Supplementary Note 1

Methods

Mice, DNA and crosses.

We obtained mouse strains and genomic DNA from The Jackson Laboratory, Taconic, Charles River or Oak Ridge National Laboratories. We obtained the V/Le-*Cdh23*^{v} strain from K. Steel (Nottingham, UK). The V/Le strain carries a loss-of-function mutation (*Cdh23*$^{834\text{-}835insG}$), shows a deaf/waltzing phenotype, originated from Asia and was previously maintained on a mixed CBA background. We serially backcrossed the *Cdh23*^{v} allele from the V/Le strain to BUB/BnJ and CBA/CaJ. The V/Le-*Cdh23*^{v} strain used for the BALB/cByJ-+/*dfw*^{2J} cross was obtained from The Jackson Laboratory (stock 000275). Genotypes were determined by direct DNA-sequencing of PCR products. Animal care and use was in accordance with NIH guidelines (ASP 991/00).

Linkage analyses

Initially we mapped *ahl* and *mdfw* between *D10Mit60* and *D10Mit48* (2.5 Mb) to middle chromosome 10 (30cM). Subsequent linkage analyses used newly developed SNP markers (*D10Ntra45*, 40, 54, 57, 42, 39, 46, 48, 29, 148, stems are omitted) to fine-map the recombination break points in eight *mdfw* and eight *ahl* recombinants (792 and 473 meioses respectively from previously described crosses). For QTL analysis we used the Map Manager QT (version Qtb8; available at http://mcbio.med.buffalo.edu.mapmgr.html). This program uses an interval mapping algorithm that computes a regression coefficient for the effect of the QTL over each marker locus. The likelihood ratio statistic, which is equivalent to the lod score, is a measure of the significance of these coefficients. Lod scores were computed for 16 kHz ABR thresholds of 473 six-month-old N2 mice combined from NOD and SKH2 backcrosses. Eight SNPs equally spaced across the recombinant interval were typed in 56 inbred strains of known hearing status. We calculated *p* values for each marker using a chi-square test.

Real-time PCR

Cochleae were dissected in RNAlater (Ambion) and stored at –80°C until use. Six to eight cochleae were homogenized in 1ml of 500mM NaCl, 10mM Tris-HCl pH 7.2, 10mM EDTA, 2% SDS using a motorized homogenizer. Samples were incubated in 20μg Proteinase K for 2hrs at 37°C. Oligo-(dT) Cellulose Type 7 (AmershamPharmacia) was added and incubated at room temperature with end-over-end rotation over night . Samples were washed three times in 500mM NaCl, 10mM Tris-HCl pH 7.2, 0.1mM EDTA, 0.2% SDS, followed by three washes in 100mM NaCl, 10mM Tris-HCl pH 7.2, 0.1mM EDTA. PolyA$^+$-enriched RNA was eluted in 4 x 100μl of 10mM Tris-HCl pH 7.2, 0.1mM EDTA. RNA was precipitated in 0.3M NaAc pH 5.3, 2.5 vol. ethanol, and presence of 10μg glycogen. PolyA$^+$-RNA was resupended in 20 μl H_2O. Eight microliter RNA was reverse transcribed using the SuperScript™ First Strand Synthesis system for RT-PCR kit (Invitrogen) with random hexamers as primer following the manufacturer's instructions. cDNA was diluted 1:10 and for PCR a 1μl aliquot was used in a 25 μl reaction using the 2x SYBR™ Green Master Mix from Applied Biosystems, amplified and analyzed on an OPTICON™ MJ Research. *Cdh23-f*, 5'-ACACCCATCTTCATCGTCAACGCCACCGAT-3'; *Cdh23-r*, 5'-GACAATAGTGTAGCCAATCCCACGGGGCCG-3'; *Gapd-f*, 5'-ATGGTGAAGGTCGGTGTGAAC-3'; *Gapd-r*, 5'-GAGTCTACTGGTGTCTTCACC-3'. Identity of bands was confirmed by direct DNA sequencing. The faint slower migrating band over the wildtype *Cdh23* band is a conformational isomer, as shown by direct sequencing and cloning. A t-test was used to calculate statistical significance (*p* value).