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Hearing loss associated with the modifier of deaf waddler (*mdfw*) locus corresponds with age-related hearing loss in 12 inbred strains of mice

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Abstract

The modifier of deaf waddler (*mdfw*) and age-related hearing loss (*Ahl*) loci were both discovered as inbred strain polymorphisms that affect hearing loss in mice. Both loci map to the same position on chromosome (Chr) 10. The *mdfw* locus interacts epistatically with the deaf waddler (*dfw*) mutation on Chr 6, and the *Ahl* locus is a major contributor to AHL in several inbred strains. To investigate the possibility of allelism, we examined the correspondence of *mdfw* and *Ahl* phenotypes among 12 inbred mouse strains. The effects of strain-specific *mdfw* alleles on hearing loss were assessed in dfw^{2J}/+ F1 hybrids produced from mating BALB-dfw^{2J}/+ mice with mice from each of 12 inbred strains. F1 hybrids were then assessed for hearing by auditory-evoked brainstem response threshold analysis and classified as dfw^{2J}/+ or +/+ by polymerase chain reaction typing. Heterozygosity for dfw^{2J} accelerated hearing loss in F1 hybrids derived from all strains tested, except those produced with the B6.CAST $+^{Ahl}$ congenic strain. dfw^{2J}/+ F1 hybrids derived from parental strains 129P1/ReJ, A/J, BUB/BnJ, C57BR/cdJ, DBA/2J, NOD/LtJ and SKH2/J exhibited a severe hearing loss by 12 weeks of age. Those derived from strains 129T2/SvEmsJ, C3H/HeJ, CBA/CaJ and NON/LtJ exhibited only a slight to intermediate hearing loss at that age. The hearing loss associated with these strain-specific *mdfw* alleles corresponds with previously determined *Ahl* allele effects, providing additional evidence that *mdfw* and *Ahl* are manifestations of the same gene. A functional relationship therefore may exist between the Ca^{2+} transporting activity of the *dfw* gene (*Atp2b2*) and AHL.

Keywords

Hearing loss; Mouse; Inbred strain; Age-related hearing loss; Noise-induced hearing loss

1. Introduction

Presbycusis (or age-related hearing loss, AHL) is a progressive, primarily sensorineural hearing loss that affects more than 40% of the human population over 65 years of age, which can lead to a diminished quality of life for the elderly (Gorlin et al., 1995; Morton, 1991). The genetic basis of presbycusis is poorly understood because of the extreme difficulty in studying such a late-onset disease in human populations. Mice offer a promising approach for the genetic study of human presbycusis because of their short life span and ease of experimental manipulation. In addition, mice provide a good model system to study human hearing because of the anatomical, functional and pathological similarities between human and mouse ears (Steel, 1991).

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AHL has been extensively studied in C57BL/6J mice (Henry and Chole, 1980; Hunter and Willott, 1987; Mikaelian, 1979; Willott et al., 1984) and a major underlying gene (*Ahl*) has been mapped to chromosome (Chr) 10 (Johnson et al., 1997). More than 20 inbred strains of mice have been discovered with AHL (Zheng et al., 1999). The same Chr 10 locus contributes to AHL in at least 10 of these strains (Johnson et al., 2000); however, the *Ahl* gene has not yet been identified at the molecular level. Susceptibility to noise-induced hearing loss (NIHL) has been shown to be associated with the propensity to develop AHL (Li et al., 1993) and with the *Ahl* gene in particular (Erway et al., 1996). Thus, the *Ahl* gene appears to play a major role in determining both AHL and NIHL susceptibility. AHL and NIHL vulnerability, however, may not be coupled in all inbred strains (Yoshida et al., 2000).

The map position of *Ahl* on Chr 10 coincides with that of another inbred strain polymorphism associated with hearing loss, the modifier of deaf waddler (*mdfw*) locus. The *mdfw* locus has been shown to interact epistatically with the deaf waddler (*dfw*) mutation on Chr 6 (Noben-Trauth et al., 1997). Mice homozygous for the deaf waddler mutation (*dfw*/*dfw*) exhibit a congenital hearing loss with an associated head bobbing and unbalanced gait. Mice that are heterozygous for *dfw* and that are also homozygous for a BALB/c-derived, recessive *mdfw* allele exhibit profound hearing loss by 10 weeks of age but have normal behavior and gait. Mice that are heterozygous for *dfw* but that have at least one copy of a CAST/Ei-derived, dominant *Mdfw* allele retain good hearing until old age. The genotype of the *mdfw* locus, regardless of allelic composition, has no affect on phenotypes of *dfw*/*dfw* or +/+ mice. While mutations of *dfw* have been shown to be alterations of the ATPase, Ca^{2+} transporting, plasma membrane 2 gene, *Atp2b2* (Street et al., 1998), the *mdfw* gene has not yet been identified at the molecular level.

The *mdfw* and *Ahl* loci were both discovered as inbred strain polymorphisms that affect hearing loss and both map to the same position on Chr 10. To investigate the possibility of allelism, we examined the correspondence of *mdfw* and *Ahl* phenotypes among 12 inbred mouse strains. For example, the *mdfw* alleles from BALB/c and C57BL/6 strains were shown to allow hearing loss to occur in *dfw*/+ mice; whereas, the dominant *Mdfw* allele from CAST/Ei prevents hearing loss (Noben-Trauth et al., 1997). Consistent with these results, both the BALB/c and C57BL/6 strains have a recessive *Ahl* allele conferring hearing loss susceptibility that is different from the dominant, AHL-resistant CAST/Ei allele. To extend this comparative analysis, we examined 12 additional mouse strains for the effects of their *mdfw* alleles on hearing loss in *dfw*/+ F1 hybrids. Here, we show that *Ahl* and *mdfw* hearing loss phenotypes do indeed correspond among all inbred strains tested, suggesting that these two independently discovered loci represent the same gene. In AHL-susceptible strains, hearing loss is greatly accelerated when mice are heterozygous for the *dfw* mutation, suggesting a functional relationship between the Ca^{2+} transporting activity of the *dfw* gene and AHL or NIHL.

2. Materials and methods

2.1. Mice and genetic crosses

The dfw^{2J} mutation arose in a congenic substrain of BALB/cByJ (Noben-Trauth et al., 1997) and this strain is hence referred to as CBy-dfw^{2J}. F1 hybrids were produced from matings of CBy-dfw^{2J}/+ heterozygotes with mice from each of the inbred strains to be tested for *mdfw* alleles. Half of the F1 hybrids from each mating are expected to be dfw^{2J}/+ (test group) and half +/+ (control group). 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 as NOD/LtJ. The care of mice used in this study was approved by the Animal Care and Use Committee of The Jackson Laboratory. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

2.2. dfw genotyping

To distinguish between *dfw*^{2J}/+ and +/+ genotypes, DNA was extracted from tail tips of F1 hybrids and assayed by polymerase chain reaction (PCR) (Street et al., 1998). The first set of PCR primers amplifies a 108 bp product from *dfw*^{2J} but not wild-type genomic DNA: forward CATCTGCTCAGACAAGACGA, reverse GCATTGATGGAGCTGGGATC. The second set of PCR primers amplifies a 162 bp product from wild-type but not *dfw*^{2J} genomic DNA: forward CATCTGCTCAGACAAGACAG, reverse GGTGTAGGCGCTGTTGATGG. The PCR reactions for each primer pair were carried out in separate 10 μl total volumes, containing 20 ng genomic DNA, using previously described methods (Ward-Bailey et al., 1996), except that the annealing temperature was 60°C.

2.3. Auditory-evoked brainstem response (ABR) threshold measurements

The parental strains, F1 hybrids and backcross mice were tested for ABR thresholds with equipment from Intelligent Hearing Systems (IHS, Miami, FL, USA) using previously described methods and equipment (Zheng et al., 1999). Subdermal needle electrodes are 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 are delivered binaurally through plastic tubes to the ear canals. Evoked brainstem responses are amplified and averaged and their wave patterns displayed on a computer screen. Auditory thresholds are obtained for each stimulus by varying the sound pressure level to identify the lowest level at which an ABR pattern can be recognized.

3. Results

CBy-*dfw*^{2J}/+ mice (which are *mdfw*/*mdfw*) were mated with mice from each of 12 inbred strains to produce F1 hybrids that are either *dfw*^{2J}/+ or +/+ at the *dfw* locus and heterozygous at the *mdfw* locus. In all F1 hybrids, the unknown *mdfw* allele of the strain to be tested is in heterozygous combination with the recessive *mdfw* allele from the CBy strain. The *dfw*^{2J}/+ and +/+ genotypes of the F1 progeny were distinguished by PCR analysis and their hearing was assessed by ABR threshold analysis.

Heterozygosity for *dfw*^{2J} caused a severe hearing impairment (ABR thresholds >60 dB above normal) by 12 weeks of age in seven of the 12 tested F1 hybrids (Table 1), derived from parental strains 129P1/ReJ, A/J, BUB/BnJ, C57BR/cdJ, DBA/2J, NOD/LtJ and SKH2/ J. Heterozygosity for *dfw*^{2J} had less of a detrimental effect on hearing in the other F1 hybrids (Table 2), derived from parental strains 129T2/SvEmsJ, C3H/ HeJ, CBA/CaJ, NON/LtJ (all with only a mild impairment, ABR thresholds 20–40 dB above normal) and B6.CAST +^{Ahl} (no impairment). B6.CAST +^{Ahl} is a congenic strain that was produced by successively backcrossing the CAST/Ei +^{Ahl} allele (conferring resistance to AHL) onto the C57BL/6J inbred strain (Johnson et al., 1997). The CAST/Ei *Mdfw* allele (which protects against hearing loss in *dfw*/+ mice (Noben-Trauth et al., 1997)) is contained within the congenic segment of the B6.CAST +^{Ahl} strain, because of the identical map positions of *Ahl* and *mdfw* on Chr 10.

Some of the F1 hybrids were tested at multiple ages to determine hearing loss onset times and progression. The *dfw*^{2J}/+ F1 hybrids derived from the 129P1/ReJ strain exhibited a mild hearing impairment at 4 weeks of age that progressed to severe impairment by 9 weeks of age (Table 1). The *dfw*^{2J}/+ F1 hybrids derived from the B6.CAST +^{Ahl} strain retained normal hearing levels even at 58 weeks of age (Table 2). The mild hearing impairment observed at 12 weeks of age in F1 hybrids derived from strains 129T2/SvEmsJ, CBA/CaJ, NON/LtJ and C3H/ HeJ progressed to intermediate impairment by 23–29 weeks of age and then to severe impairment by 34 weeks of age (in C3H/HeJ-derived *dfw*^{2J}/+ mice).

The hearing loss associated with *dfw* heterozygosity in each F1 strain hybrid was then compared with the AHL associated with its parental inbred strain. The ABR thresholds of dfw^{2J}/+ F1 hybrids at 12 weeks of age, which indicate the effect of the *mdfw* allele, correspond with the ABR thresholds of the parental inbred strain at 6 months of age, which indicate the level of AHL associated with that strain (Fig. 1). The Chr 10 *Ahl* locus has been shown by backcross linkage analysis to be a major contributor to the AHL exhibited by these same strains (Johnson et al., 2000).

4. Discussion

The relationships illustrated in Fig. 1 clearly demonstrate the phenotypic correspondence of strain-specific *mdfw* and *Ahl* alleles. Hearing loss progression is greatly accelerated in mice that are heterozygous for the dfw^{2J} mutation in AHL-susceptible strains, such as 129P1/ReJ, A/J, BUB/BnJ, C57BR/cdJ, DBA/2J, NOD/LtJ and SKH2/J (Table 1) and the previously described BALB/c and C57BL/6J strains (Noben-Trauth et al., 1997). This effect is much reduced in AHL-resistant strains, such as 129T2/SvEmsJ, C3H/HeJ, CBA/CaJ and NON/LtJ (Table 2) and absent in the congenic strain B6.CAST $+^{Ahl}$ (Table 2) and its parental donor strain CAST/Ei (Noben-Trauth et al., 1997). These results suggest there may be three allelic forms of *mdfw*: the allele conferring susceptibility to hearing loss (present in 129P1/ReJ, A/J, BALB/c, BUB/BnJ, C57BL/6J, C57BR/cdJ, DBA/2J, NOD/LtJ and SKH2/J) being recessive to alleles conferring partial resistance (present in 129T2/SvEmsJ, C3H/HeJ, CBA/CaJ and NON/LtJ) and complete resistance (present in CAST/Ei).

If *Ahl* and *mdfw* are allelic, as these results support, then a functional relationship may exist between the *dfw* gene (*Atp2b2*) and AHL or NIHL. The *Atp2b2* gene product functions as a Ca^{2+} pump in hair cells (Street et al., 1998) and acoustic over-stimulation has been shown to increase cytoplasmic Ca^{2+} concentrations in outer hair cells (Fridberger et al., 1998). Thus, consequences related to an elevation of intracellular Ca^{2+} provide a possible link to the cumulative loss of outer hair cells characteristic of AHL and NIHL.

The accelerated hearing loss in dfw^{2J}/+ hybrids potentially can be used as an early predictor of strains that are likely to exhibit late-onset AHL. For example, dfw^{2J}/+ F1 hybrids with CBA/CaJ exhibit a slight but significant hearing loss at 3 months of age (Fig. 1). Although we did not detect AHL at 6 months of age in inbred CBA/CaJ mice, AHL has been reported in this strain at a much older age (17–25 months; Parham et al., 1999). Because hearing loss onset is much earlier in dfw^{2J}/+ mice than in +/+ mice of a given strain, experiments to study AHL pathogenesis could be greatly shortened by using dfw^{2J}/+ mice. NIHL experiments might also benefit by using dfw^{2J}/+ mice. Mice that are dfw^{2J}/+ are likely to exhibit an increased vulnerability to NIHL and thus provide a more sensitive model for NIHL studies. Our results predict that NIHL susceptibility and resistance will follow the strain relationships shown in Fig. 1. To produce dfw^{2J}/+ mice for such studies, F1 hybrids can be produced as herein described, by crossing the strain of interest with CBy-dfw^{2J}/+ mice. The resulting F1 hybrids will be genetically identical except at the *dfw* locus. One half of the F1 hybrids are expected to have the +/+ genotype, and these make ideal controls for the dfw^{2J}/+ mice. As previously described, these two genotypes are easily distinguished by PCR typing.

The hearing loss in the 129P1/ReJ strain was much greater than in the 129T2/SvEmsJ strain, despite the similarity of their strain nomenclature. At 6 weeks of age, dfw^{2J}/+ F1 hybrids derived from the 129P1/ReJ strain already exhibited a severe hearing impairment (Table 1), whereas dfw^{2J}/+ F1 hybrids derived from the 129T2/SvEmsJ strain exhibited only a slight hearing impairment at 29 weeks of age (Table 2). At 6 months of age, 129P1/ReJ inbred strain mice exhibited AHL (average ABR thresholds >60 dB), whereas 129T2/SvEmsJ inbred strain mice retained normal hearing at that age (Fig. 1). The differences in hearing between these two

strains is not that surprising considering the extensive genetic variability that has been documented among 129 substrains, a result of both deliberate and accidental outcrossing (Simpson et al., 1997). Although hearing loss before 3 months of age has been reported for three of the 129-related substrains (129P3/J, 129P1/ReJ and 129X1/SvJ; Zheng et al., 1999), this is not the case for all 129 substrains, as shown by our results for the 129T2/SvEmsJ strain. In this regard, the reported resistance of the 129S6/SvEvTac strain to NIHL (Yoshida et al., 2000) is less surprising than originally thought, because this 129 substrain (like 129T2/SvEmsJ) may be resistant to AHL.

The waltzer deafness mutation (*v*) maps to the same Chr 10 position as *Ahl* and *mdfw* and, therefore, may be an allele of the same gene. The cochlear pathology (hair cell loss and spiral ganglion cell degeneration) of *v* mutants (Deol, 1956) is similar to, but more severe than, that associated with *Ahl* and *mdfw* hearing loss. The *v* mutation, however, may be more amenable to a positional cloning approach for gene identification (Bryda et al., 1997) than are the *Ahl* and *mdfw* strain polymorphisms. Once the *v* gene is identified, it could be tested as a candidate for *Ahl* and *mdfw*. The region of mouse Chr 10 containing *Ahl*, *mdfw* and *v* 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 gene or genes identified at the *v* locus in the mouse and the *DFNB12/USH1D* locus in humans may play important roles in AHL and NIHL susceptibility, as well as in the more severe deafness traits by which they were originally discovered.

In conclusion, the results presented here provide evidence that the two independently discovered loci *Ahl* and *mdfw* are different manifestations of the same gene. In AHL-susceptible strains, hearing loss is greatly accelerated in mice that are heterozygous for the dfw^{2J} mutation. Hearing assessment of dfw^{2J}/+ F1 strain hybrids, therefore, potentially can be used to predict the susceptibility of the parental strains to AHL or NIHL. The equivalency of *mdfw* and *Ahl* further suggests that a functional relationship may exist between the Ca^{2+} transporting activity of the *dfw* gene (*Atp2b2*) and AHL or NIHL.

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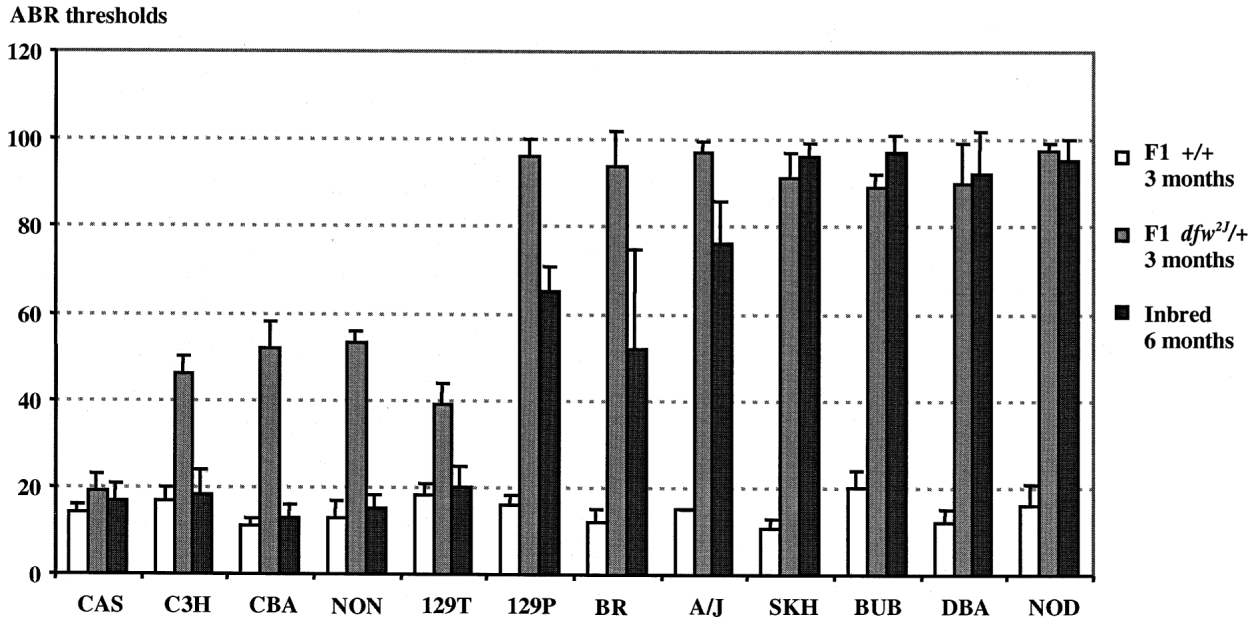

Fig. 1.

Comparison of *mdfw* and *Ahl* effects on hearing loss in 12 inbred mouse strains. The *mdfw* effects are shown as the average ABR thresholds of 3-month-old dfw^{2J}/+ F1 hybrids (Tables 1 and 2), produced by mating mice from each inbred strain with CBy-dfw^{2J}/+ mice. ABR thresholds of 3-month-old +/+ F1 hybrids are shown as controls for normal hearing. The *Ahl* effects are shown as the average ABR thresholds of 6-month-old mice from each of the inbred strains (Zheng et al., 1999 and unpublished data; at least five mice tested per strain). For clarity, only threshold responses to the 16 kHz pure-tone stimulus are shown. Strain abbreviations are CAS=B6.CAST $+^{Ahl}$, C3H=C3H/HeJ, CBA=CBA/CaJ, NON=NON/LtJ, 129T=129T2/SvEmsJ, 129P=129P1/ReJ, BR=C57BR/cdJ, SKH=SKH2/J, BUB=BUB/BnJ, DBA=DBA/2J and NOD=NOD/LtJ.

Table 1

F1 strain hybrids in which *dfw*^{2J} heterozygosity caused a severe hearing impairment by 12 weeks of age

Unique parent of F1 hybrid	Genotype	Age (weeks)	Sex	ABR thresholds			
				click	8 kHz	16 kHz	32 kHz
Normal hearing mice (Zheng et al., 1999)				<55	<40	<35	<60
129P1/ReJ	+/+	4	m	50	45	35	60
129P1/ReJ	+/+	4	m	40	35	20	45
129P1/ReJ	+/+	4	m	40	25	15	40
129P1/ReJ	+/+	4	m	40	30	15	50
129P1/ReJ	*dfw*/+	4	f	**80**	**55**	**45**	**80**
129P1/ReJ	*dfw*/+	4	m	**85**	**70**	**50**	**95**
129P1/ReJ	*dfw*/+	4	m	**85**	**65**	**50**	**90**
129P1/ReJ	*dfw*/+	4	m	55	**50**	30	**75**
129P1/ReJ	+/+	6	m	35	35	10	45
129P1/ReJ	+/+	6	m	35	35	15	45
129P1/ReJ	+/+	6	m	35	30	10	50
129P1/ReJ	*dfw*/+	6	f	**95**	**75**	**55**	**95**
129P1/ReJ	*dfw*/+	6	f	**119**	**104**	**99**	**119**
129P1/ReJ	*dfw*/+	6	m	**90**	**85**	**90**	**105**
129P1/ReJ	*dfw*/+	6	m	**85**	**80**	**90**	**105**
129P1/ReJ	+/+	9	m	40	35	15	45
129P1/ReJ	+/+	9	m	40	35	20	45
129P1/ReJ	+/+	9	m	40	40	15	60
129P1/ReJ	*dfw*/+	9	f	**115**	**95**	**90**	**119**
129P1/ReJ	*dfw*/+	9	f	**119**	**104**	**99**	**119**
129P1/ReJ	*dfw*/+	9	m	**100**	**85**	**95**	**115**
129P1/ReJ	*dfw*/+	9	m	**90**	**85**	**90**	**110**
129P1/ReJ	+/+	12	f	40	45	15	60
129P1/ReJ	+/+	12	m	40	30	15	45
129P1/ReJ	+/+	12	m	40	40	20	60
129P1/ReJ	+/+	12	f	40	35	15	60
129P1/ReJ	+/+	12	f	40	35	15	60

Unique parent of F1 hybrid	Genotype	Age (weeks)	Sex	ABR thresholds			
				click	8 kHz	16 kHz	32 kHz
129P1/ReJ	+/+	12	m	40	35	15	55
129P1/ReJ	*dfw*/+	12	f	**119**	**104**	**99**	**119**
129P1/ReJ	*dfw*/+	12	f	**119**	**104**	**99**	**119**
129P1/ReJ	*dfw*/+	12	m	**110**	**95**	**90**	**119**
129P1/ReJ	*dfw*/+	12	m	**100**	**100**	**95**	**119**
A/J	+/+	12	f	35	20	15	50
A/J	+/+	12	f	35	25	15	45
A/J	+/+	12	m	35	25	15	45
A/J	*dfw*/+	12	f	**119**	**95**	**95**	**119**
A/J	*dfw*/+	12	f	**119**	**104**	**99**	**119**
BUB/BnJ	+/+	12	f	35	25	20	45
BUB/BnJ	+/+	12	f	45	30	20	50
BUB/BnJ	+/+	12	m	40	25	20	45
BUB/BnJ	+/+	12	m	35	20	15	35
BUB/BnJ	+/+	12	m	40	25	25	40
BUB/BnJ	*dfw*/+	12	m	**115**	**95**	**85**	**119**
BUB/BnJ	*dfw*/+	12	f	**115**	**104**	**90**	**119**
BUB/BnJ	*dfw*/+	12	f	**110**	**100**	**90**	**119**
BUB/BnJ	*dfw*/+	12	f	**110**	**95**	**90**	**110**
C57BR/cdJ	+/+	12	f	35	25	10	40
C57BR/cdJ	+/+	12	f	35	20	10	35
C57BR/cdJ	+/+	12	f	35	25	15	40
C57BR/cdJ	+/+	12	f	35	20	10	35
C57BR/cdJ	+/+	12	m	35	25	15	40
C57BR/cdJ	*dfw*/+	12	f	**119**	**100**	**95**	**119**
C57BR/cdJ	*dfw*/+	12	m	**110**	**90**	**95**	**119**
C57BR/cdJ	*dfw*/+	12	m	**119**	**104**	**99**	**119**
C57BR/cdJ	*dfw*/+	12	m	**115**	**90**	**80**	**115**
C57BR/cdJ	*dfw*/+	12	m	**119**	**104**	**99**	**119**
DBA/2J	+/+	12	f	35	20	10	30
DBA/2J	+/+	12	m	35	20	15	35

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Unique parent of F1 hybrid	Genotype	Age (weeks)	Sex	ABR thresholds			
				click	8 kHz	16 kHz	32 kHz
DBA/2J	+/+	12	m	35	15	10	35
DBA/2J	*dfw*/+	12	m	**119**	**95**	**85**	**115**
DBA/2J	*dfw*/+	12	m	**115**	**95**	**80**	**110**
DBA/2J	*dfw*/+	12	f	**119**	**104**	**95**	**119**
DBA/2J	*dfw*/+	12	f	**119**	**104**	**99**	**119**
NOD/LtJ	+/+	12	f	35	25	10	45
NOD/LtJ	+/+	12	f	40	45	25	55
NOD/LtJ	+/+	12	f	40	30	15	40
NOD/LtJ	+/+	12	m	35	30	15	45
NOD/LtJ	+/+	12	f	35	25	15	40
NOD/LtJ	*dfw*/+	12	f	**119**	**104**	**99**	**119**
NOD/LtJ	*dfw*/+	12	f	**119**	**100**	**95**	**119**
NOD/LtJ	*dfw*/+	12	m	**115**	**100**	**95**	**115**

All F1 hybrids were produced from mating CBy-*dfw*^{2J}/+ mice with mice from each of the strains listed below. All ABR thresholds above normal values are shown in boldface.

Table 2

F1 strain hybrids in which *dfw*^{2J} heterozygosity caused only a slight to intermediate hearing loss at 12 weeks of age

Unique parent of F1 hybrid	Genotype	Age (weeks)	Sex	ABR thresholds			
				click	8 kHz	16 kHz	32 kHz
B6.CAST +^{Ahl}	+/+	12	f	35	25	15	40
B6.CAST +^{Ahl}	+/+	12	f	40	25	15	35
B6.CAST +^{Ahl}	+/+	12	f	40	20	10	35
B6.CAST +^{Ahl}	+/+	12	f	35	20	15	35
B6.CAST +^{Ahl}	+/+	12	m	35	20	15	35
B6.CAST +^{Ahl}	*dfw*/+	12	f	45	35	20	50
B6.CAST +^{Ahl}	*dfw*/+	12	f	40	25	20	50
B6.CAST +^{Ahl}	*dfw*/+	12	f	45	35	25	50
B6.CAST +^{Ahl}	*dfw*/+	12	f	40	30	15	35
B6.CAST +^{Ahl}	*dfw*/+	12	f	40	35	20	40
B6.CAST +^{Ahl}	*dfw*/+	12	f	40	30	15	35
B6.CAST +^{Ahl}	+/+	58	m	45	45	20	55
B6.CAST +^{Ahl}	+/+	58	m	40	40	15	45
B6.CAST +^{Ahl}	+/+	58	f	40	35	15	40
B6.CAST +^{Ahl}	+/+	58	f	40	30	20	45
B6.CAST +^{Ahl}	*dfw*/+	58	f	40	45	20	40
B6.CAST +^{Ahl}	*dfw*/+	58	f	40	45	15	45
B6.CAST +^{Ahl}	*dfw*/+	58	f	40	45	20	45
B6.CAST +^{Ahl}	*dfw*/+	58	f	45	45	20	50
B6.CAST +^{Ahl}	*dfw*/+	58	f	40	35	20	40
B6.CAST +^{Ahl}	*dfw*/+	58	f	40	30	15	40
129T2/SvEmsJ	+/+	12	f	45	45	20	60
129T2/SvEmsJ	+/+	12	f	45	40	20	55
129T2/SvEmsJ	+/+	12	m	40	30	15	45
129T2/SvEmsJ	*dfw*/+	12	f	40	35	35	55
129T2/SvEmsJ	*dfw*/+	12	m	40	30	**45**	55
129T2/SvEmsJ	*dfw*/+	12	m	40	25	**40**	50

Unique parent of F1 hybrid	Genotype	Age (weeks)	Sex	ABR thresholds			
				click	8 kHz	16 kHz	32 kHz
129T2/SvEmsJ	*dfw*/+	12	m	40	30	35	55
129T2/SvEmsJ	+/+	29	f	45	45	25	55
129T2/SvEmsJ	+/+	29	f	45	40	20	55
129T2/SvEmsJ	+/+	29	m	40	40	20	50
129T2/SvEmsJ	*dfw*/+	29	f	45	35	**50**	60
129T2/SvEmsJ	*dfw*/+	29	m	50	35	**60**	60
129T2/SvEmsJ	*dfw*/+	29	m	45	35	**70**	60
129T2/SvEmsJ	*dfw*/+	29	m	45	35	**60**	60
CBA/CaJ	+/+	12	f	35	20	15	30
CBA/CaJ	+/+	12	f	35	20	10	30
CBA/CaJ	+/+	12	f	35	20	10	30
CBA/CaJ	+/+	12	m	30	15	10	30
CBA/CaJ	+/+	12	m	35	15	10	30
CBA/CaJ	+/+	12	m	35	20	10	30
CBA/CaJ	*dfw*/+	12	f	**65**	**50**	**60**	**65**
CBA/CaJ	*dfw*/+	12	f	45	25	**45**	45
CBA/CaJ	*dfw*/+	12	f	50	30	**55**	**65**
CBA/CaJ	*dfw*/+	12	f	45	25	**45**	40
CBA/CaJ	*dfw*/+	12	f	50	25	**55**	45
CBA/CaJ	*dfw*/+	12	f	35	20	**50**	40
CBA/CaJ	+/+	28	f	40	25	10	35
CBA/CaJ	+/+	28	f	30	20	10	30
CBA/CaJ	+/+	28	m	40	35	20	35
CBA/CaJ	+/+	28	m	35	25	15	35
CBA/CaJ	+/+	28	m	35	25	15	35
CBA/CaJ	+/+	28	f	35	30	15	35
CBA/CaJ	*dfw*/+	28	f	**85**	**70**	**65**	**90**
CBA/CaJ	*dfw*/+	28	f	**90**	**75**	**70**	**95**
CBA/CaJ	*dfw*/+	28	f	**100**	**85**	**85**	**105**
CBA/CaJ	*dfw*/+	28	f	**105**	**85**	**90**	**105**
NON/LtJ	+/+	12	f	30	20	10	35

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Unique parent of F1 hybrid	Genotype	Age (weeks)	Sex	ABR thresholds			
				click	8 kHz	16 kHz	32 kHz
NON/LtJ	+/+	12	m	40	25	20	40
NON/LtJ	+/+	12	f	35	20	15	35
NON/LtJ	+/+	12	f	35	25	10	40
NON/LtJ	+/+	12	m	35	20	10	35
NON/LtJ	*dfw*/+	12	f	**85**	**65**	**50**	**90**
NON/LtJ	*dfw*/+	12	f	**75**	**55**	**55**	**80**
NON/LtJ	*dfw*/+	12	f	**80**	**70**	**50**	**80**
NON/LtJ	*dfw*/+	12	m	**80**	**75**	**55**	**90**
NON/LtJ	*dfw*/+	12	m	**90**	**75**	**55**	**85**
NON/LtJ	*dfw*/+	12	m	**80**	**65**	**55**	**75**
NON/LtJ	+/+	24	f	35	25	15	35
NON/LtJ	+/+	24	f	35	30	15	45
NON/LtJ	+/+	24	f	35	25	10	35
NON/LtJ	+/+	24	f	35	25	10	45
NON/LtJ	+/+	24	f	30	25	10	35
NON/LtJ	*dfw*/+	24	m	**85**	**50**	**80**	**90**
NON/LtJ	*dfw*/+	24	m	**95**	**70**	**70**	**95**
NON/LtJ	*dfw*/+	24	m	**119**	**100**	**90**	**119**
C3H/HeJ	+/+	12	f	35	26	15	40
C3H/HeJ	+/+	12	f	35	30	20	40
C3H/HeJ	+/+	12	f	30	30	15	35
C3H/HeJ	*dfw*/+	12	f	**65**	**55**	**50**	**80**
C3H/HeJ	*dfw*/+	12	f	55	25	**50**	55
C3H/HeJ	*dfw*/+	12	m	**65**	30	**45**	55
C3H/HeJ	*dfw*/+	12	m	**65**	**55**	**50**	**80**
C3H/HeJ	*dfw*/+	12	m	45	25	**40**	45
C3H/HeJ	*dfw*/+	12	m	**65**	**60**	**45**	**75**
C3H/HeJ	+/+	23	f	45	35	15	35
C3H/HeJ	+/+	23	f	45	35	20	35
C3H/HeJ	+/+	23	m	40	30	20	40
C3H/HeJ	*dfw*/+	23	f	**85**	**80**	**75**	**90**

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Unique parent of F1 hybrid	Genotype	Age (weeks)	Sex	ABR thresholds			
				click	8 kHz	16 kHz	32 kHz
C3H/HeJ	*dfw*/+	23	f	**80**	**70**	**75**	**90**
C3H/HeJ	*dfw*/+	23	m	**80**	**60**	**50**	**105**
C3H/HeJ	*dfw*/+	23	m	**70**	**60**	**65**	**90**
C3H/HeJ	*dfw*/+	23	m	**90**	**85**	**90**	**115**
C3H/HeJ	*dfw*/+	23	f	**110**	**90**	**95**	**119**
C3H/HeJ	+/+	34	f	40	25	20	45
C3H/HeJ	+/+	34	f	40	30	25	45
C3H/HeJ	+/+	34	f	40	30	20	45
C3H/HeJ	*dfw*/+	34	f	**119**	**100**	**95**	**119**
C3H/HeJ	*dfw*/+	34	m	**115**	**100**	**99**	**119**
C3H/HeJ	*dfw*/+	34	m	**115**	**95**	**95**	**115**
C3H/HeJ	*dfw*/+	34	m	**110**	**100**	**95**	**115**
C3H/HeJ	*dfw*/+	34	m	**110**	**90**	**85**	**110**
C3H/HeJ	*dfw*/+	34	m	115	100	85	115

Hearing remained normal in dfw^{2J}/+ hybrids with B6.CAST $+^{Ahl}$. All F1 hybrids were produced from mating CBy-dfw^{2J}/+ mice with mice from each of the strains listed below. ABR thresholds above normal values are shown in boldface.