JARO 04: 83–90 (2002)
DOI: 10.1007/s10162-002-3011-0

JARO
Journal of the Association for Research in Otolaryngology

Genetic Analyses of the Mouse Deafness Mutations Varitint-Waddler (*Va*) and Jerker (*Espn^{je}*)

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Received: 26 March 2002; Accepted: 3 July 2002; Online publication: 18 September 2002

ABSTRACT

Genetic studies on spontaneous mouse mutants with hearing defects have provided important insights into the function of genes expressed in inner ear hair cells. Here we report on our genetic analyses of the deaf mutants varitint-waddler (*Va*) and jerker (*Espn^{je}*). A high-resolution genetic map localizes *Va^{J}* to a 0.14 ± 0.08 cM region between *D3Mit85* and *D3Mit259* on distal chromosome 3. By comparative mapping, the human ortholog resides at 1p22.3 between markers *D1S3449* and *D1S2252*. To study the effect of different genetic backgrounds on the hearing phenotype, *Espn^{je}* and *Va^{J}* were crossed to various inbred strains. Auditory-evoked brainstem response tests on F2 progeny demonstrate that expression, inheritance, and penetrance of the hearing phenotype are solely controlled by the mutant allele. To test for a genetic interaction between *Espn^{je}* and *Cdh23^{v}*, auditory function was analyzed in double heterozygotes; no significant increases of thresholds of sound pressure levels were observed. The results establish the framework for cloning the *Va* gene and provide valuable insights into the genetics of deafness mutations in the mouse.

Keywords: deafness, modifying alleles, *Cdh23*, Espin, jerker, varitint-waddler

INTRODUCTION

Genetic characteristics of traits such as penetrance, inheritance, and expressivity are often coregulated (modified) by alleles segregating in the background strain (for review see Nadeau 2001). In the mouse, deafness is generally inherited as a single, autosomal, Mendelian trait with only very little variation in penetrance and expressivity. However, a few cases of multifactorial inheritance were reported in which the presence of a second genetic factor has a modifying effect on penetrance and inheritance of the disease allele (Ikeda et al. 1999; Johnson et al. 2001, Ng et al. 2001; Noben–Trauth et al. 1997). For instance, the dominant allele of modifier-of-tubby-hearing 1 (*moth1*), segregating in AKR/J, rescues hearing loss in a significant fraction of tubby homozygotes (Ikeda et al. 1999). Recently, nonsynonymous sequence polymorphisms in the microtubule-associated protein 1a gene (*Mtap1a*) were associated with *moth1* supporting previous evidence that tubby plays a role at synapses (Ikeda et al. 2002). The molecular mechanisms underlying genetic modification are currently not well understood, but further studies will undoubtedly show how modifiers exert their effects on the mutant allele. In addition, identifying and studying the mechanisms of modifiers may also help understand the genetic complexity of age-related, noise-induced, or aminoglycoside-induced hearing loss.

Varitint-waddler (*Va*) and jerker (*je*, *Espn^{je}*) are two spontaneous mutations exhibiting defects in the sensory epithelium of the organ of Corti and vestibule leading to deafness and circling behavior. *Va* is a semidominant mutation and arose in a cross between (C57black/C57brown)F1 and C57black (Cloudman and Bunker 1945). *Va* homozygotes and heterozy-

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gotes are deaf, circle, and express a variegated and dilute coat color. A second allele, *Va^J*, arose in a cross segregating for *Va* and exhibits a less severe phenotype (Lane 1969, 1972). *Va^J* homozygotes and heterozygotes show normal vestibular behavior, are less variegated, but are both deaf. Light-microscopic, electrophysiological, and ultrastructural studies of the inner ear of *Va* and *Va^J* identified defects in organ of Corti and stria vascularis (Cable and Steel 1998; Deol 1954). Correlating with the coat color phenotype, cochlea physiology and morphology are more pronounced in homozygotes.

Jerker is a recessive mutation which was first recognized by a mouse fancier in 1938 and later described by Grüneberg as a ''dancing adult female mouse'' showing erratic circling behavior, jerking head movements, hyperactivity, and deafness (Grüneberg et al. 1941). Recently, a frame-shift mutation in the Espin gene (*Espn*), encoding an actin bundling protein, was identified as the underlying molecular cause (Zheng et al. 2000). Espn localizes to stereocilia, suggesting an organizing role during formation of the actin filament network (Zheng et al. 2000). In homozygotes, auditory-evoked brainstem responses (ABR), compound action potentials, and cochlea microphonics are absent (Sjöstrom and Anniko 1992; Steel and Bock 1983). Interestingly, although jerker is inherited as recessive allele, heterozygotes develop a late onset ABR threshold shift (Sjöstrom and Anniko 1990). This partial hearing loss is accompanied by a histopathology similar to defects observed in homozygotes (Sjöstrom and Anniko 1990), which could be the result of a delayed dominant effect of jerker or the result of inheritance modification caused by the genetic background.

Identification and characterization of genes underlying deafness in mice continue to influence our understanding of how hair cells develop and function (for review see Steel and Kros 2001). Many of these genes are being identified through a genetic approach using spontaneous mouse mutants in combination with genetic mapping and molecular cloning strategies. In this article, we report on the first step towards cloning the *Va* locus and on our results from a systematic screen for *Va* and *Espn^{je}* modifier genes.

METHODS AND MATERIALS

Mice and crosses

All mouse strains were obtained from The Jackson Laboratory, Bar Harbor, ME. Animal care and use was in accordance with NIH guidelines and procedures were approved under animal study protocol 971/97.

Varitint-waddler crosses

For genetic fine-mapping, *Va^J* heterozygous were crossed against CAST/Ei and CZECHII/Ei. Heterozygous F1 progeny were phenotypically scored at 3–4 weeks of age by the absence of a preyer's reflex and their variegated coat color. Expression of the coat color phenotype in the F2 intercross varied from a large to small white belly spot. F2 progeny with a recombinant chromosome over the critical region were tested for hearing by ABR analyses. To identify modifiers, heterozygous *Va^J* mice were outcrossed and F1 progeny with a white belly spot were assumed to be *+/Va^J* and intercrossed. *Va^J/Va^J* and *+/Va^J* were classified according to their variegated coat color and tested for hearing at 12 weeks of age.

Jerker crosses

In general, homozygous *Espn^{je}* were outcrossed and mating pairs of obligate heterozygotes were established to produce the F2 offspring. To generate *Cdh23$^{v\text{-}2J}$/Espn^{je}* double heterozygotes, *Cdh23$^{v\text{-}2J}$* homozygotes were crossed to *Espn^{je}* heterozygotes and vice versa.

Genotyping by PCR

DNA was isolated from tail biopsies through digestion in 50 mM Tris/Cl, pH 8.0, 100 mM EDTA, pH 8.0, 100 mM NaCl, 1% SDS, and 500 μg proteinase K at 55°C overnight, followed by phenol/chloroform extraction and precipitation in 0.7 vol isopropanol, 0.3 M sodium acetate, pH 4.5. DNA was resuspended in TE, pH 8.5, and adjusted to a concentration of 100 ng/μL. For PCR amplification, 100 ng DNA was mixed in 500 mM KCl, 100 mM Tris/Cl, pH 8.3, 2.5 mM $MgCl_2$, 0.2 μM oligonucleotides, 20 μM dNTP (Amersham Pharmacia), and 0.02 U thermostable DNA polymerase. The 10-μL reaction was run through the following program: initial denaturation for 2 min at 95°C, 20 s at 94°C, 20 s at 52°C, 30 s at 72°C for 49 cycles; followed by a 2-min extension at 72°C. For SSLP analyses using MIT markers (Invitrogen), PCR products were separated by electrophoresis in 4% MetaPhor agarose (FMC) and visualized by staining in ethidium bromide. To identify the *Cdh23$^{v\text{-}2J}$* allele, PCR products were separated by electrophoresis in 2% agarose, bands were excised, purified (Qiagen), and directly sequenced using BigDye^{TM} terminator chemistry (Applied Biosystems, Foster City, CA) and ABI377 automated sequencer (Applied Biosystems). The jerker mutation (2426delG) abolishes a *Bsm*BI restriction enzyme recognition site. To identify the *Espn^{je}* allele, we used forward primer Espn-F20, 5′-GCACCTGCTTTGTGGGAAATC-3′, and reverse primer Espn-R19, 5′-GTCTGAAGTAATGGAGTGGT-

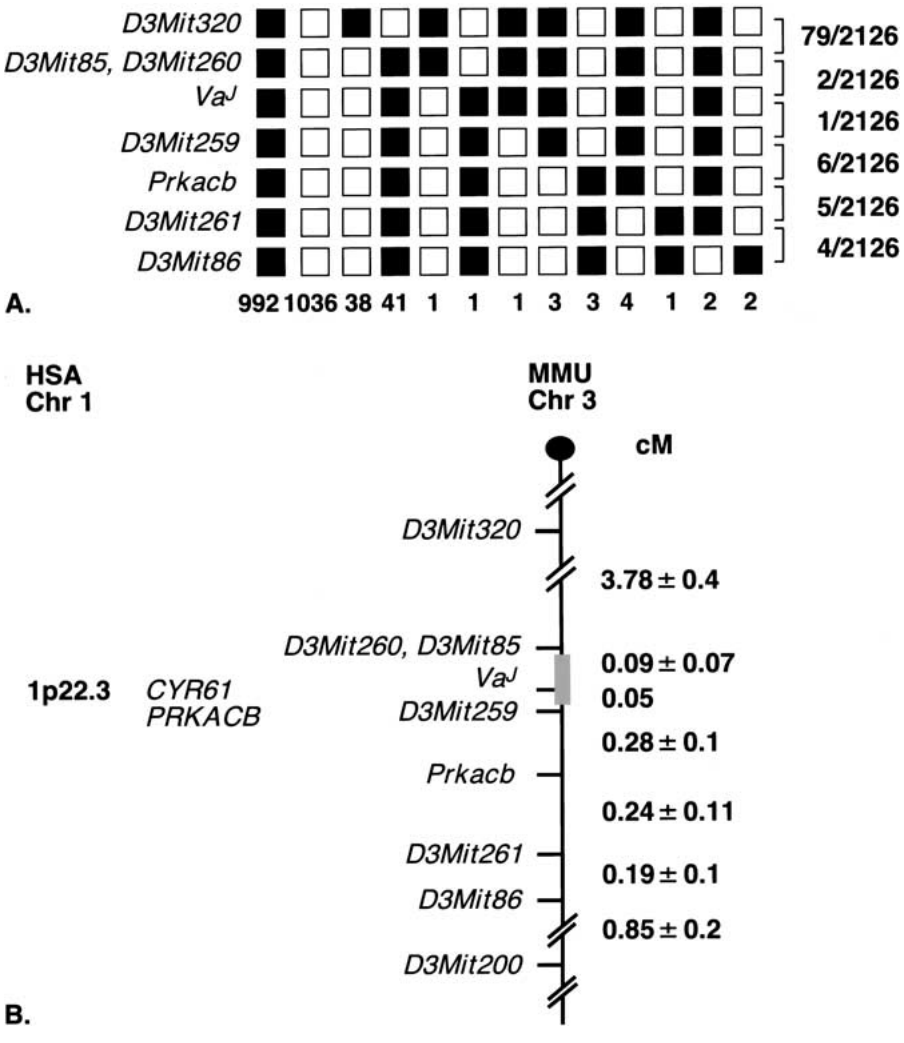


FIG. 1. Genetic map of *Va^J*. **A.** Haplotype analysis. Markers are indicated on the left and numbers of recombinations per total meioses are given on the right. Black boxes indicate the *Va^J* derived allele and the white boxes indicate the wildtype derived (CAST/Ei or CZECHII/Ei) allele. The frequency occurrence of each haplotype is shown on the bottom. **B.** Chromosomal location. Marker order on the left and distances in centiMorgan (cM) ± SE on the right are shown along the distal end of chromosome 3 (cM position 71.8–76.2). The *Va^J* critical interval is highlighted. The human homologous region is shown on the left.

TGACG-3′, to amplify a 342 bp product spanning the deletion. Restriction digest with *Bsm*BI cleaves the wild-type allele in a 211- and 131-bp product but leaves the mutant allele uncut. PCR products were precipitated in 1 vol ethanol, 0.2 μL pellet paint (Novagen), resuspended in 10 μL TE, pH 7.5, digested with the appropriate restriction enzyme, and separated on a 12% polyacrylamide gel, followed by staining in ethidium bromide.

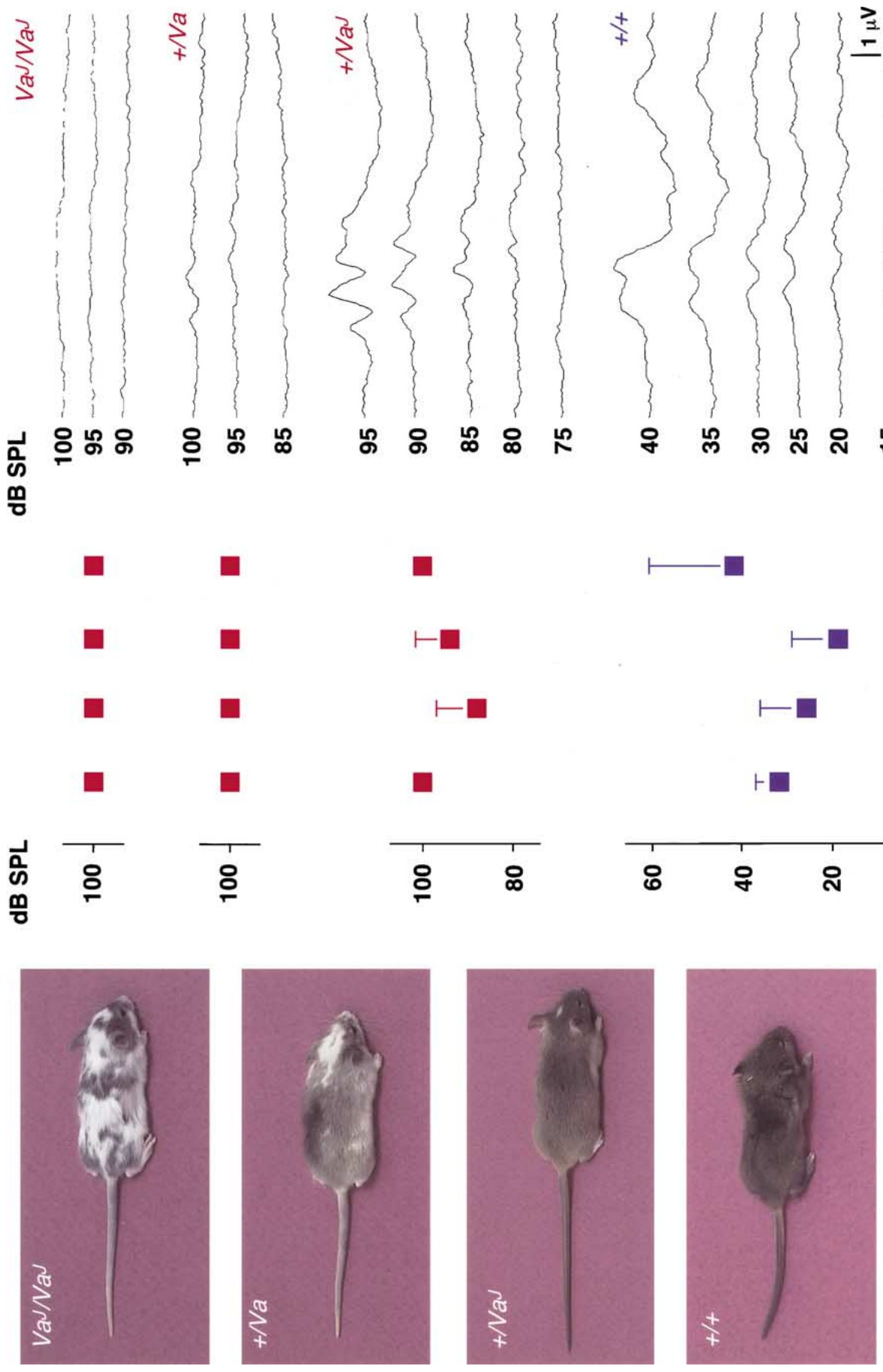
Auditory-evoked brainstem response analyses

To test mice for auditory-evoked brainstem response (ABR) thresholds, a computer-aided evoked potential system (Intelligent Hearing System, Miami, FL) was used. The Smart-EP v2.21, modified for high-frequency capability and coupled to high-frequency transducers, was used to generate specific acoustic stimuli and to amplify, measure, and display the evoked brainstem responses of anesthetized mice (concentration = 0.31 mg/g body weight). Subdermal needle electrodes were inserted at the vertex (active), ventrolaterally to the right ear (reference) and the left ear (ground). Specific acoustic stimuli were delivered monoaurally through 3-cm plastic tube channels from the high-frequency transducers. Mice were tested with click stimuli and also with 8-, 16-, and 32-kHz pure-tone pips at varying intensity from 100 dB sound pressure levels (SPL) to 10 dB SPL. Acoustic stimuli were presented at 19.1 times per second. ABR thresholds were determined for each stimulus frequency by identifying the lowest intensity producing a reproducible ABR pattern on the computer screen (at least two consistent peaks). Mice were kept on a heating pad in the soundproof chamber during testing. Samples of CBA/CaJ mice were tested periodically as reference for normal hearing.

RESULTS AND DISCUSSION

Genetic map of Va^J

Previous linkage analyses placed *Va* to the distal end of chromosome 3 (74.8 cM) (Lane et al. 1992; Mo-


Va^J/Va^J
+/Va
+/Va^J
+/+
dB SPL
100
95
90
100
95
85
95
90
85
80
75
40
35
30
25
20
15
1 μV
0
2
4
6
8 ms
dB SPL
100
100
100
80
60
40
20
0
click
8
16
32 kHz

braaten et al. 1984). With the ultimate goal of cloning *Va*, we constructed a high-resolution genetic map. Two intersubspecific intercrosses [(B6C3Fe-*a/a-Hoxa13Hd* Va^J/+ × CAST/Ei)F1 × F1] and [(B6C3Fe-*a/a-Hoxa13Hd* Va^J/+ × CZECHII/Ei)F1 × F1] were established which produced 445 and 618 F2 progeny, respectively. Initially, these 2126 meioses were genotyped with SSLP markers *D3Mit320* and *D3Mit200*, which identified 124 meiotic recombinations. To map Va^J more precisely, these recombinants were further analyzed with four intervening SSLP markers (Fig. 1). Markers *D3Mit260* and *D3Mit85* are recombinant with Va^J and define the proximal end of the interval with a recombination frequency of 2/2126 (0.09 ± 0.07 cM). On the distal end, *D3Mit259* recombines with Va^J with a frequency of 1/2126 (0.05 cM). Therefore, Va^J localizes to a 0.14 ± 0.08-cM minimal region at distal chromosome 3 (Fig. 1). Given an estimated size of the mouse genome of ~1400 cM (Copeland et al. 1993) and a physical distance of 2.7×10^9 bp (www.ensembl.org), the critical interval spans a calculated distance of approximately 270 kb. The insert size of the RPCI-23 bacterial artificial chromosome library averages 197 kb (Osoegawa et al. 2000). Thus, the 2000-meioses Va^J cross provides the resolution to delineate the critical interval to not more than two overlapping BAC clones containing approximately one to three genes. These estimates are in good agreement with distances observed in previous genetic and physical maps involving loci on different chromosomes (Bryda et al. 2001).

With the goal of identifying potential disease homologs in humans, the *Va* interval was linked to the human physical map. To develop genetic markers, genes located near Va^J were analyzed for polymorphisms. On the 5′-untranslated region (UTR) of the *Prkacb* gene encoding the beta-catalytic subunit of the cAMP-dependent protein kinase (NP_035230), a sequence length polymorphism between the C57BL/6J strain and the wild-derived parental strains CAST/Ei and CZECHII/Ei was identified. Typing the panel of recombinants with this marker placed *Prkacb* 0.32 cM distal to Va^J (Fig. 1). On the human map, *PRKACB* is found on 1p22.3 (Lander et al. 2001). On the composite mouse linkage map, the gene encoding Cysteine-rich-protein 61 (*Cyr61*, formerly *Igfbp10*) is concordant with *D3Mit113* and *D3Mit85* (MGD 2002). Provided *Cyr61* localizes centromeric to Va^J, we predict the human ortholog of *Va* to map to 1p22.3 between markers *D1S3449* and *D1S2252*. Several inherited disease loci have been localized to or near 1p22, including the dominant form of hearing loss DFNA37 (Talebizadeh et al. 2000; Van Camp and Smith 2002). Although deaf individuals of this family were not reported to express additional symptoms homologous to the *Va* phenotype, *Va* is a good candidate gene for DFNA37 as it is well established that different mutations in the same locus can produce different disease outcomes (Liu et al. 1997; Weil et al. 1995). In a recent report, *Va* was discussed as a candidate gene for the missing gene in the DFNA2 locus (Goldstein and Lalwani 2002). However, markers *D1S255* and *D1S2713*, which determine the DFNA2 critical interval in this family, map approximately 50 Mb telomeric to the predicted human homolog of *Va*.

Varitint-waddler modifier screen

Va^J heterozygotes express a slightly dilute coat color, a small white ventral spot, and sometimes a white forehead patch. Va^J homozygotes are almost entirely variegated on both the ventral and the dorsal side (Lane 1972). Coat color in *Va* heterozygotes is more dilute with an entirely white ventral side and a large white forehead spot (Fig. 2). The phenotypic baseline was established through ABR measurements. At two weeks of age, Va^J heterozygotes responded to the 8- and 16-kHz stimuli only at sound pressure levels (SPL) of 88 ± 2 dB SPL (n = 12) and 94 ± 1 dB SPL (n = 12), respectively. *Va* heterozygotes returned positive waveforms to a 8-kHz stimulus at intensities of 98 ± 2 dB SPL (n = 9). Only one of five tested Va^J homozygotes responded to a 8-kHz stimulus at 95 dB SPL. No response could be elicited with click and 32-kHz stimuli from +/Va^J, + /*Va*, and Va^J/Va^J. At three weeks of age, thresholds in Va^J heterozygotes increased to >100 dB SPL (data not shown). Severity of hearing loss and degree of variegation correlates with allele type (+/Va^J < +/*Va* < Va^J/Va^J). The reproducible and robust response of +/Va^J clearly demonstrates some hearing although only at very high intensities and diminishing from the lower to the higher frequencies. The weak response in +/*Va* lets one assume that a similar progression has occurred at earlier time points. Previous histological studies found pathological changes in the organ of Corti of +/*Va* at postnatal day 11 (P11) (Deol 1954) and in inner hair cells (IHC) of +/Va^J at P14 (Cable and Steel 1998). In light of the reported histopathology, our ABR results indicate that in +/Va^J at least some IHC, presumably located at the apex of the cochlea,

FIG. 2. Coat color and auditory defects in varitint-waddler mutants. In the left panel we show the coat color defect in *Va* and Va^J mutant mice at 2–3 weeks of age. The middle panel shows the average threshold (given in dB SPL) as a function of the stimulus (given in kHz) for each genotype. The right panel shows a representative recording obtained after a 16-kHz stimulus. Intensities and time (millisecond, ms) are shown on the y- and x-axis, respectively. Amplitude is given in μV with a scale bar given on the bottom right.

TABLE 1

ABR thresholds in various *Va^J F2* intercrosses[a]

Strain/cross	P_0 wild type	F1		F2		
		Wild type	White belly spot	Wild type	White belly spot	White spotting
Va^J/A	27 ± 6*; 3	19 ± 4; 7	>100; 8	17 ± 3; 8	>100; 8	>100; 5
Va^J/C3H	27 ± 4; 9	22 ± 8; 6	>100; 8	18 ± 3; 8	>100; 29	>100; 5
Va^J/DBA	65 ± 7; 2	35 ± 3; 7	>100; 5	19 ± 2; 12	99 ± 1; 31	>100; 10
Va^J/BALB	41 ± 3; 3	37 ± 8; 6	>100; 3	23 ± 8; 8	>100; 14	>100; 7
Va^J/CZECH	21 ± 5; 7	26 ± 4; 8	>100; 12	26 ± 6; 9	99 ± 5; 32	>100; 4
Va^J/B6	27 ± 3; 3	28 ± 6; 6	>100; 8	17 ± 5; 14	>100; 18	>100; 1

[a]*Threshold ± standard deviation for a 16-kHz stimulus is given in dB SPL followed by the number of animals tested.*

TABLE 2

Jerker phenotype on inbred and various mixed backgrounds

Strain/cross	Vestibular phenotype[b]				Auditory phenotype[a]		
					F1	F2	
	Affected	Total	% affected	$p > 0.001$[c]	Normal	Normal	Affected
JE/CZECH	167	674	25	0.09	22 ± 3; 5	30 ± 4; 33	>100; 22
JE/CAST	171	631	27	0.25	28 ± 3; 5	29 ± 5; 45	>100; 20
Je/DBA	35	127	28	0.5	32 ± 6; 12	25 ± 6; 24	>100; 13
Je/BALB	30	97	31	0.18	17 ± 3; 5	26 ± 5; 25	>100; 12
Je/A	35	108	32	0.08	35 ± 9; 7	28 ± 6; 24	>100; 15
Je/129	23	124	19	0.01	33 ± 3; 6	24 ± 4; 35	>100; 9
JE/Le[d]	44	108	41	0.06	30 ± 6; 5		>100; 8

[a]Threshold ± standard deviation for a 16-kHz stimulus is given in dB SPL followed by the number of animals tested.
[b]F2 progeny.
[c]Significance level of chi-squared test.
[d]N2 progeny.

are functionally active at P14 but degenerate rapidly. The ABR data are in agreement with the report by Cable and Steel, who obtained similar results using compound-action-potential (CAP) and summation potential (SP) measurements (Cable and Steel 1998).

To identify possible genetic background effects on the hearing phenotype, we crossed *Va^J* onto A/J, C3HeB/FeJ, DBA/2J, BALB/cByJ, CZECHII/Ei, and C57BL/6J. Some of these strains were chosen because they were recently shown to express early- and late-onset hearing loss and to segregate an age-related hearing loss allele (*ahl*) (Johnson et al. 2000; Zheng et al. 1999). We expected modifying alleles to sensitize heterozygotes, suppress the phenotype in homozygotes, and/or cause pleiotropic effects. To select for profound and robust effects, we screened 12-week-old animals. Randomly selected F2 progeny were ABR tested at 12 weeks of age. No discernible differences in degree of hearing loss were found among F2 intercrosses (Table 1). However, a marked reduction of *Va^J/Va^J* homozygotes in the F2 progeny of the C57BL/6J × *Va^J* intercross was observed. After six backcross generations (N6), we intercrossed *+/Va^J* heterozygotes. Out of 180 N6F2 progeny, only 8% were homozygotes, 34% were wild type, and 58% showed a coat color typical for heterozygotes. The cause of this reduced viability is presumably the result of an embryonic lethal effect of the C57BL/6J background that certainly relates to the perinatal mortality observed in *Va/Va* homozygotes. The coat color phenotype argues that *Va* is expressed in melanocytes and therefore *Va* might also affect other neural crest derivatives, which are vital for proper embryonic and fetal development.

Jerker modifier screen

Five- to six-week-old *je* homozygotes show no signs of hearing in auditory-evoked brainstem response tests (data not shown). To search for modifiers, the *Espn^{je}* allele was placed onto CAST/Ei, CZECHII/Ei, BALB/cByJ, 129/SvJ, A/J, and DBA/2J backgrounds.

TABLE 3

ABR thresholds in *Espn^{je}* and *Cdh23$^{v\text{-}2J}$* double heterozygotes

Strain/cross	Click[a]	8 kHz	16 kHz	32 kHz
Espn$^{+/+}$ Cdh23$^{v\text{-}2J/+}$	31 ± 4; 6	21 ± 4; 6	10; 6	36 ± 4; 5
Espn$^{je/+}$ Cdh23$^{+/+}$	35; 2	32 ± 4; 2	10; 2	40; 2
Espn$^{je/+}$ Cdh23$^{v\text{-}2J/+}$	34 ± 3; 16	22 ± 3; 16	13 ± 4; 16	39 ± 4; 14

[a]Thresholds ± standard deviation are given in dB SPL followed by the number of animals tested.

In an average sample size of 100 F2 progeny per outcross, segregation of the vestibular phenotype did not significantly deviate from the ratio expected for a recessive trait (Table 2). ABR measurements were carried out on 37–65 randomly selected 12-week-old F2 progeny from each cross. All circling F2 offspring showed an ABR pattern indistinguishable from homozygotes of the JE/Le inbred strain, and all F2 mice with a normal vestibular phenotype also returned normal thresholds (Table 2). Conversely, all animals with abnormal thresholds displayed the circling behavior. The genetic diversity between the parental strains JE/Le, CAST/Ei, and CZECHII/Ei allowed us to directly correlate phenotype with genotype. All F2 progeny from the JE/CAST-*je* and JE/CZECH-*je* intercross with vestibular defects (n = 159 and n = 170, respectively) were homozygous *je* and vice versa; out of 20 ABR-tested deaf F2 progeny, all were homozygous *je* and vice versa. In summary, vestibular and auditory phenotypes showed no variation in their expressivity, were coexpressed, and segregated as recessive traits independent of the genetic backgrounds tested.

The stereocilia defect in jerker is reminiscent of the stereocilia disorganization observed in *Cdh23$^{v\text{-}2J}$*. The actin-bundling activity of Espn and the association of classic cadherins with the actin cytoskeleton suggest a direct and/or indirect interaction between Espn and Cdh23. To test such a possible interaction on the genetic level, we determined hearing function in double heterozygotes (*Cdh23$^{v\text{-}2J}$/Espn^{je}*). All F1 offspring (n = 24) showed normal vestibular behavior and there was no statistically significant difference in the ABR thresholds between *Cdh23$^{v\text{-}2J/+}$/Espn$^{je/+}$* and *Cdh23$^{v\text{-}2J/+}$/Espn$^{+/+}$* (Table 3).

Part of this study was undertaken to identify possible genetic interactions between mutant and normal alleles segregating in different inbred strains using ABR analyses as phenotypic endpoint. Expression, inheritance, and penetrance of hearing phenotype in *je* and *Va^{J}* are controlled by the mutant allele only. However, our data do not rule out modifying effects on levels not differentiated by ABR. These include subtle accelerations or delays in pathology, pleiotropic auditory effects, or effects controlling secondary downstream events. Alternatively, alleles in the selected background strains cause weak, nondetectable effects. Some of these limitations in identifying modifiers might be overcome by performing ''sensitized screens;'' in these assays genetics of a deafness mutation is studied on the background of a randomly mutagenized genome (Balling 2001; Nadeau and Frankel 2000). This approach has the potential to produce stronger alleles, to allow for selection of pronounced effects, to screen toward saturation, and to increase probability of positively identifying the modifying locus.

CONCLUSIONS

From our study the following conclusions can be made: (1) *Va^{J}* maps to the distal end of chromosome 3 within a 0.14-cM interval flanked by *D3Mit85* and *D3Mit259*. (2) Comparative mapping localizes the human ortholog of *Va* to 1p22.3 between markers *D1S3449* and *D1S2252*. (3) Expression, penetrance, and inheritance of *je* and *Va^{J}* on different genetic backgrounds are controlled by the mutant allele. (4) Some limitations in identifying and characterizing modifiers might be overcome by performing sensitized screens.

ACKNOWLEDGMENTS

We would like to thank Elizabeth Bryda, Doris Wu, and Matt Kelley for their helpful comments on the manuscript and members of our laboratory for stimulating discussions. We thank Richard Pellegrino for technical assistance and Melissa Irby, Keith Black, and Olga Rivas for maintaining the mouse colony. This project was funded by DIR, NIDCD, DC00056-01.

REFERENCES

BALLING R. ENU mutagenesis: analyzing gene function in mice. Annu. Rev. Genomics Hum. Genet. 2:463–492, 2001.

BRYDA EC, KIM HJ, LEGARE ME, FRANKEL WN, NOBEN-TRAUTH K. High-resolution genetic and physical mapping of modifier-of-

deafwaddler (*mdfw*) and waltzer (*Cdh23*^{v}). Genomics 73:338–342, 2001.

CABLE J, STEEL KP. Combined cochleo-saccular and neuroepithelial abnormalities in the Varitint-waddler-J (*Va*^{J}) mouse. Hear. Res. 123:125–136, 1998.

CLOUDMAN AM, BUNKER LE. The varitint-waddler mouse. J. Hered. 36:258–263, 1945.

COPELAND NG, JENKINS NA, GILBERT DJ, EPPIG JT, MALTAIS LJ, MILLER WF, DIETRICH WF, WEAVER A, LINCOLN SE, STEEN RG, et al. A genetic linkage map of the mouse: current applications and future prospects. Science 262:57–66, 1993.

DEOL MS. The anomalies of the labyrinth of the mutants varitint-waddler, shaker-2 and jerker in the mouse. J. Genet. 52:562–588, 1954.

GOLDSTEIN JA, LALWANI AK. Further evidence for a third deafness gene within the DFNA2 locus. Am. J. Med. Genet 108:304–309, 2002.

GRÜNEBERG H, BURNETT JB, SNELL GB. The origin of jerker, a new gene mutation of the house mouse, and linkage studies made with it. Proc. Natl. Acad. Sci. USA 27:562–565, 1941.

IKEDA A, ZHENG QY, ROSENSTIEL P, MADDATU T, ZUBERI AR, ROOPENIAN DC, NORTH MA, NAGGERT JK, JOHNSON KR, NISHINA PM. Genetic modification of hearing in tubby mice: evidence for the existence of a major gene (moth 1) which protects tubby mice from hearing loss. Hum. Mol. Genet. 8:1761–1767, 1999.

IKEDA A, ZHENG QY, ZUBERI AR, JOHNSON KR, NAGGERT JK, NISHINA PM. Microtubule-associated protein 1A is a modifier of tubby hearing (moth 1). Nat. Genet. 30:401–405, 2002.

JOHNSON KR, ZHENG QY, BYKHOVSKAYA Y, SPIRINA O, FISCHEL–GHODSIAN N. A nuclear–mitochondrial DNA interaction affecting hearing impairment in mice. Nat. Genet. 27:191–194, 2001.

JOHNSON KR, ZHENG QY, ERWAY LC. A major gene affecting age-related hearing loss is common to at least ten inbred strains of mice. Genomics 70:171–180, 2000.

LANDER ES, LINTON LM, BIRREN B, NUSBAUM C, ZODY MC, et al. Initial sequencing and analysis of the human genome. Nature 409:860–921, 2001.

LANE PW. Two new mutations in linkage group XVI of the house mouse. Flaky tail and varitint-waddler-J. J. Hered. 63:135–140, 1972.

LANE PW. Va^{J}–varitint-waddler-jackson. Mouse News Lett. 41:32, 1969.

LANE PW, BRONSON RT, SPENCER CA. Rostral cerebellar malformation, (rcm): a new recessive mutation on chromosome 3 of the mouse. J. Hered. 83:315–318, 1992.

LIU XZ, WALSH J, TAMAGAWA Y, KITAMURA K, NISHIZAWA M, STEEL KP, BROWN SD. Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene [letter]. Nat. Genet. 17:268–269, 1997.

MGD. Mouse Genome Informatics Project The Jackson Laboratory, Bar Harbor, ME, 2002,

MOBRAATEN LE, BUNKER HP, DEMAEYER-GUIGNARD J, DEMAEYER E, BAILEY DW. Location of histocompatibility and interferon loci on chromosome 3 of the mouse. J. Hered. 75:233–234, 1984.

NADEAU JH. Modifier genes in mice and humans. Nat. Rev. Genet. 2:165–174, 2001.

NADEAU JH, FRANKEL WN. The roads from phenotypic variation to gene discovery: mutagenesis versus QTLs. Nat. Genet. 25:381–384, 2000.

NG L, RUSCH A, AMMA LL, NORDSTROM K, ERWAY LC, VENNSTROM B, FORREST D. Suppression of the deafness and thyroid dysfunction in Thrb-null mice by an independent mutation in the Thra thyroid hormone receptor alpha gene. Hum. Mol. Genet. 10:2701–2708, 2001.

NOBEN-TRAUTH K, ZHENG QY, JOHNSON KR, NISHINA PM. *mdfw*: a deafness susceptibility locus that interacts with deaf waddler (*dfw*). Genomics 44:266–272, 1997.

OSOEGAWA K, TATENO M, WOON PY, FRENGEN E, MAMMOSER AG, CATANESE JJ, HAYASHIZAKI Y, DE JONG PJ. Bacterial artificial chromosome libraries for mouse sequencing and functional analysis. Genome Res. 10:116–128, 2000.

SJÖSTROM B, ANNIKO M. Cochlear structure and function in a recessive type of genetically induced inner ear degeneration. ORL J. Otorhinolaryngol. Relat. Spec. 54:220–228, 1990.

SJÖSTROM B, ANNIKO M. Variability in genetically induced age-related impairment of auditory brainstem response thresholds. Acta Otolaryngol. (Stockh.) 109:353–360, 1992.

STEEL KP, BOCK GR. Cochlear dysfunction in the jerker mouse. Behav. Neurosci. 97:381–391, 1983.

STEEL KP, KROS CJ. A genetic approach to understanding auditory function. Nat. Genet. 27:143–149, 2001.

TALEBIZADEH Z, KENYON JB, ASKEW JW, SMITH SD. A new locus for dominant progressive hearing loss DFNA37 mapped to chromosome 1p21. Abstr. Assoc. Res. Otolaryngol. 23:1740, 2000.

VAN CAMP G, SMITH RJH (2002) Hereditary hearing loss home page: URL: http://www.uia.ac.be/dnalab/hhh.

WEIL D, BLANCHARD S, KAPLAN J, GUILFORD P, GIBSON F, WALSH J, MBURU P, VARELA A, LEVILLIERS J, WESTON MD, et al. Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature 374:60–61, 1995.

ZHENG L, SEKERKOVA G, VRANICH K, TILNEY LG, MUGNAINI E, BARTLES JR. The deaf jerker mouse has a mutation in the gene encoding the espin actin-bundling proteins of hair cell stereocilia and lacks espins. Cell 102:377–385, 2000.

ZHENG QY, JOHNSON KR, ERWAY LC. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear. Res. 130:94–107, 1999.