Short Report

A novel mutation at the DFNA36 hearing loss locus reveals a critical function and potential genotype–phenotype correlation for amino acid-572 of TMC1

Kitajiri S, Makishima T, Friedman TB, Griffith AJ. A novel mutation at the DFNA36 hearing loss locus reveals a critical function and potential genotype–phenotype correlation for amino acid-572 of TMC1. Clin Genet 2007: 71: 148–152. © Blackwell Munksgaard, 2007

We ascertained a North American Caucasian family (LMG248) segregating autosomal dominant, non-syndromic, post-lingual, progressive sensorineural hearing loss. The hearing loss begins in the second decade of life and initially affects high frequencies. It progresses to profound deafness at all frequencies by the fourth or fifth decade. The phenotype co-segregates with short-tandem repeat markers flanking the TMC1 gene at the DFNA36 locus on chromosome 9q31-q21. The affected individuals carry a novel missense substitution, p.D572H (c.G1714C), of the TMC1 gene. This mutation is at the same nucleotide and amino acid position as the only other reported DFNA36 mutation, p.D572N (c.G1714A). Our observations implicate a critical function for amino acid-572 for wild-type TMC1 function or the pathogenesis of DFNA36 hearing loss. The slower progression of hearing loss associated with p.D572H, in comparison with that caused by p.D572N, may reflect a correlation of DFNA36 phenotype with TMC1 genotype.

Hereditary non-syndromic hearing loss is genetically heterogeneous and can display autosomal, sex-linked, or mitochondrial inheritance (1). There are over 100 autosomal dominant (DFNA) and autosomal recessive (DFNB) loci, some of which are allelic (2). For example, dominant and recessive mutations of transmembrane channel-like gene 1 (TMC1) cause hearing loss at the DFNA36 and DFNB7/B11 loci, respectively, on chromosome 9q13-q21 (3, 4). TMC1 encodes a multi-pass transmembrane protein of unknown function with no significant sequence similarity to proteins of known function (3). There is a region of unknown function, termed the TMC domain, whose amino acid sequence is conserved among all known TMC homologs (5, 6).

Inactivating mutations of TMC1 are recessive alleles that cause pre-lingual, severe to profound DFNB7/B11 deafness (3, 7–11). In contrast, the only reported DFNA36 family, LMG128,
co-segregates a missense substitution of TMC1 and hearing loss with a post-lingual onset in the first decade of life and progression to profound deafness by 20 years of age (3, 12). There is a similar genotype–phenotype correlation in mice carrying mutant alleles of Tmc1. Homozygous deafness (Tmc1<sup>dn</sup>) mice have a deletion of exon 14 of Tmc1 (3) and no detectable hearing (13, 14). Heterozygous Beethoven (Tmc1<sup>Bth/+</sup>) mice have an amino acid substitution, M412K (4), of Tmc1 and an initially slight hearing loss that rapidly progresses to profound deafness (4, 14, 15). Since heterozygous carriers of recessive loss-of-function alleles of TMC1 (human) and Tmc1 (mouse) have normal hearing (7, 16, 17), the dominant missense mutations in DFNA36 (D572N) and Tmc1<sup>Bth</sup> must cause hearing loss via dominant-negative or gain-of-function effects (3, 4).

Although Tmc1<sup>Bth</sup> and Tmc1<sup>dn</sup> cochlear hair cells initiate normal or near-normal structural and biophysical development, they never functionally mature and rapidly undergo degeneration within the first few weeks and months of life (14, 18, 19). Taken together with the expression of Tmc1 RNA in hair cells of the cochlea (3, 4), these results may indicate a direct role for TMC1 in hair cell development, function, or survival.

In this study, we describe a family (LMG248) segregating post-lingual progressive hearing loss at the DFNA36 locus. Their hearing loss is caused by a novel missense substitution of TMC1 at the same nucleotide and amino acid position as the previously reported DFNA36 mutation, indicating a critical role of this amino acid position for wild-type TMC1 function or the pathogenesis of DFNA36 hearing loss. The slower progression of hearing loss in LMG248, in comparison with LMG128, may reflect a correlation of DFNA36 phenotype with TMC1 genotype.

Materials and methods

This study was approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke and the National Institute on Deafness and Other Communication Disorders, National Institutes of Health. All subjects were adult members of LMG248, a large North American Caucasian family of mixed European ancestries. Written informed consent was obtained from all study subjects.

Medical and developmental history interviews and physical examinations were performed in the field by an otolaryngologist (A. J. G.). Vestibular function was evaluated by tandem gait and Romberg tests and locomotor developmental history. Pure-tone air conduction thresholds were tested with a portable audiometer (MA25; Maico Diagnostics, Eden Prairie, MN) under ambient noise conditions. Audiological and medical records from outside facilities were reviewed when available.

We estimated progression of hearing loss as the slope of a simple linear regression of hearing threshold and age using statistiXL version 1.6 (available from http://www.statistixl.com/) with the Windows<sup>TM</sup> version of Microsoft Excel<sup>TM</sup>. We used the same software to compare regression slopes between LMG128 and LMG248 by analysis of variance (ANOVA). Since affected members of LMG128 become profoundly deaf at all frequencies before 20 years of age (12), our analysis did not include LMG128 threshold values measured at or after 20 years.

Genomic DNA was prepared from venous blood samples (Puregene; Gentra Systems, Minneapolis, MN, USA) and analyzed as described (20) for genotypes at short-tandem repeat markers flanking non-syndromic dominant hearing loss loci DFNA1 to DFNA50 (available upon request). All 24 TMC1 exons and their flanking intronic sequences were polymerase chain reaction (PCR) amplified for nucleotide sequence analysis as described (3). The ClustalW alignment was modified from Kurima et al. (5).

Results

LMG248 is a North American Caucasian family segregating autosomal dominant, post-lingual, progressive, symmetric sensorineural hearing loss (Figs 1 and 2). There are no associated signs or symptoms of abnormalities of the vestibular or other organ systems. Hearing loss appears to initially affect high frequencies with an onset in the second decade of life and progresses to profound levels by the fourth or fifth decade (Fig. 1). Although low-frequency hearing is initially spared, it eventually deteriorates to profound levels. Word recognition scores are within the expected limits for the level of pure-tone threshold elevations due to a cochlear etiology (not shown) (21). Middle ear immitance test results were within normal limits (not shown). Acoustic reflex test results were available only for III-2, who had absent reflexes consistent with her profound degree of hearing loss. Otoacoustic emissions test results were not available. Individual IV-1 does not use hearing amplification, III-4 and III-6 use hearing aids, and II-2, II-6 and III-2 have cochlear implants.
Genotype and haplotype analyses were consistent with linkage to DFNA36 (Fig. 2) and no other DFNA loci (not shown). Nucleotide sequence analysis of TMC1 identified a single variant, c.G1714C, which is predicted to result in substitution of histidine for aspartic acid at

Fig. 1. LMG248 phenotype. Serial pure-tone air conduction thresholds are shown for the right ears of affected family members. Bone conduction thresholds (not shown) are consistent with sensorineural hearing loss.

Genotype and haplotype analyses were consistent with linkage to DFNA36 (Fig. 2) and no other DFNA loci (not shown). Nucleotide sequence analysis of TMC1 identified a single variant, c.G1714C, which is predicted to result in substitution of histidine for aspartic acid at
amino acid position 572 (p.D572H; Fig. 3a). We confirmed c.G1714C by sequence analysis of subcloned PCR amplification products (not shown) and by restriction digestion analysis with TaqI, which cleaves the wild-type allele but not the mutant allele (not shown). The amino acid D572 is located in the conserved TMC domain within a predicted cytoplasmic loop (5, 6). It is the same amino acid affected by the c.G1714A (p.D572N) mutation segregating in LMG128, the original DFNA36 family (3). We previously observed no polymorphisms of this codon in 902 control chromosomes (3). D572 is conserved among human and mouse TMC1, TMC2, TMC5, and TMC6 (Fig. 3b). None of the other known TMC homologs have histidine or asparagine at this position (5, 6).

We estimated progression of hearing loss in affected members of LMG128 and LMG248 as the slope of a simple linear regression of hearing threshold and age. In comparison to p.D572N carriers in LMG128, p.D572H carriers in LMG248 have slower progression of hearing loss at all stimulus frequencies (0.25, 0.5, 1, 2, 4 and 8 kHz; p < 0.05).

Discussion

We have identified a second dominant missense mutation of TMC1 associated with DFNA36 hearing loss. Remarkably, both of these mutations affect the same nucleotide and amino acid. These DFNA36 missense substitutions must act via dominant-negative or gain-of-function effects (3), but we do not have an assay of TMC1 function to differentiate among these possibilities. It is possible that c.G1714 might be a hotspot for mutational events, but the lack of polymorphism...
at this position in normal control chromosomes, and the recurrent substitution of D572 with the weakly basic residues histidine and asparagine, likely reflect a critical function associated with either wild-type D572, the mutant residues, or both. The Taq1 restriction digestion assay we used to confirm c.G1714C will not be a useful screen for these mutations due to a common silent polymorphism (c.C1713T) in the preceding codon, which also ablates the Taq1 recognition site (not shown).

Families LMG128 and LMG248 segregate similar hearing loss phenotypes, although the rate of progression appears to be faster in LMG128 (12). This could reflect differing effects of the substitutions, different genetic backgrounds, or both. Indeed, genetic background has a modest effect on hearing thresholds in Tmc1Bth/+ mice (15). The identification of additional DFNA36 mutations could address whether there is a correlation of phenotype with TMC1 genotype, as well as cost-efficient strategies to screen TMC1 for mutations in patients with post-lingual progressive hearing loss.

Acknowledgements

This study was supported by NIH intramural research funds Z01-DC-00039-10 (T. B. F.), Z01-DC-00060-06 and Z01-DC-00064-06 (A. J. G.). S. K. was supported in part by a Japan Society for the Promotion of Science Research Fellowship for Japanese biomedical and behavioral researchers at the National Institutes of Health. The authors thank the family members for their participation, Anne Madeo for study coordination, Carmen Brewer for reviewing an outside audiologic report, and Rob Morell, Dennis Drayna, and Anne Madeo for critical review of the manuscript.

References

1. Griffith AJ, Friedman TB. Autosomal and X-linked auditory disorders. In: Keats BJB, Fay RR, Popper AN, eds. Springer handbook of auditory research, Vol. 14. New York: Springer: 2002: 121–227.
2. Friedman TB, Griffith AJ. Human nonsyndromic sensorineural deafness. Annu Rev Genomics Hum Genet 2003: 4: 341–402.
3. Kurima K, Peters LM, Yang Y et al. Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. Nat Genet 2002: 30 (3): 277–284.
4. Vreugde S, Erven A, Kros CJ et al. Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. Nat Genet 2002: 30 (3): 257–258.
5. Kurima K, Yang Y, Sorber K, Griffith AJ. Characterization of the transmembrane channel-like (TMC) gene family: functional clues from hearing loss and epidermolysis verruciformis. Genomics 2003: 82 (3): 300–308.
6. Keresztes G, Mutai H, Heller S. TMC and EVER genes belong to a larger novel family, the TMC gene family encoding transmembrane proteins. BMC Genomics 2003: 4 (1): 24.
7. Jain PK, Fukushima K, Deshmukh D et al. A human recessive neurosensory nonsyndromic hearing impairment locus is potential homologue of murine deafness (dn) locus. Hum Mol Genet 1995: 4 (12): 2391–2394.
8. Scott DA, Carmi R, Elbedour K, Yosefsberg S, Stone EM, Sheffield VC. An autosomal recessive nonsyndromic hearing-loss locus identified by DNA pooling using two inbred Bedouin kindreds. Am J Hum Genet 1996: 59 (2): 385–391.
9. Meyer CG, Gasmelseed NM, Mergani A et al. Novel TMC1 structural and splice variants associated with congenital nonsyndromic deafness in a Sudanese pedigree. Hum Mutat 2005: 25 (1): 100.
10. Santos RL, Wajid M, Khan MN et al. Novel sequence variants in the TMC1 gene in Pakistani families with autosomal recessive hearing impairment. Hum Mutat 2005: 26 (4): 396.
11. Kalay E, Karaguzel A, Caylan R et al. Four novel TMC1 (DFNB7/DFNB11) mutations in Turkish patients with congenital autosomal recessive nonsyndromic hearing loss. Hum Mutat 2005: 26 (6): 591.
12. Makishima T, Kurima K, Brewer CC, Griffith AJ. Early onset and rapid progression of dominant nonsyndromic DFNA36 hearing loss. Otol Neurotol 2004: 25 (5): 714–719.
13. Steel KP, Bock GR. The nature of inherited deafness in deafness mice. Nature 1980: 288 (5787): 159–161.
14. Marcotti W, Erven A, Johnson SL, Steel KP, Kros CJ. Tmc1 is necessary for normal functional maturation and survival of inner and outer hair cells in the mouse cochlea. J Physiol 2006: 574 (Pt 3): 677–698.
15. Noguchi Y, Kurima K, Makishima T et al. Multiple quantitative trait loci modify cochlear hair cell degeneration in the Beethoven (Tmc1Bth) mouse model of progressive hearing loss DFNA36. Genetics 2006: 173 (4): 2111–2119.
16. Kirsch JP, Money MK, Webster DB. Mice heterozygous for the deafness gene have normal auditory thresholds. Hear Res 1993: 67 (1–2): 51–54.
17. Huang JM, Money MK, Berlin CI, Keats BJ. Auditory phenotyping of heterozygous sound-responsive (+/dn) and deafness (dn/dn) mice. Hear Res 1995: 88 (1–2): 61–64.
18. Bock GR, Steel KP. Inner ear pathology in the deafness mutant mouse. Acta Otolaryngol 1983: 96 (1–2): 39–47.
19. Webster DB. Degeneration followed by partial regeneration of the organ of Corti in deafness (dn/dn) mice. Exp Neurol 1992: 115 (1): 27–31.
20. Bork JM, Peters LM, Riazuddin S et al. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. Am J Hum Genet 2001: 68 (1): 26–37.
21. Yellin MW, Jerger J, Fifer RC. Norms for disproportionate loss in speech intelligibility. Ear Hear 1989: 10 (4): 231–234.