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Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss

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Abstract

Age-related hearing loss (AHL) in common inbred mouse strains is a genetically complex quantitative trait. We found a synonymous single-nucleotide polymorphism in exon 7 of *Cdh23* that shows significant association with AHL and the deafness modifier *mdfw* (modifer of deafwaddler). The hypomorphic *Cdh23*^{753A} allele causes in-frame skipping of exon 7. Altered adhesion or reduced stability of CDH23 may confer susceptibility to AHL. Homozygosity at *Cdh23*^{753A} or in combination with heterogeneous secondary factors is a primary determinant of AHL in mice.

Inbred mouse strains vary greatly in their susceptibility to age-related (AHL) and noise-induced hearing loss (NIHL; refs. 1–3). BALB/cByJ, BUB/BnJ and C57BL/6J strains develop early- or late-onset sensorineural hearing impairment and are highly susceptible to acoustic overstimulation2,4; in comparison, CBA/CaJ and MOLF/Ei have normal hearing throughout life and are fairly resistant to noise trauma. Quantitative and qualitative linkage analyses linked predisposition to AHL and NIHL to the *ahl* locus on chromosome 10 (ref. 4,5). The *ahl* interval coincides with the map location of the deafness modifier *mdfw* (modifier of deafwaddler). The recessive *mdfw* allele accelerates hearing loss in heterozygous *Atp2b2* (plasma membrane Ca^{2+} ATPase 2)-deficient BALB/cByJ-*Atp2b2*$^{dfw\text{-}2J/+}$ and C57BL/6J-*Atp2b2*$^{dfw\text{-}2J/+}$ mice; on wild-type backgrounds, *mdfw* has little effect[6]. Genetic complementation tests have shown allelism between *ahl* and *mdfw*[7].

We localized *mdfw* to a 830-kb and *ahl* to a 630-kb interstitial genomic region between markers *D10Ntra57* and *D10Ntra46* (Fig. 1a). Four genes localize to this interval: *Spock2, Chst3, Psap* and *Cdh23* (encoding cadherin 23; Fig. 1b). Mutations in *Cdh23* cause deafness in humans and in mouse models[8]. We screened for nucleotide differences by sequencing all exons of these genes and flanking intronic sequences (≤20 bp) in CBA/CaJ and C57BL/6J mice. We found two sequence changes, both in *Cdh23:* a deletion of 11 bp in the 3′ untranslated region of exon 69 (*Cdh23*^{10497del11}) and a G→A transition at nucleotide 753 in exon 7 (*Cdh23*$^{753G\rightarrow A}$). To investigate association with AHL, we sequenced exon 7 and exon 69 in an additional 54 inbred strains. We found *Cdh23*^{10497del11} in strains R/J and C57BL/6J only. The 753G→A polymorphism showed nearly perfect correlation with AHL ($P = 2 \times 10^{-5}$ by χ^2 test; Fig. 1c and Supplementary Table 1 online). Of 31 strains classified with AHL, 27 carry the 753A allele, and of 25 AHL-negative strains, 22 segregate the 753G variant. All strains for which

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we genetically linked AHL to the *ahl* interval and those for which we showed allelism with *mdfw* carry the *Cdh23*^{753A} allele. Cby-*Atp2b2*$^{dfw-2J}$, which segregates *mdfw*, also has the *Cdh23*^{753A} variant. The few strains that did not correlate may show incomplete penetrance, may develop hearing loss later in life (BDP/J, SEC/Re1J, SHR/GnJ), may segregate a mutation in *Ednrb* (I/LnJ) or may have acquired susceptibility allele(s) other than *ahl* (MRL/MpJ, C3H/HeSnJ, YBR/Ei). At seven marker loci across the *ahl* interval, laboratory strains share the same haplotype, which is derived from an ancient *Mus musculus domesticus* chromosome (Supplementary Table 2 online). Given the origin of these strains from a few founder mice, the association of the *Cdh23*^{753A} allele with one common haplotype argues in favor of an ancestral mutation.

The synonymous G→A substitution occurs at the last position of exon 7. To test whether the substitution alters splicing, we carried out PCR analysis on reverse-transcribed cochlea mRNA. In 11 strains tested, the *Cdh23*^{753A} allele perfectly correlated with in-frame skipping of exon 7 (Fig. 2a). Strains with the *Cdh23*^{753G} allele preferentially produced wild-type transcripts. We next asked whether increasing levels of alternatively spliced transcripts parallel the onset and progression of AHL. Using the quantitative real-time PCR assay, we did not find a statistically significant difference ($P > 0.05$) in the accumulation of alternatively spliced mRNA with increasing thresholds in C57BL/6J, C57BR/cdJ, NOD/LtJ, DBA/2J and CBy-*Atp2b2*$^{dfw-2J}$ (Fig. 2b).

To test the functionality of the *Cdh23*^{753A} allele, we studied its *trans* effect on the frame-shift allele *Cdh23*$^{834–835insG}$ (*Cdh23*^{v}). We determined by SNP marker analysis that *Cdh23*^{v} arose on an ancestral *Mus musculus molossinus* chromosome and that the retained congenic interval contains both *ahl* and *mdfw* loci (Fig. 1a). Because MOLF/Ei mice have normal hearing and are resistant to NIHL, we assumed that if *Cdh23* and *ahl* were different genes, then the *Cdh23*^{v} allele would be in coupling phase with the protecting allele of *ahl* in the V/Le strain. If so, hybrid mice derived from matings between AHL-susceptible strains and V/Le would be protected from AHL by the dominant V/Le-derived resistance allele at *ahl*. If *Cdh23*^{753A} underlies the hearing loss associated with *ahl*, however, then such hybrid mice would have AHL because the *Cdh23*^{753A} allele from the AHL-susceptible strain would be combined with the *Cdh23*^{v} null allele from the V/Le strain. *Cdh23*^{753A}/*Cdh23*^{v} compound heterozygotes had significantly higher auditory-brainstem response (ABR) thresholds to a series of acoustic stimuli (Supplementary Table 3 online). In comparison, *Cdh23*^{753G}/*Cdh23*^{v} had normal waveforms and thresholds.

The stereocilia hair bundle has a highly organized staircase-like architecture, which is central to the function of cochlea and vestibular hair cells. Mice deficient in *Cdh23* develop a structurally disorganized hair bundle[9]. Recent data provide evidence that cadherin 23 forms a complex with harmonin b and myosin 7A that localizes to stereocilia and is a component of interciliary links[10],[11]. The peptide of 43 amino acids encoded by exon 7 is part of the second and third ectodomain and lies in the potential homodimerization site of cadherin 23. The *CDH23*^{5712A} mutation in humans is associated with Usher syndrome type 1, and its mechanism of action is similar to that of the *Cdh23*^{753A} allele[12]. Together, the data suggest that *Cdh23*^{753A} is a pathological, hypomorphic allele; predisposition to AHL and NIHL may be conferred through altered adhesion or intracellular targeting of misfolded protein.

Homozygosity with respect to *Cdh23*^{753A} significantly increases susceptibility to AHL but is not the only cause of its phenotypic manifestation. Predisposition to early-onset AHL conferred by *Cdh23*^{753A} depends on the effects of several strain-specific genetic factors, including the mitochondrial mutation *mt-Tr*^{9827ins8} (as in A/J; ref. 13), *ahl2* (as in NOD/LtJ; ref. 14) and *ahl3* (K.R.J and Q.Y.Z, unpublished data). Combination of any of these `accelerating alleles' with *Cdh23*^{753A} is sufficient to induce AHL expression (Supplementary Fig. 1 online). An

additional genetic factor is the null allele of *Atp2b2*, which is an important regulator of intrastereocilia Ca^{2+} levels[15]. Haploinsufficiency at *Atp2b2* and homozygosity with respect to *Cdh23*^{753A} together, but neither alone, cause early-onset hearing loss in *mdfw* mice (*Atp2b2*$^{+/dfw\text{-}2J}$ *mdfw/mdfw*; Fig. 2b). The heterogeneity of secondary factors suggests additive or stochastic interactions with *Cdh23*^{753A}. The genetic architecture of AHL and NIHL may provide a paradigm for predisposition to AHL and NIHL in human and defines a presbyacusis model to explore therapeutic avenues, such as stem cell therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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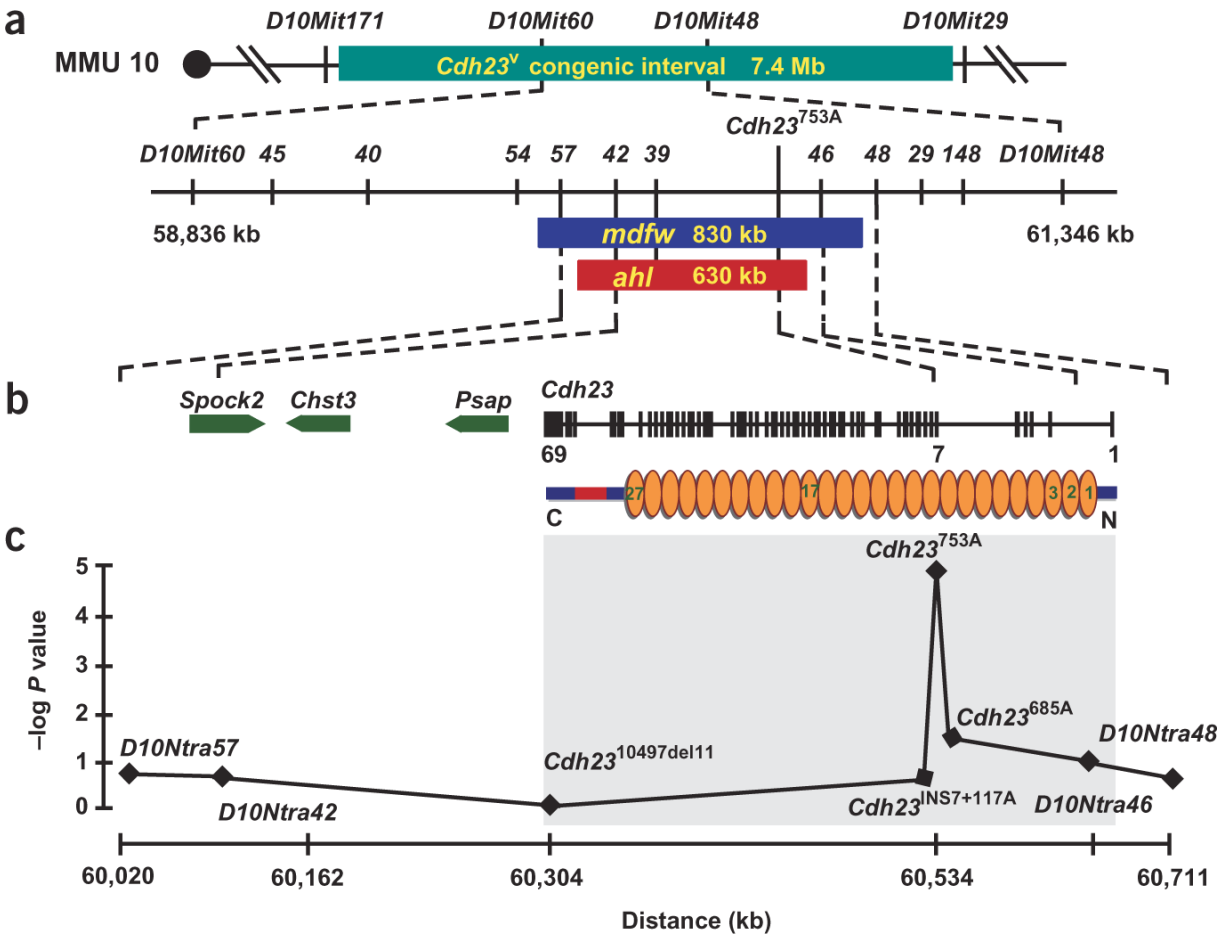

Figure 1.

Positional cloning of *ahl* and *mdfw*. (**a**) Physical map of *ahl* and *mdfw*. The *Cdh23*^{v} congenic interval (7.4 Mb) is defined by *D10Mit171* and *D10Mit29*. Positions of newly developed SNP markers (D10Ntra45, D10Ntra40, D10Ntra54, D10Ntra57, D10Ntra42, D10Ntra39, D10Ntra46, D10Ntra48, D10Ntra29 and *D10Ntra148*; stems omitted in figure) in relation to *mdfw* and *ahl* intervals are shown. *D10Ntra54* and *D10Ntra48* are recombinant (*D10Ntra54* - 0.26 ± 0.18 cM - *mdfw* - 0.13 ± 0.13 cM - *D10Ntra48*) with *mdfw*. The highest lod score for *ahl* (108.4) was in the region between *D10Ntra57* and *D10Ntra46*. Primer sequences are available on request. (**b**) Four genes localize to the critical interval: sparc/osteonectin 2 (*Spock2*), carbohydrate sulfotransferase 3 (*Chst3*), prosaposin (*Psap*) and cadherin 23 (*Cdh23*). Transcription orientation is indicated. Genomic structure, from telomere to centromere, of *Cdh23* (black vertical lines) and the domain structure of cadherin 23, including transmembrane domain (red) and ectodomains (orange, 1–27), are shown. (**c**) Profile of probability scores. Negative logarithm of *P* is plotted against marker location on the physical map (available at http://genome.cse.ucsc.edu; February 2002 assembly).

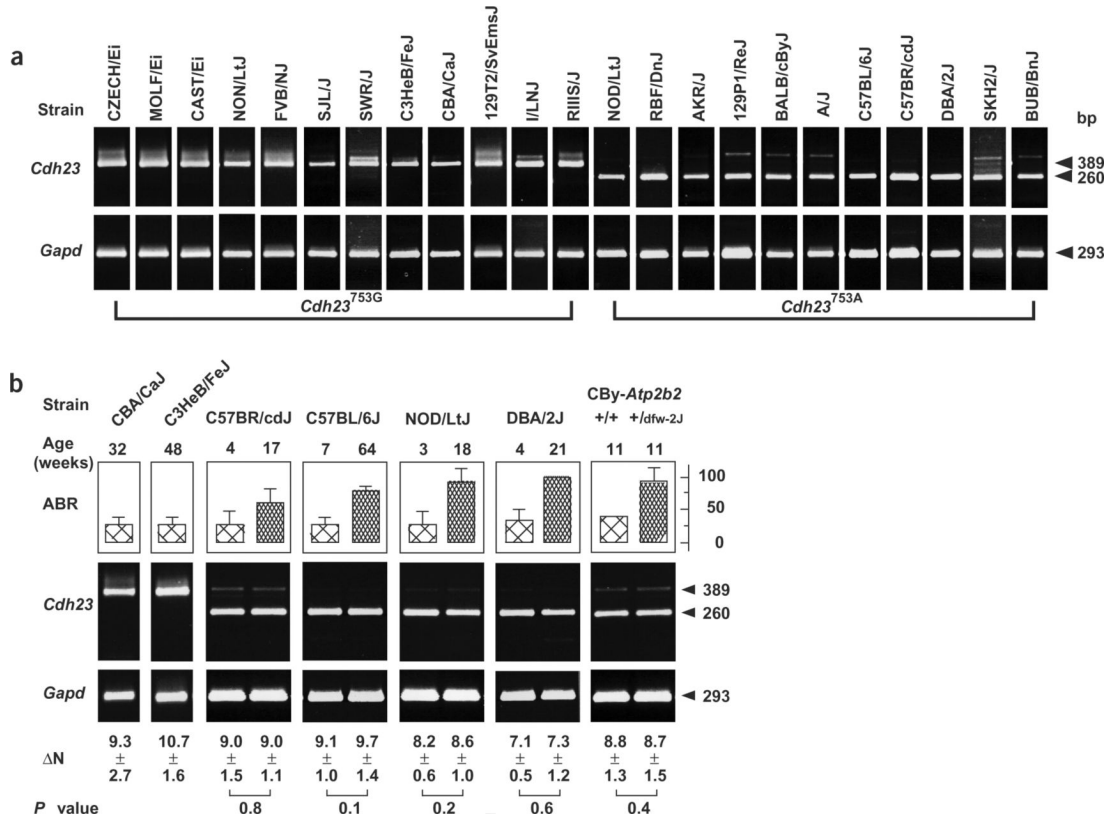

Figure 2.

Cdh23^{753A} affects splicing of exon 7. *Cdh23*-specific primers located in exon 6 and exon 8 amplify wild-type (389 bp) and alternatively spliced transcripts (260 bp) from cochlea cDNA (see Supplementary Note online). *Gapd* was included as reference. (**a**) RT-PCR analyses of exon 7 in 23 common inbred strains. Strains, allele status at *Cdh23*^{753} and amplified PCR fragments are shown. Strains within genealogical subgroups of mice (Swiss mice, Castle's mice) have different allele status and alternative splicing; compare NOD/LtJ with NON/LtJ and 129P1/ReJ with 129T2SvEmsJ. (**b**) ABR analysis was used to assess hearing function in the indicated strains (at the indicated ages), and the averaged response (dB SPL) to a click stimulus is plotted. $\Delta N = N_{Gapd} - N_{Cdh23}$, where N is the cycle number at which a significant increase of fluorescence signal above background (usually 0.01 units) was observed. The mean ± s.d. ($n \geq 10$ assays) and statistical significance (*P* value) are given. Both Cby-*Atp2b2*$^{+/+}$ and Cby-*Atp2b2*$^{+/dfw\text{-}2J}$ are homozygous with respect to *mdfw*.