Beethoven, a mouse model for dominant, progressive hearing loss DFNA36

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Despite recent progress in identifying genes underlying deafness, there are still relatively few mouse models of specific forms of human deafness. Here we describe the phenotype of the Beethoven (*Bth*) mouse mutant and a missense mutation in *Tmc1* (transmembrane cochlear-expressed gene 1). Progressive hearing loss (DFNA36) and profound congenital deafness (DFNB7/B11) are caused by dominant and recessive mutations of the human ortholog, *TMC1* (ref. 1), for which *Bth* and deafness (*dn*)[1] are mouse models, respectively.

The semidominant *Bth* mutation arose in a large-scale ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis program[2]. *Bth* was mapped by a standard backcross approach (Fig. 1*a*) to a 4.4-cM region on proximal chromosome 19. This region includes the *dn* locus[3] as well as *Bth*. Based on the deafness mutations identified in *TMC1* by Kurima and colleagues[1] and its predicted conserved linkage on chromosome 19 within the *Bth* interval, the mouse ortholog, *Tmc1*, was a good candidate. We used cDNA from *Bth/Bth* and wildtype brain to sequence the coding region of *Tmc1*, and we used genomic DNA to sequence splice sites. We identified a T→A transversion (1235T→A) in exon 13 of the *Bth* sequence, predicted to change a methionine to a lysine residue (M412K; Fig. 1*b*). Sequencing of *Bth*/+ DNA confirmed the presence of two alleles, A and T, at this site. The *Bth* mutation was generated on a C3HeB/FeJ background[2], and DNA from this strain and an additional fourteen wildtype strains all showed a T at this position. To confirm the location of *Tmc1* in the *Bth*-linked interval, we mapped the gene to chromosome 19, between *D19Mit128* and *D19Mit60*, using the Jackson Laboratory Mapping Panels[4]. This 4.25-cM interval overlaps the non-recombinant region around *Bth*.

Whole-mount *in situ* hybridization revealed *Tmc1* expression in cochlear hair cells (Fig. 1*c*,*d*). There was no detectable expression in embryos at embryonic day (E) 9.5, nor in cochleas from E12.5 to postnatal day (P) 1. *Tmc1* expression was first detected at P3, and expression was seen from P5 to P90, in agreement with the inner-ear expression analysis using quantitative real-time RT–PCR carried out by Kurima *et al.*[1].

We found that *Bth*/+ mutants show progressive loss of the Preyer reflex from around P30. The middle ear and gross structure of the inner ear appeared normal (data not shown). Scanning electron microscopy of *Bth*/+ cochleas, however, revealed progressive hair-cell degeneration from P20 onwards (Fig. 2*a*). Most remaining hair cells looked normal, judging from the appearance of their stereocilia bundle. In *Bth*/+ mutants at P30–35, inner hair cells (IHCs) in the middle turn of the cochlea were severely depleted, whereas outer hair cells (OHCs) showed scattered loss (Fig. 2*d*). Compound action-potential thresholds, reflecting cochlear nerve activity, were raised at frequencies corresponding to maximum loss of IHCs (Fig. 2*b*–*d*). *Bth/Bth* cochleas at P30 showed almost complete degeneration of IHCs (Fig. 2*d*). *Bth/Bth* mice showed little or no sign of a Preyer reflex at any age, similar to earlier reports of *dn/dn* mutants[5].

Membrane-protein topology algorithms[6] predicted the presence of 6–11 transmembrane domains in Tmc1, suggesting that Tmc1 might be a channel or a transporter. The M412K mutation found in *Bth* occurs either in the middle of a predicted transmembrane domain or in one of the extracellular domains. The insertion of a highly charged residue in a hydrophobic TM region could lead to abnormal folding or assembly of Tmc1 with other proteins.

As the *Bth* mutation may affect the function of a channel expressed in cochlear hair cells, we assessed hair cell function using whole-cell patch clamp to record currents in individual IHCs and OHCs from *Bth*/+ and wildtype mice before the onset of hair cell degeneration. Transducer[7] and basolateral calcium and potassium currents[8–10] developed normally in *Bth*/+ hair cells between P6 and P15 (data not shown), although this does not rule out the possibility that Tmc1 might be a channel. Further investigations using hair cells from *Bth* homozygotes or Tmc1/Bth expression in *Xenopus* oocytes may help determine the function of Tmc1.

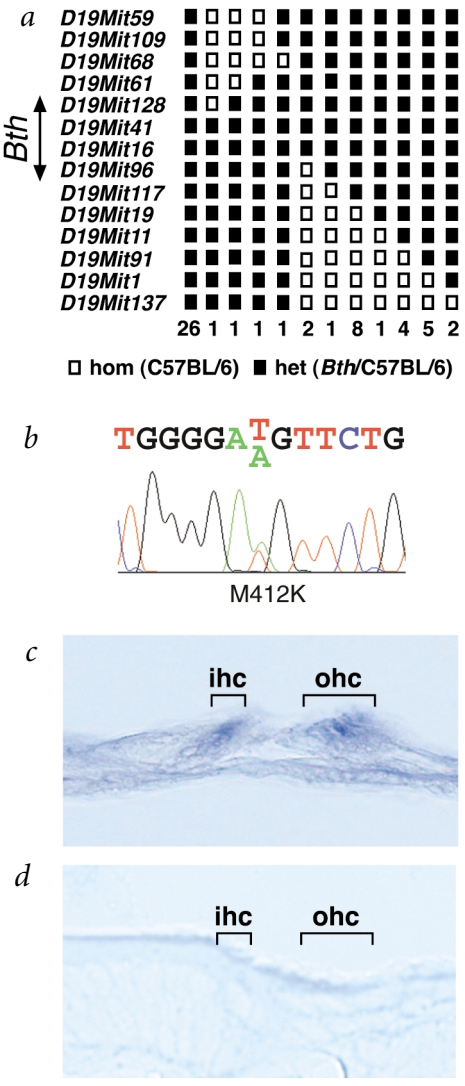

Fig. 1 Beethoven chromosomal location, mutation identification and expression. ***a***, *Bth* maps to a 4.4-cM region on mouse chromosome 19 between *D19Mit128* and *D19Mit96*, located 10.9 cM and 15.3 cM from the centromere, respectively. A loss of Preyer reflex was used to distinguish *Bth*/+ (het) from +/+ (hom) mice. ***b***, Sequence of *Tmc1* in a *Bth*/+ mouse showing the T→A mutation and the predicted protein sequence change. ***c***, Mouse *Tmc1* is expressed in inner (ihc) and outer hair cells (ohc), as shown in a section of a whole-mount *in situ* hybridization of a wildtype P15 cochlea. ***d***, Negative control for nonspecific hybridization.

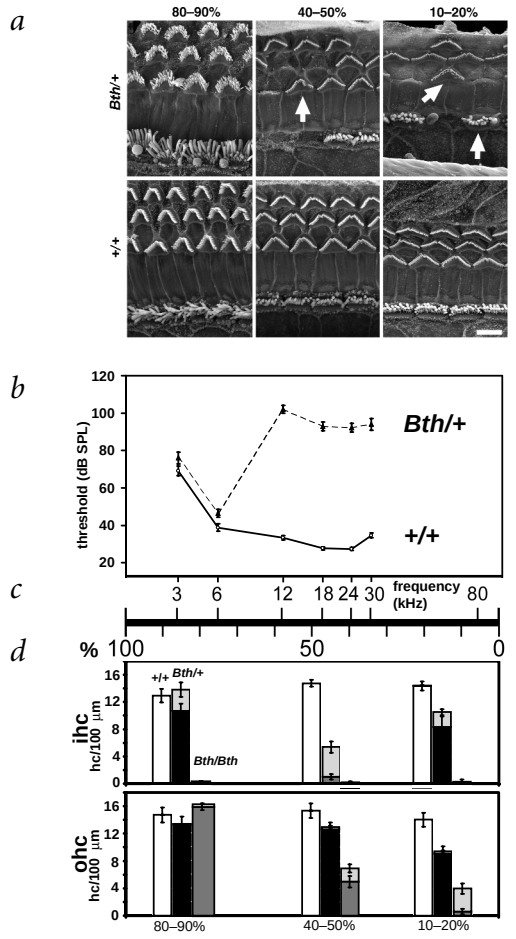

Fig. 2 Morphological and physiological characteristics of *Bth* inner ears. ***a***, Scanning electron micrographs of *Bth*/+ and +/+ organ of Corti at P30. The *Bth*/+ sample shows hair-cell degeneration (indicated by arrows) in the basal (10–20%) and middle (40–50%) regions (percentage of total distance from base). Scale bar=5 µm. ***b***, Mean (± s.e.m.) compound action potential thresholds measured from the round window[12] in *Bth*/+ (*n*=9) and +/+ (*n*=7) mice aged P29–31. ***c***, Frequency-place map of mouse cochlea showing the organ of Corti aligned with the best frequency response at each point, adapted from Ehret[13]. ***d***, Average hair-cell counts (± s.e.m.) per 100 µm in three areas of the cochlear duct. White bars represent +/+ mice (*n*=5), black bars represent *Bth*/+ samples (*n*=5), dark gray bars represent *Bth*/*Bth* mutants (*n*=2) and light gray bars represent degenerating hair cells in all samples. All experiments were carried out in full compliance with UK Home Office conditions, with the Tel Aviv University Animal Care and Use Committee (11-00-65) and with the German Law on Animal Protection.

Seven recessive mutant alleles have been found in *TMC1* that are associated with human nonsyndromic hearing loss, as well as in the recessive deafness (*dn*) mouse mutant, which shows no auditory response and has secondary hair-cell degeneration[1,5,11]. One dominant allele has been found in *TMC1* that is associated with human nonsyndromic progressive hearing loss, DFNA36 (ref. 1), making Beethoven an invaluable model for studying postlingual deafness. The dominant alleles in *TMC1/Tmc1*, both of which are missense mutations, may act through dominant-negative or gain-of-function mechanisms. In DFNA36 and *Bth*, the dominant phenotypes may also result in part from a modifier, contributed by the genetic background. Beethoven mutants are unusual among deaf mouse mutants in that their hair cells seem to function normally before they degenerate. Beethoven may thus provide insight into the factors needed for long-term survival of hair cells and may increase our understanding of the hair-cell degeneration assumed to be associated with progressive hearing loss with ageing (presbyacusis) in a large proportion of the human population.

GenBank accession number. Mouse *Tmc1*, AF417579.

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Competing interests statement

Some authors declare competing financial interests (T.B.F., E.R.W. and A.J.G.). Details accompany the paper on the website of Nature Genetics (http://genetics.nature.com).

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