Mutations in *OTOF, CLDN14 & SLC26A4* genes as major causes of hearing impairment in Dhadkai village, Jammu & Kashmir, India

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Background & objectives: A high incidence of hearing impairment is reported from the village of Dhadkai in the State of Jammu and Kashmir, India. Prevalence of endogamy in this community suggested a common genetic basis for the disorder. A genetic study was undertaken to ascertain the basis for the high incidence of hearing impairment in this region.

Methods: In a two-step approach to identify the causative mutation/s, a whole-genome-based linkage analysis of an extended family of 45 members was carried out, which included 23 affected and 22 unaffected members. Mutational analysis for the candidate deafness genes helped reveal causative mutations in the family. In addition, seven deafness-causing genes, *Cx26, SLC26A4, CLDN14, TMPRSS3, TMC1, TMIE* and *USH1C,* were analyzed in smaller families with hearing impairment.

Results: In the 45-member extended family, the critical chromosomal region mapped to 2p24-p22. The c.2122C>T (p.R708X) mutation in *OTOF* in 2p24-p22 was identified as being the causal change. Linkage to 2p24-p22 locus was not observed in a particular branch of this extended family. Analysis of seven known deafness-causing genes in this branch revealed a mutation, c.254T>A (p.V85D), in *CLDN14*. Among seven small families unrelated to the 45-member extended family, hearing loss was attributable to p.R708X in *OTOF* in three families and to p.V85D in *CLDN14* in one family; a new mutation c.1668T>A (p.Y556X) *SLC26A4* was identified in two families and the causative change could not be identified in one family.

Interpretation & conclusions: This study suggested considerable genetic heterogeneity in the causation of hearing loss in Dhadkai. Recessive mutations were observed in at least three genes causing hearing loss: *OTOF* (p.R708X), *SLC26A4* (p.Y556X) and *CLDN14* (p.V85D). Mutation p.R708X appeared to be the major cause of hearing impairment in Dhadkai.

Key words Autosomal recessive non-syndromic hearing loss - *CLDN14* - genetic heterogeneity - hearing impairment - *OTOF* - *SLC26A4*

Hearing loss constitutes the most common form of sensory defect, affecting one in 2000 newborns. Nearly 50 per cent of hearing impairment has a genetic basis to its aetiology. About 85 per cent of hereditary hearing loss is recessive in inheritance and non-syndromic in manifestation. To date, 88 genes and 145 loci implicated in non-syndromic hearing loss (NSHL) have been identified (hereditary hearing loss website: *http://hereditaryhearingloss.org/*). Mutations in a single gene connexin 26 (*Cx26*) are the most common cause of hearing loss worldwide[1].

Dhadkai is a remote village located in Doda district of Jammu and Kashmir, India. According to the 2011 census, the village had a total population of 1774 inhabitants as 253 families (*http://censusindia.gov.in*). A majority of individuals in this village belong to the *Gujjar* tribe, which is an endogamous community. A high incidence of hearing loss has been reported from Dhadkai. In 2005, 72 cases of hearing-impaired individuals were reported in a survey conducted by the State's Social Welfare Department[2]. Other reports indicated an increase in the number of hearing-impaired individuals in this village[2,3]. To understand the genetic basis of this disorder, one extended family and seven small families were studied from this village.

Material & Methods

This study was conducted during 2004-2009 as a part of a deafness project carried out at Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bengaluru, India. Selection of families and collection of blood samples were carried out by scientists from University of Kashmir, Srinagar, and department of Audiology and Ali Yavar Jung National Institute for the Hearing Handicapped (AYJNIHH), Mumbai. Audiological assessment of members was carried out by AYJNIHH. This study was approved by the Institutional Human Bioethics and Biosafety Committee of JNCASR, the main coordinating centre for the study. Up to 10 ml of peripheral blood was collected after obtaining written informed consent for participation. DNA was extracted using the phenol-chloroform method.

A selected set of eight densely affected families comprising about 50 per cent of the affected individuals from Dhadkai village were examined. Families with one or more members exhibiting prelingual NSHL were selected. A large family (KSH01) was enrolled for genome-wide linkage analysis on the bases of its multi-generational nature and the presence of multiple affected and unaffected members. In case of singleton-affected families, samples of both parents and, in families with more than one affected member, sample of one parent were necessary. The mean age of hearing impaired members was 14 yr (range between 3 and 45 yr). The male/female ratio for affected members was 1.2:1. In KSH01 with 45 members, there were 23 affected and 22 unaffected members (Fig. 1A). A three-generation branch of KSH01 comprising 18 members (8 affected and 10 unaffected) was subjected to whole-genome linkage mapping (Fig. 1A). Seven small families, KSH02-08 (Fig. 1B) examined, included four families with at least two affected members (KSH02, KSH03, KSH04 and KSH08) and three families (KSH05, KSH06 and KSH07) each with a single affected member. Detailed clinical histories of the affected members were collected to evaluate age of onset and to examine the possibility of a non-genetic cause for hearing loss, such as a birth defect, acoustic trauma or ear infections. The degree of hearing loss was ascertained through pure-tone audiometric evaluations for air and bone conduction. Members exhibited hearing impairment of profound, bilateral and sensorineural type (Fig. 2C). No apparent additional clinical feature was noted segregating with the hearing loss, indicating its non-syndromic nature.

Genome-wide scan and linkage analysis: Genome-wide scan was carried out by genotyping 18 members of KSH01 using ABI PRISM Linkage mapping set v2.5 MD-10 (ThermoFisher Scientific Inc., USA). In the KSH01 extended family, 27 additional members were also examined for the seven microsatellite markers D2S162, D2S168, D2S305, D2S165, D2S367, D2S2259 and D2S391 from the mapping set, for a 52.5cM genetic region in 2p25-p21. Polymerase chain reactions (PCRs) were performed with fluorescence-labelled primers and True allele™ PCR premix on a Gene Amp PCR system 9700 (Thermo Fisher Scientific Inc.), according to the manufacturer's protocol. Aliquots from the fluorescence-labelled products were pooled and denatured at 94°C for five minutes, snap chilled and electrophoresed on an ABI3730 DNA Analyzer (Thermo Fisher Scientific Inc.). A reference individual CEPH 1347-02 with known genotypes was used as an internal control. Genotypes were called using ABI PRISM GeneMapper™ v3.7 (Thermo Fisher Scientific Inc.). Genotypes were checked for Mendelian inconsistencies if any, and haplotypes were generated manually, allowing for minimum number of possible recombination events. Two-point linkage analysis was carried out using
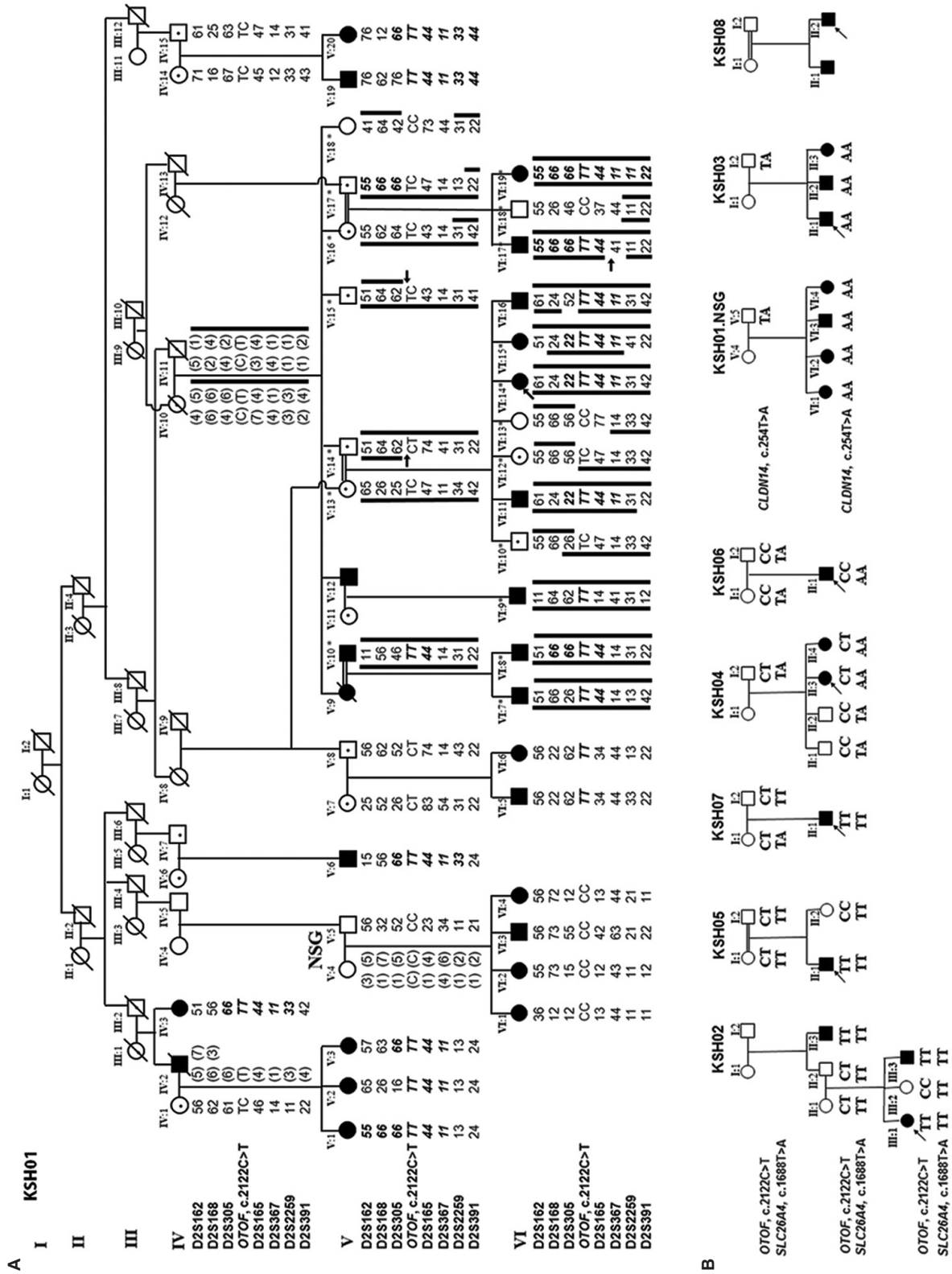
Fig.1. Pedigree analysis of KSH01-KSH08. (**A**) 2p24-p22 marker haplotypes and *OTOF*, c.2122C>T in KSH01. Members taken up for genome-wide scan (asterisk), additional affected members genotyped and their parents/ancestors are depicted. Of the 45 members of this family, all 24 affected members and 16 of 22 unaffected members (excluding 6 unaffected members from the extended family) are shown. Microsatellite markers (left-side), affected chromosomes (black bars), critical recombination boundaries (arrows), autozygous genotypes (bold italics) and inferred genotypes (parenthesis) are indicated. NSG (non-segregating) denotes the branch where linkage to 2p24-p22 was absent. (**B**) KSH02-KSH08 and KSH01.NSG showing the segregating mutations: c.2122C>T, c.254T>A and c.1668T>A. Squares, circles, filled and unfilled symbols denote males, females, affected and unaffected individuals, respectively.
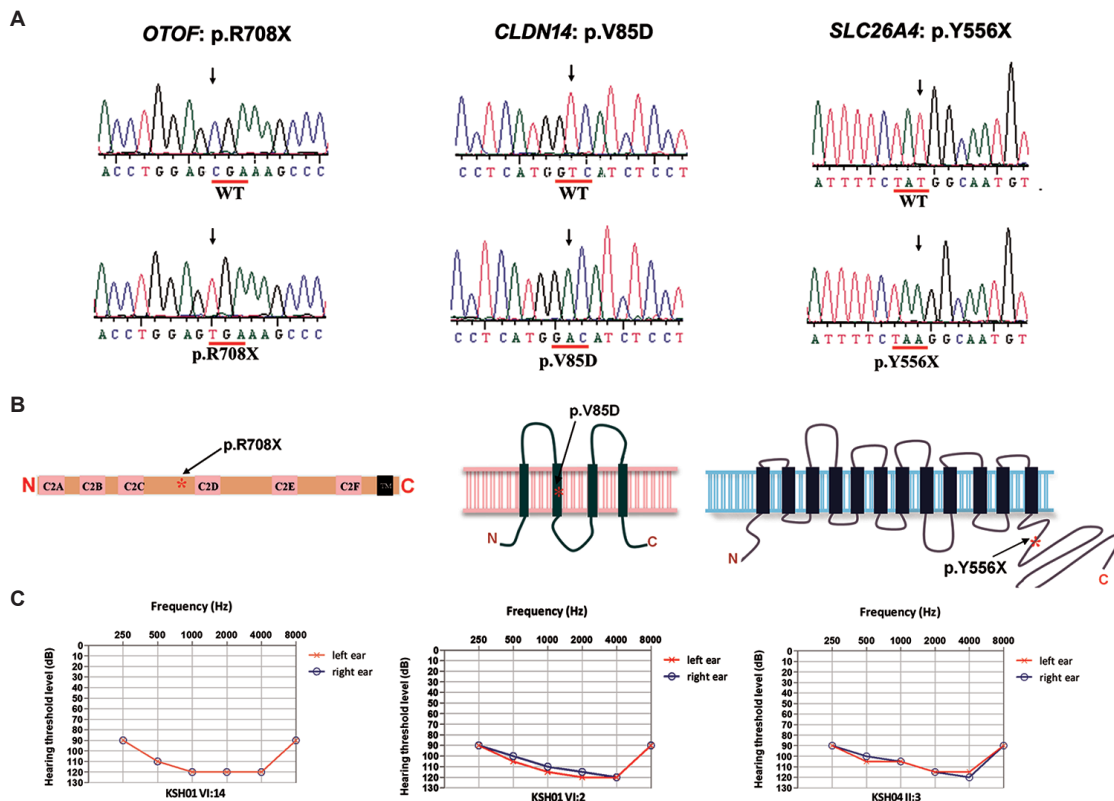
Fig. 2. Mutations *OTOF* p.R708X (c.2122C>T); *CLDN14* p.V85D (c.254T>A) and *SLC26A4* p.Y556X (c.1668T>A). (**A**) Representative sequence traces from the hearing impaired individuals exhibiting the mutations (lower panel) are shown. The corresponding wild-type alleles are shown in upper panel. (**B**) Protein schematics of *OTOF*, *CLDN14* and *SLC26A4* depicting approximate locations (asterisk) of mutations. (**C**) Audiograms of affected members KSH01-VI:14, KSH01.NSG-VI:2 and KSH04-II:3.Curves indicate the hearing thresholds for left (red) and right (blue) ears.

MLINK v5.2 of linkage[4]. Disease allele frequency was taken as 0.001 and equal frequencies were assumed for the marker alleles. Logarithm of odds (LOD) scores were computed under a recessive model with consanguinity loops at 99 per cent penetrance value and 0 per cent phenocopy.

Mutation analysis: The proband, KSH01-VI:14, was examined for mutation in *Cx26* by sequencing its coding and non-coding exons. Mutation in the otoferlin gene (*OTOF*) in VI:14 was detected by sequencing its 48 exons including the coding exons, 5'- and 3'-UTRs (untranslated regions) and intronic flanks (National Center for Biotechnology Information, *http://www.ncbi. nlm.nih.gov/mapview/*). Primer pairs were designed using Primer3 Input (*http://primer3.ut.ee/*). PCR amplification was carried out using 100 ng of genomic DNA, 10 pmol primers, 800 μM dNTPs (nucleoside triphosphates, New England Biolabs Inc., USA), 1.5 mM $MgCl_2$ and 1 U of *Taq* DNA polymerase (Thermo Fisher Scientific Inc., USA) in a 20 μl reaction volume. The amplified products were purified by Montage PCR_{96} clean-up

reagents (EMD Millipore, USA). Cycle sequencing was performed using 20 ng of purified PCR products, 2.5 pmol of primer and ABI PRISM BigDye™ Terminator cycle sequencing reagents. The sequenced products were ethanol washed, denatured in formamide, snap chilled and electrophoresed using a 3730 DNA Analyzer. Each amplicon was sequenced in both directions and analyzed using SeqManII 5.01 (DNASTAR Inc., USA).

To examine the KSH01-non-segragating (NSG) branch and KSH04 for possible mutations in *Cx26, transmembrane inner ear* (*TMIE*) and claudin 14 (*CLDN14*), bidirectional sequencing of the exons and flanking intronic regions was done in an affected member. For analysis of solute carrier family 26, member 4 (*SLC26A4)*, transmembrane protease, serine 3 (*TMPRSS3)*, transmembrane cochlear-expressed gene 1(*TMC1*) and Usher syndrome type 1 C(*USH1C*) genes, a concordance/discordance test using polymorphic microsatellite markers closely linked to the gene of interest was performed in KSH04 and KSH01.NSG (Table I). PCR was carried out with 50 ng of genomic DNA, 2.5

Table I. Autosomal recessive non-syndromic hearing loss genes analyzed in KSH01.non-segragating (NSG) and KSH04

Gene	Deafness form	OMIM	Microsatellite markers for concordance-discordance test (locations)
Cx26[5]	DFNB1	121011	-
SLC26A4[6]	DFNB4	605646	D7S2420 (425 kb-cen), D7S2459 (intragenic), D7S2456 (368 kb-tel), D7S692 (1Mb-tel)
CLDN14[7]	DFNB29	605608	-
TMPRSS3[8]	DFNB8/DFNB10	605511	D21S1260 (900 kb-cen), D21S1225 (128 kb-cen), D21S49 (83 kb-tel), D21S1411 (344 kb-tel)
TMC1[9]	DFNB7/DFNB11	606706	D9S789 (1.3 Mb-cen), D9S1822 (300 kb-tel), D9S1876 (intragenic)
TMIE[10]	DFNB6	607237	-
USH1C[11]	DFNB18		D11S902 (25 kb-tel), D11S4130 (180 kb -cen), D11S1888, (190 kb-cen), D11S4138 (175 kb-cen)

Superscript numerals denote reference numbers. Cen, centromeric; tel, telomeric, OMIM, Online Mendelian Inheritance in Man accession number; *Cx26*, Connexin 26; *SLC26A4*, Solute carrier family 26 member 4; *CLDN14*, Claudin 14; *TMPRSS3*, Transmembrane protease, serine 3; *TMC1*, Transmembrane cochlear-expressed gene 1; *TMIE*, Transmembrane inner ear; USH1C, Usher syndrome type 1C

Table II. Logarithm of odds scores for certain markers in the 2p24-p22 region in KSH01

Marker[a]	Genetic position[c] (cM)	LOD scores (Z) at recombination fraction (θ) values				
		0	0.1	0.2	0.3	0.4
D2S162	21.3	−12.60	−1.08	−0.34	−0.10	−0.03
D2S168	28.6	−1.08	0.83	0.71	0.46	0.18
D2S305[b]	40.7	−0.22	1.97	1.44	0.80	0.26
D2S165[b]	50.7	4.03	3.05	2.01	0.99	0.25
D2S367[b]	58.3	−2.34	1.09	0.73	0.32	0.05
D2S2259	67.4	−4.50	0.35	0.39	0.22	0.06
D2S391	73.8	−12.43	−1.07	−0.37	−0.11	−0.01

[a]Marker order is as per the human genome physical map (human genome map viewer build 37.3 database, NCBI, NIH, USA); [b]Markers in the linked interval; [c]Positions are according to the genethon linkage map (human genome map viewer build 37.3 database). LOD, logarithm of odds; NCBI, National Center for Biotechnology Information; NIH, National Institutes of Health

pmol primers, 1.5 mM MgCl$_2$ and 2.5 U *Taq* DNA polymerase on a GeneAmp PCR9700 and genotyping was done on a 3730 DNA Analyzer. Allele sizing was performed by GeneMapper v3.7. In those families which could not be excluded for a possible mutation in the gene on basis of concordance/discordance tests, complete *SLC26A4* (21 exons), *TMPRSS3* (13 exons), *TMC1* (24 exons) and *USH1C* (28 exons) transcripts comprising exons and flanking intronic regions were analyzed by sequencing. All members included in the study were examined for the identified mutations. In KSH04 proband, heterozygous for mutation p.R708X, the possibility of a second *OTOF* mutation leading to compound heterozygous condition was examined by sequencing all the exons of *OTOF*.

Results

Major deafness locus in family KSH01 maps to chromosome 2p24-p22: KSH01 was an extended,

six-generation family (Fig. 1A) exhibiting hearing loss transmitted in an apparently autosomal recessive manner. No *Cx26* mutation was detected in the proband. In the genome-wide scan involving 18 members of KSH01,the highest two-point LOD score obtained was 4.03 (θ=0) for D2S165 (Fig. 1A and Table II).

Haplotyping analysis identified a co-segregating marker, D2S165 amongst all the affected siblings. The recombination boundaries for the linked interval were defined by markers D2S305 and D2S367 (Fig. 1A). Genotyping the remaining members in the family for D2S165 and its neighbouring markers revealed autozygous regions in the affected siblings extending upto nearly 33.1cM (Fig. 1A: Individual V:20). The alleles shared by these siblings included D2S165 and D2S367. In affected sibs VI:5 and VI:6, though autozygosity was not noted, linkage to the same region was noted. Among the family members who underwent

genome-wide scan, autozygous regions were observed for all the members except individual VI:9. In one of the branches of KSH01, named KSH01.NSG, comprising an available parent and four affected offsprings, linkage to D2S165 or its flanking region was not observed (Fig. 1A). This suggested the presence of a second deafness-causing locus elsewhere in the genome in the NSG branch.

p.R708X mutation in OTOF in KSH01: Sequence analysis of *OTOF* in the proband identified a homozygous mutation c.2122C>T (Fig. 2A and Table III). c.2122C>T transition created a premature stop codon at amino acid position 708 (p.R708X) of the long isoform of the protein (Fig. 2B). Except in the KSH01.NSG branch, p.R708X was found to co-segregate with hearing loss in the family (Figs 1A and 2C).

p.V85D mutation in CLDN14 in the KSH01.NSG branch: To identify the possible deafness-causing gene in KSH01.NSG, seven autosomal recessive NSHL (ARNSHL) genes, namely *Cx26, SLC26A4, CLDN14, TMPRSS3, TMC1, USH1C* and *TMIE*, were taken up

for mutation analysis (Table I). These analyses revealed a homozygous mutation, c.254T>A (p.V85D), in *CLDN14*[7] (Table III and Fig. 2A). c.254T>A was found to segregate with hearing loss in KSH01.NSG (Figs 1B and 2C).

Presence of p.R708X, p.V85D as well as a new mutation p.Y556X in SLC26A4 in the Dhadkai families: Additional seven families (Fig. 1B, KSH02-08) were analyzed for p.R708X (*OTOF*) and p.V85D (*CLDN14*). Mutation p.R708X accounted for hearing loss in KSH02, KSH05 and KSH07 and p.V85D accounted for hearing loss in a single family, KSH03 (Fig. 1B and Table IV). However, in family KSH04, p.R708X was present in heterozygous condition in the two affected siblings (Fig. 1B). This suggested two possibilities: (*i*) KSH04 members had another *OTOF* mutation present elsewhere in the gene, or (*ii*) these members were carriers for p.R708X and were affected due to genetic defects elsewhere in the genome. To test the first possibility, the remaining exons of *OTOF* were sequenced in the KSH04 proband which revealed 11 known polymorphisms and a new intronic deletion,

Table III. Sequence variants observed in *OTOF* (KSH01 proband, VI: 14), *CLDN14* (KSH01.non-segragating (NSG) member, VI: 2) and *SLC26A4* (KSH04 proband, II: 3)

Nucleotide change	Location	Homozygous/heterozygous	SNP/mutation	Effect on protein
OTOF: KSH01 proband, VI: 14				
c. 244C > T	Exon 4	Homozygous	SNP: rs13031859	
c. 509+39A > T	Exon 5	Homozygous	SNP: rs7575020	
c. 2122C > T	Exon 18	Homozygous	Mutation[12]	p.R708X
c. 5655C > T	Exon 44	Homozygous	SNP: rs45442103	
CLDN14:KSH01.NSG, member VI: 2				
c. 254T > A	Exon 7	Homozygous	Mutation[7]	p.V85D
SLC26A4: KSH04 proband, II: 3				
c. 1668T > A	Exon 16	Homozygous	Mutation: this study	p.Y556X
c. 2035-210C > T	Intron 17	Homozygous	SNP: rs6955309	

OTOF, OMIM 603681, Gene ID 9381, NM_194248.2, NP_919224.1; *CLDN14*, OMIM 605608, Gene ID 23562, NM_144492.2, NP_652763.1; SLC26A4, OMIM 605646, Gene ID 5172, NM_000441.1, NP_000432.1. SNP, single nucleotide polymorphism. Superscript numerals denote reference numbers.

Table IV. Summary of study on Dhadkai families: KSH01-KSH08

Gene, mutation	Families with occurrence of mutation: 7 of 8	Families accounted by mutation: 7 of 8	Homozygous in affected: 36 (%)	Heterozygous in affected: 5 of 36 (%)	Heterozygous in unaffected: 29 of 38 (%)
OTOF, p.R708X	5	4−KSH01.NSG	24 (66.7)	2 (5.6)	24 (63.2)
CLDN14, p.V85D	2	1+KSH01.NSG	7 (19.4)	0	2 (5.3)
SLC26A4, p.Y556X	4	2	3 (8.3)	3 (8.3)	9 (23.7)
NSG, non-segregating					

c.6011+66_67del. This deletion was a benign change as it did not segregate with the phenotype in the family. A haplotype analysis with c.6011+66_67del and three polymorphisms (c.2215-291G>C, c.2215-203_-204del and c.5655C>T) confirmed that the affected members did not share a second mutation in *OTOF*.

Hearing impairment in KSH04, KSH06 and KSH08 could not be attributed to either p.R708X or p.V85D (Fig. 1). In KSH04, analysis was done for the possibility of a mutation in *Cx26, SLC26A4, TMPRSS3, TMC1, TMIE* or *USH1C*. In *SLC26A4,* a new mutation c.1668T>A (p.Y556X) was found to segregate with hearing loss in this family (Table III and Figs 1B, 2A, C). c.1668T>A was absent in 96 chromosomes of normal hearing individuals examined.This mutation introduces a stop codon in place of tyrosine at the 556 position, thereby prematurely truncating the SLC26A4 protein in its C-terminal tail (Fig. 2B). Besides KSH04, p.Y556X also accounted for hearing loss in KSH06 (Fig. 1B). In KSH08, no mutation was detected.

Among the eight families (KSH01-08) of Dhadkai studied here, there was a predominance of *OTOF*, p.R708X mutation which was present in five families and could account for hearing impairment in 24 of the 36 (66.7%) affected individuals (Table IV). Among the unaffected members from these families, the carrier frequency for p.R708X was 0.61 (Table IV). In comparison, *CLDN14*, p.V85D mutation was not frequent. p.V85D was observed in two families (Table IV). *SLC26A4*, p.Y556X mutation accounted for hearing loss in two families, and in families KSH01 and KSH07, carriers of p.Y556X were observed (Fig. 1B and Table IV). Co-occurrence of *SLC26A4,* p.Y556X and *OTOF,* p.R708X mutations was observed in three families (KSH01, KSH02 and KSH04). Family members with both p.Y556X and p.R708X mutations included six unaffected individuals who were heterozygous for both the mutations and five affected individuals who were homozygous for one mutation and were coincidentally carrying a mutant allele of the second mutation.

Discussion

The Dhadkai clan with its endogamous culture was expected to be a genetically homogeneous population with a single founder mutation in a known or novel gene. However, this study involving one large and seven small families identified mutations in three known NSHL genes: *OTOF, SLC26A4* and *CLDN14.*

OTOF mutations have been studied in deaf cohorts from Spain[13,14], Turkey[15], USA[16,17], Colombia[14], Argentina[14], Pakistan[18], Brazil[19], China[20,21], Taiwan[22], Iran[23], Japan[24,25] and Korea[26]. *OTOF* is frequently implicated in ARNSHL and many of its pathogenic alleles include stop-codon changes and deletions or duplications that result in premature truncation of the protein. p.R708X, the mutation found in KSH01, results in an abnormal termination of the long isoform of OTOF protein exclusively. The prevalence of p.R708X mutation was about 0.36 per cent in 557 families from Pakistan exhibiting prelingual, recessive, severe-to-profound hearing loss[18].

The contribution of *OTOF* mutations to the load of hereditary hearing loss in India remains largely unexplored. Previously, a splice-site mutation, IVS8-2A>G in *OTOF*, was identified in a family from Southwest India with three affected sibs[27]. In the present study, *OTOF*, p.R708X was found to be the most common mutation in Dhadkai. As evident from the KSH01 family structure, p.R708X was transmitted from a common ancestor that was at least six generations old.

Mutations in *OTOF* are frequently associated with the clinical phenotype of non-syndromic auditory neuropathy[16] or more specifically auditory synaptopathy[28]. These hearing-impaired individuals do not have pure-tone audiometric and auditory brainstem response (ABR). However, transiently evoked otoacoustic emissions (TEOAEs), either unilateral or bilateral, and/or cochlear microphonics are present in them. Individuals have unusually poor reception of speech in proportion to the degree of hearing loss, and hearing aids may provide limited benefit[16]. Cochlear implants are found to enhance sound detection and improve communication skills in such cases[29]. Thus, patients with the *OTOF*, p.R708X in Dhadkai are likely to benefit from cochlear implantation.

CLDN14, p.V85D was the second mutation observed in the clan. Although mutations in *CLDN14* have not been examined in any Indian cohort so far, p.V85D has been reported as a frequent *CLDN14* mutation of a founder origin in Pakistan[7,30]. In a cohort of 800 NSHL families from Pakistan, 12 families exhibited p.V85D mutation (prevalence of 1.5%)[30].

The third mutation noted in Dhadkai was a new mutation, p.Y556X in *SLC26A4*. Mutations in *SLC26A4* are a frequent cause of hearing loss and are causally

linked to a syndromic form of hearing impairment, Pendred syndrome[31] (MIM 274600) and NSHL with enlarged vestibular aqueduct[6] (MIM 600791).

The Dhadkai clan exemplified a case of genetic heterogeneity underlying hearing loss in a highly endogamous population. Presence of more than one mutation in a family/individual suggested assortative mating: all the three mutations p.R708X, p.Y556X and p.V85D occurred in KSH01extended family; and in smaller families KSH04 and KSH07, both p.Y556X and p.R708X were noted. The KSH08 family, wherein p.R708X (*OTOF*), p.V85D (*CLDN14*) and p.Y556X (*SLC26A4*) were absent, represented a fourth, as yet, unidentified genetic cause for hearing loss in Dhadkai.

From a sub-population perspective, allelic diversity and contribution of the NSHL genes to hearing impairment seem distinct in Dhadkai from the situation in the overall Indian population. In Dhadkai, *OTOF, SLC26A4* and *CLDN14* mutations accounted for hearing loss in 94 per cent of the cases examined. There are several possible approaches to reduce the incidence of hearing loss in Dhadkai: (*i*) appropriate genetic counselling about the risks involving deaf-deaf and intra-community marriages, and (*ii*) examining the carrier status in the Dhadkai population and where necessary offering prenatal screening for the mutations identified. Further, TEOAEs routinely conducted to evaluate hearing impairment in newborns are apparently misleading in cases of *OTOF* mutations. Genetic screens for neonates and use of suitable audiological tests (such as TEOAE coupled with ABR and tympanometry) should aid in early intervention and better management of the disability.

Conflicts of Interest: None.

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