The Genetic Basis of Nonsyndromic Hearing Loss in Indian and Pakistani Populations

Denise Yan,^{1,*} Abhiraami Kannan-Sundhari,^{1,2,*} Subramanian Vishwanath,^{1,2}
Jie Qing,¹ Rahul Mittal,¹ Mohan Kameswaran,³ and Xue Zhong Liu¹

Deafness encompasses a series of etiologically heterogeneous disorders with mutations in more than 400 independent genes. However, several studies indicate that a large proportion of both syndromic and non-syndromic forms of deafness in the racially diverse Indian and Pakistani populations are caused by defects in just a few genes. In these countries, there is a strong cultural preference for consanguineous marriage and an associated relatively high prevalence of genetic disorders. The current Indian population is approximately 1.2 billion and it is estimated that 30,000 infants are born with congenital sensorineural hearing loss (HL) each year. The estimated rate of profound bilateral HL is 1.6 per 1000 in Pakistan and 70% of this HL arises in consanguineous families. Knowledge of the genetic cause of deafness within a distinct population is important for accurate genetic counseling and early diagnosis for timely intervention and treatment options. Many sources and technologies are now available for the testing of hearing efficiency. Population-based screening has been proposed as one of the major strategies for translating genetic and genomic advances into population health gains. This review of the genetics of deafness in Indian and Pakistani populations deals with the major causes of deafness in these countries and perspectives for reducing the incidence of inherited deafness.

Introduction

HEARING LOSS (HL) is a sensory disorder that affects millions of people worldwide. The prevalence of childhood permanent congenital HL is 1.2 to 1.7 cases per 1000 live births (Korver *et al.*, 2010). About 50% of these cases are hereditary and most of them have nonsyndromic hearing loss (NSHL) (genetic deafness not associated with other clinical signs and symptoms) (Morton, 1991; Marazita *et al.*, 1993). To date, approximately 80 NSHL genes have been cloned in 150 loci (Hereditary Hearing Loss Homepage; <http://webh01.ua.ac.be/hhh/>). Autosomal recessive inheritance is responsible for about 80% of cases of NSHL, while autosomal dominant genes cause 20%; less than 2% of cases are caused by X-linked and mitochondrial genetic malfunctions.

Despite the obstacles of locus and allelic heterogeneity, genetic screening to identify genes and specific variants that contribute to NSHL has been widely implemented worldwide, especially with regard to identifying the spectrum of genes and variants associated with deafness across various populations.

The genetic structure of human populations is mainly determined by geography, history, and culture. The marriage

patterns have significant implication for these factors underlying the biological phenomenon over time (Burchard *et al.*, 2003). In particular, interfamily marriages are recognized as being associated with a higher risk of passing an autosomal recessive genetic disorder (Al-Gazali, 2005; Bittles, 2005a). It is indeed estimated that about 10% of congenital and genetic disorders worldwide are associated with customary consanguineous marriage (WHO, 2000). In addition, populations that have a small number of founders and/or are geographically or culturally isolated from other populations have been a precious resource for genetic mapping studies of inherited disorders (Arcos-Burgos and Muenke, 2002; Heutink and Oostra, 2002).

The huge population of the Indian subcontinent (Pakistan, India, and Bangladesh) has provided an opportunity for studies of genetic disorders (Gadgil *et al.*, 1998). Due to their unique geography and history, the population of India (over 1.21 billion) and Pakistan (approximately 140 million) is a goldmine for these studies. In addition, it is an admixture of ethnically diverse populations with unique familial and social structure (Mehdi *et al.*, 1999). From a genetic perspective, the population of India is unique, because of the traditional status consanguineous unions regarded as customary for the

¹Departments of Otolaryngology-Head and Neck Surgery, Leonard M. Miller School of Medicine, University of Miami, Miami, Florida.

²SRM University, SRM Nagar, Chennai, India.

³Madras ENT Research Foundation (MERF) Chennai, India.

*These authors contributed equally to this work.

people of Southern India. For example, in Tamil Nadu, a South Indian state, 47% of all marriages were consanguineous, compared to 25% in Maharashtra (West India) and less than 10% of marriages in the Northern and Eastern states of India were consanguineous (IIPS, 1995). It is estimated that as many as 45–60% of marriages in Pakistan are between close relatives (the vast majority of marriages are between first cousins) (Bittles, 2005b).

Consanguineous families have contributed significantly to the identification of mutated genes associated with HL (Friedman and Griffith, 2003). Autosomal recessive forms (DFNB) of isolated deafness are higher than the world average in the Indian and Pakistani populations due to a preponderance of consanguineous marriage, caste barriers in marital relations, and assortative mating, whereas autosomal dominant, X-linked, and mitochondrial inheritance of deafness are less prevalent (Bhalla *et al.*, 2009; Padma *et al.*, 2012). In this study, we review the genetics of familial deafness in Indian and Pakistani populations. A summary of these genes is given in Tables 1 and 2, and more about the most common mutations causing HL is discussed in the text. Genes and geographic maps of population sampling locations for both countries are shown in Figures 1 and 2.

Most of the DFNB forms cause a phenotypically identical severe to profound, prelingual nonprogressive HL. However, mutations at a few loci, DFNB1 (*GJB2*), DFNB3 (*MYO15A*), and DFNB29 (*Claudin 14*), can result in variation in severity even within families (Cohn *et al.*, 1999; Bashir *et al.*, 2012, 2013). This is suggestive of other factors modifying the effects of mutations in the involved genes. Deafness is usually stable, but progression has been reported. DFNB2 (*MYO7A*) and DFNB8 (*TMPRSS3*) cause a delayed childhood-onset HL. Also of note is that HL in DFNB4 due to mutations in *SLC26A4* may be associated with dilated vestibular aqueducts and endolymphatic sacs, and there is a link of auditory neuropathy with mutations in DFNB9 (*OTOF*). In addition, vestibular symptoms have been noted in DFNB2 (*MYO7A*), DFNB4 (*SLC26A4*), and DFNB12 (*CDH23*) (Bitner-Glindzicz, 2002).

The overall incidence of *GJB2* mutations in the Indian population is around 25%. Several other DFNB genes to recessive, severe to profound, congenital, or prelingual-onset deafness in the Indian population include *TMC1* (DFNB7/11 (1.6%), *CDH23*/DFNB12 (1.8%), DFNB8/10/*TMPRSS3* (1.2%), and *TMIE* (1.6%) (Table 1). In the Pakistani population, 6.1–53% of inherited deafness is caused by mutations in *GJB2*. Other important contributors to genetic deafness are *SLC26A4* (7.2%), *OTOF* (2.3%), *MYO15A* (3.3–13%), *TMC1* (3.4–5.4%), *TMPRSS3* (1.8–2.5%), and *TMIE* (1.7%) (Table 2).

Nonsyndromic Recessive Forms of Deafness in Indian Families

The prevalence of deafness in India is fairly significant. It is the second most common cause of disability. Approximately 63 million people (6.3%) in India suffer from significant auditory loss, based on population surveys in 2003 using the WHO protocol (WHO, 2009a). The estimated prevalence of adult-onset deafness was found to be 7.6% and childhood-onset deafness was found to be 2%. Molecular genetic epidemiology studies have revealed that deafness is widely prevalent in the country, especially in Southern India where the incidence is six times that of the global average.

DFNB1/GJB2/GJB6

Gap-junction proteins expressed in the cochlea have been shown to be essential for normal hearing as they are involved in the recycling of potassium ions that flow into sensory hair cells during the process of mechanotransduction. Mutations in the *GJB2* (gap-junction protein beta 2) gene that encodes the connexin-26 protein have been unambiguously cited as the leading cause of nonsyndromic prelingual congenital deafness throughout the world, with certain mutations having higher incidences in specific populations. For instance, the 35delG mutation is the leading genetic cause of deafness in Europe and the United States (Lucotte and Mercier, 2001; Roux *et al.*, 2004; Lucotte and Dieterlen, 2005; Putcha *et al.*, 2007). Instead, the 235delC mutation has been observed widely in patients from East Asia (i.e., China, Japan, and Korea) (Abe *et al.*, 2000; Ohtsuka *et al.*, 2003; Yan *et al.*, 2003). Other deafness-associated *GJB2* mutations include 167delT in Ashkenazi Jews and the R143W in Africans (Morell *et al.*, 1998).

The overall incidence of *GJB2* mutations in cohorts of Indian family studies was observed to be 22.5%, with a regional variation from 15.4% in Western (Maharashtra) to up to 36% in Southern (Kerala) parts of India. Biallelic W24X was being the major mutation identified. Its carrier frequency was estimated to be 0.024 (RamShankar *et al.*, 2003; Godbole *et al.*, 2010; Nayyar *et al.*, 2011). A possible founder effect for this mutation in the Indian population has subsequently been suggested (RamShankar *et al.*, 2003). The W24X mutation typically manifests in the majority of deaf patients across most regions of India. However, North India seemed to have a lower prevalence of biallelic W24X mutations. In addition, W77X and Q124X appeared to be more common in the north (Ghosh *et al.*, 2004). The variation in the incidence of the W24X mutation between the northern and southern populations is further highlighted by a study by Ramchander *et al.* (2004).

The 35delG mutation has also been known to occur in the heterogeneous Indian population (Mani *et al.*, 2009). In a cohort of North Indian patients, the 35delG mutation has indeed been found to surpass the occurrence of W24X, accounting for close to 10.9% incidence over the erstwhile predominant W24X (3.8%), confirming the genetic complexity that is the result of extensive genetic diversity in the Indian population (Bhalla *et al.*, 2009).

Ethnic differences between the South (indigenous Dravidians) and North Indians (Indo-European migrants from Russia) based on linguistic genealogy have also been discussed (Pasarino *et al.*, 1996). This theory has been reinforced by studies on South Indian patients showing variation in the occurrence of W24X ranging from 32.5% to 72.72% in deaf patients with *GJB2* mutations (Joseph and Rasool, 2009; Padma *et al.*, 2009). W24X has also been found in 4–5% of 603 unrelated indigenous Romanians believed to have once migrated to the Balkan and Vlach Romani territories from India (Bouwver *et al.*, 2007). Reports from Slovakia (Minarik *et al.*, 2003) and Spain (Alvarez *et al.*, 2005) indicate 39% occurrence of W24X, consolidating the theory of emigration of the ancestral Romanians from India and a consequent founder effect.

W77X is the second most frequent mutation with affected individuals dispersed in most regions in India. Incidences for this mutation vary from 0.5% (Padma *et al.*, 2009) to 5.9–6.89% (Bhalla *et al.*, 2009; Godbole *et al.*, 2010) of patients

TABLE 1. (CONTINUED)

Gene	Locus	Protein & function	Nucleotide change	AA* change	Prevalence	Phenotype
DFNB18/ <i>USH1C</i>	11p15.1	Pdz domain-containing protein is part of a transmembrane complex that connects stereocilia into a bundle necessary for normal hearing	c.187C>T c.267G>A c.388-8T>A c.496+1G>A c.598G>A c.876+6T>C c.1084C>T c.1858C>T c.2410G>A c.2611G>A IVS12+5G→C IVS12+5G→C IVS5+1G→A 769_ins36bp_770 238_239insC c.189_190insC c.415G>A c.429+4G>A c.2752G>A c.2968G>A c.5101G>A c.5660C>T c.7580C>T c.92A>G 125-126insCGCC 250C>T	p.R63H p.R89H p.G200S p.Q362X p.R620C p.A804T p.A871T	51 families ^{10,12}	Prelingual profound hearing loss
DFNB12/ <i>CDH23</i>	10q22.1	Cadherin 23, expressed in the stereocilia of hair cells, supports calcium-dependent cell–cell adhesion	c.415G>A c.429+4G>A c.2752G>A c.2968G>A c.5101G>A c.5660C>T c.7580C>T c.92A>G 125-126insCGCC 250C>T	p.V139I p.D918N p.D990N p.E1701K p.T1887I p.S2527L p.E31G R84W	1.8% ¹⁰	Prelingual profound deafness. Allelic with type 1D Uher
DFNB6/ <i>TMIE</i>	3p21.31	Transmembrane inner ear-expressed gene involved in development of hair cells. May be required for correct development of stereocilia bundles, essential for mechanotransduction of sound	889C→T 3202_3203delCG 3225_3226insC 3349C→T	Q297X D1069fsX1082 R1078fsX1083 R1117X	1.6% ¹⁰ 2 families ¹³	Severe to profound prelingual deafness
DFNB28/ <i>TRIOBP</i>	22q13.1	In the inner ear, the function of TrioBP-1 is important for formation or stabilization of the cytoskeletal structure of stereocilia and/or the cuticular plate, both actin-rich structures in sensory hair cells	c.482+1986 _1988delTGA c.785T>G	L262R	4 families ¹⁴	Prelingual severe to profound hearing loss
DFNB39/ <i>HGF</i>	7q21.11	Hepatocyte growth factor			2 families ¹⁵	Profound prelingual deafness involving all frequencies
DFNB15/ <i>GIPC3</i>	19p13.3	GAIP c-terminus-interacting protein 3 required for postnatal maturation of the hair bundle and long-term survival of hair cells and spiral ganglion in the ear.			1 family ¹⁶	Prelingual-onset hearing loss

¹Bhalla *et al.* (2009); ²Padma *et al.* (2010); ³Godbole *et al.* (2010); ⁴Ramshankar *et al.* (2003); ⁵Mani *et al.* (2009); ⁶Joseph and Rasool (2009); ⁷Wang *et al.* (1998); ⁸Ali *et al.* (2013); ⁹Yasunaga *et al.* (2000); ¹⁰Ganapathy *et al.* (2014); ¹¹Kurima *et al.* (2002); ¹²Ahmed *et al.* (2002); ¹³Naz *et al.* (2002); ¹⁴Rizuddin *et al.* (2006); ¹⁵Shultz *et al.* (2009); ¹⁶Charizopoulou *et al.* (2002).
*Amino acids.

TABLE 2. MUTATIONS UNDERLYING NONSYNDROMIC HEARING LOSS IN PAKISTANI POPULATION

<i>Gene</i>	<i>Locus</i>	<i>Protein & function</i>	<i>Nucleotide change</i>	<i>AA* change</i>	<i>Prevalence</i>	<i>Phenotype</i>
DFNB8/10/ <i>TMPRSS3</i>	21q22.3	Transmembrane serine protease 3 involved in the development and maintenance of the inner ear or the contents of the perilymph and endolymph	207delC IVS8 + 8insT 1219T>C 581G>T 726C>G	T70Xfs C407R C194F C242W	1.8% ¹	Childhood onset (10–12 years) affecting all frequencies or prelingual profound deafness
DFNB9/ <i>OTOF</i>	2p23.3	Oroferlin, involved in vesicle membrane fusion	1103_1104del insC 1273C>T 1607G>>A 2122C>>T 4809C>A 2295_2297del 1718T>G 5197G>A 5567G>A 5815C>T	G368AfsX2 R425X W536X R708X Y1603X E766del L573R E1733K R1856Q R1939W W24X W77X G12Vfs*2 E147K R127H R127Cfs*85	2.3% ²	Prelingual profound deafness. May be associated with auditory neuropathy
DFNB1/ <i>GJB2</i>	13q12.11	Connexin 26 gap-junction protein involved in recycling of ions	71G>A 231G>A 35delG 439G>A 380G>A 377_378ins ATGCGGA 598G>A 167delT 95G>A 358-360delGAG 269T>C 71G->T 416G->T 694G->A 716T->A 269C->T 1363A->T 1667A->G 1337A->G 1115C->T 170C->A 2145G->T 304 + 2T->C 1341 + 3A->C 1692_1693insA -23177_164 + 1027 del24368ins ⁷ 1264-477_2090-4927 del11202	W24X W77X G12Vfs*2 E147K R127H R127Cfs*85 G200R R32H delE120 L90P R24L G139V V231M V239D S90L I455F Y556C Q446R A372V S57X K715N C565MfsX8	6.1–53% ^{3,4}	Prelingual usually severe to profound deafness (can be variable)
DFNB4/ <i>SLC26A4</i>	7q22.3	Solute carrier family 26, member 4 encodes an anion transporter			4.7–7.2% ^{5,6}	Prelingual with profound high-frequency deafness. May be progressive and frequently associated with enlarged vestibular aqueducts and endolymphatic ducts

(continued)

TABLE 2. (CONTINUED)

<i>Gene</i>	<i>Locus</i>	<i>Protein & function</i>	<i>Nucleotide change</i>	<i>AA* change</i>	<i>Prevalence</i>	<i>Phenotype</i>
DFNB7/11/ <i>TMC1</i>	9q21.13	Transmembrane channel-like protein 1; may have a role in mechanoelectrical transduction of sound by cochlear hair cells	100C>T 2004T>G 1543T>C IVS5+1G>T 1541C>T 1534C>T 362+18A>G 1114G>A IVS3_IVS5del127kb IVS13+1G→A IVS10-8T→A 36-37insT 3496C>T 647A>T	R34X S668R C515R R512X E122Yfs*10 V372M	3.4–10% ^{7,4}	Prelingual profound hearing loss
DFNB37/ <i>MYO6</i>	6q14.1	MYO6 is an actin-based molecular motor involved in intracellular vesicle and organelle transport and may have a role in membrane trafficking, recycling, cell movement, and endocytosis	36-37insT 3496C>T 647A>T	R1166X E216V	1.2% ⁸	Congenital/prelingual onset of severe to profound sensorineural hearing loss
DFNB29/ <i>Claudin14</i>	121q22.13	Tight junction protein expressed in the inner ear, plays a role in selective paracellular permeability	398delT 254 T>A 242G>A 256A>G 259_260TC>AT 281C>T 8486G>A IVS4+1G>T 8486G>T 9948G>A 3866+1G>A 8767C>T 8222T>C 3313G>T 3334delG 4176C>A 4669A>G 5117_5118GC>TT 5189T>C 6052G>A 6061C>T 6614C>T 6731G>A 6796G>A 8158G>C 8821_8822insTG	M133RfsX23 V85D R81H I86V S87I A94V Q1229X Q3316Q T1253fsX1277 R2923X F2741S E1105X G1112fsX1124 Y1392X K1557E G1706V L1730P G2018R Q2021X T2205I G2244E V2266M D2720H V2940fsX3034	0.2–2.5% ^{9,10}	Prelingual profound deafness. May be associated with marked inter- and intrafamilial variability in hearing thresholds
DFNB3/ <i>MYO15A</i>	17p11.2	Myosin15, a motor molecule involved in actin organization in the hair cells of the cochlea and is important for hair cell maturation	398delT 254 T>A 242G>A 256A>G 259_260TC>AT 281C>T 8486G>A IVS4+1G>T 8486G>T 9948G>A 3866+1G>A 8767C>T 8222T>C 3313G>T 3334delG 4176C>A 4669A>G 5117_5118GC>TT 5189T>C 6052G>A 6061C>T 6614C>T 6731G>A 6796G>A 8158G>C 8821_8822insTG	Q3316Q T1253fsX1277 R2923X F2741S E1105X G1112fsX1124 Y1392X K1557E G1706V L1730P G2018R Q2021X T2205I G2244E V2266M D2720H V2940fsX3034	3.3–13% ^{11,4}	Prelingual severe to profound sensorineural deafness. May be associated with a less severe hearing loss with some residual hearing at low frequencies

(continued)

TABLE 2. (CONTINUED)

<i>Gene</i>	<i>Locus</i>	<i>Protein & function</i>	<i>Nucleotide change</i>	<i>AA* change</i>	<i>Prevalence</i>	<i>Phenotype</i>
DFNB12/ <i>CDH23</i>	10q22.1	Cadherin 23, expressed in the stereocilia of hair cells, supports calcium-dependent cell–cell adhesion	9478C>T	L3160F	9 families ¹²	Prelingual profound deafness. Allelic with type 1D Usher
			10474C>T	Q3492X		
			9229 + 1G>A			
			7395 + 3G>C			
			6555C>T			
			9957_9960delTGAC			
			IVS44 + 1G>A			
			IVS23 + 1G>A			
			8486G>T			
DFNB15/ <i>GIPC3</i>	19p13.3	GAP c-terminus-interacting protein 3. Required for postnatal maturation of the hair bundle and long-term survival of hair cells and spiral ganglion in the ear.	136G>A	G46R	7 families ¹³	Prelingual-onset hearing loss
			264G>A	M88I		
			281G>A	G94D		
			565C>T	R189C		
			662C>T	T221I		
			685dupG	A229GfsX10		
			767G>A	G256D		
			785T>G	L262R		
			903G>A	W301X		
			20T>G	L7R		
DFNB74/ <i>MSRB3</i>	12q14.3	Methionine sulfoxide reductase b3 repairs oxidatively damaged proteins			<1% ⁴	Prelingual bilateral profound deafness
DFNB39/ <i>HGF</i>	7q21.11	Hepatocyte growth factor	S		39 families ^{4,14}	Profound prelingual deafness involving all frequencies
			482 + 1986_1988delTGA			
			495G>A	S165S		
			241C>T	R81C		
			92A>G	E31G	1.7% ¹⁵	Severe to profound prelingual deafness
			98delAGCCCAAGinsC			
			274C>T	R92W		
DFNB36/ <i>ESPN</i>	1p36.31	Espin necessary for actin bundle formation	1988delAGAG		2 families ¹⁶	Recessively inherited deafness associated with vestibular areflexia.
			2469delGTCA			
			1135G>T	Q379X		
			1032delG	T345ProfsX20		
			290G>A	R97Q	10 families ¹⁷	Prelingual (and possibly congenital), bilateral, and of moderate to profound severity.
			1358G>A	R453Q		
			1387C>T	R463C		
			499 + 1G>A			
			1180delG	Q394SfsX15		
			411delG	W137CfsX25		
			3G>A	M1?		

(continued)

TABLE 2. (CONTINUED)

<i>Gene</i>	<i>Locus</i>	<i>Protein & function</i>	<i>Nucleotide change</i>	<i>AA* change</i>	<i>Prevalence</i>	<i>Phenotype</i>
DFNB49/ MARVELD2/ TRIC	5q13.2	Tricellulin encodes tricellulin, tight junction protein that contributes to the structure and function of tricellular contacts of neighboring cells in many epithelial tissues Required for sound transduction TPRN encodes taperin, which is concentrated at the taper region of stereocilia with possible involvement in maintenance of ankle links of stereocilia Important for proper cohesion and positioning of the stereociliary tips	IVS4 + 2T → C IVS4 + 2delTGAG IVS3-1G → A 1498C → T IVS4 + 1G > A 328G > A 44_54dup 1244 delC 1056G > A 42_52 del	R500X E110K W352X	1.06% ¹⁸ 1 family ¹⁹ 4 families ²⁰	Congenital, bilateral, prelingual, and profound deafness Profound deafness Profound deafness
DFNB63/LRTOMT DFNB79/TPRN (C9orf75)	11q13.4 9q34.3					
DFNB16/STRC	15q15.3		3157insC		1 family ²¹	Early childhood (at 3–5 years) as having a hearing loss increased in higher frequencies Moderate to severe prelingual hearing loss.
DFNB21/TECTA	11q23.	α-tectorin is one of the major glycoproteins of the tectorial and α-tectorin and has predicted structural domains with similarity to protein modules important for cross linking with other proteins Belongs to the cadherin superfamily of calcium-dependent cell–cell adhesion molecules, involved in a variety of functions, including neural circuit formation and synapse formation	6037delG		1 family ²²	
DFNB23/PCDH15	10q21.1		533A > G 785G > A 400C > G	D178G G262D R134G	2.3% ²³	Profound deafness
DFNB24/RDX	11q22.3	Radixin is expressed along the length of the stereocilia, suggesting its role in organizing or anchoring membrane proteins to the central actin filaments. A role in actin organization in hair cells has been suggested because of the localization of GRXCR1 in the hair bundle and stereocilia In the inner ear, the function of Triobp-1 is important for formation or stabilization of the cytoskeletal structure of stereocilia and/or the cuticular plate, both actin-rich structures in sensory hair cells	1732G > A 1404_1405insG 463C > T	D578N A469fsX487 Q155X	0.3% ²⁴	Profound hearing loss
DFNB25/GRXCR1	4p13		229C > T 412C > T	Q77X R138C	2 families ²⁵	Profound hearing loss and vestibular dysfunction
DFNB28/TRIOBP	22q13.1		2362C → T 3202C → T 1193_1195delAAC	R788X R1068X delQ398	1.6% ²⁶	Prelingual severe to profound hearing loss

(continued)

TABLE 2. (CONTINUED)

<i>Gene</i>	<i>Locus</i>	<i>Protein & function</i>	<i>Nucleotide change</i>	<i>AA* change</i>	<i>Prevalence</i>	<i>Phenotype</i>
DFNB67/ <i>TMHS</i>	6p21.31	Tetraspan membrane protein of hair cell stereocilia	246delC 380A>G 252C>G 398_399insC 471_1G>A 495delG 640G>A 977T>A 1309G>A 1591C>T 193511G>A 2476G>A 2914C>T 3135_3136insC 3508G>A 3631delT 4838delA 5146_5148delGAG 536611G>A 5944G>A IVS8+2T→G 238-239insC 238-239insC	83fsX84 Y127C N84K H133fsX139 E166fsX170 G214R L326Q D437N Q531X A826T R972X L1046fsX1054 E1170K Y1211fsX1231 E1613fsX1644 E1716del G1982R	2 families ²⁷ 1 family ²⁸	Profound hearing loss Profound hearing loss. Age of onset can be variable, ranging from birth to late in childhood
DFNB2/ <i>Myo7A</i>	14q24.3	An unconventional myosin presumed to be tethered to different macromolecular structures that move relative to actin filaments, thus enabling them to transport cargo				
DFNB18/ <i>USH1C</i>	11p15.1	PDZ domain-containing protein is part of a transmembrane complex that connects stereocilia into a bundle necessary for normal hearing			3 families ^{29, 30}	Profound prelingual deafness
DFNB35/ <i>ESRRB</i>	14q24.3	Estrogen-related receptor-beta is essential for inner ear development and function	329C>T 959T>C 1024G>T 1040C>T c.35T>C c.10G>T c.97G>C	A110V L320P V342L L347P I12T E4X V33L	4 families ³¹	Profound prelingual hearing loss
<i>BSND</i>	1p32.3	Crucial for renal salt reabsorption and potassium recycling in the inner ear			3 families ³²	Congenital sensorineural deafness

¹Ahmed *et al.* (2004); ²Choi *et al.* (2009); ³Santos *et al.* (2005); ⁴Shafique *et al.* (2014); ⁵Park *et al.* (2003); ⁶Anwar *et al.* (2009); ⁷Kitajiri *et al.* (2007); ⁸Ahmed *et al.* (2003a); ⁹Wilcox *et al.* (2001); ¹⁰Bashir *et al.* (2013); ¹¹Nal *et al.* (2007); ¹²Bork *et al.* (2001); ¹³Rehman *et al.* (2001); ¹⁴Schultz *et al.* (2009); ¹⁵Santos *et al.* (2006); ¹⁶Naz *et al.* (2004); ¹⁷Borek *et al.* (2011); ¹⁸Riazuddin *et al.* (2006a); ¹⁹Ahmed *et al.* (2008); ²⁰Rehman *et al.* (2011); ²¹Verpy *et al.* (2001); ²²Naz *et al.* (2003); ²³Ahmed *et al.* (2003b); ²⁴Khan *et al.* (2007); ²⁵Schraders *et al.* (2010); ²⁶Riazuddin *et al.* (2006b); ²⁷Shabbir *et al.* (2006); ²⁸Riazuddin *et al.* (2008); ²⁹Ouyang *et al.* (2002); ³⁰Bitner-Glindzicz *et al.* (2000); ³¹Collin *et al.* (2008); ³²Riazuddin *et al.* (2009).

*Amino acid.

FIG. 1. Genes and geographic maps of Indian population sampling locations.

with *GJB2* mutations. Q124X has also been detected in the Indian population and with W77X appeared to be more common in North India. The 235delC mutation detected in 0.5–3% of deaf patients also has been documented in Southern India (Ramchander *et al.*, 2004; Padma *et al.*, 2009).

Occurrence of the IVS1 + 1G > A mutation among Indians at a prevalence of 0.3–4.68% was documented in a study from Southern India (RamShankar *et al.*, 2003) (Table 1).

An in-frame deletion, delE120 in *GJB2*, was first identified in a consanguineous Indian Muslim family and has been

FIG. 2. Genes and areas of samples collection in Pakistan. NWFP, North West Frontier Province.

shown to occur in 0.8–1.7% of patients, pointing to a higher prevalence in the North (Bhalla *et al.*, 2009; Mani *et al.*, 2009). The mutation was subsequently reported in Pakistan, Germany, and Turkey. Other potentially pathogenic mutations found include the R143W mutation, I35S, I33T, and W172R (Mani *et al.*, 2009).

Surprisingly, an absence of *GJB6* mutations has been reported in North Indian patients, in a study aiming to find individuals with DFNB1, due to *GJB6* mutations concordant with the high allele frequency of 35delG observed previously in a similar cohort (Bhalla *et al.*, 2009).

DFNB3/MYO15A

Mutations in the gene coding for myosin XVA cause DFNB3 (Wang *et al.*, 1998; Belyantseva *et al.*, 2005). DFNB3/*MYO15A* is not a common cause of deafness in the Indian population with 600 families screened, only 6 showed linkage

to DFNB3, thus contributing only 1% (Ali *et al.*, 2013). Studies in mice suggest that *MYO15A* is necessary for actin organization in the hair cells of the cochlea and its interactions with other proteins, besides WHIRLIN, are important for hair cell maturation (Mustapha *et al.*, 2007). Originally mapped in deaf individuals born in the village of Bengkala in Bali (Wang *et al.*, 1998), DFNB3 has subsequently been shown to segregate in two unrelated deaf Indian families. The haplotypes of affected individuals in these two Indian families were different from each other and different from the Bengkala haplotype, suggesting that these three DFNB3 mutations arose independently. The two mutations identified in the Indian families include N890Y and K1300X (Table 1).

DFNB8/DFNB10/TMPRSS3

The gene encoding a transmembrane serine protease, *TMPRSS3*, has been shown to be responsible for both the

DFNB8 and DFNB10 phenotypes (Scott *et al.*, 2001). The deafness DFNB8 locus was mapped by linkage analysis on a large Pakistani family consisting of 41 members (Veske *et al.*, 1996). Studies by Guipponi *et al.* (2002) suggested the existence of autocatalytic processing, by which TMPRSS3 would become active, and the epithelial sodium channel (ENaC) could be a substrate of TMPRSS3 in the inner ear. Its contribution to hereditary deafness in the Indian population has recently been evaluated in a screening of 1739 individuals from 374 families with NSHL (Ganapathy *et al.*, 2014). Analysis led to the identification of 3 TMPRSS3 novel mutations, including p.V116M, p.G243R, and p.C386R. TMPRSS3 mutations have been noted as the cause of deafness in 1.2% of the families included in this study.

DFNB7/11/TMC1

Mutations in the transmembrane channel-like 1 (TMC1) gene cause DFNB7/11. All TMC family members contain a conserved 120-amino acid sequence, which is called the TMC domain. TMC proteins may act as ion channels, ion pumps, or transporters depending on their structure. Data obtained from the *tmc1* mutant mice suggest a possible role for TMC1 in mechanoelectrical transduction of sound by cochlear hair cells (Kawashima *et al.*, 2015). In their screening of 374 families, Ganapathy *et al.* (2014) identified eight TMC1 mutations. Among these, five were novel. The identified variants include the splice site mutations c.237-6T>G, c.453+2T>C, c.1566+1G>A, the deletion mutation p.I210del, and the missense mutations (p.V372M, p.R445C, and p.G267E). The nonsense mutation p.R34X, a commonly occurring mutation in multiple world populations (saïd *et al.*, 2010), was also observed in one family. TMC1 mutations have been pinpointed as the cause of deafness in 1.6% of the families included in this study.

DFNB6/TMIE

Mutations in the transmembrane inner ear-expressed gene (TMIE) cause DFNB6. Studies in mice suggest that this gene is required for normal postnatal maturation of sensory hair cells in the cochlea, including correct development of stereocilia bundles, essential for mechanotransduction of sound (Chung *et al.*, 2007). Ganapathy *et al.* (2014) identified three known TMIE mutations in their study. These include two missense mutations (p.E31G and p.R84W) and one insertion mutation (c.125_126insCGCC). In this cohort, HL is attributed to these TMIE mutations in 1.6% of families.

Nonsyndromic Recessive Forms of Deafness in Pakistani Families

The Pakistani population is one of the richest genetic sources to study hereditary disorders due to a mixture of diverse ethnicities with unique familial and social characteristics (Mehdi *et al.*, 1999) and its unique sociocultural trends, where out of every ten marriages, six are consanguineous and four are between first cousins (Hussain and Bittles, 1998). It is estimated that the prevalence of hearing impairment in Pakistan is 1.6 per 1000 live births (Elahi *et al.*, 1998), which is higher than the world average of 1 per 1000 live births (Atar and Avraham, 2005).

DFNB1/GJB2

The contribution of GJB2 variants to NSHL in the Pakistani population has been reported to vary from 6.1% to 50%. Screening of 430 affected subjects from 196 families for GJB2 variations by Santos *et al.* (2005) showed that only 6.1% of the families had GJB2-related HL. W24X and W77X were found to be the most common. The 167delT, R2H, delE120, and L90P mutations have also been detected. Of the families that were studied, 70% come from the Punjab province, the largest province in Pakistan. The native languages of these families are Punjabi and Saraiki. The remaining 30% of the families in the study were equally representative of the other provinces and their corresponding language groups. There was no association found between the presence of a particular GJB2 mutation and the region of origin or linguistic background. A recent study by Bukhari *et al.* (2013) also established that GJB2 does not contribute to a high proportion of isolated HL in the Hazara population. Hazara is a province of Khyber Pakhtunkhwa of Northern Pakistan. Their sequencing analysis of 70 patients led to the identification of three GJB2 mutations in three individuals (4.28%). These include p.W24X and two other mutations (p.I35S and c.35delG) identified for the first time in Pakistan. However, recent studies indicate that the frequency of GJB2 mutations is higher in Pakistani deaf patients; of the 20 large consanguineous families from different areas of Pakistan screened for GJB2, 10 (50%) were reported to have GJB2-related HL (Anjum *et al.*, 2014). A total of six different mutations were identified in the study, with W24X as the major mutation found, followed surprisingly by c.35delG being the second most prevalent, surpassing Q124X, W77X, V153I, and T8M. Furthermore, sequence analysis of GJB2 in 30 consanguineous families with NSHL from different regions of Punjab, by Shafique *et al.* (2014), revealed the presence of mutations in this gene in 16 families (53%). W24X and W77X were the most common mutations found. In addition, a novel homozygous change p.G200R was detected in one family. Other known mutations identified include c.35delG, E147K, and c.377_378insATGCGGA (R127Cfs*85).

DFNB9/OTOF

Mutations in OTOF (DFNB9), encoding otoferlin, usually lead to prelingual profound deafness (Yasunaga *et al.*, 2000). OTOF-related HL can also be associated with intact otoacoustic emissions as part of a distinctive phenotype referred to as auditory neuropathy/dyssynchrony (AN/AD) (Varga *et al.*, 2003). Otoferlin has been proposed to act as a calcium sensor that regulates synaptic vesicle exocytosis in cochlear hair cells (Roux *et al.*, 2006). Most of the pathogenic alleles of OTOF are private, each one being reported in only one family. A total of 13 (2.3%) families linked to DFNB9 were identified in a screening of 557 large consanguineous Pakistani families (Choi *et al.*, 2009). Probable pathogenic variants in the OTOF gene were found among all 13 families. These include the previously reported nonsense mutation p.R708X (Rodriguez-Ballesteros *et al.*, 2003) and 10 unreported variants: 3 nonsense mutations (p.R425X, p.W536X, and p.Y1603X), 1 frameshift (c.1103_1104delinsC), 1 deletion mutation (p.E766del), and 5 missense substitutions (p.L573R, p.A1090E, p.E1733K, p.R1856Q, and p.R1939W). A novel splice site variant (c.766-3C>T) in the cis configuration with p.E766del was also found in one Pakistani family (Table 2).

DFNB4/SLC26A4

DFNB4/PDS is the most common locus in the Pakistani population with a prevalence of 7.2% (Anwar *et al.*, 2009). Pendrin is the transmembrane protein encoded by *SLC26A4* that functions primarily as a transporter of chloride and iodide across the membranes of the cell. In the cochlea, *SLC26A4* is mainly expressed in regions of endolymph resorption; therefore, mutations in *SLC26A4* may disturb endolymphatic fluid homeostasis (Borck *et al.*, 2003). Anwar *et al.* (2009) analyzed the *SLC26A4* gene in 46 large consanguineous Pakistani families linked to DFNB4/PDS. Their study revealed 16 probable pathogenic variants, 8 of which were not previously reported. These include three missense substitutions (p.R24L, p.G139V, and p.V231M), two splice site variants (c.304+2T>C and c.1341+3A>C), and one frameshift (c.1692_1693insA) resulting in premature protein (p.C565MfsX8) truncation. Each of the six pathogenic variants (p.V239D, p.Q446R, p.S90L, p.Y556C, p.R24L, and p.K715N) was found in more than one family and haplotype analyses suggest that they are founder mutations. Two genomic deletions were also identified: the c.-23177_c.164+1027del24368ins7 and c.1264-477_2090-4927del11202. Eight earlier reported pathogenic variants (p.V239D, p.S90L, p.I455F, p.Y556C, p.Q446R, p.A372V, p.S57X, and p.K715N) were also observed (Reardon *et al.*, 1997; Park *et al.*, 2003; Tsukamoto *et al.*, 2003) (Table 2).

DFNB7/11/TMC1

Mutations in the *TMC1* gene cause DFNB7 (Fukushima *et al.*, 1995; Kurima *et al.*, 2002). Kitajiri *et al.* (2007) reported 9 different mutations in the *TMC1* gene in 19 (3.4%) of the 557 Pakistani families with NSHL (Table 2). A single mutation p.R34X causes deafness in 10 (10/557; 1.8%) of the families. A shared haplotype in different families strongly suggests a single ancestral origin of the mutation. Other variants were also detected, including IVS5+1G>T, p.P514L, and p.C515R. Santos *et al.* (2005) reported a prevalence of 4.4% of NSHL due to *TMC1* in the Pakistani population. We previously estimated that *TMC1* mutations account for approximately 5% of recessive deafness in a mixed cohort of 230 Pakistani and Indian families (6/10 Punjabi families) (Kurima *et al.*, 2002). Of the 30 families analyzed by Shafique *et al.* (2014), 3 (10%) were found to carry *TMC1* mutations.

DFNB29/CLDN14

Deafness at the *DFNB29* locus is caused by recessive mutations of *CLDN14*, encoding claudin 14, a tight junction protein. Loss of claudins has been reported to result in increased paracellular permeability of K⁺ in the reticular lamina and an elevation in the K⁺ concentration around the basolateral regions of hair cells, which is toxic. Thus, claudins likely act as selective electrostatic barriers for anions and cations. The relative contribution of *CLDN14* mutations in the Pakistani deaf population is approximately 2.25% (18/800 families) and is associated with marked inter- and intrafamilial variability in hearing thresholds (Bashir *et al.*, 2013). The identified mutations include two unreported missense substitutions (p.S87I and p.A94Val) and three known variants (p.R81H, p.V85D, and p.M133RfsX23) (Wilcox *et al.*, 2001). Haplotype analyses indicate that p.V85D and p.M133RfsX23 are founder mutations. The

p.V85D accounts for approximately 67% of the mutant alleles of *CLDN14* in the study.

DFNB3/MYO15A

DFNB3 is responsible for 3.3–13% of recessive deafness in the Pakistani population (Nal *et al.*, 2007). A total of 17 mutations (IVS4+1G>T, Q2716H, Q1229X, p.T1253fsX1277, p.Y1392X, p.K1557E, p.G1706V, p.L1730P, p.G2018R, p.Q2021X, p.T22051, p.G2244E, p.V2266M, p.2720H, p.V2940fsX3034, p.L3160F, and p.Q3492X) were identified in Pakistani deaf patients in the *MYO15A* gene by two independent groups (Liburd *et al.*, 2001; Nal *et al.*, 2007). These seventeen missense, nonsense, and splice site mutations are located in exons encoding motor and first FERM domains of *MYO15A*. In a more recent study, 4/30 (13%) families were found to have mutation in *MYO15A* (Shafique *et al.*, 2014). These include four unreported variants, of which two were splice site mutations (c.9948G>A and c.3866+1G>A), one was a nonsense (p.R2923X), and one missense mutation (p.F2741S). Interestingly, Bashir *et al.* (2012) reported a duplication of cytosine in a stretch of seven repetitive C nucleotides (c.1185dupC) in exon 2 of *MYO15A*, leading to a frameshift mutation (p.E396fsX431), which was identified in a Pakistani family with moderate to severe HL. A less severe HL with some residual hearing at low frequencies or moderate deafness has only been reported if mutations in *MYO15A* lie in the second exon of this gene (Nal *et al.*, 2007; Cengiz *et al.*, 2010). A role of modifier genes in determining the HL phenotype has also been proposed since individuals with identical mutations in exon 2 of *MYO15A* may exhibit different degrees of HL (Liburd *et al.*, 2001; Nal *et al.*, 2007).

Conclusion

Work during the past decade has generated a wealth of information connecting mutations with health. This has dramatically enhanced our understanding of the pathogenetic basis of inherited diseases. However, due to extreme clinical and genetic heterogeneity, full comprehension of these correlations still remains a great challenge in the case of NSHL. Studies have been particularly successful when investigating the fundamental genetic cause of NSHL in families from regions of the world with high consanguineous marriage rates. Despite progress made in the identification of deafness genes, the population distribution for many of the newly identified HL genes still remains unclear. It is well known that the molecular epidemiology of deafness can vary considerably among populations.

We have summarized the various genes and mutations associated with HL in the Indian and Pakistani populations. In these studies, the spectra of sequence variations in the deafness genes were frequently different from those observed outside the Indian subcontinent. For instance, the W77X variant in *GJB2* has only been reported in people originating from the Indian subcontinent. The W24X variant has been observed in Europeans, but its prevalence is about three times higher in Pakistanis and at least 20 times higher in Indians. Thus, the occurrence of the same deafness loci and spectrum of variants in several genes in both populations may reflect shared origins of HL alleles within the Indian subcontinent. The reports also show that there is a need for awareness and accurate genetic counseling to families with high genetic risk, for which it is

necessary to establish genetic testing for the common deafness mutations prevalent in the ethnic populations. A promising technique for genetic testing is the use of DNA microarrays. This approach offers the possibility of testing for many known mutations and/or a complete mutation analysis of one or more genes in parallel in a single experiment.

Homozygosity mapping has proven successful with consanguineous families and homogeneous populations sharing similar clinical features. However, due to limiting meiotic crossing-over events, these locus mapping approaches typically identify large chromosomal regions that include hundreds of genes. These loci could now undergo parallel sequencing of all linked genes through either custom targeted capture and massively parallel sequencing of the single locus or whole-exome sequencing (WES). In addition, small families unsuitable for conventional approaches can now be alleviated through the use of next-generation sequencing powered methods such as WES.

Deafness remains a high burden for the Indian subcontinent. A lack of skilled manpower and human resources makes this problem a huge challenge. According to the WHO (2009b) statistics, half the causes of deafness are preventable in India. About 30%, although not preventable, are treatable or can be managed with assistive devices. To address the issue of deafness, the Government of India launched the National Programme for Prevention and Control of Deafness in 2006, with a focus on manpower development for prevention, early identification, and ear service provision, including rehabilitation (Garg *et al.*, 2009). Since the programme is also being implemented at the primary healthcare level, it envisages a reduction in the burden of deafness and prevention of future HL in India.

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Author Disclosure Statement

The authors declare no conflicts of interest.

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Address correspondence to:

Xue Zhong Liu, PhD, MD, FACS

Departments of Otolaryngology-Head and Neck Surgery

Leonard M. Miller School of Medicine

University of Miami

1601 NW 12th Avenue, Mailman Center, Room 6023

Miami, FL 33136

E-mail: xliu@med.miami.edu