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Mutations of TMC1 cause deafness by disrupting mechanoelectrical transduction

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Abstract

Objective—Mutations of transmembrane channel-like 1 gene (*TMC1*) can cause dominant (DFNA36) or recessive (DFNB7/B11) deafness. In this article, we describe the characteristics of DFNA36 and DFNB7/B11 deafness, the features of the *Tmc1* mutant mouse strains, and recent advances in our understanding of TMC1 function.

Methods—Publications related to *TMC1*, DFNA36 or DFNB7/B11 were identified through PubMed.

Results—All affected DFNA36 subjects showed post-lingual, progressive, sensorineural hearing loss (HL), initially affecting high frequencies. In contrast, almost all affected DFNB7/B11 subjects demonstrated congenital or prelingual severe to profound sensorineural HL. The mouse *Tmc1* gene also has dominant and recessive mutant alleles that cause HL in mutant strains, including *Beethoven*, *deafness* and *Tmc1* knockout mice. These mutant mice have been instrumental for revealing that *Tmc1* and its closely related paralog *Tmc2* are expressed in cochlear and vestibular hair cells, and are required for hair cell mechanoelectrical transduction (MET). Recent studies suggest that TMC1 and TMC2 may be components of the long-sought hair cell MET channel.

Conclusion—*TMC1* mutations disrupt hair cell MET.

Keywords

DFNA36; DFNB7/B11; Hearing loss; Mechanoelectrical transduction; *TMC1*; *TMC2*

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Conflict of interest

Kiyoto Kurima and Andrew J. Griffith hold the following U.S. patents: 7,166,433 (Transductin-2 and Applications to Hereditary Deafness), 7,192,705 (Transductin-1 and Applications to Hereditary Deafness), and 7,659,115 (Nucleic Acid Encoding Human Transductin-1 Polypeptide).

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Introduction

Hearing loss (HL) affects approximately one in 500 newborns and 4% of people 45 years of age and younger [1], reaching 50% by 80 years of age [2]. It is estimated that 278 million people worldwide have HL [3,4].

More than 50% of cases of congenital deafness are thought to have a genetic etiology [5], while the other cases are caused by environmental factors including exposure to ototoxic drugs or congenital cytomegalovirus infection [6]. Within the group that is considered to have a genetic cause, approximately 30% of cases present with other clinical features in addition to HL and is termed syndromic HL. The remaining 70% shows HL without additional overt clinical features, and is called nonsyndromic HL [7]. Approximately 70% of cases of nonsyndromic HL are autosomal recessive in inheritance, 25% are autosomal dominant, and X-linked and mitochondrial inheritance account for the remaining few percent [8,9]. Depending on the inheritance mode, the nonsyndromic phenotypes and loci are classified as: DFNA (autosomal dominant), DFNB (autosomal recessive), DFNX (X-linked) or DFNY (Y-linked). DFNM denotes modifier loci for which there are alleles that modify the HL phenotypes caused by mutations at another locus.

The strategy to identify genes for HL through a positional cloning approach has been successful over the past two decades. Linkage analysis with microsatellite markers and single nucleotide polymorphisms (SNPs) has been used widely, allowing the chromosomal locations of deafness genes to be mapped in large families. Following identification and refinement of the linkage loci, dideoxy (Sanger) sequencing has been used to analyze the genes within the critical interval, which has led to the identification of the causative mutations. In addition to studies of human families, some deafness genes have been identified from analogous analyses of spontaneous or chemically induced mutations in the mouse. Candidate gene approaches, in the absence of statistically significant linkage or association analyses or a closely corresponding animal model, have not yet yielded conclusive results. To date, 74 genes are known or proposed to be involved in nonsyndromic HL (<http://hereditaryhearingloss.org/> accessed on February 12, 2014). More genes will more certainly be discovered, since the causative genes at over 50 deafness loci have not yet been identified. The genes identified to date encode many different proteins with a large variety of functions in the inner ear, including gene regulation, development, ionic and osmotic homeostasis, synaptic transmission, hair cell bundle morphology, generation of the endocochlear potential, and mechanoelectrical transduction (MET) [10,11].

Hair cells have a critical role in transducing mechanical forces evoked by sound, or linear or angular accelerations of the head into an electrical signal. The apical surface of hair cells contains stereocilia, which play a pivotal role in this mechanism. The stereocilia have a typical staircase arrangement, and are connected with lateral and tip links stabilizing the mature hair bundle structure [12,13]. The tip link connects the top of a stereocilium with the side of its taller neighbor [14–16], and gates the opening of the MET channel(s) located at the tip of the shorter stereocilium [17–21]. Numerous candidates for the hair cell MET have emerged over the past 15 years, but none have withstood rigorous scientific scrutiny [22].

In this review, we highlight the transmembrane channel-like 1 (*TMC1*) gene, which was identified as the gene mutated in DFNA36 and DFNB7/B11 deafness [23]. First, we introduce the identification of *TMC1* as a deafness-causing gene, and describe the phenotype and mutation spectrum of DFNA36 and DFNB7/B11 patients. Next, we review mutant mouse models of human DFNA36 and DFNB7/B11 deafness, which have been instrumental for revealing the hair cell expression and function of *TMC1*. Recent experiments using these mouse models revealed that *Tmc1* and the closely related *Tmc2* genes are required for MET and might encode components of the MET channel [24,25].

Identification of *TMC1* as a causative gene for DFNA36 and DFNB7/B11 deafness

TMC1 was identified as the causative gene of DFNA36 and DFNB7/B11 deafness through positional cloning [23]. The DFNA36 interval had been mapped to chromosome 9q13-q21 by linkage analysis of a large North American family, LMG128, segregating autosomal dominant, nonsyndromic, sensorineural HL. Genotype analysis of markers linked to known nonsyndromic recessive deafness loci had revealed that the DFNA36 region overlapped the DFNB7/B11 linkage interval. Linkage analysis of approximately 230 Indian or Pakistani consanguineous families segregating autosomal recessive, nonsyndromic, sensorineural HL identified 11 additional families showing linkage to the DFNB7/B11 locus. Within this linkage interval, dideoxy sequencing of the *TMC1* gene revealed p.D572N (c.1714G>A) segregating in family LMG128, as well as one of eight other pathogenic mutations segregating among each of the ten DFNB7/B11 families. These findings showed that DFNA36 and DFNB7/B11 were allelic disorders caused by mutations of *TMC1*.

TMC1 spans approximately 300 kb on chromosome 9q21, and consists of 24 exons that make up a coding region of 2283 nucleotides [23]. It is a member of the transmembrane channel-like (*TMC*) gene family that includes seven other paralogs in mammals (*TMC2* to *TMC8*) [26]. At the time, the function of the *TMC* genes was unknown, and *in silico* translation products showed no significant sequence similarity to proteins or domains of known function. However, all were predicted to encode membrane proteins with at least six membrane-spanning domains [26]. The six-pass transmembrane topology was experimentally confirmed for mouse *TMC1* expressed in heterologous systems and suggested that it might function as a receptor, transporter, pump, or channel [27].

TMC genes have been implicated in other human diseases and disorders. Recessive mutations of *TMC6* (also designated as *EVER1*) or *TMC8* (*EVER2*) cause epidermodysplasia verruciformis (EV), which is characterized by abnormal susceptibility to infection by specific subtypes of human papillomaviruses with a high risk of progression to skin carcinoma [28]. The disease was reviewed in detail by Orth [29] and Patel et al. [30]. It has been proposed that the site of the functional lesion of EV is either in keratinocytes or T lymphocytes [29,31]. However the molecular pathogenesis of EV and the function of *TMC6* and *TMC8* thus remain unknown, although one report described an abnormality in zinc transport [32]. It is unclear if this observation was a direct or indirect effect of heterologous overexpression in these cells.

Phenotype and mutation spectrum of DFNA36 subjects

Three different missense mutations, p.G417R (c.1249G>A), p.D572H (c.1714G>C) and p.D572N (c.1714G>A), have been reported to cause autosomal dominant HL at the DFNA36 locus (Table 1) [23,33–35]. Families L1754 and LMG248 segregate p.G417R and p.D572N, respectively. Families LMG248 and H segregate p.D572H.

All of the affected members of families L1754, LMG248, LMG128 and H show post-lingual, progressive and symmetrical sensorineural HL initially affecting high frequencies, although there are variations in the age of onset and rate of progression [33–36]. In LMG128 family members carrying p.D572N, HL became evident in the first decade of life and rapidly progressed to severe to profound deafness by the second decade of life (Fig. 1a). The calculated rate of progression was 5.9 dB/year before 20 years of age. After the age of 20 years, the rate of progression was less than 1 dB/year. This slower rate at older ages reflects a ceiling effect due to the elevated thresholds [36]. In contrast, HL in LMG248 family members with the p.D572H mutation began in the second decade of life and progressed to profound levels by the fourth or fifth decade (Fig. 1b). The rate of progression in family LMG248 was significantly slower than that in family LMG128 for all stimulus test frequencies, although the calculated progression rate was not reported [33]. This difference may reflect differing effects of these substitution mutations, different genetic backgrounds, or both.

Affected subjects with DFNA mutations typically do not have vestibular symptoms. Although three of 13 affected members of family LMG128 reported various vestibular symptoms, they were not recurrent and were unaccompanied by tinnitus or fluctuations of hearing [36]. In combination with incomplete penetrance of the vestibular phenotype, Makishima et al. [36] suggested that vestibular symptoms were not related to DFNA36. Their observation was consistent with a lack of associated vestibular signs or symptoms in family LMG248 [33].

Remarkably, two different missense mutations, p.D572H and p.D572N, affect the same nucleotide and amino acid of *TMC1*. Of these, p.D572N was identified in families LMG128 and H. To determine if the p.D572N mutation was derived from a common founder, Hilgert et al. [34] compared *TMC1*-linked haplotypes of short tandem repeat (STR) markers and SNPs between the two families. They demonstrated several differences in the linked haplotypes and concluded that families LMG128 and H most likely did not have a common founder. The result indicates that c.1714G is a possible hot spot for mutations that arose independently in DFNA36 families, the p.D572 residue or the mutated residues are associated with a critical function or gain-of-function, or a combination of these possibilities.

Phenotype and mutation spectrum of DFNB7/B11 subjects

To date, 39 mutations have been reported to be associated with autosomal recessive HL at the DFNB7/B11 locus among 60 families. These families have their origins in Pakistan, Turkey, Tunisia, Iran, Lebanon/Jordan, North America, China, Europe, Sudan and Morocco (Table 2) [23,35,37–48]. These 39 mutations are widely distributed throughout the entire

coding region of *TMC1*. Some mutations, including c.64+2T>A, p.R34X, c.536-8T>A, p.R389X, p.R445H, c.1763+3A>G, p.R604X and p.S668R, were identified in multiple unrelated families [23,37,38,40–47]. p.R34X was identified in 10 different Pakistan families in addition to 6 families from Tunisia, Iran, Lebanon/Jordan and Turkey [23,40–43]. To examine if p.R34X arose from a common founder, Kitajiri et al. [40] studied the *TMC1*-linked haplotypes of STR markers and SNPs for the 10 independent p.R34X chromosomes. They demonstrated that all of the 10 chromosomes have identical combinations of STR markers and suggested that the Pakistani p.R34X mutation was derived from a common founder.

Mutations in *TMC1* are a common cause of autosomal recessive nonsyndromic deafness (Table 3). Mutation analysis of *TMC1* among Pakistani and North American families segregating autosomal recessive nonsyndromic HL identified *TMC1* mutations in 11 (4.8%) of 230 families [23]. The Pakistani portion of this mixed cohort was later increased to 557 families and mutations in *TMC1* were identified in 19 (3.4%) of 557 families [40]. Another study in a different Pakistani population identified *TMC1* mutations in eight (4.4%) in 180 families [37]. Mutations in *TMC1* were also identified in other populations of hearing loss patients of different ethnicity at a similar prevalence: 4.3% (4/93) in Turkey [38]; 5.9% (5/85) in Tunisia [42]; 8.1% (7/86) in another study among Turkish population [43]; and 4.2% (1/24) in Europe [46]. Recently, high-throughput sequencing of 79 known deafness-causing genes in 190 Chinese patients revealed *TMC1* mutations in three (1.6%) of 190 [47]. Since this latter study included patients with prelingual or early-onset severe to profound deafness, regardless of a family history of hearing loss, the prevalence may be low due to inclusion of non-genetic cases. These findings indicate that recessive mutations of *TMC1* mutations are distributed worldwide and are a comparatively common cause of nonsyndromic HL.

Generally, DFNB7/B11 patients have congenital or prelingual non-progressive severe to profound hearing loss (Table 2) [23,35,37–43,45–48]. Interestingly, in one family (W06-792) segregating DFNB7/B11, HL was noticed in the first to second decade of life, starting at high frequencies and rapidly progressing to severe to profound levels by the third or fourth decade (Fig. 1c) [44]. Genome-wide linkage analysis and dideoxy sequencing of *TMC1* identified c.1763+3A>G in the family, leading to a frameshift and a premature stop codon due to the utilization of a cryptic splice donor site 47 bp downstream in intron 19. The delay in the onset of HL may result from residual wild-type *TMC1* transcripts escaping the aberrant splicing or from residual function of mutant truncated proteins.

Although vestibular function was formally examined in only some affected subjects, none reported vestibular symptoms or a developmental delay in gross motor skills [23,37,39,41,43,48]. These findings collectively suggest that *TMC1* mutations do not affect vestibular function.

Mouse models for DFNA36 and DFNB7/B11 deafness

The laboratory mouse is an invaluable model organism for investigating the genetic basis of human disease because of its short life span, ease of experimental manipulation and limited

genetic heterogeneity. Mice have additional advantages for use in the analysis of HL. There are strong structural similarities between the human and the mouse auditory system [49]. Furthermore, mouse models allow histopathological and ultrastructural studies and electrophysiological measurements to investigate the underlying pathogenesis of HL.

The mouse genes involved in hearing exhibit strong sequence similarities and similar functions with their human counterparts [50]. Most information about protein function in the auditory system originates from the study of mouse mutants carrying alterations in the genes corresponding to those in human HL. These mouse mutants are derived from spontaneous mutagenic events, chemical mutagens such as N-ethyl-N-nitrosourea (ENU), as well as gene-targeting techniques.

The mouse ortholog *Tmc1* spans approximately 170 kb on mouse chromosome 19. It consists of 21 exons with a coding region of 2274 nucleotides [23]. The amino acid sequence of mouse TMC1 protein has 95% identity and 96% similarity with that of human TMC1 [26]. *Tmc1* mRNA is expressed in mouse cochlear and vestibular hair cells from the late embryonic stages of development [23,24]. Mouse *Tmc1* also has dominant and recessive mutant alleles that cause hearing loss in mutant mouse strains, including *Beethoven* (*Bth*) [51], *deafness* (*dn*) [23,52], *baringo*, *nice*, *stitch* [53] and a targeted deletion of *Tmc1* (*Tmc1*^{-/-}) mice [24] (Table 4).

Phenotypic, ultrastructural and electrophysiological characteristics of *Beethoven* (*Bth*) mice

The *Bth* mutation of *Tmc1*, p.M412K, arose in a large-scale ENU mutagenesis program [51]. Heterozygous mice (*Tmc1*^{Bth/+}) show progressive sensorineural HL analogous to that of human DFNA36 [51]. The measurement of compound action potentials (CAPs) showed a small but significant elevation of thresholds for stimulus test frequencies 18 kHz at postnatal day 15 (P15), and a large increase in CAP thresholds for frequencies 12 kHz at P30 [54]. Scanning electron microscopy of *Tmc1*^{Bth/+} organs of Corti revealed progressive hair cell degeneration from P20 onward. At P30, *Tmc1*^{Bth/+} mice showed hair cell damage and a significant reduction of number of hair cells in the basal and middle turns of the cochlea. There was an unusual pattern with inner hair cells (IHCs) lost at an earlier age than outer hair cells (OHCs), but no significant hair cell loss in the apical region [51,55]. There was a correlation between hair cell loss and elevated CAP thresholds in *Tmc1*^{Bth/+} mice. In contrast, homozygous mice (*Tmc1*^{Bth/Bth}) showed profound deafness at all time points at which hearing can be detected in wild-type mice [54]. CAP responses could not be detected in P15 *Tmc1*^{Bth/Bth} ears exposed to maximum stimulus intensities. Scanning electron microscopy showed hair cell damage and a significant reduction in the number of hair cells in the basal and middle turns even at P15, but no significant hair cell loss in the apical region. Endocochlear potentials (EPs) were present and normal in *Tmc1*^{Bth/+} and *Tmc1*^{Bth/Bth} mice. Furthermore, MET currents could be recorded from OHCs at P6 to P7 under whole-cell voltage clamp [54].

Phenotypic, ultrastructural and electrophysiological characteristics of deafness (*dn*), *baringo*, *nice*, *stitch* and *Tmc1* mice

Homozygous *dn*, *baringo*, *nice*, *stitch* and *Tmc1* mice show severe to profound deafness at P30, whereas heterozygotes display no differences in hearing thresholds from those of wild-type mice [24,52,53]. These findings reveal that these mouse strains are models of human recessive deafness DFNB7/B11. The model mice were generated in different ways: *dn*, a 1,656-bp deletion encompassing the 171-bp exon 14 and adjacent introns, is a spontaneous mutation [23,52]; *baringo* (p.Y182C), *nice* (p.Y449H) and *stitch* (p.W554L) arose in a large-scale ENU mutagenesis program [53]; and *Tmc1* was generated using homologous recombination to replace the region encompassing exons 8–9 and adjacent introns with a gene trap construct fused to a *LacZ* reporter gene [24].

Of these DFNB7/B11 models, *dn* mice have been the most extensively examined. This strain was first described by Doel and Kocher in 1958 as an autosomal recessive mutant arising from the curly-tail stock [52]. *Tmc1^{dn/dn}* mice lacked any cochlear responses (CAPs, summing potentials or cochlear microphonics) to sound stimuli between the ages of P12 and P20 [54,56]. Scanning electron microscopy revealed that *Tmc1^{dn/dn}* mice had normal hair bundles at P14 but showed significant hair cell damage and loss in middle and basal cochlear turns at P30, where IHCs and OHCs appeared equally affected. In the apical turn, many hair cells retained a normal, or near-normal, bundle appearance at P30 [54]. The EPs were present and normal in *Tmc1^{dn/dn}* mice [56]. Furthermore, MET currents could be recorded from *Tmc1^{dn/dn}* OHCs at P6–7 under whole-cell voltage clamp [54]. *Tmc1^{dn/dn}* mice do not show circling behavior or head tilting/tossing that suggest the existence of congenital severe vestibular dysfunction [52].

Homozygous *baringo*, *nice* and *stitch* mice show auditory and morphological characteristics similar to those of *Tmc1^{dn/dn}* mice with some variations that may be caused by differences in the mutations [53]. Homozygous *Tmc1* (*Tmc1^{-/-}*) mice showed the same auditory and morphological characteristics as *Tmc1^{dn/dn}* mutants [24].

The association of *Tmc1* and *Tmc2* with mechanoelectrical transduction (MET) in mouse hair cells

The absence of CAPs in *Tmc1^{Bth/Bth}* and *Tmc1^{dn/dn}* mice at P15 in spite of their normal hair bundle appearance at the apical cochlear turn indicated that HL might not be an indirect consequence of a hair cell structural defect but a functional deficit. Furthermore, the absence of cochlear microphonics in *Tmc1^{dn/dn}* mice, in spite of the presence of an intact EP, suggested that the underlying mutation might affect the MET channels [54,56,57]. However, this hypothesis seemed unlikely when MET currents were recorded from P6–7 OHCs from *Tmc1^{Bth/Bth}* and *Tmc1^{dn/dn}* mice [54].

Kawashima et al. [24] revealed a contribution of *Tmc1* and the closely related *Tmc2* gene to MET using mice segregating the targeted deletion alleles *Tmc1* and *Tmc2*. *Tmc2*, the mouse ortholog of human *TMC2*, is expressed in mouse cochlear and vestibular hair cells, but neither *Tmc2* nor *TMC2* has been implicated in inner ear dysfunction. Its temporal

expression pattern in hair cells was different from that of *Tmc1*. *Tmc2* expression was transient in early postnatal cochlear hair cells but persisted in vestibular hair cells, whereas *Tmc1* expression was persistent in mature cochlear and vestibular hair cells. While *Tmc1*^{-/-} mice were deaf and *Tmc2*^{-/-} mice were phenotypically normal, double homozygous (*Tmc1*^{-/-};*Tmc2*^{-/-}) mice showed deafness and complete loss of peripheral vestibular function. These observations suggest that persistent *Tmc2* expression in vestibular hair cells may preserve vestibular function in mature *Tmc1*^{-/-} mice. Scanning electron microscopic analysis of *Tmc1*^{-/-};*Tmc2*^{-/-} inner ear tissue demonstrated that cochlear and vestibular hair cells had intact hair bundles, tip links and tip-link tension at P3 and had normal structure during the early postnatal period. These morphologically normal cochlear and vestibular hair cells were used to evaluate mechanosensory function. While MET currents were detected in vestibular and cochlear hair cells from early postnatal *Tmc1*^{-/-} or *Tmc2*^{-/-} mice, MET currents were completely absent in *Tmc1*^{-/-};*Tmc2*^{-/-} hair cells. Furthermore, MET currents were rescued by the expression of exogenous *Tmc1* or *Tmc2* in cultured *Tmc1*^{-/-};*Tmc2*^{-/-} hair cells. These findings indicate that *Tmc1* and *Tmc2* are necessary for hair cell MET. They also suggest that the MET currents in early postnatal *Tmc1*^{-/-} cochlear hair cells are mediated by *Tmc2*.

The spatiotemporal expression patterns of *Tmc1* and *Tmc2* mRNA were examined by quantitative RT-PCR and *in situ* hybridization. In the vestibular organs, a *Tmc2* expression rises just before the onset of MET around E16-17, and the rise of *Tmc1* expression lagged MET acquisition by several days [24]. In the cochlea, the rise in *Tmc2* expression just preceded the spatiotemporal onset of MET in OHCs in the base at P0 and the apex at P2. The rise in *Tmc1* expression lagged MET acquisition by 2–3 days in all regions of the cochlea. Moreover, *Tmc2* expression declined from the base to the apex during this developmental period, revealing a switch from *Tmc2* to *Tmc1* expression [24]. Importantly, this developmental decrease of *Tmc2* is consistent with the decrease of calcium permeability (P_{Ca}) in OHC MET channels from the base to the apex [58]. In wild-type mice, the P_{Ca} of OHCs decreases from the cochlear base to the apex before P6. In *Tmc1*^{dn/dn} mice, P_{Ca} in basal OHCs was larger than that in wild-type mice, whereas in *Tmc2* mutant mice (*Tmc2*^{tm1Lex/tm1Lex}), P_{Ca} in apical and basal OHCs was decreased compared with that in wild-type OHCs. On the basis of these results, it was postulated that differences in P_{Ca} reflected different expression of *Tmc1* and *Tmc2*, with the latter conferring higher P_{Ca} [24,58]. These results further support the association of *Tmc1* and *Tmc2* with MET.

Function of *Tmc1* and *Tmc2* in mouse hair cells

Several findings revealed that *Tmc1* and *Tmc2* are required for mouse hair cell MET [24,58], but the precise molecular function of their protein products is unknown. Considering the data described above, there are at least four hypotheses about their function: first, TMC1 and TMC2 proteins may be pore-forming subunits of the MET channel; second, they may be accessory subunits physically associated with the pore-forming subunits of the MET channel; third, they may be components of the MET complex, but not directly contacting the MET channel; finally, they may be required for assembly, trafficking, development, or function of MET channel or complex without being stably physically associated with them. These four hypotheses are not mutually exclusive: more than one may be true.

Recently, Pan et al. [25] measured whole-cell and single-channel currents from mouse cochlear hair cells of *Tmc1*^{+/-}; *Tmc2*^{-/-}, *Tmc1*^{-/-}; *Tmc2*^{+/-} or *Tmc1*^{Bth/-}; *Tmc2*^{-/-} mice: these mutant hair cells expressed only wild-type TMC1, only wild-type TMC2, or only mutant TMC1 (p.M412K) molecules. This allowed direct comparison of MET channel properties associated with the presence of only TMC1, TMC2 or mutant TMC1 by measuring MET currents of hair cells from each compound mutant mouse. IHCs that expressed wild-type *Tmc2* but not *Tmc1* had high P_{Ca} and large single-channel currents in comparison to those of hair cells expressing only *Tmc1*. In contrast, hair cells that expressed mutant *Tmc1* had reduced P_{Ca} and reduced single-channel currents in comparison to those of cells that expressed wild-type *Tmc1*. These findings demonstrated that a missense amino acid substitution, p.M412K, in TMC1 alters the ion permeation properties of hair cell MET channels, consistent with the hypothesis that TMC1 and TMC2 are components of the hair cell MET complex, if not the channel itself.

An alternative technique was used by Kim et al. [59], who recently reported the existence of a mechanically elicited current in the OHCs from double homozygous mutant mice (*Tmc1*^{dn/dn}; *Tmc2*^{tm1Lex/tm1Lex}) using an oscillating fluid jet. After disruption of tip links with a calcium chelator, they observed currents in OHCs of neonatal wild-type and the double homozygous mutant mice. However, the characteristics of the stimulus were anomalous in that the currents were activated by displacements of the hair bundle away from its tallest row of stereocilia. They were unable to elicit currents when the bundle was displaced toward the tallest row of stereocilia, as occurs in wild-type hair cells whose bundles have not been disrupted with calcium chelation. These “reverse” MET currents had similar characteristics to conventional MET currents in wild-type hair bundles: the novel currents were blocked by styryl dye FM1-43, dihydrostreptomycin, and extracellular calcium at concentrations similar to those that block wild-type hair cell MET channels. In contrast, the P_{Ca} of the reverse transduction current in the double homozygous mutant hair cells was lower than that of conventional wild-type MET. On the basis of these findings, Kim et al. concluded that, in the absence of TMC1 and TMC2, the MET channel is not associated with the tip link and cannot be activated by tip-link tension, but can be activated by reverse displacement. This led them to conclude that neither TMC1 nor TMC2 are pore-forming components of MET channel. They hypothesized that TMC1 and TMC2 are required for targeting the MET channel to the tips of the stereocilia where they can interact with other constituents of the transduction complex, including the tip link.

There are several caveats to these conclusions. First, the targeted deletion alleles of *Tmc1* and *Tmc2* used by Kim et al. may encode functional or partially functional TMC proteins. Even if the mutant TMC1 or TMC2 proteins cannot traffic to tips of stereocilia to contribute to conventional MET, they may nevertheless contribute to the non-canonical reverse transduction that may be occurring at other locations at the apical region of the hair cell. Second, the non-canonical MET currents could be mediated by another protein(s), including another TMC protein(s), because the inhibitors used by Kim et al. are not specific for the MET channel of hair cell stereocilia. The precise role of TMC1 and TMC2 in hair cell MET remains an open question at this time.

Several criteria have to be met for a protein to be considered the *bona fide* hair cell MET channel [60,61]. First, the channel must be expressed in the appropriate temporal and spatial domains to allow for hair cell MET function. Second, the channel must be required for the hair cell MET response to mechanical stimuli. Third, missense substitutions of the pore region of the protein, which do not affect its assembly or folding, should alter the core biophysical properties of the channel pore. Fourth, the MET channel activity should be reconstituted by expression of the candidate protein(s) *in vitro* or in a heterologous expression system.

For the first criteria, the rise in *Tmc2* mRNA expression immediately preceded the onset of MET, and exogenous fluorophore-tagged TMC1 and TMC2 proteins are localized to the tips of the stereocilia [24]. For the second criteria, the conclusions are disparate: Kawashima et al. [24] and Pan et al. [25] showed that hair cells in *Tmc1*^{-/-};*Tmc2*^{-/-} mutant eliminates conventional MET currents and exogenous TMC1 and TMC2 can restore MET currents in cultured *Tmc1*^{-/-};*Tmc2*^{-/-} hair cells. While Kim et al. [59] demonstrated non-canonical MET currents in the double homozygous mutant hair cells, the relevance of those currents is unknown in the absence of information about the expression and localization of TMC proteins encoded by the *Tmc* mutant alleles they studied. For the third criteria, Pan et al. [25] showed that a missense mutation in TMC1 altered the permeation properties of the hair cell MET channel. This is consistent with TMC1 being a component of the channel, but it is inconclusive evidence. For the fourth criteria, reconstitution of the MET channel in a heterologous system has not yet been successful. When TMC1, TMC2, or both are expressed in heterologous systems, they fail to localize to the plasma membrane where they could be tested by electrophysiologic methods for channel activity. Reconstitution of MET may require yet to be identified beta subunits, if any, and other protein or lipid partners necessary for stretch-activated channel function in hair cells. A recent report concluded that *C. elegans tmc-1* encodes a sodium-sensitive ion channel, but this study lacked a mutated control and did not, in our opinion, adequately demonstrate plasma membrane localization of the protein. Moreover, this observation has not been replicated for vertebrate TMC1 [62]. Expression of functional TMC1 and TMC2 *in vitro* or in a heterologous system will be helpful to test the hypothesis that they are components of the hair cell MET channel. Given the long journey to identify the hair cell MET channel, we do not expect this to be easy.

Conclusion

A large number of *TMC1* mutations have been identified by linkage analysis and DNA sequencing of DFNA36 and DFNB7/B11 patients worldwide, indicating that *TMC1* mutations are comparatively common causes of genetic deafness. Typically, DFNA36 subjects show post-lingual, progressive sensorineural HL, resulting in severe to profound deafness. In contrast, DFNB7/B11 subjects show congenital or prelingual severe to profound sensorineural HL. While the precise function of *Tmc1* remains unknown, recent studies of *Tmc1* and *Tmc2* mutant mouse models revealed that they are required for MET in cochlear and vestibular hair cells. There is a possibility that TMC1 and TMC2 are actual components of the elusive hair cell MET channel, whose molecular identity remains unknown.

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Fig. 1.

Age-related typical audiograms for three families, LMG128, LMG248 and W06-792. (a) In LMG128, hearing loss was evident in the first decade of life and rapidly progressed to severe to profound deafness by the second decade of life. (b) In LMG248, hearing loss was reported in the second decade of life and progressed to profound levels by the fourth or fifth decade. (c) In family W06-792, hearing loss was noticed in the first to second decade, starting at high frequencies and rapidly progressing to severe to profound levels in the third or fourth decade. Numbers near the lines in the graph indicate ages in decade steps. We estimated age-matched hearing thresholds by plotting audiogram data of all the affected subjects in each frequency and depicting a polynomial trendline using the Mac version of Microsoft Excel.

Table 1

Clinical phenotypes of DFNA36 patients

Family	Origin	Nucleotide mutation ^a	Predicted effect	Exon number	Onset of HL	Severity of HL	Reference
L1754	Iran	c.1249G>A	p.G417R	Exon 16	Postlingual	Severe to profound 2nd decade: all frequencies Rapid progression ^b	Yang et al. [35]
LMG248	North America (Caucasian)	c.1714G>C	p.D572H	Exon 19	Postlingual	Profound 2nd decade: high frequencies 4th–5th decade: all frequencies	Kitajiri et al. [33]
LMG128	North America (Caucasian)	c.1714G>A	p.D572N	Exon 19	Postlingual	Profound 1st decade: mid and high frequencies 2nd decade: all frequencies	Kurima et al. [23] Makishima et al. [36]
Family H	North America (Caucasian)	c.1714G>A	p.D572N	Exon 19	Postlingual	Severe to profound 1st decade: mid and high frequencies Rapid progression ^b	Hilgert et al. [34]

Abbreviation: HL, hearing loss.

^aThe numbering is based on cDNA sequence (GenBank accession number: NM_138691.2).

^bDetailed time course was not described.

Table 2

Clinical phenotypes of DFNB7/B11 patients

Family	Origin	Nucleotide mutation ^a	Predicted effect	Exon/Intron number	Onset of HL	Severity of HL	Reference
<i>Homozygous state^b</i>							
PKDF22	Pakistan	c.-195_16del ^d	Genomic deletion		Prelingual	Severe to profound	Kurima et al. [23]
PKDF274	Pakistan	c.16+1G>T	Splice disruption	Intron 5	Prelingual	Severe to profound	Kitajiri et al. [40]
734, 763	Turkey	c.64+2T>A	Splice disruption	Intron 6	Congenital/Prelingual	Profound	Sirmaci et al. [43]
PKSR9, PKSN9, PKSN24, PKDF7, PKDF75	Pakistan	c.100C>T	p.R34X	Exon 7	Prelingual	Severe to profound	Kurima et al. [23]
PKDF69, PKDF178, PKDF243, PKDF319, PKDF401	Pakistan	c.100C>T	p.R34X	Exon 7	Prelingual	Severe to profound	Kitajiri et al. [40]
Family A, B, C							
935	Tunisia	c.100C>T	p.R34X	Exon 7	Congenital	Profound	Thili et al. [42]
	Iran	c.100C>T	p.R34X	Exon 7	Congenital	Severe to profound	Hilgert et al. [41]
Nas	Lebanon/Jordan	c.100C>T	p.R34X	Exon 7	Congenital	Severe to profound	Hilgert et al. [41]
685	Turkey	c.100C>T	p.R34X	Exon 7	Congenital/Prelingual	Severe to profound	Sirmaci et al. [43]
L787	Iran	c.150delT	p.N50KfsX26	Exon 7	Congenital	Profound	Yang et al. [35]
M36	Iran	c.236+1G>A	Splice disruption	Intron 7	Congenital	Severe to profound	Hilgert et al. [41]
IN-DKB6	North America (Indian)	c.295delA	p.K99KfsX4	Exon 8	Prelingual	Severe to profound	Kurima et al. [23]
PKSR25	Pakistan	c.536-8T>A	Splice disruption	Intron 10	Prelingual	Severe to profound	Kurima et al. [23]
4090	Pakistan	c.536-8T>A	Splice disruption	Intron 10	Prelingual	Severe to profound	Santos et al. [37]
DFI39	Turkey	c.767delT	p.F255FfsX14	Exon 13	Congenital	Severe to profound	Hilgert et al. [41]
TR56	Turkey	c.821C>T	p.P274L	Exon 13	Prelingual	Profound	Kalay et al. [38]
4049	Pakistan	c.830A>G	p.Y277C	Exon 13	Prelingual	Severe to profound	Santos et al. [37]
PKSR1a	Pakistan	c.884+1G>A	Splice disruption	Intron 13	Prelingual	Severe to profound	Kurima et al. [23]
TR56	Turkey	c.1083_1087delCAGAT	p.R362PfsX6	Exon 15	Prelingual	Profound	Kalay et al. [38]
4119, 4160	Pakistan	c.1114G>A	p.V372M	Exon 15	Prelingual	Severe to profound	Santos et al. [37]
Family D							
Fay	Tunisia	c.1165C>T	p.R389X	Exon 15	Congenital	Profound	Thili et al. [42]
	Lebanon/Jordan	c.1165C>T	p.R389X	Exon 15	Congenital	Severe to profound	Hilgert et al. [41]
DFI35	Turkey	c.1166G>A	p.R389Q	Exon 15	Congenital	Severe to profound	Hilgert et al. [41]
D555	China	c.1209G>C	p.W403C	Exon 15	Prelingual	Severe to profound	Yang et al. [47]
232	Turkey	c.1330G>A	p.G444R	Exon 16	Congenital/Prelingual	Profound	Sirmaci et al. [43]

Family	Origin	Nucleotide mutation ^a	Predicted effect	Exon/Intron number	Onset of HL	Severity of HL	Reference
647	Turkey	c.1333C>T	p.R445C	Exon 16	Congenital/Prelingual	Severe to profound	Sirmaci et al. [43]
TR56	Turkey	c.1334G>A	p.R445H	Exon 16	Prelingual	Profound	Kalay et al. [38]
PKSR20a	Pakistan	c.1534C>T	p.R512X	Exon 17	Prelingual	Severe to profound	Kurima et al. [23]
PKDF431	Pakistan	c.1541C>T	p.P514L	Exon 17	Prelingual	Severe to profound	Kitajiri et al. [40]
PKDF329, PKDF511	Pakistan	c.1543T>C	p.C515R	Exon 17	Prelingual	Severe to profound	Kitajiri et al. [40]
W06-792	Netherlands	c.1763+3A>G	p.W588W/IsX81	Intron 19	Postlingual	Profound 1st decade: high frequencies 2nd–3rd decade: all frequencies	de Heer et al. [44]
Family E	Tunisia	c.1764G>A	p.W588X	Exon 20	Congenital	Profound	Tlili et al. [42]
GRE	Greece	c.1810C>T	p.R604X	Exon 20	Congenital	Severe to profound	Hilgert et al. [41]
IN-M17	North America (Indian)	c.1960A>G	p.M654V	Exon 20	Prelingual	Severe to profound	Kurima et al. [23]
4070, 4138	Pakistan	c.2004T>G	p.S668R	Exon 21	Prelingual	Severe to profound	Santos et al. [37]
PKDF419	Pakistan	c.2004T>G	p.S668R	Exon 21	Prelingual	Severe to profound	Kitajiri et al. [40]
675	Turkey	c.2030T>C	p.I677T	Exon 21	Congenital/Prelingual	Profound	Sirmaci et al. [43]
4008, 4033	Pakistan	c.2035G>A	p.E679K	Exon 21	Prelingual	Severe to profound	Santos et al. [37]
551	Turkey	c.1696_2283del ^e	Genomic deletion		Congenital/Prelingual	Profound	Sirmaci et al. [43]
<i>Compound heterozygous state^c</i>							
D419	China	c.150delIT c.1107C>A	p.N50KfsX25 p.N369K	Exon 7 Exon 9	Prelingual	Severe to profound	Yang et al. [47]
D472	China	c.236+1G>C c.1334G>A	Splice disruption p.R445H	Intron 7 Exon 16	Prelingual	Severe to profound	Yang et al. [47]
21	Europe	c.458G>A c.1763+3A>G	p.W153X Splice disruption	Exon 10 Intron 19	Prelingual	Moderate to profound	Schrauwen et al. [46]
1953	China	c.589G>A c.1171C>T	p.G197R p.Q391X	Exon 11 Exon 15	Prelingual	Severe to profound	Gao et al. [48]
Not described	Sudan	c.1165C>T c.1763+5G>A	p.R389X Splice disruption	Exon 13 Intron 19	Congenital	Profound	Meyer et al. [39]
D28C	Morocco (Jewish)	c.1810C>T c.1939T>C	p.R604X p.S647P	Exon 20 Exon 20	Not available	Profound	Brownstein et al. [45]

Abbreviation: HL, hearing loss.

^aThe numbering is based on cDNA sequence (GenBank accession number: NM_138691.2).

^bHomozygous state indicates that the patients have same mutations on both alleles.

^cCompound heterozygous state indicates that the patient have different mutations on each allele.

^dThe mutation is a 27-kb deletion encompassing exons 4–5 and adjacent introns.

^eThe mutation is a 431-kb deletion encompassing exons 19–24 with intron 18 and downstream sequences of 3' untranslated region.

Table 3

Prevalence of DFNB7/B11 among deaf populations

Reference	Study family/patient criteria	Origin	Number of families/patients with <i>TMC1</i> mutations (%)	Method to detect <i>TMC1</i> mutations
Kurima et al. [23]	Consanguineous families segregating autosomal recessive, nonsyndromic, prelingual, severe to profound SNHL	Pakistan and North America (Indian)	11/230 families (4.8%)	Linkage analysis and dideoxy sequencing ^b
Kitajiri et al. [40]	Consanguineous families segregating autosomal recessive, prelingual, severe to profound SNHL	Pakistan	19/557 families (3.4%)	Linkage analysis and dideoxy sequencing ^b
Santos et al. [37]	Families segregating autosomal recessive, nonsyndromic, prelingual, severe to profound SNHL Each family includes at least two affected individuals	Pakistan	8/180 families (4.4%)	Linkage analysis and dideoxy sequencing ^b
Kalay et al. [38]	Consanguineous families segregating autosomal recessive, nonsyndromic, prelingual, severe to profound SNHL Families with GJB2 mutations were excluded	Turkey	4/93 families (4.3%)	Linkage analysis and dideoxy sequencing ^b
Tilli et al. [42]	Consanguineous families segregating autosomal recessive, nonsyndromic, congenital, moderate to profound SNHL Families with GJB2 mutations were excluded	Tunisia	5/85 families (5.9%)	Linkage analysis and dideoxy sequencing ^b
Sirmaci et al. [43]	Consanguineous families segregating autosomal recessive, nonsyndromic, congenital or prelingual, severe to profound SNHL Families with GJB2 mutations were excluded	Turkey	7/86 families (8.1%)	Linkage analysis and dideoxy sequencing ^b
Schrauwen et al. [46]	Patients with autosomal recessive, nonsyndromic, moderate to profound SNHL Patients with GJB2 mutations were excluded	Europe	1/24 patients (4.2%)	High-throughput sequencing of 34 deafness genes
Yang et al. [47]	Patients with nonsyndromic, prelingual or early onset, severe to profound SNHL ^a	China	3/190 patients (1.6%)	High-throughput sequencing of 79 deafness genes

Abbreviation: SNHL, sensorineural hearing loss.

^aThis study included 137 simplex and 53 multiplex probands. Of the multiplex probands, seven were from families with dominant segregation; 42 from families with probable recessive inheritance and four from families with maternal inheritance.

^bLinkage analysis to DFNB7/B11 interval was performed among family members and dideoxy sequencing of coding exons and flanking introns of *TMC1* was performed among the linked families.

Table 4

Tmc1 mutant mouse strains

Mouse mutant	Mutagenesis method	Nucleotide mutation ^a	Predicted effect	Exon number	Reference
<i>Beethoven (Bth)</i>	ENU	c.1235T>A	p.M412K	Exon 13	Vreugde et al. [51]
<i>deafness (dn)</i>	Spontaneous	c.1387_1557del ^c	p.(1463_Q519del)		Kurima et al. [23] Deol et al. [52]
<i>baringo</i>	ENU	c.545A>G	p.Y182C	Exon 8	Manji et al. [53]
<i>nice</i>	ENU	c.1345T>C	p.Y449H	Exon 13	Manji et al. [53]
<i>stitch</i>	ENU	c.1661G>T	p.W554L	Exon 15	Manji et al. [53]
<i>Tmc1 b</i>	Targeted deletion				Kawashima et al. [24]

Abbreviation: ENU, N-ethyl-N-nitrosourea.

^aThe numbering is based on cDNA sequence (GenBank accession number: NM_028953.2).

^bExons 8–9 and adjacent introns were replaced with a gene trap construct fused to a *LacZ* reporter gene.

^cThe mutation is a 1656-bp deletion encompassing 171-bp exon 14 with adjacent introns.