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A locus on distal Chromosome 11 (*ahl8*) and its interaction with *Cdh23*^{ahl} underlie the early onset, age-related hearing loss of DBA/2J mice

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Abstract

The DBA/2J inbred strain of mice is used extensively in hearing research, yet little is known about the genetic basis for its early onset, progressive hearing loss. To map underlying genetic factors we analyzed recombinant inbred strains and linkage backcrosses. Analysis of 213 mice from 31 BXD recombinant inbred strains detected linkage of auditory brainstem response (ABR) thresholds with a locus on distal Chromosome 11, which we designate *ahl8*. Analysis of 225 N2 mice from a backcross of (C57BL/6J × DBA/2J) F1 hybrids to DBA/2J mice confirmed this linkage (LOD>50) and refined the *ahl8* candidate gene interval. Analysis of 214 mice from a backcross of (B6.CAST-*Cdh23*$^{Ahl+}$ × DBA/2J) F1 hybrids to DBA/2J mice demonstrated a genetic interaction of *Cdh23* with *ahl8*. We conclude that *ahl8* is a major contributor to the hearing loss of DBA/2J and that its effects are dependent on the predisposing *Cdh23*^{ahl} genotype of this strain.

Introduction

Age-related hearing loss or presbycusis negatively affects the quality of life for many elderly individuals [1]. About one third of adults older than 60 and about half the population by age 80 suffer from a significant hearing loss [2]. Genetic factors have been shown to contribute to the progression and severity of age-related hearing loss (AHL) [3], but variable environmental factors (such as exposures to loud noise, ototoxic drugs, and disease) coupled with the late onset of the disorder make gene identification studies extremely difficult in human populations [4]. Nonetheless, large-scale association studies of candidate genes have begun to identify a few gene variants that may be causative, including variants of the *NAT2* [5], *KCNQ4* [6], and *GRHL2* genes [7].

The laboratory mouse offers a complementary approach for studying the genetic basis and pathophysiology of AHL. Many inbred mouse strains exhibit AHL [8] and are easily amenable to genetic analysis [9]. Several quantitative trait loci (QTL) associated with AHL in mice have been genetically mapped by inbred strain crosses, including *ahl* [10,11], *ahl2* [12], *ahl3* [13, 14], *ahl4* [15], *ahl5* and *ahl6* [16], and *Phl1* and *Phl2* [17]. Refined genetic and molecular analyses have provided strong evidence that *ahl* is an inbred strain-specific dimorphism of the *Cdh23* gene [18]. The recessive AHL susceptibility allele for this locus is now designated *Cdh23*^{ahl} and the dominant resistance allele *Cdh23*$^{Ahl+}$. The causative gene variants for the

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other mapped AHL loci have yet to be identified; however, once identified they can be tested as candidate genes for human presbycusis susceptibility.

DBA/2J is a well established mouse strain that was derived from DBA, the oldest of all inbred strains of mice [19]. Mice of the DBA/2J strain (henceforth designated D2) begin to exhibit a high frequency (>16 kHz) hearing loss as early as three weeks of age that progresses to a severe broad frequency hearing loss by 2–3 months and is accompanied by a rapid, progressive degeneration of the organ of Corti and spiral ganglia [20,21]. D2 mice have been used extensively to study various aspects of both peripheral and central hearing loss and related pathologies. A PubMed search retrieved more than 100 articles related to the use of this strain in auditory research. D2 mice have been used to study susceptibility to audiogenic seizures and response properties and morphologies of auditory neurons [20,22–25]. D2 mice also have been used to study the effects of an augmented acoustic environment on the auditory system [21,26]. D2 mice recently were used in a microarray study of cochlear gene expression, which detected significant age-related changes in over 4000 genes, including many that are involved in mitochondrial respiratory chain complexes [27]. Determining the genetic basis of the hearing loss exhibited by D2 mice would aid in the interpretations of these and other auditory studies involving this strain.

In contrast to the early onset and rapid progression of hearing loss exhibited by D2 mice, high frequency hearing loss in mice of the C57BL/6J inbred strain (henceforth designated B6) is not observed until three months of age and does not progress to a broad frequency, profound hearing loss until l0 months of age or older [8,28–30]. The *Cdh23*^{ahl} allele is an important contributor to AHL in many inbred mouse strains, but because B6 and D2 share this susceptibility allele [18], other genetic factors must be responsible for their hearing loss differences. To identify these additional genetic factors, we analyzed recombinant inbred strains and a linkage backcross derived from B6 and D2 parental strains. We detected highly significant linkage of auditory brainstem response (ABR) thresholds with a locus on distal Chromosome (Chr) 11, which we designate *ahl8*. To evaluate the genetic interaction of *ahl8* with *Cdh23*^{ahl}, we analyzed an independent backcross that segregates alleles at both loci and found that the phenotypic effects of *ahl8* genotypes are dependent on a predisposing *Cdh23*^{ahl} genotype.

Results

We assessed hearing in mice by analyzing ABR thresholds to broad-band click and pure-tone 8 kHz, 16 kHz, and 32 kHz stimuli [8]. We first tested mice of the B6 and D2 parental strains and their F1 hybrids. Hearing loss in D2 mice is progressive with a much earlier onset than in B6 mice (Fig. 1). For example, at 10 weeks of age the average 16 kHz thresholds of D2 mice are about 50 decibels (dB) higher than those of 30-week-old B6 mice, which still retain normal thresholds around 20 dB SPL (sound pressure level). The ABR thresholds of 10 B6D2F1/J hybrid mice tested at 12 weeks of age were not statistically different from the normal thresholds of 30-week-old B6 mice.

ABR thresholds and linkage analysis of BXD recombinant inbred strains

As a first approach to genetically map loci that underlie the hearing threshold differences between B6 and D2 mice, we analyzed linkage in BXD recombinant inbred (RI) strains. RI lines are useful for obtaining rough estimates of the number of major genes affecting a complex trait and for determining their approximate map locations [31]. The BXD set of RI strains were derived by crossing B6 and D2 mice and then inbreeding separate progeny lines for over 21 generations. We retested 22 of the 25 BXD RI strains that previously had been assessed by Willott and Erway [32] and evaluated nine new strains [33]. In total, we measured ABR thresholds of 213 mice (5–12 mice per strain) from 31 BXD RI strains. The RI strain mice

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were tested at 6–9 months of age, an age when ABR thresholds of the parental strains differ by more than 50 dB (Fig. 1). Means, standard errors, and standard deviations for click, 8 kHz, 16 kHz, and 32 kHz threshold measurements are given in Supplementary Table 1. A high proportion of the total ABR threshold variation is explained by the between-strain variance component (ANOVA adjusted R-square values of 0.76, 0.82, 0.83, and 0.81 for click, 8 kHz, 16 kHz, and 32 kHz thresholds, respectively) and is evident by the highly variable RI strain means (Fig. 2A). The high level of between-strain variance indicates a strong genetic influence, which improves the likelihood of detecting linkage.

We used the web-based mapping program WebQTL (http://www.genenetwork.org/) to analyze linkage of ABR thresholds with the set of over 750 markers that already have been typed for these RI strains [31]. A statistically significant linkage association (LOD score 4.3) of 16 kHz ABR thresholds was found with *D11Mit48*, a marker on distal Chr 11 (Fig. 2B). Linkage associations with all other chromosome regions were below the 95th percentile significance level, which was determined empirically from 1000 permutations (Manly et al., 2001). We assigned the symbol *ahl8* to designate this quantitative trait locus (QTL) on distal Chr 11 that contributes to the progressive hearing loss of DBA/2J mice.

ABR thresholds and linkage analysis of (B6 × D2) × D2 backcross mice

To confirm and refine the map position of *ahl8* ascertained from the BXD RI lines, we analyzed a linkage backcross. The ABR thresholds of F1 hybrids between B6 and D2 mice do not differ from those of B6 mice (at least up to 12 weeks of age, Fig. 1). We backcrossed the F1 hybrids to D2 mice because a backcross is more efficient than an intercross for detecting dominance effects [34]. The frequency distribution of ABR thresholds among N2 mice from this backcross, designated (B6 × D2) × D2, was roughly bimodal rather than continuous (Fig. 3A). At 13 weeks of age, about 50% of mice had 16 kHz thresholds less than 45 dB SPL and about 50% had thresholds greater than 45 dB SPL, suggesting a primary effect of a single locus.

ABR thresholds for click, 8 kHz, 16 kHz, and 32 kHz stimuli were evaluated as quantitative traits and QTL linkage analysis was performed using the computer program Map Manager QTX [35]. Analysis of 225 progeny from the (B6 × D2) × D2 backcross confirmed a strong linkage association of ABR thresholds with distal Chr 11. The strongest association was with the distal-most marker, *D11Mit104* (Fig. 4; Table 1). The magnitude of the *ahl8* (*D11Mit104*) effect was much greater at 13 weeks than at 6 weeks of age and greater for the high frequency 16 and 32 kHz thresholds than for the 8 kHz or click thresholds (Table 1). The *ahl8* locus can explain up to 70% of the total high frequency threshold variation among 13-week-old backcross mice. The average 16 kHz ABR thresholds of N2 mice that were homozygous for the D2-derived allele at *D11Mit104* (genotype *ahl8/ahl8*) were about 20 dB higher than those of heterozygous mice (genotype +/*ahl8*, where the "+" allele derives from B6) at 6 weeks and about 50 dB higher at 13 weeks of age (Fig. 5).

ABR thresholds and linkage analysis of (B6.CAST-Cdh23^{Ahl+} × D2) × D2 backcross mice

Because B6 and D2 mice share the same *Cdh23*^{ahl} susceptibility allele, neither the BXD RI lines nor the (B6 × D2) × D2 backcross could be used to investigate genetic interactions of *Cdh23* and *ahl8*. To produce F1 hybrids that are heterozygous for both loci, we mated D2 mice with mice of the B6.CAST-*Cdh23*$^{Ahl+}$ congenic strain (which has the *Cdh23*$^{Ahl+}$ AHL resistance allele derived from the CAST/EiJ strain introgressed into an otherwise B6 genetic background) rather than B6 mice. The (B6.CAST-*Cdh23*$^{Ahl+}$ × D2) F1 hybrids then were backcrossed to D2 mice to produce 214 N2 progeny that were informative for allelic segregation at *Cdh23* and *ahl8*. This backcross segregates CAST and D2 alleles in the *Cdh23* congenic region of Chr 10, but B6 and D2 alleles throughout the rest of the genome. Although the frequency distribution of ABR thresholds among N2 mice from this backcross

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was roughly bimodal, there were unequal numbers of mice in the two modes (Fig. 3B). At 13 weeks of age, about 70% of N2 mice had 16 kHz ABR thresholds less than 45 dB SPL and about 30% had thresholds greater than 45 dB SPL, suggesting the influence of more than one gene, consistent with the expectations for this cross.

QTL linkage analysis of the ABR thresholds of N2 mice from the (B6.CAST-*Cdh23*$^{Ahl+}$ × D2) × D2 backcross was performed as described above for the (B6 × D2) × D2 backcross progeny, and statistically significant associations were found with both the *Cdh23* locus on Chr 10 and the *ahl8* locus on Chr 11 (Table 2; Fig. 6). As with the (B6 × D2) × D2 backcross, a greater degree of ABR threshold variation and stronger linkage associations were observed at the older test age and with the higher frequency test stimuli. The main effect of the *Cdh23* locus was greater than that of *ahl8*, and there was a significant interaction component (Table 2). Most of the interaction was due to the epistatic effect of the CAST-derived *Cdh23*$^{Ahl+}$ allele, which prevented hearing loss regardless of *ahl8* genotype (Fig. 6). When this locus was homozygous for the D2-derived *Cdh23*^{ahl} allele, however, the *ahl8* genotype had a highly significant effect on thresholds. In the subset of backcross mice with *Cdh23*^{ahl}/*Cdh23*^{ahl} genotypes (N = 106), the *ahl8* locus (*D11Mit104*) could explain 37% of the 16 kHz ABR threshold variation at 13 weeks of age (LOD 10.8).

Candidate genes for ahl8

There are approximately 150 named genes in the current candidate gene interval for *ahl8* (Chr 11 position 113–122 Mb, NCBI build m37). Two of these, *Actg1* (actin, gamma, cytoplasmic 1, position 120.2 Mb) and *Ush1g* (Usher syndrome 1G homolog, position 115.2 Mb), are known hearing-related genes. Mutations in *ACTG1*, the human homolog of *Actg1*, cause the dominant nonsyndromic hearing loss associated with the DFNA20/26 locus [36,37]. Mutations in the mouse *Ush1g* gene are responsible for the deafness of Jackson shaker (*js*) mice [38], and mutations in the human homolog, *USH1G*, underlie Usher syndrome type 1G, which includes deafness as a major defining characteristic [39]. To test *Actg1* and *Ush1g* as candidates for *ahl8*, we designed PCR primer pairs (Supplementary Table 2) to amplify the exons and exonintron splice sites of these genes. The sequences of PCR products amplified from D2 genomic DNA were then compared with the corresponding B6 DNA sequences from the public database; however, no sequence differences were found for either gene. A search of SNP databases (http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=snpQF) also failed to identify any protein-coding or splice site sequence differences. These results indicate that neither *Actg1* nor *Ush1g* is likely to be responsible for the *ahl8* phenotype.

Discussion

The linkage results from the BXD RI strains, the (B6 × D2) × D2 backcross, and the (B6.CAST-*Cdh23*$^{Ahl+}$ × D2) × D2 backcross provide independent corroborating evidence for a QTL on distal Chr 11 (*ahl8*) that has a major effect on AHL in DBA/2J mice. In contrast to the *Cdh23*^{ahl} hearing loss susceptibility allele, which is common to multiple inbred strains [18], the *ahl8* susceptibility allele appears to be unique to the DBA/2J inbred strain. The *ahl8* locus showed no association with ABR thresholds in previously described linkage backcrosses involving CAST/Ei and the following inbred strains: B6, A/J, BUB/BnJ, NOD/ShiLtJ, 129P1/ReJ, SKH2/J, and BALBcByJ ([11]; our unpublished data). Analysis of the (B6.CAST-*Cdh23*$^{Ahl+}$ × D2) × D2 backcross showed that the effect of *ahl8* on hearing loss is limited to mice with predisposing *Cdh23*^{ahl} genotypes. We have noted a similar epistatic effect of *Cdh23* strain variants on hearing loss attributable to other AHL-related factors, including *ahl2* [12], *ahl4* [15], and *mt-Tr* [40].

Recent evidence has implicated cadherin 23 (CDH23) as an essential component of the interstereociliary tip links of inner ear hair cells [41]. The hypomorphic CDH23 isoform

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encoded by the inbred strain-specific *Cdh23*^{ahl} variant [18] may weaken these links, thus resulting in an increased likelihood of breakage. Tip link breakage could be exacerbated by other genetic factors that weaken hair bundle integrity or by environmental exposures to loud sounds. The accumulation of broken tip links over time could increase the susceptibility of a hair cell to further damage (perhaps related to increased levels of Ca^{2+} or increased ATP demand and free radical generation) that eventually results in its degeneration. The genetic interaction that we detected between *Cdh23*^{ahl} and *ahl8* suggests that the gene underlying *ahl8* is involved in some aspect of this proposed mechanism of cumulative hair cell degeneration, which is consistent with the cochlear pathology associated with the hearing loss of D2 strain mice [20,21].

Two other hearing-related QTLs have been mapped to Chr 11 by crosses involving D2 strain mice. A QTL (*Tmc1m2*) that modifies the auditory phenotype of Beethoven mutant mice (*Tmc1*^{Bth}) was mapped to the 55 cM position of Chr 11 [42]; however, the map position for *ahl8* is distal to *D11Mit333* (Fig. 4), which is at the 66 cM position. Thus, it is unlikely that *ahl8* and *Tmc1m2* are allelic. A QTL for startle response to loud noise was mapped to distal Chr 11 by a high resolution genome-wide association study of complex traits in a heterogeneous stock of mice, which included D2 as one of the parental strains [43]. The 95% confidence interval (118–120 Mb) for this startle response QTL lies within the *ahl8* candidate interval (113–122 Mb; Fig. 4), so it is possible that they represent the same underlying D2 strain variant.

An earlier study by Willott and Erway [32] described cochlear pathologies and ABR thresholds of mice from 25 BXD RI strains. Consistent with our findings, they reported greater threshold elevations for higher frequencies and older ages. They classified each strain into B6-like or D2-like categories on the combined basis of spiral ganglion cell densities and ABR thresholds, but they failed to find any statistically significant chromosomal association by strain distribution pattern (SDP) analysis. Our success in detecting a significant linkage with distal Chr 11 is probably due to our inclusion of additional BXD RI strains and because we used quantitative trait analysis rather than categorical SDP analysis. Using the Genetic Correlation Analysis feature of GeneNetwork (http://www.genenetwork.org/home.html), we found highly significant correlations (−0.69 to −0.77, p values < 0.0001) of our ABR threshold measurements with the basal spiral ganglion cell densities reported by Willott and Erway [32], verifying the consistency of the two studies in their assessments of the auditory phenotypes of the BXD RI strains.

QTL mapping has proven to be a productive method for identifying genes underlying other complex traits in mammals [44]. Because *ahl8* has such a large phenotypic effect (it can explain up to 70% of the total ABR threshold variation of 13-week-old backcross mice), higher resolution genetic mapping and congenic strain analysis should enable the eventual molecular identification of the causative molecular alteration [34]. Identification of the underlying gene and further studies of the *ahl8* phenotype will lead to a better understanding of the molecular mechanisms responsible for age-related hearing loss and provide a new candidate gene for presbycusis and other hearing disorders in humans.

In conclusion, we mapped a modifier locus on distal Chr 11 (*ahl8*) that is a major contributor to the progressive hearing loss of DBA/2J mice. Its effects are dependent on the predisposing *Cdh23*^{ahl} genotype of this strain. The unique epigenetic inheritance pattern makes this model a valuable tool in the study of gene interactions underlying complex traits. Age-related hearing loss in humans is likely a multifactorial condition as well. This study illustrates the importance of animal models in the analysis of such complex diseases and provides evidence that identification of genetic determinants of multigenic traits is possible with modern molecular genetic tools.

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Materials and methods

Mice

The BXD recombinant inbred strains and the DBA/2J, C57BL/6J, and B6.CAST-*Cdh23*$^{Ahl+}$/Kjn (Stock Number 002756) inbred strains used to generate the backcross mice examined in this study originated from The Jackson Laboratory (http://www.jax.org/). Experimental mice were housed in the Research Animal Facility of the Jackson Laboratory, and 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.

ABR analysis

Hearing in mice was assessed by ABR threshold analysis, as previously described [8]. 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. 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 click, 30 dB for 8 kHz, 20 dB for 16 kHz, and 45 dB 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 or deafness.

RI strain QTL mapping

Because mice within individual RI strains are genetically identical, the ratio of the between-strain variance to the total variance can be used to roughly estimate trait heritability. This variance ratio is equivalent to the adjusted R-square value derived from one-way analysis of variance (ANOVA) with RI strain as a single factor. We calculated means, standard errors, standard deviations, and adjusted R-square values for click, 8 kHz, 16 kHz, and 32 kHz thresholds of BXD RI strain mice (Supplementary Table 1) using the ANOVA program of the JMP (version 7.0) statistics software package (SAS Institute, Cary, NC, USA). The online WebQTL mapping program (http://www.genenetwork.org/home.html) was used to perform quantitative linkage analysis of ABR thresholds with hundreds of previously typed SNP and microsatellite chromosomal markers.

Linkage backcross mapping

Individual DNA samples from linkage backcross mice were genotyped for multiple polymorphic MIT microsatellite markers located on Chromosome 11. PCR primer pairs designed to amplify specific markers were purchased from Integrated DNA Technologies (Coralville, IA, USA). PCR reactions and PCR product visualization methods were as previously described [45]. ABR thresholds for click, 8 kHz, 16 kHz, and 32 kHz stimuli were evaluated as quantitative traits and QTL linkage analysis was performed using the computer program Map Manager QTX [35]. This program uses a fast regression method to detect and localize quantitative trait loci within intervals defined by genetic markers and can perform pair-wise locus analysis to search for QTL interactive effects.

Candidate gene analysis

PCR for comparative DNA sequence analysis between D2 and B6 mice was performed according to the conditions described above for linkage backcross mapping. PCR primers used to amplify specific regions of the *Actg1* and *Ush1g* genes are given in Supplementary Table 2. PCR products from genomic DNA were purified with the QIAquick PCR Purification Kit

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(Qiagen Inc., Valencia, CA). Primers used for DNA amplification were also used for DNA sequencing on an Applied Biosystems 3700 DNA Sequencer with an optimized Big Dye Terminator Cycle Sequencing method.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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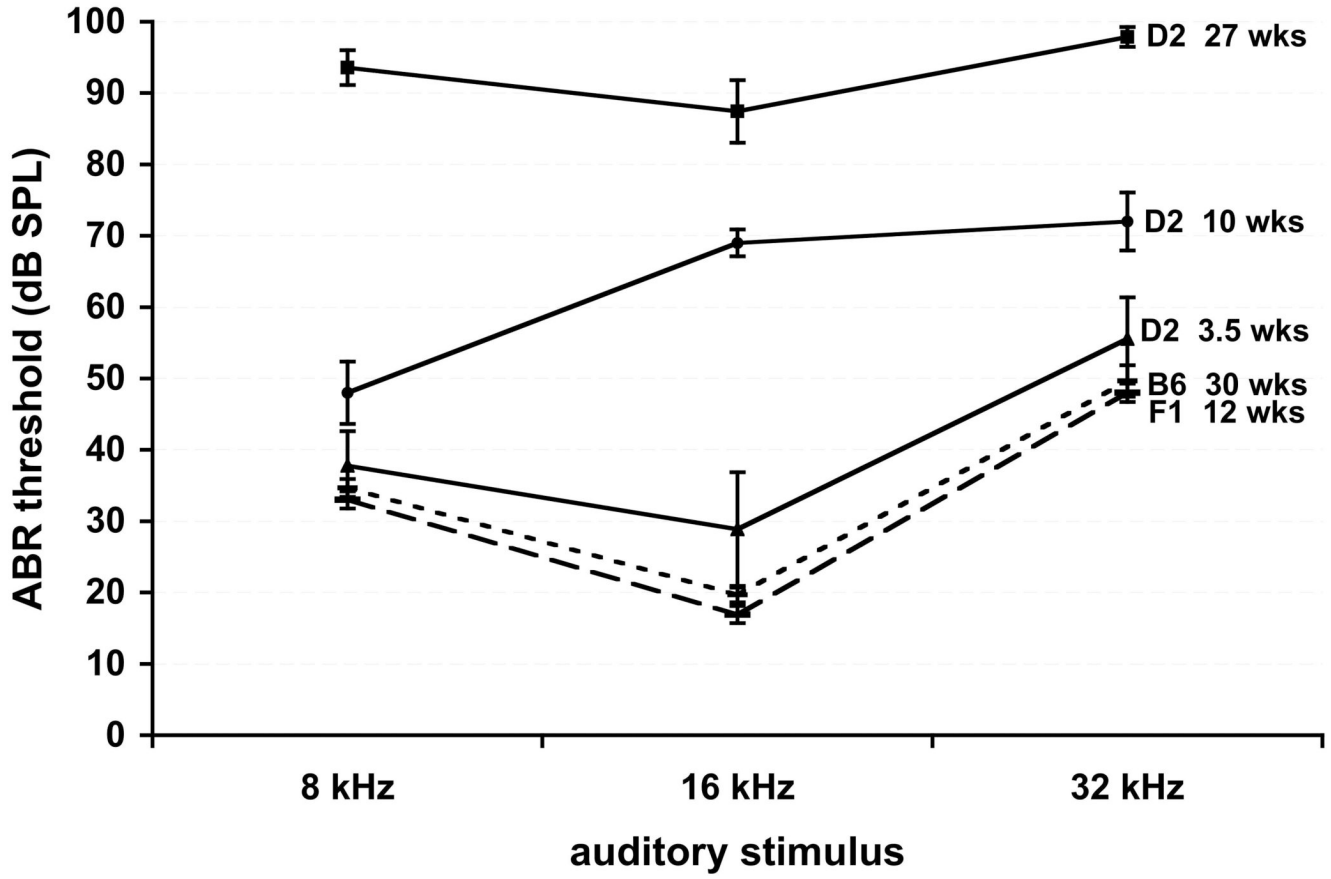

Fig. 1. ABR thresholds of D2, B6, and F1 hybrid mice

D2 mice exhibit an early onset, progressive hearing loss as shown by the average ABR thresholds of mice tested at 3.5 (N=9), 10 (N=5), and 27 (N=7) weeks of age (solid lines). B6D2 F1 hybrids tested at 12 weeks of age (N=10, dashed line) had normal ABR thresholds that were not significantly different from those of B6 mice (N=15) tested at 30 weeks of age (N=15, dotted line). ABR thresholds are given in dB SPL (decibels, sound pressure level). Standard error bars are shown for each threshold mean.

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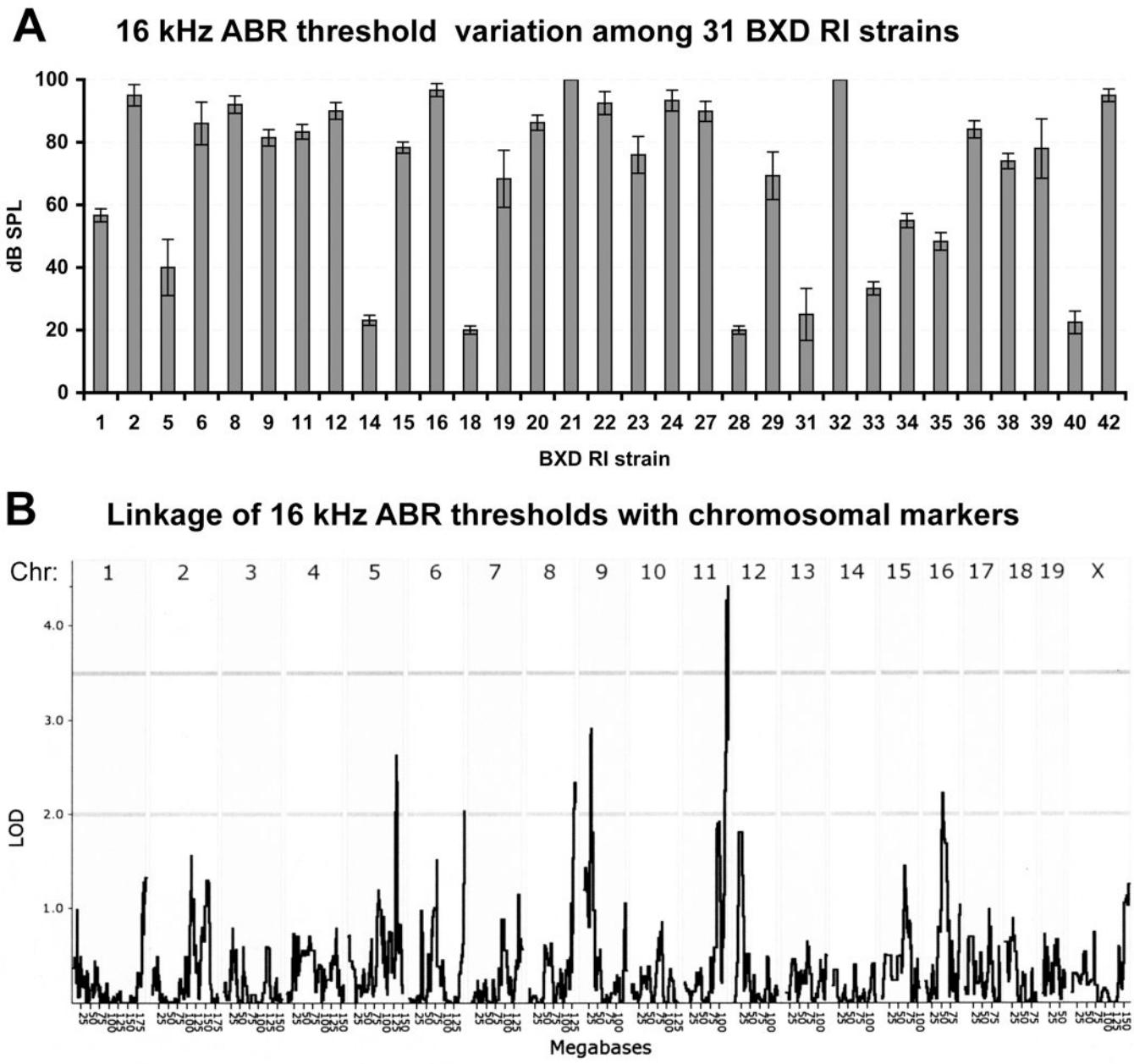

Fig. 2. Genetic analysis of hearing loss in BXD recombinant inbred strains

A. Average 16 kHz ABR thresholds (dB SPL) and their standard errors are shown for 5–12 mice from each of 31 BXD strains (a total of 213 mice) tested at 6–9 months of age. **B.** The WebQTL program was used to calculate linkage associations of chromosomal markers with the ABR threshold averages shown in Fig 2A above. Chromosomes are numbered across the top of the figure and megabase positions are given below each chromosome. In a genome-wide scan, only loci on distal Chr 11 exhibited statistically significant associations (LOD = 4.3 for *D11Mit48*). The lower dotted line indicates suggestive (N = 0.63) and the upper dotted line indicates significant (N = 0.05) genome-wide linkage associations, determined from 1000 permutations.

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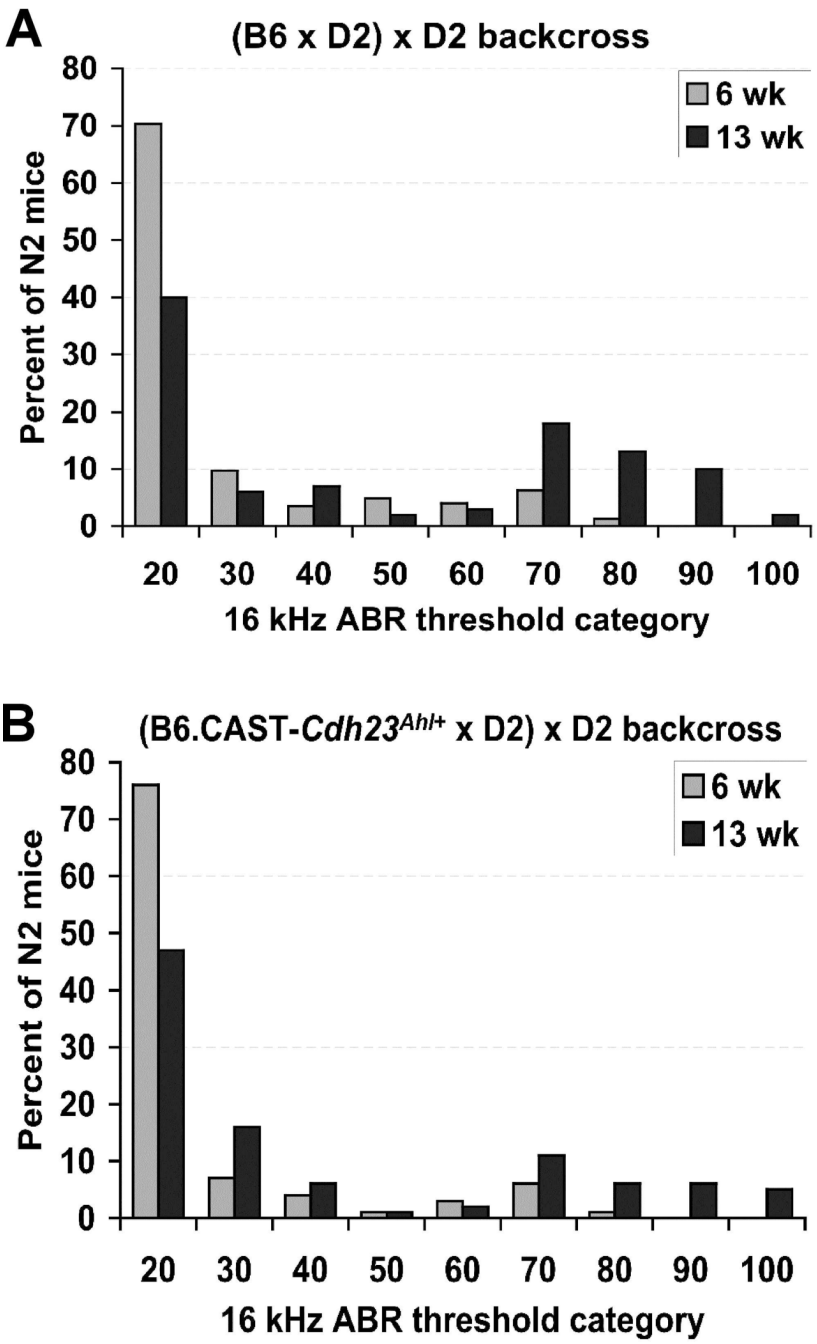

Fig. 3. Frequency distributions of ABR thresholds among progeny from two linkage backcrosses

The histograms show the distributions of 16 kHz ABR thresholds (dB SPL) among backcross mice tested at 6 and 13 weeks of age. Progression of hearing loss is reflected by an increase in the percentage of mice with higher thresholds at the older test age. **A.** Thresholds of 225 N2 mice from the (B6 × D2) × D2 backcross. **B.** Thresholds of 214 N2 mice from the (B6.CAST-*Cdh23*$^{Ahl+}$ × D2) × D2 backcross, which segregates alleles at *Cdh23* and *ahl8*.

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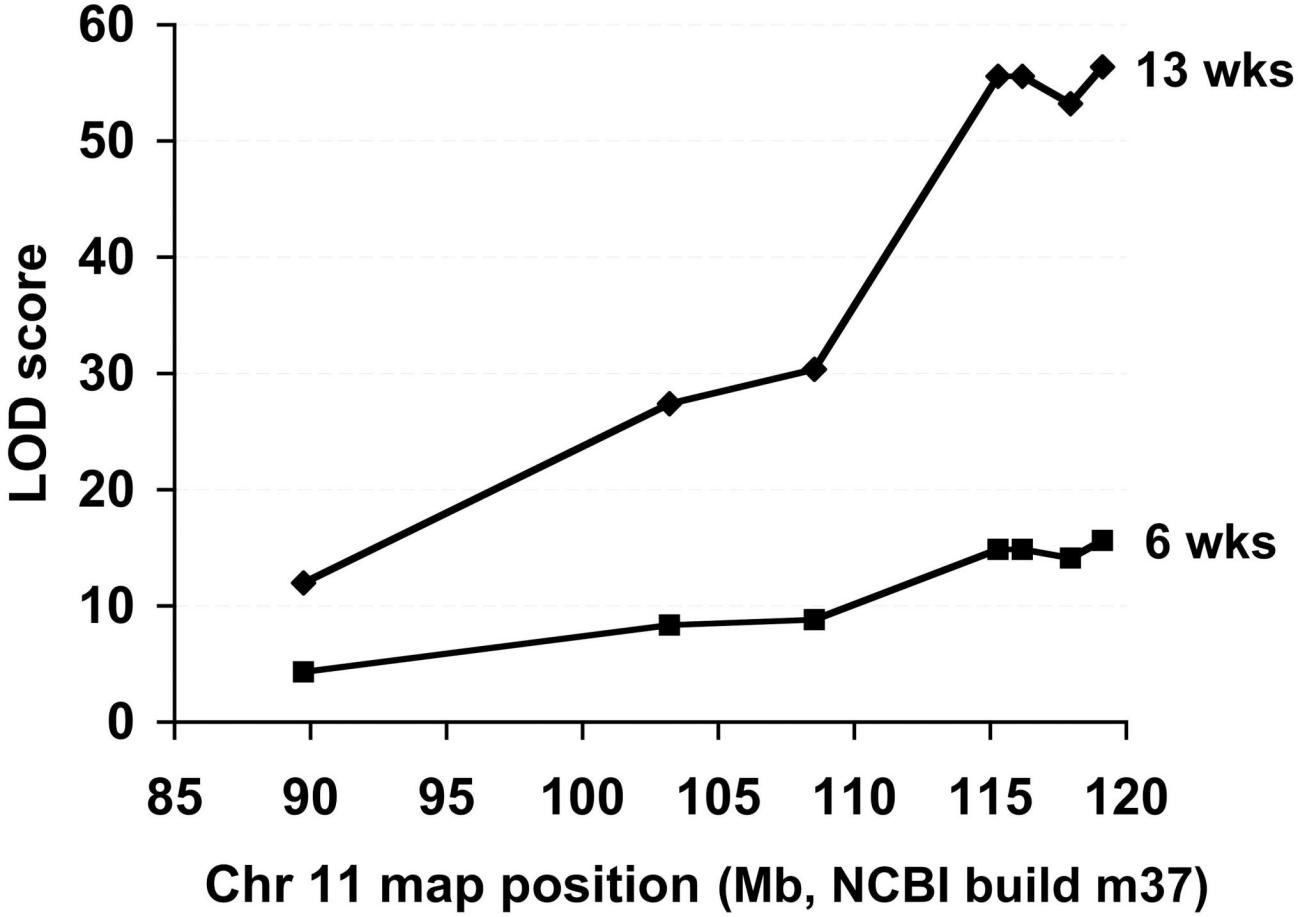

Fig. 4. Refined map position for *ahl8* determined from the (B6 × D2) × D2 backcross

The 16 kHz ABR thresholds of 225 (B6 × D2) × D2 N2 backcross mice were analyzed for linkage associations with markers on distal Chr 11. Diamonds (13 week test age) and squares (6 week test age) denote LOD scores for the following markers: *D11Mit285* (89.8 Mb), *D11Mit360* (103.2 Mb), *D11Mit333* (108.6 Mb), *D11Mit338* (115.3 Mb), *D11Mit48* (118.0 Mb), and *D11Mit104* (119.2 Mb). The highest LOD score was obtained for the most distal marker, *D11Mit104* (56.4 at 13 weeks of age). The candidate gene interval for *ahl8* extends from approximately 113 Mb to the most distal end of Chr 11.

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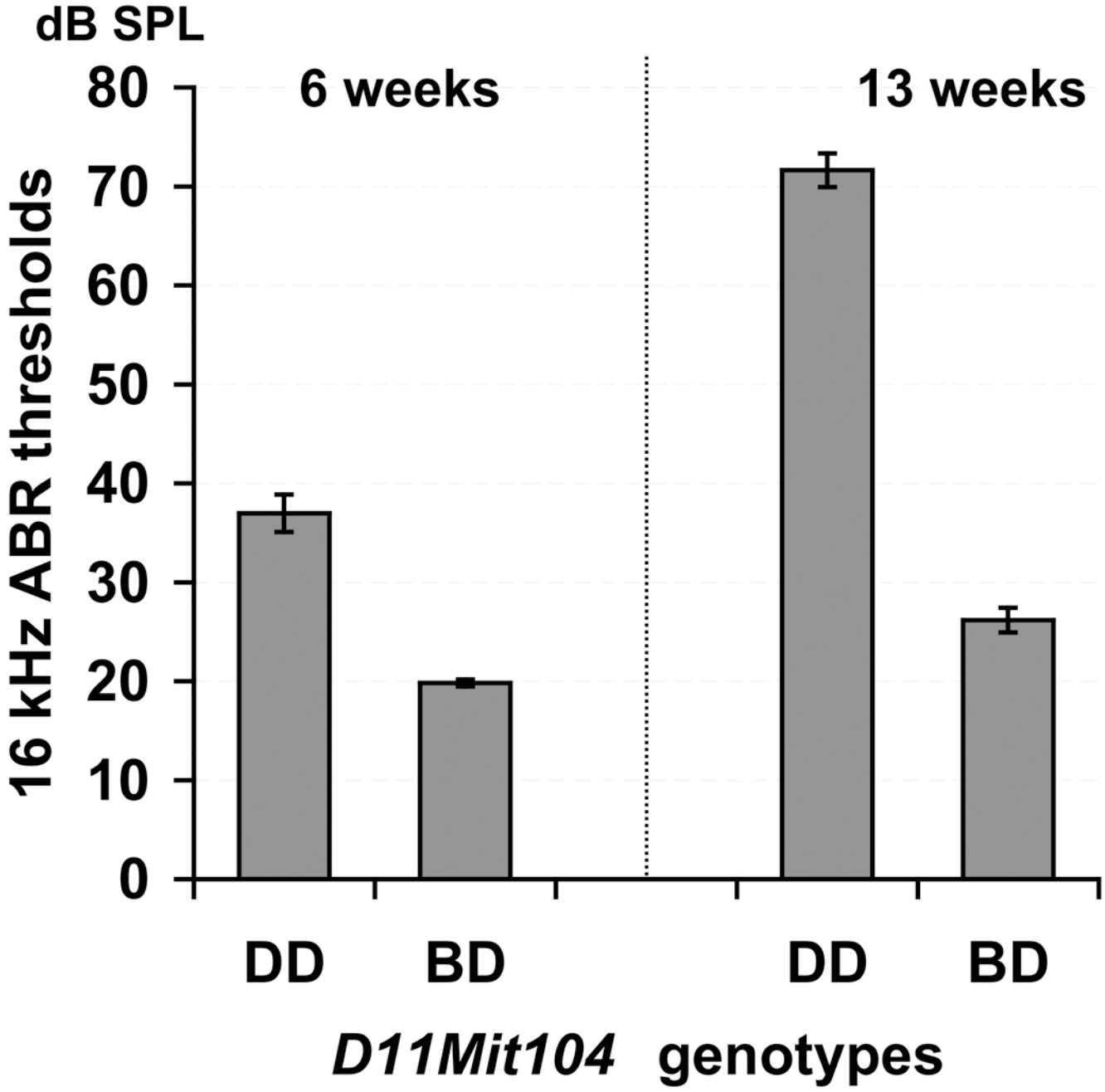

Fig. 5. Effects of *ahl8* on ABR thresholds of N2 mice from the (B6 × D2) × D2 backcross

We used *D11Mit104* (Chr 11, 119.2 Mb, NCBI build m37) as a marker for the *ahl8* locus. Average 16 kHz ABR thresholds (dB SPL) and their standard error bars are shown for each genotype at 6 and 13 weeks of age. *D11Mit104* genotypes of mice are represented as DD if homozygous for the D2 allele (N=110) and BD if heterozygous for B6 and D2 alleles (N=115).

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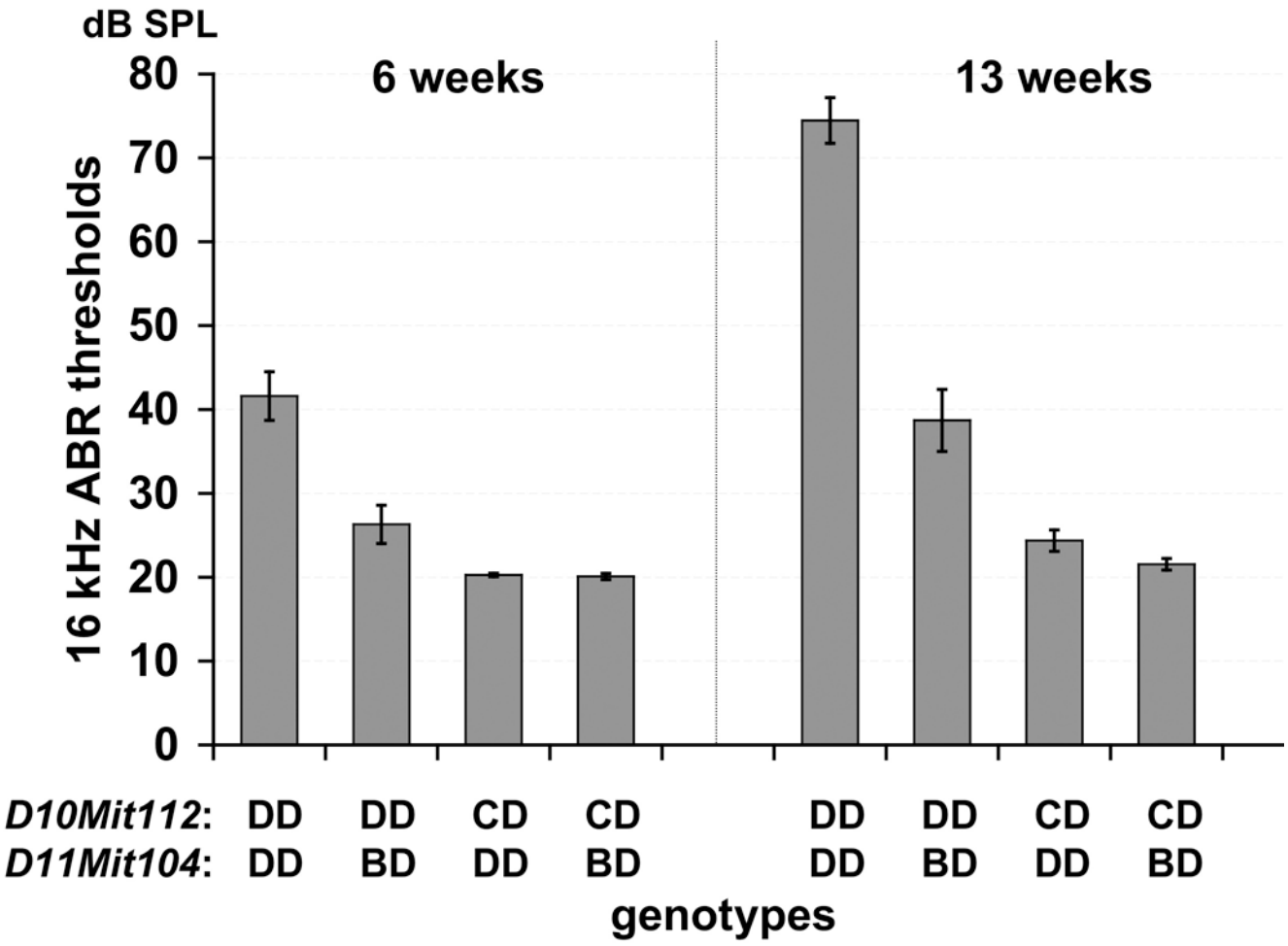

Fig. 6. Digenic effects of *Cdh23* and *ahl8* loci on ABR thresholds of N2 mice from the (B6.CAST-*Cdh23*$^{Ahl+}$ × D2) × D2 backcross

D10Mit112 (60.9 Mb position on Chr 10, NCBI build m37) was used as a marker for the *Cdh23* locus (59.8–60.2 Mb position) and *D11Mit104* as a marker for *ahl8*. Average 16 kHz ABR thresholds (dB SPL) and their standard error bars are shown for each *D10Mit112*/*D11Mit104* (*Cdh23*/*ahl8*) digenic genotype at 6 and 13 weeks of age. *D10Mit112* genotypes are designated as DD if homozygous for the D2 allele and CD if heterozygous for CAST and D2 alleles. *D11Mit104* genotypes are designated as DD if homozygous for the D2 allele and BD if heterozygous for B6 and D2 alleles. The number of mice (N) with each *D10Mit112*/*D11Mit104* digenic genotype are as follows: DD/DD (N=56), DD/BD (N=50), CD/DD (N=55), and CD/BD (N=53). Note that the effect of *D11Mit104* genotypes on ABR thresholds is restricted to mice with the permissive DD genotype at *D10Mit112*.

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Table 1

LOD scores of the associations of Chr 11 markers with ABR thresholds of 225 N2 mice from the (B6 × D2) × D2 backcross. Chromosomal map positions in megabases (Mb) are from NCBI build m37. The threshold mean and standard deviation are given for each age and stimulus. The percentages of total ABR threshold variation that can be explained by *D11Mit104*, the most highly associated locus, are shown in the right-most column.

	LOD scores of associations with ABR thresholds						**ABR thresholds**		**% threshold**
marker: **position:**	***D11Mit285*** **89.8 Mb**	***D11Mit360*** **103.2 Mb**	***D11Mit333*** **108.6 Mb**	***D11Mit338*** **115.3 Mb**	***D11Mit48*** **118 Mb**	***D11Mit104*** **119.2 Mb**	**mean** **dB SPL**	**standard** **deviation**	**variation** ***D11Mit104***
6 weeks									
click	2.3	4.7	5.1	7.7	7.0	8.2	41.8	9.1	15
8 kHz	1.5	3.1	3.8	7.3	7.0	7.3	43.4	8.6	14
16 kHz	4.3	8.4	8.8	14.9	14.1	15.6	28.1	16.4	27
32 kHz	6.1	11.0	12.2	20.7	19.5	21.1	51.6	18.2	35
13 weeks									
click	5.1	12.2	14.2	20.3	19.6	21.5	50.0	16.4	35
8 kHz	4.6	12.1	13.9	21.6	20.9	21.8	50.5	16.7	36
16 kHz	12.0	27.4	30.3	55.6	53.2	56.4	48.8	27.8	68
32 kHz	12.0	29.8	32.5	59.9	57.1	59.9	70.5	25.0	70

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Table 2

Main effects and interaction of *Cdh23* (marked by *D10Mit112*) and *ahl8* (marked by *D11Mit104*) loci on ABR thresholds of 214 N2 mice from the (B6.CAST-*Cdh23*$^{Ahl+}$ × D2) × D2 backcross tested at 13 weeks of age. The threshold mean (dB SPL) and standard deviation (std dev) are given for each auditory stimulus along with the percentages of the total threshold variation (% var) that can be explained by each effect and their corresponding LOD scores of association.

	click thresholds		**8 kHz thresholds**		**16 kHz thresholds**		**32 kHz thresholds**	
	mean 47.5, std dev 17.1		**mean 49.9, std dev 16.9**		**mean 40.2, std dev 27.5**		**mean 61.1, std dev 25.0**	
Effect	**% var**	**LOD**	**% var**	**LOD**	**% var**	**LOD**	**% var**	**LOD**
ahl main	28	15.1	19	9.6	40	23.4	46	28.3
ahl8 main	11	5.6	10	4.8	13	6.4	12	5.9
interaction	13	6.7	7	3.8	19	9.5	17	8.5
Combined	52	29.2	36	19	72	43.5	75	46.7