to interpret because of strain differences in times of onset and because of the confounding effects of hybrid vigor. Backcross segregation analysis is a more definitive method for determining the genetic basis of hearing loss among inbred strains. In this analysis, F1 hybrids between AHL-positive and AHL-negative strains are backcrossed to the parental strain with AHL, and the N2 progeny are examined for linkage associations of hearing loss with known marker loci.

Using such backcross analysis, we previously mapped a gene responsible for AHL in C57BL/6J mice (Johnson *et al.*, 1997). Here, we show that this same gene (symbol *Ahl*) is a major contributor to AHL in nine other inbred mouse strains. We chose to examine the BALB/cByJ strain because, like C57BL6J, it is used as a model for progressive hearing loss (Willott *et al.*, 1998). Eight other inbred strains with significant hearing loss (Zheng *et al.*, 1999) were chosen for further genetic analysis on the basis of their divergent strain histories and genetic distinctness: 129P1/ReJ, A/J, BUB/BnJ, C57BR/cdJ, DBA/2J, NOD/LtJ, SKH2/J, and STOCK760. Linkage analysis of more than 1500 N2 mice from these nine backcrosses refined the map position for *Ahl* to a small interval on Chromosome (Chr) 10. This result places *Ahl* near the waltzer mutation (*v*) and the modifier of deaf waddler locus (*mdfw*), suggesting the possibility of allelism. The common occurrence of a single gene with major effects on AHL in so many inbred strains of mice suggests that individual genes with large effects may also underlie human presbycusis. The human homolog of *Ahl* is a likely candidate for such a gene.

MATERIALS AND METHODS

Mice and genetic crosses. To map age-related hearing loss genes, mice from the wild-derived inbred strain CAST/Ei (both males and females) were first mated with mice from each of the inbred strains 129P1/ReJ, A/J, BALB/cByJ, BUB/BnJ, C57BR/cdJ, DBA/2J, NOD/LtJ, SKH2/J, and STOCK760. To alleviate husbandry difficulties encountered with diabetic mice, we used the resistant NOD.NON-$H2^{nb1}$ congenic strain, rather than its diabetic NOD/LtJ progenitor, but for brevity have designated these mice NOD/LtJ. F1 hybrids (both males and females) from these matings were then backcrossed to mice from the non-CAST/Ei parental strain. After weaning, the backcross mice from the 129P1/ReJ, BALB/cByJ, and DBA/2J hybrids were shipped from The Jackson Laboratory and maintained under barrier conditions in the Department of Biological Sciences at the University of Cincinnati for subsequent ABR testing. All other backcross mice used in these studies were reared and tested under modified barrier conditions in the Mouse Mutant Resource at The Jackson Laboratory. The Animal Care and Use Committee of The Jackson Laboratory approved the care and use of the animals reported on in this study. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

ABR threshold measurements. The parental strains, F1 hybrids, and backcross mice were tested for ABR thresholds with equipment from Intelligent Hearing Systems (Miami, Florida) using previously described methods and equipment (Zheng *et al.*, 1999). Subdermal needle electrodes were inserted at the vertex and ventrolaterally to both ears of anesthetized mice. Specific auditory stimuli (broadband click and pure-tone pips of 8, 16, and 32 kHz) from high-frequency transducers were delivered binaurally through plastic tubes to the ear canals. Evoked brain-stem responses were amplified and averaged and their wave patterns displayed on a computer screen. Auditory thresholds were obtained for each stimulus by varying the sound pressure level (SPL) at 5-dB steps up and down to identify the lowest level at which an ABR pattern can be recognized.

Heritability estimates. Inbred strains of mice and their F1 hybrids comprise genetically uniform populations and therefore can be used to estimate the environmental variance of a trait. Statistical variance estimates of ABR thresholds among mice within each inbred strain and among F1 hybrids with CAST/Ei were calculated and used as estimates of nongenetic variance (V_{NG}). For all strains and ages, the ABR threshold variance of F1 hybrids was less than the within-strain variance; therefore, to conservatively estimate heritability, we used the V_{NG} estimate obtained from the parental inbred strain rather than the smaller variance estimate obtained from its F1 hybrid. Statistical variance estimates were calculated for ABR thresholds among mice from each backcross population and used as estimates of the total phenotypic variation (V_P). General heritability (H) was then estimated as V_G/V_P, where V_G is the total genetic variation and is equal to $V_P - V_{NG}$.

QTL linkage analysis. DNA was extracted from spleen tissue of backcross mice. Ten microsatellite markers (surrounding the *Ahl* locus previously identified in C57BL/6J mice)—*D10Mit4, D10Mit5, D10Mit21, D10Mit31, D10Mit60, D10Mit90, D10Mit112, D10Mit130, D10Mit138,* and *D10Mit299* (DNA segments, Chr 10, Massachusetts Institute of Technology)—were typed by modification of standard PCR methods (Dietrich *et al.*, 1992). PCR were carried out for 30 cycles, and products were separated on 3% agarose gels (Metaphor; FMC BioProducts, Rockland, ME) and visualized by ethidium bromide staining. Primer pairs for these loci were purchased from Research Genetics, Inc. (Huntsville, AL).

The Macintosh microcomputer program Map Manager QT (Manly and Olson, 1999) was used for data management and for linkage analysis. Map Manager QT (version QTb8; available at the WWW address http://mcbio.med.buffalo.edu/mapmgr.html) uses an interval mapping procedure that fits a regression equation for the effect of a quantitative trait locus (QTL) at the position of each marker locus and at regular intervals between the marker loci. It reports the resulting regression coefficients and a likelihood ratio statistic that measures the significance of the coefficients. This likelihood statistic is based on natural logarithms; values were converted to conventional base 10 logarithms (lod scores) by dividing by 4.61.

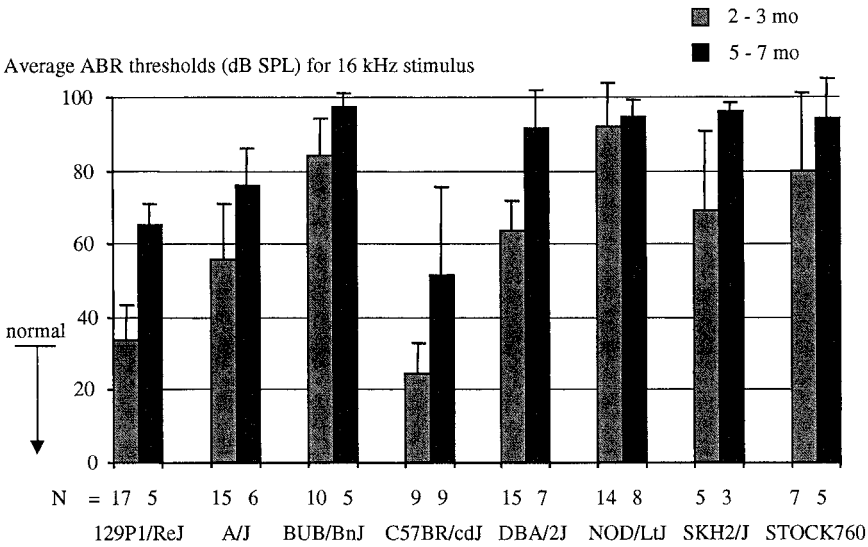

FIG. 1. Progressive hearing loss in eight inbred mouse strains. For each inbred strain, average ABR thresholds (dB SPL) for a 16-kHz stimulus (with associated standard errors) are plotted for mice tested at 2–3 months and 5–7 months of age. The numbers of mice tested are given under the bars for each strain and age group. ABR thresholds below 40 dB are considered normal, as indicated by the arrow. Detectable hearing loss in BALB/cByJ and C57BL/6J mice (not shown) does not occur before 10 months of age.

RESULTS

Inbred Mouse Strains with Progressive Hearing Loss

ABR thresholds of all mice were measured for broadband clicks and pure-tone frequencies of 8, 16, and 32 kHz. For all of the nine inbred strains examined in this study and for the C57BL/6J strain previously reported (Johnson *et al.*, 1997), age-related increases in ABR threshold responses to the 16- and 32-kHz test frequencies were always higher than for click or 8-kHz stimuli. These results indicate a greater progressive loss of auditory sensitivity at high frequencies. Because ABR audiometric profiles indicate that threshold responses to the 16 kHz stimuli show the greatest elevations above normal values (Zheng *et al.*, 1999), we used them as the best indicators for measuring AHL in all of the inbred strains and hybrids. Similar results were obtained for all test stimuli; however, to simplify presentations of data, all tables and figures refer only to the more sensitive 16-kHz response thresholds.

All of the inbred mouse strains chosen for genetic analyses except BALB/cByJ have significantly elevated ABR thresholds before the age of 3 months (Zheng *et al.*, 1999). Hearing loss in mice from the BALB/cByJ strain does not develop until after 10 months of age, as in C57BL/6J mice (Erway *et al.*, 1993; Willott *et al.*, 1998). The hearing loss in all of the strains is progressive, but the time of onset varies extensively among strains (Fig. 1). In contrast to the late time of onset for hearing loss in C57BL/6J, BALB/cByJ, and C57BR/cdJ mice, most mice of the strains BUB/BnJ, DBA/2J, NOD/LtJ, SKH2/J, and STOCK760 exhibit a severe hearing loss before 3 months of age. The onset time for hearing loss in most mice of the 129P1/ReJ and A/J strains is intermediate, at around 3–5 months of age.

The inbred strain CAST/Ei was derived from a wild population of *Mus musculus castaneus* and retains very good hearing beyond 18 months of age (Johnson *et al.*, 1997; Zheng *et al.*, 1999). F1 hybrids between each of the inbred strains with AHL and CAST/Ei also retain good hearing, indicating that inheritance of AHL is recessive in these strains. For example, the average ABR threshold of mice from the parental NOD/LtJ strain is greater than 80 dB SPL by 3 months of age (Fig. 1), whereas ABR thresholds of 6-month-old (NOD/LtJ × CAST/Ei) F1 hybrids are normal (15–35 dB). To analyze meiotic segregation and to genetically map the postulated recessive gene(s) responsible for AHL, F1 hybrids with CAST/Ei were backcrossed to the parental strains with AHL, and ABR thresholds of N2 mice were analyzed as quantitative traits.

Heritability and Segregation of Hearing Loss in Backcross Populations

For all strain backcrosses, at least two different age groups of N2 mice were tested for ABR thresholds. Because of late-onset hearing loss, N2 mice from the BALB/cByJ backcross were tested at 12, 15, and 18 months of age; all other backcross mice were tested at 2–9 months of age. General heritability estimates for 16-kHz ABR thresholds ranged from 0.60 to 0.85 at 3 months of age and from 0.86 to 0.98 at 6 months for all backcross populations except C57BL/6J, BALB/cByJ, and C57BR/cdJ (which exhibit later onset hearing loss). Heritability estimates of hearing loss in C57BL/6J, BALB/cByJ, and C57BR/cdJ backcross populations become similarly high when mice are tested at older ages (>12 months). These very high heritability estimates indicate that most of the ABR threshold variation observed in the backcross populations is genetic in origin.

The frequency distributions of ABR thresholds

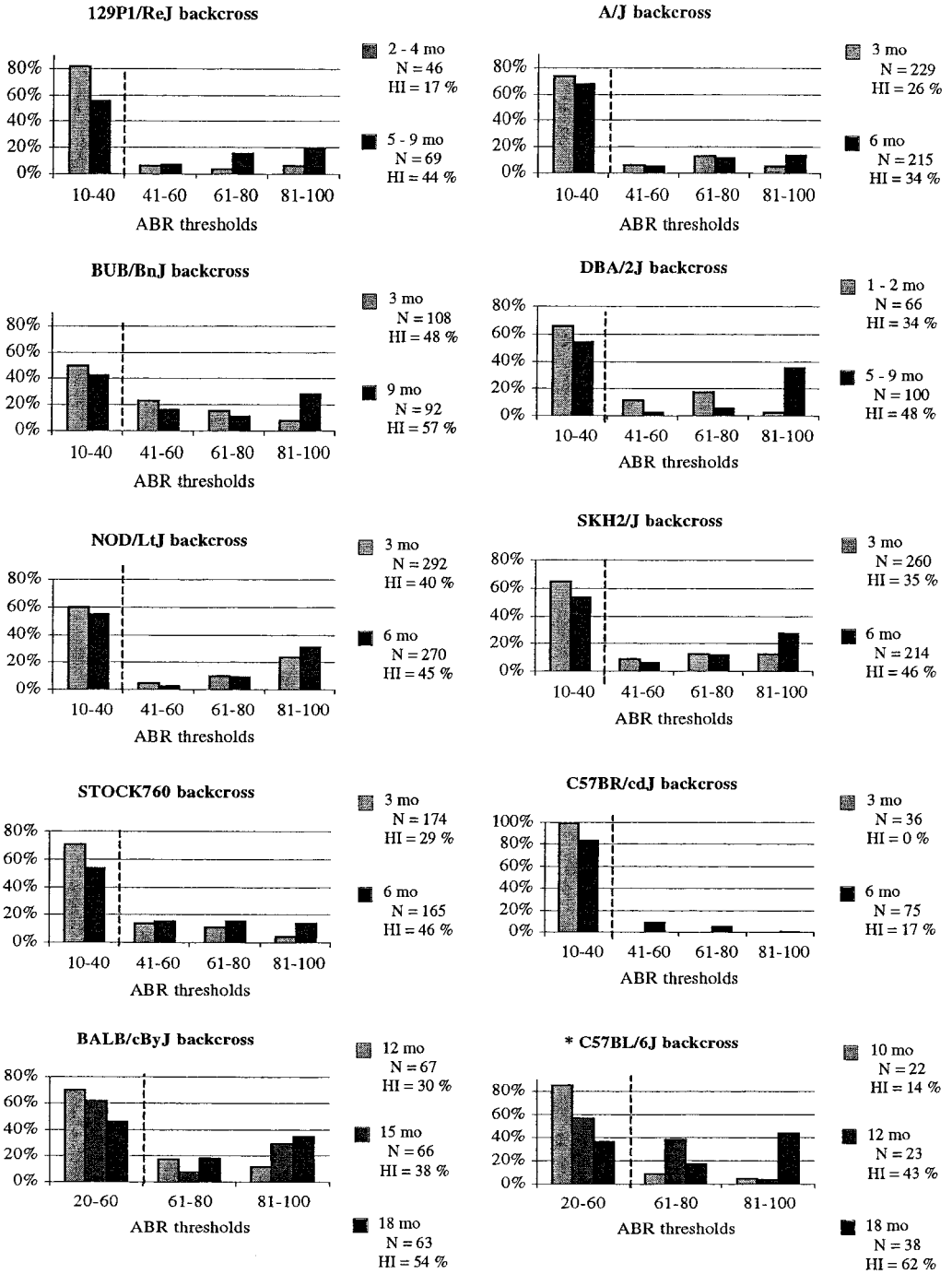

FIG. 2. Frequency distributions of 16 kHz ABR thresholds among 10 backcross populations tested at various ages. For each backcross and test age, N = the total number of N2 mice analyzed and HI = the total percentage of mice with hearing impairment (ABR thresholds >40 dB SPL). The demarcation between normal and hearing-impaired mice is indicated in each diagram by a vertical dashed line. Bar heights indicate the percentage of N2 mice at each test age with 10–40 dB thresholds (normal hearing), 41–60 dB thresholds (mild hearing loss), 61–80 dB thresholds (intermediate hearing loss), and 81–100 dB thresholds (severe hearing loss). ABR threshold categories for the BALB/cByJ and C57BL/6J backcrosses are slightly altered to compensate for the older test ages. The data for the C57BL/6J backcross are from a previous publication (Johnson *et al.*, 1997) but presented here for comparison.

among N2 progeny from each of the backcrosses, at each test age, are shown in Fig. 2. The onset time and severity of hearing loss varied among the backcrosses, occurring earliest in NOD/LtJ backcross mice and latest in BALB/cByJ backcross mice. The progression of hearing loss can be clearly seen in all backcross populations. Eventually, there are nearly equal frequencies of normal hearing (thresholds less than 40 dB) and hearing-impaired mice (thresholds greater than 40 dB). This approximate 1:1 segregation suggests the influence of a single major gene that differs between the parental strain with AHL and the CAST/Ei strain. To test if this postulated gene is the same as that originally identified in strain C57BL/6J and mapped to

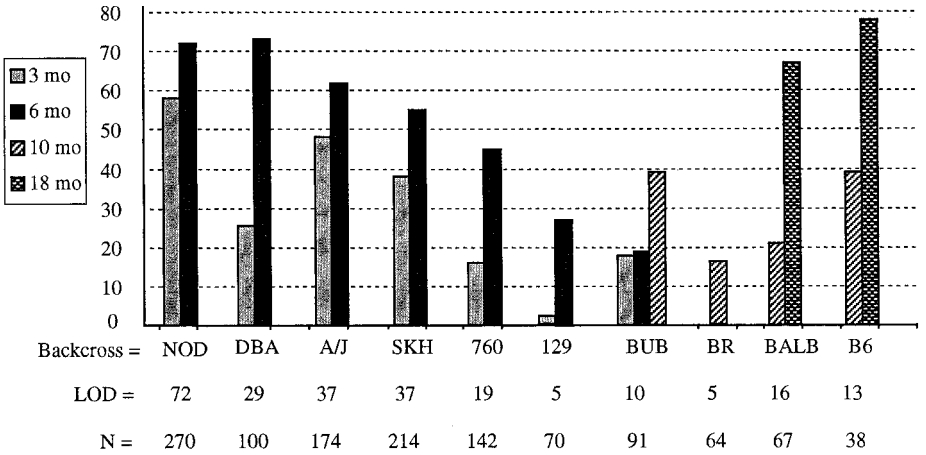

FIG. 3. Percentage of ABR threshold variation attributable to a QTL in the *D10Mit138–D10Mit31* interval of Chr 10 in 10 backcross populations. Backcross mice were tested at various ages as indicated. Maximum lod scores and sample sizes are shown for each backcross at the oldest age tested. The data for the C57BL/6J backcross are from a previous publication (Johnson *et al.*, 1997).

Chr 10 (*Ahl*), we typed mice from the other nine backcrosses for multiple Chr 10 markers and looked for linkage associations with their ABR thresholds. At older ages, most backcross mice could be separated into two groups on the basis of whether their measured ABR thresholds were higher or lower than the cut-off values selected on the basis of bimodal frequency distributions (Fig. 2). However, because some mice with intermediate thresholds could not be confidently classified, we chose to treat ABR thresholds as quantitative traits and analyzed them accordingly.

The Same Chr 10 Locus Is Associated with AHL in All Ten Strain Backcrosses

The response thresholds to the 16-kHz stimulus were used as measures of hearing loss for QTL linkage analysis. Likelihood ratio statistics for associations with Chr 10 loci were calculated for each age tested. All nine backcrosses examined showed highly significant associations of ABR thresholds with markers on Chr 10 near the location where the *Ahl* gene was originally mapped with C57BL/6J backcross mice. Base 10 log-likelihood probabilities of association (lod scores) varied depending on the number and age of mice tested, but all were highly significant (Fig. 3), especially considering that only a single chromosomal region was examined for associations rather than a genome-wide linkage screen. The highest lod score was 72, for the 270 NOD/LtJ backcross mice tested at 6 months of age. The percentage of total ABR threshold variation that could be attributed to a QTL in this region of Chr 10 varied from more than 70% for the NOD/LtJ and DBA/2J backcrosses to zero for the BALB/cByJ and C57BL/6J backcrosses at 6 months of age. At 18 months of age, however, this same QTL could explain about 70% of the ABR threshold variation in the BALB/cByJ and C57BL/6J backcrosses.

Plots of lod scores for ABR threshold associations with 10 MIT markers and additional hypothetical loci at 1-cM intervals along Chr 10 are shown in Fig. 4 for the NOD/LtJ and SKH2/J backcrosses, the two largest backcrosses examined at 6 months of age. In both backcrosses, the highest lod scores were obtained for a QTL near *D10Mit112*. Plots for the other backcrosses (not shown) were of lower resolution, but all were consistent with this localization for *Ahl*, in the interval between *D10Mit138* and *D10Mit31*. In the NOD/LtJ backcross, the peak lod score was 71.8 at *D10Mit112* and the lod scores for the nearest flanking markers *D10Mit60* and *D10Mit130* were 66.6 and 59.7, respectively, indicating a high probability that the *Ahl* gene is located within this estimated 2.9-cM genetic interval. Outside of this interval, probability drops at least 10^5-fold (lod score of 71.8 for *D10MIt112* − lod score of 66.6 for *D10Mit60* = lod score difference of 5.2, a 10^5 difference in probability). In the SKH2/J backcross, the peak lod score was 37.0 at *D10Mit112* with lod scores of 35.8 and 30.9 for the flanking markers *D10Mit60* and *D10Mit130*, respectively.

Ahl Alleles from Different Strains Have Similar Effects

The *Ahl* gene is a major contributor to AHL in all 10 inbred strains examined; however, the severity and time of onset for AHL varies extensively among strains and backcrosses (Figs. 1 and 2). This variation could be caused either by strain-specific allelic differences of the Chr 10 *Ahl* gene itself (allelic heterogeneity) or by contributions from additional genes. To distinguish between these two possibilities, we analyzed crosses between C57BL/6J mice (late onset hearing loss, beyond 10 months) and NOD/LtJ mice (early onset, before 3 months). Analysis of 6-month-old F2 progeny from an intercross and N2 progeny from reciprocal backcrosses showed no significant linkage associations of ABR thresholds with the *Ahl* locus on Chr 10 (Table 1). We used the closely linked marker *D10Mit257* (<3 cM from *D10Mit112*, the most likely position for *Ahl*; Fig. 4) to distinguish between NOD/LtJ- and C57BL/6J-derived *Ahl* alleles. The lod score for linkage of this

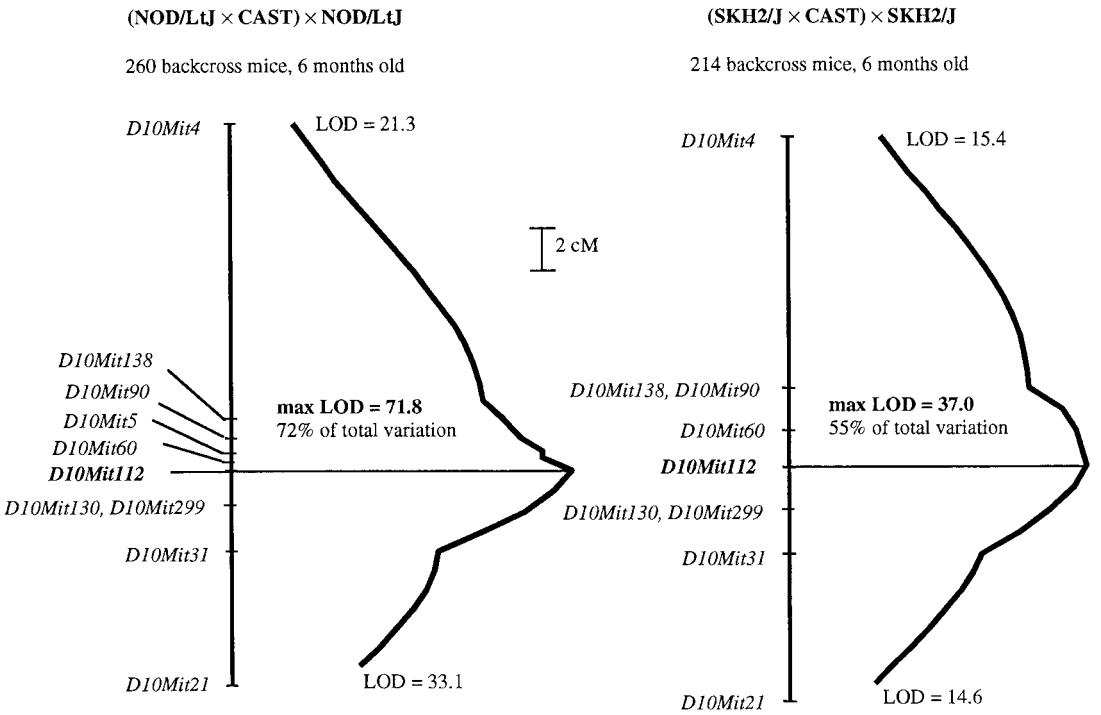

FIG. 4. Genetic maps of Chr 10 from the two largest backcrosses analyzed in this study, showing the most likely position for a major QTL affecting AHL. Probabilities of association calculated as lod scores are plotted along the length of the chromosome (the vertical line represents a lod score of zero). Strongest associations occurred at *D10Mit112* for both the NOD/LtJ and the SKH2/J backcrosses. The mapping results from the other eight backcrosses analyzed in this study were consistent with this position for *Ahl*.

marker with 16-kHz ABR thresholds of 166 6-month-old F2 progeny was only 0.56. The average ABR threshold for F2 mice homozygous for the C57BL/6J-derived *Ahl* allele (32.7) is not significantly different from that of heterozygous mice (39.2) or mice homozygous for the NOD-derived *Ahl* allele (39.3). Similarly, the lod score for association of ABR thresholds with the *Ahl* locus was only 0.07 in the (C57BL/6J × NOD/LtJ) × C57BL/6J backcross. The average ABR threshold for heterozygous N2 mice (60.0) is not significantly different from that of N2 mice homozygous for the NOD/LtJ-derived *Ahl* allele (65.5).

The high degree of ABR threshold variation observed among both the F2 and the N2 mice of these two crosses (standard deviations 20.7 and 28.7, respectively) thus cannot be attributed to allelic heterogeneity at the Chr 10 *Ahl* locus. Most of the observed variation in the C57BL/6J × NOD/LtJ crosses, therefore, must be the result of other contributing genes. These additional genes that contribute to early onset must be

TABLE 1

Nonassociation of ABR Threshold Variation with *Ahl* Gene Segregation in C57BL/6J × NOD/LtJ Intercross and Backcross Populations

Cross	Lod score	*D10Mit257* genotype	*N*	ABR thresholds (dB SPL)	
				Average	Standard dev
(C57BL/6J × NOD/LtJ); intercross	0.56	All	166	37.9	20.7
		BB	33	32.7	18.1
		BN	86	39.2	19.8
		NN	47	39.3	23.8
(C57BL/6J × NOD/LtJ) × NOD/LtJ; backcross	0.07	All	112	62.5	28.7
		BN	61	60.0	29.1
		NN	51	65.5	28.2
(C57BL/6J × NOD/LtJ) × C57BL/6J; reciprocal backcross	0.02	All	18	18.1	3.9
		BB	9	17.8	2.6
		BN	9	18.3	5.0

Note. The closely linked marker *D10Mit257* was used to infer genotypes for *Ahl*. ABR thresholds for a 16-kHz stimulus were obtained from 6-month-old F2 and N2 mice.

TABLE 2

Inbred Strains Resistant and Susceptible to AHL and Their Genetic Marker Haplotypes in the *Ahl* Region of Chr 10

Locus	Genetic position	AHL-resistant strains					AHL-susceptible strains								
		CAST	C3H	AKR	NON	CBA	NOD	A/J	B6	DBA	BALB	SKH	760	BUB	129
D10Mit129	23	71	77	77	77		77	77	77	77	77				
D10Mit149	23	111	145	145	145		145	145	145	145	145				
D10Mit185	23	171	151	151	151		151	151	151	151	151				
D10Mit195	23	148	146	146	146		146	146	146	146	146				
D10Mit196	23	210	204	204	204		204	206	204	204	204				
D10Mit220	23	137	125	125	125		125	125	125	125	125				
D10Mit221	23	116	110	110	110		110	110	110	110	110				
D10Mit29	23	178	168	168	168		168	168	168	168	168				
D10Mit30	23	292	288	290	288		288	288	288	288	288				
D10Mit48	23	156	192	192	186		192	192	192	—	192				
*D10Mit112**	23	125	133	133	133		133	133	133	133	133				
D10Mit130	**24**	**170**	**158**	**158**	**158**	**158**	***154***	**150**	**150**	**150**	**150**	**150**	**150**	***158***	**150**
D10Mit299	**24**	**192**	**185**	**185**	**185**	**185**	**188**	**188**	**188**	**188**	**188**	**188**	**188**	**188**	**188**
D10Mit61	24	140	148	148	148		148	148	148	140	148				
D10Mit15	25.1	135	180	180	180		182	180	180	184	171				
D10Mit20	25.1	186	228	228	228		230	228	228	238	226				

Note. Marker allele sizes for CAST, C3H, AKR, NON, NOD, A, B6, DBA, and BALB and genetic map positions are from MIT Whitehead. Allele sizes for additional strains—CBA/CaJ (CBA), SKH2/J (SKH), STOCK 760 (760), BUB/BnJ (BUB), and 129P1/ReJ (129)—were determined for the two markers shown in boldface. An asterisk indicates the marker most closely linked to *Ahl* as shown in Fig. 4.

primarily recessive in nature, whereby only homozygosity for NOD/LtJ-derived alleles increases ABR thresholds. At 6 months of age, all N2 mice from the reciprocal backcross (C57BL/6J × NOD/LtJ) × C57BL/6J had normal ABR thresholds (Table 1), presumably because none could be homozygous for NOD alleles at loci contributing to AHL. The average ABR threshold observed in N2 mice from the (C57BL/6J × NOD/LtJ) × C57BL/6J backcross (62.5) was higher than in F2 mice from the intercross (37.9). This higher value likely reflects the greater proportion of mice that are expected to be homozygous for NOD/LtJ-derived alleles in the backcross (1/2) than in the intercross (1/4). Conversely, the C57BL/6J-derived alleles at these loci can be thought of as acting in a dominant manner to prevent early AHL onset. Genome-wide screens of the C57BL/6J × NOD/LtJ crosses are in progress to identify these postulated additional recessive genes that contribute to the early onset AHL in the NOD/LtJ strain.

Possible Linkage Disequilibrium of AHL Phenotype with Chr 10 Markers

The identical Chr 10 map position for a QTL affecting AHL in all 10 inbred strains examined suggests that either (1) a susceptibility allele at this locus originated from a single founder or (2) different susceptibility alleles arose independently in different strains. The similarity in effects of *Ahl* alleles from different strains with AHL suggests that these alleles may be identical by descent. If a single founder event underlies the strain distribution of AHL phenotypes, then linkage disequilibrium methods could be used to localize the underlying gene. Linkage disequilibrium in a population is the nonrandom association of alleles of two or more genes. Its presence may reveal ancestral allele combinations of linked genes that, because of their close proximity, have not yet randomly assorted. The linkage disequilibrium of genetic markers with a disease phenotype has been used to obtain precise locations of disease genes in isolated human populations (Jorde, 1995) and to map a lung cancer susceptibility locus shared by several inbred mouse strains (Manenti *et al.*, 1999).

To test for linkage disequilibrium, we compared the allele sizes of Chr 10 microsatellite markers between inbred strains that exhibit AHL and those that do not (Table 2). Strain-specific allele sizes and genetic positions for markers presented in Table 2 are from the final release of the Mouse Genetic Map at the Whitehead Institute/MIT Center for Genome Research (Dietrich *et al.*, 1996). Comparing the haplotypes for Chr 10 markers in the interval where we mapped *Ahl* suggests that the markers *D10Mit130* and *D10Mit299* may represent a possible region of linkage disequilibrium. Allele sizes for these two loci correspond with the AHL phenotypes of the inbred strains, whereas allele sizes for all other loci in the *Ahl* region of Chr 10 show no association. AHL-resistant strains have allele sizes of 158 for *D10Mit130* and 185 for *D10Mit299;* AHL-susceptible strains have allele sizes of 150 for *D10Mit130* and 188 for *D10Mit299.* To test this hypothesis further, we typed these two markers in additional strains known to be AHL resistant (CBA/CaJ) and AHL susceptible (SKH2/J, STOCK760, BUB/BnJ, and 129P1/ReJ). Except for the genetically divergent CAST/Ei

strain, the strain-specific allele distribution for the marker *D10Mit299* was completely concordant with strain-specific AHL phenotypes. Allele sizes for the marker *D10Mit130* were discordant only for the AHL-susceptible strains NOD/LtJ and BUB/BnJ.

DISCUSSION

For each inbred strain with AHL, there appears to be an age-specific transition period during which most hearing loss occurs. During this period, ABR thresholds may vary extensively among individual mice, but either before or after this period of transition, there is little within-strain variance. The timing and duration of this transition period vary among inbred strains with AHL, as shown in Fig. 1 and previously described (Zheng *et al.*, 1999). For each of the strain backcross populations, about one-half of the N2 mice eventually develop a hearing loss approaching that of the parental strain with AHL; the other half retain normal hearing to older ages, similar to the parental CAST/Ei strain (Fig. 2). At older ages, high heritability estimates and bimodal frequency distributions of ABR thresholds indicate the primary influence of a single major gene. This gene, *Ahl*, was originally mapped in C57BL/6J mice and, on the basis of identical genetic map positions, appears to be a major contributor to AHL in all other inbred strains so far examined. If measured after the strain-specific transition period for hearing loss, about 70% of ABR threshold variation can be attributed to this single gene in backcross mice derived from strains with either early (NOD/LtJ, DBA/2J) or late (BALB/cByJ, C57BL/6J) onset times (Fig. 3).

Homozygosity for the *Ahl* susceptibility allele appears necessary for AHL manifestation in these inbred strains, but other genes probably contribute to the different times of onset and severity of hearing loss. Our results from crosses of C57BL/6J (late onset of AHL) and NOD/LtJ (early onset) mice indicate that *Ahl* alleles from AHL-susceptible strains with markedly different onset times have similar effects. Therefore, the ABR threshold variation observed in F2 and N2 progeny from these crosses must primarily be caused by allelic differences of other contributing genes, perhaps acting epistatically with *Ahl*. These segregation results are consistent with the evidence for linkage disequilibrium. Both indicate that a common *Ahl* allele is shared among AHL-susceptible strains, regardless of hearing loss severity and time of onset. The map positions of the markers showing linkage disequilibrium with AHL (Table 2) are in approximate agreement with the map position for *Ahl* obtained from backcross linkage analysis (Fig. 4). Once the *Ahl* gene has been identified at the molecular level, the possibility of its origin from a single founder can be directly tested by comparing DNA sequences among AHL-resistant and AHL-susceptible strains.

Our previously determined location for the *Ahl* gene was within an approximately 12-cM interval on Chr 10 with peak probablity at *D10Mit5* (Johnson *et al.*, 1997). In the present study, by segregation analysis of more than 1000 backcross mice, we refined this genetic region to approximately 2 cM, between *D10Mit60* and *D10Mit130*, with peak probability at *D10Mit112*. The mouse waltzer mutation (*v*), which causes circling behavior and deafness (Deol, 1956), has been localized 1.1 cM distal to *D10Mit60* and 0.2 cM proximal to *D10Mit112* (Bryda *et al.*, 1997), very near the most likely map position for *Ahl* (Fig. 4). Thus, *Ahl* and *v* may be alleles of the same gene. The nonsyndromic AHL of inbred strains could be caused by a less severe, allelic form of the same gene that is altered by waltzer mutations. The cochlear pathology (hair cell loss and spiral ganglion cell degeneration) observed in old mice from inbred strains with AHL (Johnson *et al.*, 1997; Li and Hultcrantz, 1994; Spongr *et al.*, 1997; Willott and Erway, 1998) is similar to but less severe than that described in mice homozygous for *v* (Deol, 1956). The *Ahl* gene and *v* mutation map near another hearing-related gene—the modifier of deaf waddler (*mdfw*) (Noben-Trauth *et al.*, 1997)—that affects deafness susceptibility in mice that are heterozygous for a different mutation, deaf waddler (*dfw*) on Chr 6. The similarities of their genetic map positions and their effects on hearing suggest that *Ahl*, *mdfw*, and *v* may be alleles of the same gene. They are located in a region of mouse Chr 10 that has homologies with human Chr 10q21–q22, where the recessive, nonsyndromic deafness gene *DFNB12* and the Usher syndrome type ID gene (*USH1D*) have been mapped (Chaib *et al.*, 1996; Wayne *et al.*, 1996).

The refined genetic mapping of *Ahl* described in this study provides the first step toward its molecular identification by the positional-candidate gene approach. Because of the recessive nature of AHL manifestation, complementation analysis using large-insert transgenic clones can be used to test candidate genes for *Ahl*. Furthermore, the analysis of a congenic line of C57BL/6J containing a CAST/Ei-derived *Ahl* allele (Johnson *et al.*, 1997) has demonstrated that the AHL resistance conferred by the dominant *Ahl* allele is independent of genetic background effects. Thus, resistance to AHL conferred by transgenic introduction of the wildtype allele of a candidate gene (for example from CAST/Ei) into an inbred strain with AHL (for example NOD/LtJ) could be considered evidence for gene identity. This *in vivo* complementation approach has been used successfully in several positional cloning efforts (Antoch *et al.*, 1997; Hamilton *et al.*, 1997; Probst *et al.*, 1998). The DNA sequence of *Ahl* candidate genes can also be compared between AHL-resistant and AHL-susceptible strains to see if there are consistent differences indicating causation.

Ahl is the first gene affecting late-onset, nonsyndromic hearing loss that has been mapped in the mouse. Here we show that it is common to at least 10 inbred strains with AHL. Homozygosity for a susceptibility allele at this Chr 10 locus confers a necessary

predisposition to AHL in these inbred strains, but other genes contribute to interstrain differences in severity and onset times. Susceptibility of certain inbred mouse strains to noise-induced hearing loss (NIHL) is associated with their propensity to develop AHL (Li *et al.*, 1993) and with the *Ahl* gene in particular (Erway *et al.*, 1996). The common occurrence and magnitude of the *Ahl* gene's contribution to AHL (and probably NIHL) in mice suggest that its homolog may likewise play an important role in human presbycusis and NIHL. Molecular identification of this gene could be used in tests to identify individuals genetically predisposed toward presbycusis and NIHL so that preventative measures can be taken, such as minimizing exposure to loud sounds. Gene identification could also provide entry to molecular pathways of pathogenesis and suggest possible means of intervention.

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