Journal of Biological Sciences and Medicine
Available online at www.jbscim.com

ISSN: 2455-5266

Research Article **Open Access**

Identification of nonsense mutation in *TMC1* gene inducing hearing loss by clinical exome sequencing

Maryam Alobathani[1], Abdullah Al Mutery[1,2*], Walaa Kamal Eddine Ahmad Mohamed[2,3] and Abdelaziz Tlili[1,2]

[1]Department of Applied Biology, College of Sciences, University of Sharjah, Sharjah, United Arab Emirates
[2]Molecular Genetics Research Laboratory, University of Sharjah, Sharjah, United Arab Emirates
[3]Universitat Autónoma de Barcelona, Spain

*Corresponding Author: aalmutery@sharjah.ac.ae

ARTICLE INFO

Article History:
Received
18 November 2018

Accepted
20 December 2018

Available online
31 December 2018

Key words:
Autosomal recessive non-syndromic hearing loss; Next-generation sequencing; Nonsense mutation; *TMC1* gene

ABSTRACT

Hearing loss is one of the most common sensorineural disorders, affecting one in 1000 individuals that can be classified into syndromic and non-syndromic. *TMC1* gene has been identified as a non-syndromic gene for both autosomal and recessive forms. In this study, UAE consanguineous family with congenital profound non-syndromic hearing loss was characterized. By using clinical exome sequencing, Sanger sequencing and PCR-RFLP, the p.Arg34X as the disease-associated variant was identified. The screening of other families with deafness revealed the presence of this nonsense mutation in one additional family and suggested that p.Arg34X is major contributor to DFNB7/11 form of deafness in UAE population. The present investigation reports to be the first study associating *TMC1* mutations to hearing loss in the GCC region.

Introduction

Hearing loss is one of the most common sensorineural disorders, affecting one in 1000 individuals; and can be classified into syndromic and non-syndromic forms. Approximately, 80% of hearing loss is non-syndromic and implicates more than 100 genes (Egilmez et al. 2016). Almost, 70% of mutations responsible for non-syndromic hearing loss (NSHL) are autosomal recessives (Belguith et al. 2009). Today, 108 loci associated with autosomal recessive non-syndromic hearing loss (ARNSHL) have been reported and 71 genes have been identified (Tlili et al. 2017b).

Mutations in the *TMC1* gene are responsible for both dominant and recessive NSHL (DFNB7/11 and DFNA36). In 2002, Kurima et al., narrowed the critical overlapping interval between DFNB7/11 and DFNA36 to 3 Mb. The bioinformatics analysis of the candidate region and Sanger sequencing of a new gene (*TMC1*) in the affected members DFNA36 and DFNB7/11, revealed the presence of mutations in the new gene *TMC1*. This gene is one of the transmembrane channels-like (*TMC*) genes that share a conserved 120 amino-acids (*TMC*

domain) of unknown function (Kurima et al. 2002a). In the cochlea, *TMC1* is required for the normal function of mechanically-activated channels (Kawashima et al. 2015; Pan and Holt. 2015; Maeda et al. 2014; Pan et al. 2013). The hearing defect has also been reported in *Tmc1* mutant mice *dn* (deafness) and *Bth* (Beethoven) (Keats et al. 1995; Vreugde et al. 2002). Electronic microscopy studies of both animal models showed a degeneration of cochlear hair cells (Vreugde et al. 2002).

TMC1 gene is one of the five major genes causing profound recessive deafness worldwide (Kitajiri et al. 2007; Kurima et al. 2002b; Riazuddin et al. 2012; Yang et al. 2013). Mutations in this gene account 3% to 6% of deafness in Turkish Tunisian, European, Indian and Pakistani populations (Kalay et al. 2005; Tlili et al. 2008; Schrauwen et al. 2013). In the present study implication of *TMC1* gene in two different consanguineous families from UAE were described for the first time.

Materials and Methods

Family recruitment and DNA extraction

In this study, two UAE families with ARNSHL were recruited. Clinical examinations and analysis excluded environmental factors and other symptoms. Informed consent from all participants and parents of subjects younger than 18 years were obtained before sample (saliva) collection. Genomic DNA has been extracted by Oragene-DNA kit (DNA Genotek, Canada). In total, DNA has been extracted from 8 family members, 92 unrelated deaf patients and 120 healthy individuals from UAE population. All experiments were already approved by the University of Sharjah Ethics Committee.

Clinical exome analysis

After checking the quality control for the DNA, capture-based method was used to prepare the clinical exome sequencing (CES) library. Biotinylated oligonucleotide capture probes (Roche Life sciences) also called as baits that were designed for the exonic regions of ~ 6800 clinically relevant genes were used to enrich by hybridization. Qubit High Sensitivity reagent was used to quantify that prepared libraries. The obtained libraries were diluted to final concentration of 2nm in 10 ul and were subjected for Cluster amplification. Once the cluster generation was completed, the flow cells were loaded on to the sequencer. Hi Seq X ten was carrying the sequencing results to generate 2X150 bp sequence reads at 100X sequencing depth (~4 GB). Sequenced data were processed to generate FASTQ files and uploaded on the FTP server for download. Data received from CES was compressed in BAM files that was processed by SAM tools (samtools-1.2), as well hg19/GRCh37 was built by mapping the paired-end (2x100 bases) DNA sequence reads that proceed the quality control to the human reference genome by BWA tools (bwa-0.7.12). Analyzing data from BAM files, to identify SNP/point mutations and short indels in the exome samples, realignment and recalibration were performed using Genome analysis toolkit (GATK) (v2.3-9). To rise the accuracy with a more precession, different variants were filtered according to specific sequencing depth (100b) with the minimum reads =10b.

Sanger sequencing

To sequence *TMC1* gene with exon 7, the following primers were designed: Forward 5'CACGATGTGGAGAATTGCTAGA3' and Reverse 5'GCATCATCAGATTAAGGCTCTC3'. Wizard SV Gel was used to purify the PCR products. Big Dye Terminator V3.1 cycle sequencing kit and the genetic analyzer ABI 3500 genetic analyzer (Applied Biosystems, Thermo Fisher Scientific, USA) were used to sequence PCR products.

PCR-RFLP

In order to check the segregation of the c.100 C>T mutation in the two affected UAE families, and to screen 92 unrelated deaf patients and 120 healthy individuals, the *TaqI* restriction enzyme has been used. PCR reaction products were digested according to manufacturer's instructions

(New England Biolabs, USA) and run on agarose 2% gel.

Results

In this study, one UAE consanguineous family with ARNSHL was recruited. Audiogram analysis showed that both affected individuals have profound deafness (Figure 1). First *GJB2* gene mutations which are the most common deafness mutations in UAE population were analyzed (Tlili et al. 2017a), and absence of the *ILDR1* mutations previously reported in one UAE deaf family were verified (Tlili et al. 2017b). These analyses showed the absence of pathogenic variants in the tested chromosomal regions. Next, target genomic capturing and clinical exome sequencing for a panel of ~6800 genes including 124 genes related to hereditary deafness, was conducted using the DNA from the proband (Fig 1).

The clinical exome analysis using the proband's DNA revealed 407727 variants. To determine the causing variant, several filtrations were performed like: i) only variants located within genes responsible for deafness were considered, ii) as the family is consanguineous, only homozygous variants were kept, and iii) variants with a frequency more than 0.01 were removed. This filtration resulted in only 126 candidate DNA variations: 3 in UTR regions, 122 intronic and one nonsense (Table 1). The last one was located in the *TMC1* gene (c.100C>T) and it substitutes the Arginine amino acid at position 34 into a stop codon (p.Arg34X) (Table 1). As this variant has been associated with deafness in previous studies, it corresponds to the responsible mutation for ARNSHL observed in the proband. This finding has been confirmed by Sanger and RFLP analyses that showed the co-segregation of the c.100C>T mutation with deafness in the proband's family (Fig 1 & 2). The screening of additional 93 deaf and 120 control individuals revealed the presence of this mutation in one unrelated patient with deafness. The co-segregation of c.100C>T mutation with the disease in this patient's family has been confirmed by PCR-RFLP. Obtained data suggested that this mutation has a frequency of 2% in the UAE individuals with ARNSHL.

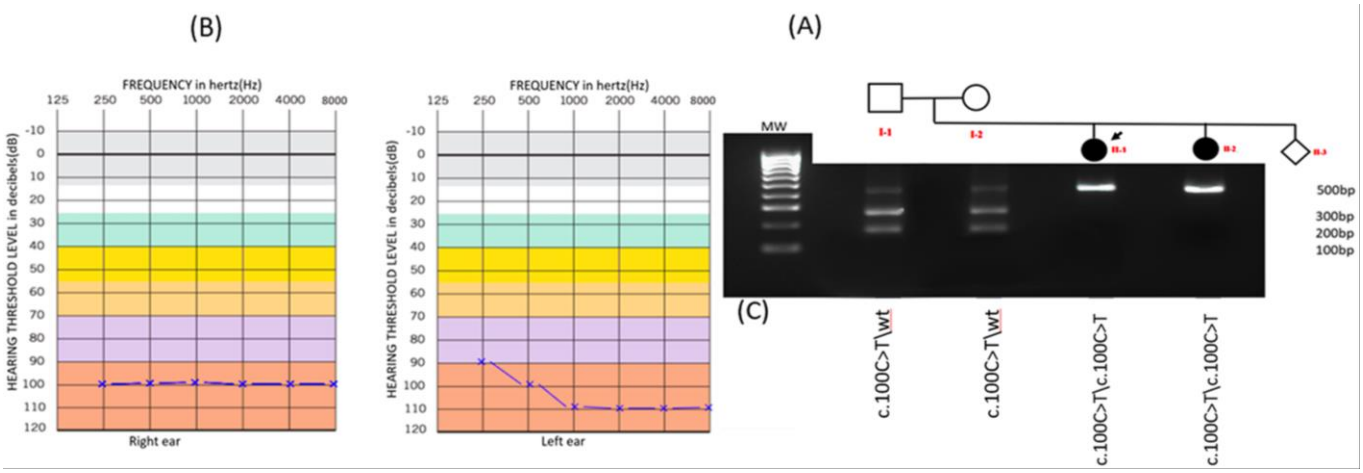

Fig 1. Pedigree of the affected family, Audiogram and PCR-RFLP analysis. (A) Pedigree of the affected family with non-syndromic hearing loss. Arrow denotes the proband. (B) Audiogram of the proband individual shows a profound sensorineural hearing loss. (C) Results of PCR-RFLP analysis of DNA of the affected family with non-syndromic hearing loss. A 427 bp PCR fragment is digested with Taq1 restriction enzyme. The wild-type DNA is cleaved into three fragments 264 and 163 bp, whereas the c.100C>T mutant allele is not cleaved. MW: DNA Ladder (100bp DNA Ladder, REF G2101).

Table 1: Filtration results of the 407727 variants obtained by the clinical exome sequencing.

Variant Class	Gene Name	c.DNA variants
3UTR	ADCY1 (+)	(c.*4682G>A)
	DCDC2 (-)	(c.*884A>T)
	TMEM132E (+)	(c.*34_*35insA)
Intronic	36 genes	122 variants
Nonsense	TMC1 gene	(c.100C>T)

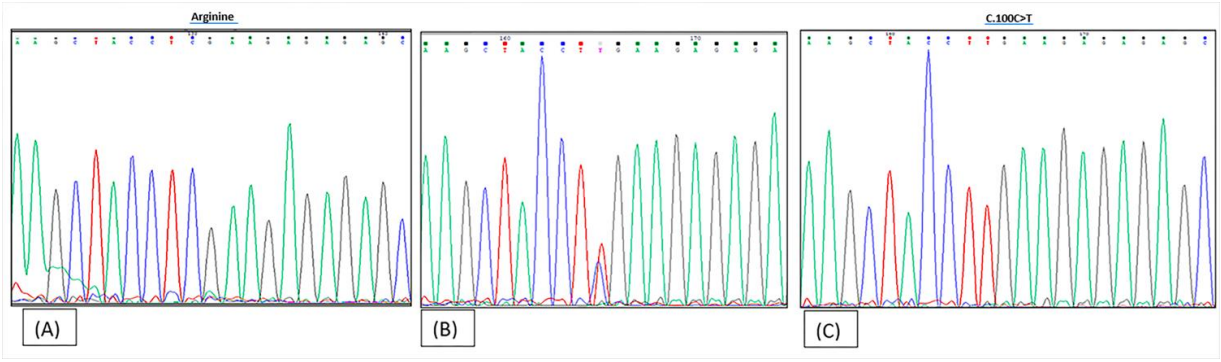

Fig 2. Electropherograms. (A) Wild-type homozygous normal individual, (B) Heterozygous individual. (C) Affected individual with the c.100C>T pathogenic variant in the *TMC1* gene (NC_000009.12).

Discussion

ARNSHL is a monogenic disorder with a high genetic heterogeneity. Mutation screening of common variants and predominant genes by classical and conventional methods can be sometimes efficient to identify the responsible mutation in unclassified affected individuals (Lu et al. 2018). However, to screen the total of 71 genes associated today with ARNSHL is time-consuming and expensive (Wang et al. 2018). Therefore, it was confirmed that the absence of mutations in the GJB2 gene and the *ILDR1* mutation c.804delG reported in previous studies in UAE population (Tlili et al. 2017b, Tlili et al. 2017a), finally summarizing a clinical exome of ~6800 genes including 124 deafness-related genes. Hence, homozygous nonsense mutation in the *TMC1* gene of the proband was identified which is corresponds to a transition T to C at position 100 of the coding region (c.100 C>T) substituting the Arginine 34 by a stop codon (p.Arg34X). This pathogenic variant has been previously reported in several populations with different frequencies and it was characterized by a marker founder effect (Kurima et al. 2002b,Kitajiri et al. 2007,Hilgert et al. 2008,Tlili et al. 2008,Ben Said et al. 2010,Sirmaci et al. 2009). In the present study, mutation was found in an additional UAE family and suggested that it contributes to 2% of ARNSHL in UAE population. Moreover, the audiogram revealed profound hearing loss similar to previous phenotypes associated to this mutation in many others populations (Scott et al. 1996; Tlili et al. 2008; Kitajiri et al. 2007; Sirmaci et al. 2009; Kurima et al. 2002a) which confirmed the deleterious effect of the p.Arg34X mutation.

Conclusion

To conclude, this study reports for the first time an association between *TMC1* gene mutations and ARNSHL in UAE and the GCC region. The identified mutation p.Arg34X has been detected in two different families, which suggests that it can be a major contributor to DFNB7/11 form of deafness in the UAE population.

Conflict of interest

There is no conflict of interest.

Acknowledgements

The authors are grateful to all participants for their cooperation and support. This work was supported by UoS.

References

Belguith H, Tlili A, Dhouib H, Dhouib H, Rebeh I , Lahmar I,Charfeddine I, Driss N, Ghorbel A, Ayadi H ,Masmoudi S (2009) Mutation in gap and tight junctions in patients with non-syndromic hearing loss. Biochem Biophys Res Commun 385:1-5.

Ben Said M, Hmani-Aifa M, Amar I, Mahmood Baig S,Mustapha M, Delmaghani S, Tlili A, Ghorbel A, Ayadi H, Van Camp G, J.H. Smith R, Tekin M, and Masmoudi S (2010) High frequency of the p.R34X mutation in the TMC1 gene associated with nonsyndromic hearing loss is due to founder effects. Genet Test Mol Biomarkers 14:307-311.

Egilmez OK, Kalcioglu MT (2016) Genetics of Nonsyndromic Congenital Hearing Loss. Scientifica (Cairo). 2016: 7576064.

Hilgert N, Alasti F, Dieltjens N, Pawlik B, Wollnik B, Uyguner O, Delmaghani S, Weil D, Petit C, Danis E, Yang T, Pandelia E, B. Petersen M, Goossens D, Del Favero J, Hossein Sanati M, JH Smith R, and Van Camp G (2008) Mutation analysis of TMC1 identifies four new mutations and suggests an additional deafness gene at loci DFNA36 and DFNB7/11. Clin Genet 74:223-232.

Kalay E, Karaguzel A, Caylan R , Heister A, Cremers FP, Cremers CW, Brunner HG, de Brouwer AP, Kremer H.(2005) Four novel TMC1 (DFNB7/DFNB11) mutations in Turkish patients with congenital autosomal recessive nonsyndromic hearing loss. Hum Mutat 26:591.

Kawashima Y, Kurima K, Pan B, Griffith AJ, Holt JR.(2015) Transmembrane channel-like (TMC) genes are required for auditory and vestibular mechanosensation. Pflugers Arch 467:85-94.

Keats BJ, Nouri N, Huang JM, Money M, Webster DB, Berlin CI. (1995) The deafness locus (dn) maps to mouse chromosome 19. Mamm Genome 6:8-10.

Kitajiri SI, McNamara R, Makishima T, Husnain T, Zafar AU, Kittles RA, Ahmed ZM, Friedman TB, Riazuddin S, Griffith AJ.(2007) Identities, frequencies and origins of TMC1 mutations causing DFNB7/B11 deafness in Pakistan. Clin Genet 72:546-550.

Kurima K, Peters LM, Yang Y, Riazuddin S, Ahmed ZM, Naz S, Arnaud D, Drury S, Mo J, Makishima T, Ghosh M, Menon PS, Deshmukh D, Oddoux C, Ostrer H, Khan S, Riazuddin S, Deininger PL, Hampton LL, Sullivan SL, Battey JF Jr, Keats BJ, Wilcox ER, Friedman TB, Griffith AJ. (2002a) Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. Nat Genet 30:277-284.

Kurima K, Peters LM, Yang Y, Riazuddin S, Ahmed ZM, Naz S, Arnaud D, Drury S, Mo J, Makishima T, Ghosh M, Menon PS, Deshmukh D, Oddoux C, Ostrer H, Khan S, Riazuddin S, Deininger PL, Hampton LL, Sullivan SL, Battey JF Jr, Keats BJ, Wilcox ER, Friedman TB, Griffith AJ. (2002b) Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. Nat Genet 30:277-284.

Lu Y, Yao J, Wei Q, Lu YYao JXu J, Xing G, Cao X (2018) Genetic analysis of CLDN14 in the Chinese population affected with non-syndromic hearing loss. Int J Pediatr Otorhinolaryngol 105:6-11.

Maeda R, Kindt KS, Mo W, Morgan CP, Erickson T, Zhao H, Clemens-Grisham R, Barr-Gillespie PG, Nicolson T. (2014) Tip-link protein protocadherin 15 interacts with transmembrane channel-like proteins TMC1 and *TMC2*. Proc Natl Acad Sci U S A 111:12907-12912.

Pan B, Geleoc GS, Asai Y, Horwitz GC, Kurima K, Ishikawa K, Kawashima Y, Griffith AJ, Holt JR.(2013) TMC1 and *TMC2* are components of the mechanotransduction channel in hair cells of the mammalian inner ear. Neuron 79:504-515.

Pan B, Holt JR (2015) The molecules that mediate sensory transduction in the mammalian inner ear. Curr Opin Neurobiol 34:165-171.

Riazuddin S, Belyantseva IA, Giese AP, Lee K, Indzhykulian AA, Nandamuri SP, Yousaf R, Sinha GP, Lee S, Terrell D, Hegde RS, Ali RA, Anwar S, Andrade-Elizondo PB, Sirmaci A, Parise LV, Basit S, Wali A, Ayub M, Ansar M, Ahmad W, Khan SN, Akram J, Tekin M, Riazuddin S, Cook T, Buschbeck EK, Frolenkov GI, Leal SM, Friedman TB, Ahmed ZM. (2012) Alterations of the CIB2 calcium- and integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48. Nat Genet 44:1265-1271.

Schrauwen I, Sommen M, Corneveaux JJ, Reiman RA, Hackett NJ, Claes C, Claes K, Bitner-Glindzicz M, Coucke P, Van Camp G, Huentelman MJ. (2013) A sensitive and specific diagnostic test for hearing loss using a microdroplet PCR-based approach and next generation sequencing. Am J Med Genet A 161A:145-152.

Scott DA, Carmi R, Elbedour K, Yosefsberg S, Stone EM, Sheffield VC (1996) An autosomal recessive nonsyndromic-hearing-loss locus identified by DNA pooling using two inbred Bedouin kindreds. Am J Hum Genet 59:385-391.

Sirmaci A, Duman D, Ozturkmen-Akay H, Erbek S, Incesulu A, Ozturk-Hismi B, Arici ZS, Yuksel-Konuk EB, Tasir-Yilmaz S, Tokgoz-Yilmaz S, Cengiz FB, Aslan I, Yildirim M, Hasanefendioglu-Bayrak A, Aycicek A, Yilmaz I, Fitoz S, Altin F, Ozdag H, Tekin

M. (2009) Mutations in TMC1 contribute significantly to nonsyndromic autosomal recessive sensorineural hearing loss: a report of five novel mutations. Int J Pediatr Otorhinolaryngol 73:699-705.

Tlili A, Al Mutery A, Kamal Eddine Ahmad Mohamed W, Mahfood M, Hadj Kacem H (2017a) Prevalence of GJB2 Mutations in Affected Individuals from United Arab Emirates with Autosomal Recessive Nonsyndromic Hearing Loss. Genet Test Mol Biomarkers 21:686-691.

Tlili A, Fahd Al Mutery A, Mahfood M, Kamal Eddine Ahmad Mohamed W, Bajou K (2017b) Identification of a novel frameshift mutation in the ILDR1 gene in a UAE family, mutations review and phenotype genotype correlation. PLoS One 12:e0185281.

Tlili A, Rebeh IB, Aifa-Hmani M, Dhouib H, Moalla J, Tlili-Chouchène J, Said MB, Lahmar I, Benzina Z, Charfedine I, Driss N, Ghorbel A, Ayadi H, Masmoudi S. (2008) *TMC1* but not *TMC2* is responsible for autosomal recessive nonsyndromic hearing impairment in Tunisian families. Audiol Neurootol 13:213-218.

Vreugde S, Erven A, Kros CJ, Marcotti W, Fuchs H, Kurima K, Wilcox ER, Friedman TB, Griffith AJ, Balling R, Hrabé De Angelis M, Avraham KB, Steel KP. (2002) Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. Nat Genet 30:257-258.

Wang X, Wang L, Peng H, Yang T, Wu H. (2018) A Novel p.G141R Mutation in ILDR1 Leads to Recessive Nonsyndromic Deafness DFNB42 in Two Chinese Han Families. Neural Plast 2018:7272308.

Yang T, Wei X, Chai Y, Li L, Wu H. (2013) Genetic etiology study of the non-syndromic deafness in Chinese Hans by targeted next-generation sequencing. Orphanet J Rare Dis 8:85-1172-8-85.