Published in final edited form as:

Ann Otol Rhinol Laryngol. 2010 December ; 119(12): 830–835.

Mutations in *TMC1* are a common cause of DFNB7/11 hearing loss in the Iranian population

Michael S. Hildebrand, PhD^{1,*}, Kimia Kahrizi, MD^{2,*}, Catherine J. Bromhead, BSc^{3,*}, A. Eliot Shearer, BSc¹, Jennifer A. Webster, BA⁴, Hossein Khodaei⁵, Rezvan Abtahi⁶, Niloofar Bazazzadegan², Mojgan Babanejad², Nooshin Nikzat², William J. Kimberling, PhD⁷, Dietrich Stephan, PhD^{4,8,9}, Partick L.M. Huygen, PhD¹⁰, Melanie Bahlo, PhD³, Richard J.H. Smith, MD^{1,11,*}, and Hossein Najmabadi, PhD^{2,*}

¹ Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, IA, USA

² Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

³ Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

⁴ Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA

⁵ Yazd Welfare Organization, Yazd, Iran

⁶ Omid Counseling Center, Saveh, Markazi, Iran

⁷ Department of Genetics, Boys Town National Research Hospital, Omaha, NE, USA

⁸ Arizona Alzheimer's Consortium, Phoenix, AZ, USA

⁹ Banner Alzheimer's Institute, Phoenix, AZ, USA

¹⁰ Department of Otorhinolaryngology, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, The Netherlands

¹¹ Interdepartmental PhD Program in Genetics, Department of Otolaryngology, University of Iowa, Iowa City, Iowa City, IA, USA

Abstract

Objectives—To investigate the cause of autosomal recessive non-syndromic hearing loss (ARNSHL) segregating in two consanguineous Iranian families.

Methods—Otologic and audiometric examinations were performed on affected members of each family. Genome-wide parametric multipoint linkage mapping using a recessive model was performed with Affymetrix 50K GeneChips or short tandem repeat polymorphisms (STRPs). Direct sequencing was used to confirm the causative mutation in each family.

Results—In two Iranian families, L-1651 and L-8600606, segregating ARNSHL that mapped to the DFNB7/11 locus, homozygosity for a reported splice site mutation (c.776+1G>A) and a novel deletion (c.1589_1590delCT; p.S530*) were identified in the *TMC1* gene, respectively.

Conclusions—Consistent with the previously reported phenotype in DFNB7/11 families, the two Iranian families segregate congenital, profound hearing impairment. However, in family L-1651 one affected family member (IV:3) has milder hearing impairment than expected, suggesting a potential genetic modifier effect. These results indicate that DFNB7/11 is a common form of genetic hearing loss in Iran since this population is the source of six of the 29 *TMC1* mutations reported worldwide.

Keywords

DFNB7/11; *TMC1* gene; deletion; splice site mutation

*Corresponding authors: Dr. Richard J.H. Smith, MD Department of Otolaryngology – Head and Neck Surgery University of Iowa, 5270 CBRB Building, Iowa City, IA 52242, USA Tel: +319 335 6501, Fax: +613 353 5869 richard-smith@uiowa.edu Dr. Hossein Najmabadi, PhD Genetics Research Center University of Social Welfare and Rehabilitation Sciences, Tehran, Iran hnajm12@yahoo.com.

*These two authors contributed equally to this work

INTRODUCTION

Hearing impairment is a common sensory and genetically heterogeneous disorder. Recessively inherited deafness accounts for ~80% of genetic hearing loss cases. To date, 34 genes and 85 genetic loci have been implicated in autosomal recessive non-syndromic hearing loss (ARNSHL)¹. Most of these ARNSHL genes have been identified by linkage analysis of consanguineous ARNSHL families.

Mutations in the *TMC1* gene at the DFNB7/11 locus on chromosome 9q31-21 are a common cause of ARNSHL. To date, 30 recessive mutations have been reported in 48 families worldwide²⁻¹⁰. This number makes DFNB7/11 the sixth most common cause of ARNSHL¹¹. *TMC1* has also been implicated in ADNSHL at the DFNA36 locus, however, this type of hearing loss is much less common with only three mutations reported^{2, 9, 12, 13}. Recessive mutations in *TMC1* lead to congenital and profound hearing loss, while dominant mutations are associated with progressive hearing loss that initially affects the high frequencies⁹.

The function of the transmembrane channel-like 1 (TMC1) protein in the inner ear is unknown although its expression localizes to outer hair cells of the mouse inner ear after P3¹⁴. *Tmc1* deficiency in the recessive mouse mutant *dn* triggers hair cell degeneration and leads to profound hearing loss, while semi-dominance for the *Tmc1* p.M412K allele in the heterozygous *Bth* mutant causes progressive hearing loss, consistent with the genotype-phenotype correlations observed in human patients^{14, 15}.

In this study we report genetic and phenotypic analyses of two consanguineous Iranian families segregating ARNSHL. A novel deletion, a previously reported splice site mutation and a potential genetic modifier effect are identified and described in these families. This report provides further evidence that mutations at the DFNB7/11 loci are a frequent cause of NSHL.

MATERIALS AND METHODS

Families

Families L-1651 and L-8600606 are consanguineous Iranian families segregating ARNSHL (Fig.1). Audiologic testing was completed to document the degree of hearing loss in all affected family members (Fig.2). Vestibular dysfunction and a syndromic presentation were excluded through examination by an otolaryngologist and clinical geneticist.

All participating family members gave written informed consent. Ten milliliters of whole blood was obtained from each family member by venipuncture and genomic DNA was extracted as described previously¹⁶. Human research institutional review boards at the Welfare Science and Rehabilitation University and the Iran University of Medical Sciences, Tehran, Iran, and the University of Iowa, Iowa City, Iowa, USA approved all procedures.

SNP Genotyping and Linkage Analysis of Family L-1651

Genomic DNA from individuals III:2, IV:1, IV:2, IV:3, IV:4, IV:5, V:1 and V:2 in family L-1651 (Fig.1) was genotyped for 50,000 SNPs using Affymetrix 50K XBA GeneChips at the Translational Genomics Research Institute (TGEN, Phoenix, Arizona). Genotypes were determined using the BRLMM genotyping algorithm^{13, 14}. A subset of 7,993 single nucleotide polymorphisms (SNPs) with high heterozygosity spaced approximately 0.3 cM apart across the genome was chosen from the 50K XBA set to satisfy the linkage equilibrium requirements of the Lander-Green algorithm for multipoint linkage analysis¹⁷. Selection and assembly of the data files were performed with linkdatagen

(<http://bioinf.wehi.edu.au/software>)¹⁷. Mendelian inheritance errors were identified and removed with linkdatagen, and MERLIN¹⁶ was used to remove genotyping errors identified based on inferred unlikely double recombination events between tightly linked markers.

An autosomal recessive, genome-wide parametric linkage analysis was performed with MERLIN since males and females appeared equally affected. Haplotypes inferred with Merlin were imported into HaploPainter¹⁸.

STRP analysis of Family L-8600606

Short tandem repeat (STRP) analysis was completed using four markers developed by the Cooperative Human Linkage Center and made available through Research Genetics Incorporated (Invitrogen, Carlsbad, CA) according to a published methodology¹⁸. Markers with high heterozygosity were selected for this analysis.

PCR and Sequencing

The *TMC1* gene was amplified using previously reported gene-specific primers¹⁰. Amplification reactions were cycled using a standard protocol on a GeneMate Genius thermocycler (ISC BioExpress, UT, USA). Sequencing was completed with BigDye™ v3.1 Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Sequencing products were read using an ABI 3730s sequencer (Perkin Elmer, Waltham, MA). All sequencing chromatograms were compared to published cDNA sequence; nucleotide changes were detected using Sequencher v4.5 (Gene Code Corporation, Ann Arbor, MI).

RESULTS

Clinical Data

In consanguineous Iranian family L-1651 two individuals were diagnosed with ARNSHL (Fig.1). The hearing loss is congenital, moderate-to-profound across all frequencies and stable over time. Variation in the severity of hearing impairment is apparent between individuals IV:3 and IV:5 (Fig.2A). Individual IV:5 has profound hearing impairment across all frequencies that is typical of ARNSHL, whereas individual IV:3 has a significantly milder hearing impairment that is moderate at the higher frequencies.

Five individuals in consanguineous Iranian family L-8600606 were diagnosed with ARNSHL (Fig.1). Audiometric data were available from three affected family members. The hearing loss is congenital, profound across all frequencies and stable over time (Fig. 2B).

Linkage Mapping

Genome wide linkage analyses of GeneChip data from family L-1651 detected 511 Mendelian errors. Due to the small number of affected individuals, the highest parametric LOD score was only 1.76. This score was obtained for a single region on chromosome 9 that spans approximately 12 cM and is flanked by markers SNP_A-1744539 (rs4086590) and SNP_A-1673794 (rs10491841) at chromosomal position 9p11.2-p21.13 (43.4-79.2 Mb) (Fig.3A). Haplotype analysis revealed that the critical region coincided with the region identified in the linkage analysis (Fig.3B). The critical region overlaps the DFNB7/11 locus.

STRP analysis using four highly polymorphic markers suggested that DFNB7/11 was also the likely disease locus for family L-8600606 based on homozygosity for two of these markers shared by affected individuals (data not shown).

Mutation Analysis

The entire coding region and splice sites of the *TMC1* gene were sequenced in both families. In family L-1651 a previously reported homozygous splice site mutation (c.776+1G>A) was identified that changes the conserved 5' splice donor site of exon 7 from GT to AT (Fig.4A). This mutation segregated with the affected members of this family and has been shown to be absent in 100 Iranian control individuals¹⁰.

In family L-8600606, we identified a novel homozygous two base-pair deletion (c.1589-1590delCT) in exon 18 leading to a predicted frameshift that immediately introduces a premature stop codon (p.S530*; Fig.4B). This mutation segregated with affected members of family L-8600606 and has been shown to be absent in 56 Iranian control individuals.

DISCUSSION

Our data provide further evidence that *TMC1* mutations are common amongst ARNSHL cases worldwide. We report here the 31st recessive mutation in *TMC1*, c.1589-1590delCT, and the second incidence of the c.776+1G>A splice site mutation. These findings mean that mutations in *TMC1* are the sixth most frequent cause of ARNSHL, only behind *GJB2*, *SLC26A4*, *MYO15A*, *OTOF* and *CDH23*¹¹. DFNB7/11 mutations also appear to be common in the consanguineous Iranian ARNSHL population, as a total of six Iranian DFNB7/11 cases have now been reported^{2, 10}.

The c.776+1G>A mutation in L-1651 was first reported in Iranian family M36¹⁰. It is therefore possible that this mutation arose as a founder effect in the Iranian population since it accounts for one-third of the DFNB7/11 cases reported in Iran and has not been observed elsewhere. However, family L-1651 originates from the Yazd province in central Iran, while family M36 is from the Mashad province in northern Iran. Although these provinces are large and distinct, they are located adjacent to each other in north western Iran, providing the possibility of common ancestry between these families.

The alteration of the 5' splice donor site of exon 7 from a GT to an AT could lead to partial exon skipping, intron retention or a significantly truncated transcript depending on whether the cryptic splice site is used¹⁰. These possibilities have not been investigated and RNA studies are not possible in this study because we cannot obtain fresh peripheral blood samples. Individuals in family M36 were reported to have congenital, severe-to-profound hearing loss¹⁰, consistent with other DFNB7/11 families. An identical phenotype was observed for individual IV:5 (Fig.2A) in family L-1651 reported here. However, the hearing impairment of the other affected family member, IV:3 (Fig.2A), was significantly less severe, in the moderate-to-severe range. In addition, the high frequency hearing of IV:3 was much better preserved. High frequency hearing loss is a hallmark of both DFNB7/11 and DFNA36 hearing loss⁹. Individual IV:3 also carries the c.776+1G>A mutation and his milder phenotype may reflect an unidentified genetic modifier effect, which would be the first such report for a recessive *TMC1* (DFNB7/11) mutation. Notably, this phenotypic variation was not reported in family M36 in which the equivalent mutation was found to segregate with hearing loss¹⁰.

The novel indel mutation c.1589-1590delCT identified in Iranian family L-8600606 is predicted to lead to a frameshift and introduction of a premature stop codon (p.S530*), the consequence of which could be a shortened transcript that is susceptible to nonsense-mediated decay. If resistant, a severely truncated protein lacking the last 231 amino acid residues of the 760 amino acid TMC1 protein would be generated. The truncated protein would lack the last two predicted transmembrane domains, suggesting that its channel activity would be reduced or abolished. Unfortunately, we were also unable to investigate

these possibilities by RNA or protein studies since fresh peripheral blood samples are not available from patients.

The milder hearing loss exhibited by individual IV:3 in family L-1651 may be the result of a genetic modifier. Such a modifier has not been reported for DFNB7/11 hearing impairment. Other possibilities are that the c.776+1G>A mutation does not completely abolish normal splicing of *TMC1* or that the mutant transcript is not subject to nonsense-mediated decay and the truncated protein retains some residual function. This type of 'leaky splicing' has been observed for other types of deafness as well as DFNB7/11 (Patrick Huygen, personal communication). These effects are difficult to measure due to differential splicing of genes in different tissues. Even if we could obtain fresh blood samples from affected members it is possible that splicing of *TMC1* is differentially regulated in lymphocytes compared to inner ear cells.

In summary, we have identified two new DFNB7/11 families in the Iranian population. Of these families, one segregates a novel mutation and the other, a reported mutation in *TMC1*. In family L-1651, a possible genetic modifier effect is suggested based on the milder hearing impairment in one person. The results of our study suggest that mutation at the DFNB7/11 locus is a common cause of ARNSHL in the Iranian population and that the c.776+1G>A mutation may have risen due to a founder effect in this population.

Acknowledgments

We would like to thank the families for their participation in this research. Khadijeh Jalalvand and Sanaz Arzhanghi (Genetics Research Center, Tehran, Iran) are acknowledged for their contribution to this study. RJH Smith is the Sterba Hearing Research Professor, University of Iowa College of Medicine, who supported the project with National Institutes of Health (NIH)-NIDCD grant R01 DCO02842. K. Kahrizi supported this project with Iranian National Science Foundation Grant 85033/10. M. Bahlo is supported by an Australian National Health and Medical Research Council (NHMRC) Career Development Award. M. Hildebrand is supported by an NHMRC Postdoctoral Training Fellowship. AE. Shearer is supported by a Doris Duke Clinical Research Fellowship. This project was partially supported by the Iranian National Science Foundation. No researchers involved in this study report a conflict of interest.

Funding: NIH NIDCD grants R01 DC002842 (RJHS), Iranian National Science Foundation Grant 85033/10 (KK), Australian National Health and Medical Research Council (NHMRC) Career Development Award (MB), NHMRC Overseas Biomedical Postdoctoral Training Fellowship (MH) and a Doris Duke Clinical Research Fellowship (AS).

Abbreviations

TMC1	transmembrane channel-like 1 gene
ARNSHL	autosomal recessive non-syndromic hearing loss
SNHL	sensorineural hearing loss

REFERENCES

1. Van Camp, G.; Smith, RJ. Hereditary Hearing Loss Homepage. 2010. <http://hereditaryhearingloss.org/>
2. Yang T, Kahrizi K, Bazazzadeghan N, et al. A novel mutation adjacent to the Bth mouse mutation in the TMC1 gene makes this mouse an excellent model of human deafness at the DFNA36 locus. Clin Genet. 2010; 77:395–8. [PubMed: 20447146]
3. Sirmaci A, Duman D, Ozturkmen-Akay H, et al. Mutations in TMC1 contribute significantly to nonsyndromic autosomal recessive sensorineural hearing loss: a report of five novel mutations. Int J Pediatr Otorhinolaryngol. 2009; 73:699–705. Epub 2009 Feb 1. [PubMed: 19187973]

4. 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; 26:396. [PubMed: 16134132]
5. Tlili A, Rebeh IB, Aifa-Hmani M, et al. TMC1 but not TMC2 is responsible for autosomal recessive nonsyndromic hearing impairment in Tunisian families. *Audiol Neurotol.* 2008; 13:213–8. Epub 2008 Feb 7. [PubMed: 18259073]
6. 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; 25:100. [PubMed: 15605408]
7. Kitajiri SI, McNamara R, Makishima T, et al. Identities, frequencies and origins of TMC1 mutations causing DFNB7/B11 deafness in Pakistan. *Clin Genet.* 2007; 72:546–50. Epub 2007 Sep 17. [PubMed: 17877751]
8. 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; 26:591. [PubMed: 16287143]
9. 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; 30:277–84. Epub 2002 Feb 19. [PubMed: 11850618]
10. Hilgert N, Alasti F, Dieltjens N, et al. Mutation analysis of TMC1 identifies four new mutations and suggests an additional deafness gene at loci DFNA36 and DFNB7/11. *Clin Genet.* 2008; 74:223–32. Epub 2008 Jul 9. [PubMed: 18616530]
11. Hilgert N, Smith RJ, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? *Mutat Res.* 2009; 681:189–96. Epub 2008 Aug 29. [PubMed: 18804553]
12. Kitajiri S, Makishima T, Friedman TB, et al. 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; 71:148–52. [PubMed: 17250663]
13. Hilgert N, Monahan K, Kurima K, et al. Amino acid 572 in TMC1: hot spot or critical functional residue for dominant mutations causing hearing impairment. *J Hum Genet.* 2009; 54:188–90. Epub 2009 Jan 30. [PubMed: 19180119]
14. Vreugde S, Erven A, Kros CJ, et al. Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. *Nat Genet.* 2002; 30:257–8. Epub 2002 Feb 19. [PubMed: 11850623]
15. Marcotti W, Erven A, Johnson SL, et al. Tmc1 is necessary for normal functional maturation and survival of inner and outer hair cells in the mouse cochlea. *J Physiol.* 2006; 574:677–98. Epub 2006 Apr 20. [PubMed: 16627570]
16. Grimberg J, Nawoschik S, Belluscio L, et al. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Res.* 1989; 17:8390. [PubMed: 2813076]
17. Bahlo M, Bromhead CJ. Generating linkage mapping files from Affymetrix SNP chip data. *Bioinformatics.* 2009; 25:1961–2. [PubMed: 19435744]
18. Brown MR, Tomek MS, Van Laer L, et al. A novel locus for autosomal dominant nonsyndromic hearing loss, DFNA13, maps to chromosome 6p. *Am J Hum Genet.* 1997; 61:924–7. [PubMed: 9382104]

Figure 1. Pedigrees Iranian families

A. Family L-1651; genotypes for individuals carrying the c.776+1G>A mutation are shown.

B. Family L-8600606; genotypes for individuals carrying the c.1589-1590delCT mutation are shown. Open symbols = unaffected; filled symbols = affected; diagonal line = deceased.

Figure 2. Audiometric data from Iranian families

A. Family L-1651. B. Family L-8600606. Age at time of audiogram is shown in brackets.

Figure 3. Linkage mapping results for family L-1651

A. Graph showing LOD score for the region on chromosome 9. LOD scores of 0 (blue line) and 3 (green line) are indicated. B. Haplotype analysis results. Affymetrix SNP markers are shown. The aqua colored haplotype is the susceptibility haplotype. Both affecteds are homozygous by descent for this haplotype at this locus.

Figure 4. Mutation analysis results for Iranian families

A. Reported *TMC1* splice site mutation in family L-1651. B. Novel *TMC1* deletion in family L-8600606. The predicted frameshift and introduction of a premature stop codon are shown.