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Global genetic insight contributed by consanguineous Pakistani families segregating hearing loss

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Abstract

Consanguineous Pakistani pedigrees segregating deafness have contributed decisively to the discovery of 31 of the 68 genes associated with nonsyndromic autosomal recessive hearing loss (HL) worldwide. In this study, we utilized genome-wide genotyping, Sanger and exome sequencing to identify 163 DNA variants in 41 previously reported HL genes segregating in 321 Pakistani families. Of these, 71 (43.6%) variants identified in 29 genes are novel. As expected from genetic studies of disorders segregating in consanguineous families, the majority of affected individuals (94.4%) are homozygous for HL-associated variants, with the other variants being compound heterozygotes. The five most common HL genes in the Pakistani population are *SLC26A4, MYO7A, GJB2, CIB2* and *HGF*, respectively. Our study provides a profile of the genetic etiology of HL in Pakistani families, which will allow for the development of more efficient genetic diagnostic tools, aid in accurate genetic counseling and guide application of future gene-based therapies. These findings are also valuable in interpreting pathogenicity of variants that are potentially associated with HL in individuals of all ancestries. The Pakistani population, and its infrastructure for studying human genetics, will continue to be valuable to gene discovery for HL and other inherited disorders.

Keywords

Deafness; genetic spectrum; Pakistan; allelic heterogeneity; autosomal recessive hearing loss; DFNB; pathogenic variant

Introduction

Normal hearing ability requires many specialized cell types. In the inner ear there are intricately-structured mechanosensory hair cells overlaid by an acellular tectorial membrane, the stria vascularis, which generates the potassium-rich endolymph, and several supporting cell types, which in concert are necessary for transduction of sound (Burns, Kelly, Hoa, Morell, & Kelley, 2015). A failure of normal development, loss of any one of several essential physiological or biochemical functions or degeneration of one or more of these cell types in the inner ear can cause hearing loss (HL).

Today, ~466 million people have disabling HL (WHO, March 2018), including 1 in 500 newborns (Morton & Nance, 2006), 34 million children and one-half of all individuals over the age of 75 (Yamasoba et al., 2013). HL can be one feature of a more complex clinical syndrome (Gettelfinger & Dahl, 2018). Isolated hearing loss, referred to as nonsyndromic autosomal recessive hearing loss (NSARHL), is also genetically heterogenous. As of June 2018, 105 NSARHL loci have been mapped and 68 identified (http://hereditaryhearingloss.org). Close to half of these genes (31 out of 68) were identified through studies of large consanguineous families from Pakistan. Subsequently, additional mutations in these same genes were recognized in diverse populations across the world.

The genetic heterogeneity of hearing disorders can present a challenge for identification of causative molecular lesions and expeditious molecular diagnosis which is critical for prognosis and informed genetic counseling (Askew et al., 2015; Shibata et al., 2016). The

availability of next generation sequencing (NGS), particularly exome sequencing, has improved the success for the identification of possible pathogenic variants for inherited disorders, including HL (Gao & Dai, 2014; Sloan-Heggen & Smith, 2016; Vona, Nanda, Hofrichter, Shehata-Dieler, & Haaf, 2015). Using a combination of experimental strategies from classical homozygosity mapping (Friedman et al., 1995) to exome sequencing, we identified 163 variants (71 of which are novel) in 41 reported hearing-associated genes co-segregating with HL in 321 Pakistani families. Our present and previous genetic profiling provides an improved understanding of the causes of inherited HL in the Pakistani population while revealing genes with high population-specific prevalence rates.

Material and Methods

Human subjects

This study followed the tenets of the Declaration of Helsinki. Approval for this study was obtained from institutional review boards at the University of Maryland, School of Medicine, Baltimore, Maryland, USA (HP-00059851), the Baylor College of Medicine and Affiliated Hospitals (H-17566), the Combined Neurosciences Blue Panel at the National Institutes of Health (OH-93-N-016), the National Centre of Excellence in Molecular Biology at the University of the Punjab, Quaid-i-Azam University, and Liaquat University of Medical and Health Sciences. Written informed consent was obtained from all participating individuals in this study.

Screening and identification of HL variants

Three new cohorts of Pakistani families segregating HL were examined by NGS. Cohorts 1, 2 and 3 have 261, 40, and 20 families, respectively, for a total of 321 families. For cohort 1, DNA samples from two affected individuals were first screened for variants in *HGF* (Schultz et al., 2009), *CIB2* [c.272T>C; p.(Phe91Ser)] (Riazuddin et al., 2012), and *GJB2* (Santos, Wajid, Pham, et al., 2005), using Sanger sequencing. HL-associated variants were found in 94 of these families. DNA samples from the family members of these 94 families were also Sanger-sequenced to test co-segregation of variants with HL. For the remaining 167 families that were negative for variants of *GJB2, CIB2* or *HGF*, DNA samples for 129 families were genotyped using microsatellite or single nucleotide polymorphism (SNP) markers. Genotype data were checked for errors using PedCheck (O'Connell and Weeks, 1998) and MERLIN (Abecasis, et al. 2002). Analysis was performed using HomozygosityMapper (Seelow, Schuelke, Hildebrandt, & Nurnberg, 2009) and Allegro for linkage analysis (Gubjartsson, et al., 2005). Out of 129 families with genotype data, 46 families were mapped to a region containing a known HL gene and the putatively pathogenic variant was identified and co-segregation with HL confirmed using Sanger sequencing.

The remaining 121 families from cohort 1 included those who were not mapped to known HL genes and were negative when followed-up with Sanger sequencing. Forty additional families (cohort 2) did not have genome-scan genotype data. A DNA sample from an affected individual from each of these families (total 161 DNA samples) were exome sequenced either at the University of Maryland School of Medicine (UMSOM), University of Washington Center for Mendelian Genomics (UWCMG), or at the National Institute of

Deafness and Other Communication Disorders (NIDCD) Genomic and Computational Biology Core.

Aside from the first two cohorts of 301 families, an additional 20 families (cohort 3) were screened using a gene panel that includes all known nonsyndromic and selected syndromic HL genes (n=101) (Zein et al., 2014) and the segregation of pathogenic variants identified from this panel was verified by Sanger sequencing the DNA of all informative family members.

Variants were considered further if (a) they have allele frequency <1% and (b) Combined Annotation Dependent Depletion (CADD; (Kircher et al., 2014)) scaled score was ≥20 and/or (c) at least two of the following algorithms predict the mutation to be deleterious: PROVEAN (Y. Choi & Chan, 2015), SIFT (Kumar, Henikoff, & Ng, 2009), MutationTaster (Schwarz, Rodelsperger, Schuelke, & Seelow, 2010), MutationAssessor (Reva, Antipin, & Sander, 2007), FATHMM (Shihab et al., 2013; Shihab et al., 2015) and PolyPhen2 (Adzhubei et al., 2010). Splice site and splice region variants were evaluated using splice site-altering scoring algorithms such as dbscSNV (Jian, Boerwinkle, & Liu, 2014) and Human Splice Finder (Desmet et al., 2009). Further details of bioinformatics analyses are described in Supp. Tables S1 and S2. All variants were tested for co-segregation with HL in the corresponding family via Sanger sequencing. All novel variants have been submitted to ClinVar public databasis (https://www.ncbi.nlm.nih.gov/clinvar/).

Results

Genetic spectrum

For 41 of the 68 reported NSARHL genes, we identified 163 variants co-segregating with severe-to-profound HL in 321 families (Fig. 1, Table 1). All of these consanguineous families segregate NSARHL associated genes either with homozygous (94.4%; Fig. 2, Table 1) or compound heterozygous (5.6%) variants (Fig. 3, Supp. Table S3). While 92 (56.4%) of the variants have been previously reported to cause HL in humans, we also identified 71 (43.6 %) novel variants associated with HL (Fig. 1). Almost 80% (127 of 163) of the identified variants are present only once in our cohort (Table 1). However, 57.1% of all causative variants identified in this study are found in five genes: *SLC26A4, GJB2, MYO7A, HGF*, and *TMC1* (Fig. 4A).

Causal variants

Of the causative variants identified in this study 48.7% are missense, 23% are nonsense, 21.6% are small insertion or deletions and 6.7% are predicted to alter splice sites (Fig. 4B). In the five most common HL genes in this study, the distribution of mutation types appears to be gene-specific (Fig. 4B). Pathogenic alleles of *HGF* are all small deletion variants of an alternative 3'UTR (Schultz et al., 2009), while nonsense variants represent more than 70% of the HL alleles for *GJB2* with two nonsense variants [c.71G>A, p.(Trp24*); c.231G>A, p.(Trp77*)] representing 38.9% and 33.3%, respectively, of the *GJB2*-associated HL mutations (Fig. 4).

A homozygous genomic deletion spanning exons 14 and 15 of *PCDH15* was identified in family PKDF627, which causes a shift in the wild type translation reading frame and is predicted to result in a premature truncation of the encoded protein. This deletion has not been previously reported in humans. In mice that are homozygous for the *Pcdh15*$^{av-5J}$ (*Ames Waltzer*) allele, skipping of exon 14 due to a splice site variant results in disorganized stereocilia bundles, hearing loss, balance problems (Zheng et al., 2006) and delayed development of retinal sensory function (Haywood-Watson et al., 2006). CNV analyses of other families in our HL cohorts did not reveal any known or potentially HL causal indels. Thus, contrary to previous observations in other cohorts (Shearer et al., 2014), large indels probably rarely cause HL within the Pakistani population.

An unreported variant of *ADGRV1* (also known as *VLGR1*, *MASS1*, *KIAA0686* and *GPR98* MIM# 602851*)*, c.1055C>T (p.(Pro352Leu)), co-segregates with HL in family PKDF1551 (Fig. 5A). So far, mutations in *ADGRV1* have been exclusively associated with Usher syndrome type 2 (USH2C, MIM# 605472) (Besnard et al., 2012; Reddy et al., 2014; Weston, Luijendijk, Humphrey, Moller, & Kimberling, 2004), characterized by congenital mild to severe sensorineural hearing loss, intact vestibular responses and progressive retinitis pigmentosa. In family PKDF1551, affected individuals exhibit mild to severe bilateral sensorineural hearing loss with no vision defects revealed by electroretinogram (Fig. 5B). Usher syndrome type 2 is a progressive disorder and the oldest affected individual in the family was only 17 years old at the time of clinical evaluation. However, USH2C, due to a large deletion in *ADGRV1* was previously diagnosed in a 14-year old patient with vision defects (Hilgert et al., 2009).

Out of 321 families, a total of 19 families (5.9%) have evidence of intra-familial locus heterogeneity in that some deaf siblings or deaf relatives in other branches of the family are not segregating the same HL-causal variant (data not shown). For instance, two families (HL007 and DEM4372) display locus heterogeneity, within the same or different branches, segregating homozygous variants in two different known NSARHL genes (Fig. 2), which further highlights the unexpected complexity of the genetic diagnosis of HL in consanguineous families. In family HL007, most of the affected individuals are homozygous either for a reported c.1056G>A, p.(Trp352*) variant of *TPRN*, (Rehman et al., 2010), or another reported c.7814A>G, p.(Asn2605Ser) variant in *CDH23* (Naz et al., 2017) a gene known to be involved in either NSARHL or Usher syndrome type 1D (MIM# 601067) (Bork et al., 2001). Affected individual VII:3 is homozygous for both of the likely pathogenic variants of *TPRN* and *CDH23* (Fig. 2). Family DEM4372 is segregating variants for two different known NSARHL genes: *OTOG* and *TMPRSS3* (Fig. 2). Affected individuals V:2 and V:3 are homozygous for a novel *OTOG* deletion [c.7235delG, p.(Arg2412Hisfs*77)]. Four affected individuals, IV:2, IV:6, IV:8, and IV:9, are homozygous for the recurrent c. 1219T>C [p.(Cys407Arg)] variant (Ben-Yosef et al., 2001) in *TMPRSS3*. These two families (HL007 and DEM4372) exhibit intra-familial locus heterogeneity, which we previously demonstrated in several consanguineous Pakistani families with NSARHL (Rehman, et al., 2015).

Regardless of whether causal variants of one or more HL genes are segregating in a single family, the variants associated with HL are usually but not always homozygous. Affected

individuals of family DEM4652 are compound heterozygous for three variants c.1258A>T [p.(Lys420*)], c.3502C>T [p.(Arg1168Trp)] and c.4505A>G [p.(Asp1502Gly)] variants in *MYO7A* (Fig. 3). While predicted damaging by various algorithms (Mutation Assessor, Mutation Taster, PolyPhen2, SIFT and PROVEAN), we cannot conclude that *MYO7A* c.4505A>G is pathogenic because normal hearing father III:2 is heterozygous for two different *MYO7A* variants: the known HL-causing variant c.1258A>T and the newly identified c.4505A>G variant. This family shows that even though multiple rare, predicted-as-damaging variants co-segregate with HL, it is likely that only a subset of these variants are causal, which further highlights the need to evaluate pathogenicity of variants based on multiple lines of evidence even when the variants occur in reported HL genes.

In order to obtain a more precise assessment of the genetic spectrum of HL in this population, we included in our counts not only the data from this specific study but also already published HL variants identified in our previously reported cohorts of Pakistani families (61 publications). Together, our studies have revealed 875 mutant alleles (368 unique variants) in 54 genes (Supp. table S4) and the 10 most prevalent genes are *SLC26A4* (14.2%), *MYO7A* (10.5%), *GJB2* (9.9%), *CIB2* (8.6%), *HGF* (8%), *TMC1* (6.4%), *MYO15A* (5.7%), *CDH23* (4.6%), *TMPRSS3* (4.3%) and *OTOF* (3.1%). Mutations in these ten genes represent 75.3% of all known causative variants identified in Pakistani families we have studied (Fig. 6, Supp. table S4).

Discussion

Consanguineous families with recessively inherited HL have been a valuable resource for genetic mapping, gene and variant identification and genotype-phenotype correlations. Subsequently, functional studies and knockout animal models that follow gene identification aid in understanding the biology of inner ear development and function. In Pakistan, 45 to 60% of marriages are between relatives (Bittles, 2005; E. M. Scott, 1973), which has increased the coefficient of inbreeding in this population. According to a 2012 World Health Organization report, the prevalence of recessive disorders such as HL is higher in Pakistan (2.4%) compared to a world prevalence of 1.7%. In this study that includes a large cohort of 321 unpublished Pakistani families, we identified 163 unique variants in 41 genes responsible for autosomal recessive hearing loss. As expected, autosomal recessive inheritance due to homozygous variants is demonstrated in the majority of our HL cohort (94.4%), however 5.6% of the variants segregate in a compound heterozygous fashion even in consanguineous families (Fig. 3). Of the 163 identified variants, 71 (43.6%) are novel. In addition, 98.6% of these novel variants are found only once in our cohort. Given the rarity of these private variants, reporting them as damaging based on bioinformatics tools and co-segregation with HL in mostly consanguineous families aids in determining variant pathogenicity (Supp. Table S5) (Richards et al., 2015). The data provided here will also help variant curation in diagnostic laboratories where physician-referred genetic testing is usually performed on singletons.

Despite the heterogeneity of genetic causes of HL, in our study, variants in five known HL genes, *SLC26A4* (58), *GJB2* (54), *MYO7A* (39), *HGF* (27), and *TMC1* (18), account for 57.1% (95% CI: 51.7–62.4%) of the identified alleles (Table 1). Variants in *SLC26A4* alone

account for 16.9% (95% CI: 13.2–21.4%) of variants in our 321 families. *SLC26A4* encodes pendrin, a transmembrane anion exchanger for chloride, iodide, bicarbonate and other ions, essential for the development of inner ear and ionic homeostasis (Chattaraj et al., 2017; B. Y. Choi et al., 2011; Everett et al., 2001; Rehman, Friedman, & Griffith, 2017). Consistent with our previous findings (Anwar et al., 2009), c.716T>A [p.(Val239Asp)] is the prevalent variant responsible for *SLC26A4*-associated HL in Pakistani families. The c.2106delG [p.(Lys702Asnfs*19)] variant, which so far has only been reported in the Iranian population (Yazdanpanahi et al., 2015), is the third most common variant of *SLC26A4* in our study, segregating with HL in six Pakistani families from Baluchistan, a province that shares a border with neighboring Iran. Besides these and other common alleles, we also found four novel variants [c.42delC, p.(Glu15Serfs*51); c.154A>T, p.(Lys52*); c.317C>T, p.(Ala106Val); c.1264–3C>G] of *SLC26A4*. Taken together, our results further confirm the high prevalence of *SLC26A4* alleles in Pakistani families with HL (Anwar et al., 2009; Tsukada, Nishio, Hattori, & Usami, 2015).

GJB2 variants are the second most common (15.7%; 95% CI: 12.1–20.1%) cause of HL in our cohort of 321 families. The *GJB2* variant spectrum is ancestry-specific and varies significantly around the world. Consistent with previous studies in the Pakistani population, two variants [c.71G>A, p.(Trp24*); c.231G>A, p.(Trp77*)] represent the majority of the *GJB2*-associated HL (Anjum et al., 2014; Bukhari, Mujtaba, & Naz, 2013; Salman et al., 2015; Santos, Wajid, Pham, et al., 2005; Shafique et al., 2014). We also identified a new nonsense allele [c.355G>T, p.(Glu119*)] segregating with HL in one family (DEM4614), which expands the list of more than 200 variants in the single protein-coding exon of *GJB2* (Duman & Tekin, 2012).

Variants of *MYO7A* account for 11.4% (95% CI: 8.3–15.3%) of HL in our cohort of 321 families, slightly more frequent than in previous reports (Riazuddin et al., 2008; Shahzad et al., 2013). Contrary to *SLC26A4* and *GJB2*, no predominant *MYO7A* variant in the Pakistani population was uncovered in our study. However, six *MYO7A* variants (c.496delG, c.722G>A, c.1183C>T, c.1258A>T c.3502C>T, c.4838delA) were present in more than one family (Table 1 and Supp. Table S3). We also identified eight families harboring causative compound heterozygous variants in *MYO7A* (Supp. Table S3).

Similar to *MYO7A*, no prevalent allele of *TMC1* was revealed in our study and 14 out of 18 identified variants of this gene were found in single families. Seven new likely pathogenic variants (c.945G>A [p.(Trp315*)], c.1143C>G [p.(Tyr381*)], c.1209G>A [p.(Trp403*)], c.1224+2T>C, c.1259G>A [p.(Cys420Tyr)], c.1728C>G [p.(Asn576Lys)] and c.1753_1754insA [p.(Asn407Lysfs*2)]) were identified in our cohort (Table 1 and Supp. Table S3). In contrast, the c.482 +1986_88delTGA allele of *HGF* accounts for HL segregating in 26 families (Table 1), which was already identified as a founder allele in 50 other Pakistani families in our collaborative studies (Naz et al., 2017; Rehman et al., 2015; Schultz et al., 2009). Similarly, the c.272T>C allele of *CIB2* accounts for the HL phenotype segregating in 15 families (Table 1). Studies in mouse have highlighted the crucial role of CIB2 in the mechanotransduction process and the deleterious effect of this specific missense mutation on hearing (Giese et al., 2017; Michel et al., 2017). Variants of *CIB2* have been found in hearing-impaired individuals of Turkish, Caribbean Hispanic, Iranian, Palestinian

Arab, European and Dutch origin (Booth et al., 2017; Michel et al., 2017; Patel et al., 2015; Riazuddin et al., 2012; Seco et al., 2016; Shaikh et al., 2017). Together with previously published studies, the *CIB2* c.272T>C allele has been found in 85 hearing-impaired Pakistani families (Rehman et al., 2015; Riazuddin et al., 2012; Seco et al., 2016; Shaikh et al., 2017) and is likely to be a founder allele (Riazuddin et al., 2012).

Likely pathogenic variants of other genes associated with hearing loss in our cohort of 321 families include *CDH23* (n=16 variants), *TMPRSS3* (n=16), *OTOF* (n=10) and *BSND* (n=10) (B. Y. Choi et al., 2009; Naz et al., 2017; Rehman et al., 2015; Riazuddin et al., 2012; Shafique et al., 2014). This study highlights the genetic heterogeneity of HL-associated variants as 127 out of 163 (77.1%) identified alleles occur only once in our cohort. The diversity and the growing numbers of identified rare and non-recurrent variants increase the complexity of a molecular genetic diagnosis of HL (Gao & Dai, 2014). These data also predict that the Pakistani population will continue to be valuable for discovering the molecular genetics not only of HL but also of a multitude of other understudied monogenic and complex disorders such as persistent stuttering (Raza, Amjad, Riazuddin, & Drayna, 2012; Raza, Riazuddin, & Drayna, 2010; Riaz et al., 2005).

To better evaluate the genetic spectrum of recessive nonsyndromic HL in the Pakistani population, we combined data from these 321 new families with data from 61 of our previously published collaborative studies in Pakistan since 1999 (Fig. 6, Supp. table S4). Out of 875 identified mutant alleles in 54 genes (368 unique variants), more than 75% (75.3%) of the pathogenic alleles were found in 10 genes: *SLC26A4*, *MYO7A, GJB2, CIB2*, *HGF*, *TMC1*, *MYO15A, CDH23*, *TMPRSS3* and *OTOF*. *MYO15A* variants were recently reviewed (Rehman et al., 2016) and represent 5.7% of the total predicted causative mutations, while alleles in *BSND* a gene involved in either Bartter syndrome or sensorineural HL with mild renal dysfunction (MIM# 602522), occur in 1.8% of the identified variants. We emphasize that the families in these studies were recruited on the initial assumption that they were segregating non-syndromic HL although a syndromic deafness often becomes evident only after identifying the responsible variants and completing phenotypic analyses and functional studies of the gene in mutant and wild type animal models (Imtiaz et al., 2018).

When the Pakistani population is compared to other populations i.e. Iranian (Ghasemnejad, Shekari Khaniani, Zarei, Farbodnia, & Mansoori Derakhshan, 2017; Sloan-Heggen et al., 2015), Indian (Yan et al., 2015), Chinese (Jiang et al., 2015; Xin et al., 2013; Zhang et al., 2016), African-descent (Rudman et al., 2017), European ancestry (Yan et al., 2017) or multi-ethnic (Sloan-Heggen et al., 2016; Yan et al., 2016), the distribution of genes associated with nonsyndromic HL and the variants reveal differences and commonalities. Some of the most prevalent “HL genes” in all of these populations are *SLC26A4, GJB2* and *MYO7A*.

In these three genes, hundreds of pathogenic variants associated with HL have been identified while only 7 to 50 variants have occurred in the other most prevalent genes in our cohort with the exception of *CDH23* (Human Gene Mutation Databases). Increased mutational events might explain the higher incidence of HL-associated variants, independently of the ethnicity of the considered population.

In conclusion, this study expands our understanding and knowledge of the allelic spectrum of HL genes in the Pakistani population, highlighting the extreme locus and variant heterogeneity of this disorder, sometimes even among siblings (e.g. see Fig. 3 in (Rehman et al., 2015)). This implies that even though we identified the genetic etiology of HL in hundreds of families within coding regions of known NSARHL genes, due to intra-familial genetic heterogeneity, we expect to identify at least 1000 additional variants in perhaps hundreds more novel genes in our unsolved families. Given the complexity of inner ear structure and function of the mechano-chemical transduction of sound, it has been speculated that there will be as many as 1000 genes that are involved in HL in mammals (Steel, 2014), which is further supported by yet undiscovered genetic lesions in hundreds of individuals and families with HL in our cohorts. With the rapid evolution of sequencing technologies and bioinformatics tools (He et al., 2017; Ott, Wang, & Leal, 2015), and with whole-genome sequencing more affordable, we anticipate the identification of more rare variants within HL genes. Identifying the molecular lesions will be critical for a more complete understanding of how we hear and for accomplishing personalized therapies (Askew et al., 2015; Chien et al., 2016; Shibata et al., 2016).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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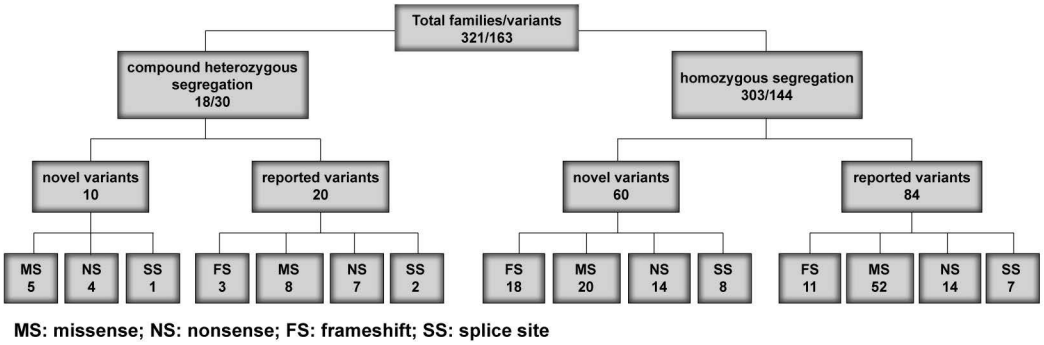

Figure 1. Overview of genetic studies of 321 Pakistani families segregating recessive hearing loss.

Each box has the number of families over the number of variants found in our study. Of 163 variants, 11 of these variants were identified in multiple families as either homozygous or compound heterozygous. In the bottom two rows, only the number of variants is indicated. MS: missense, NS: nonsense, FS: frameshift, SS: splice site.

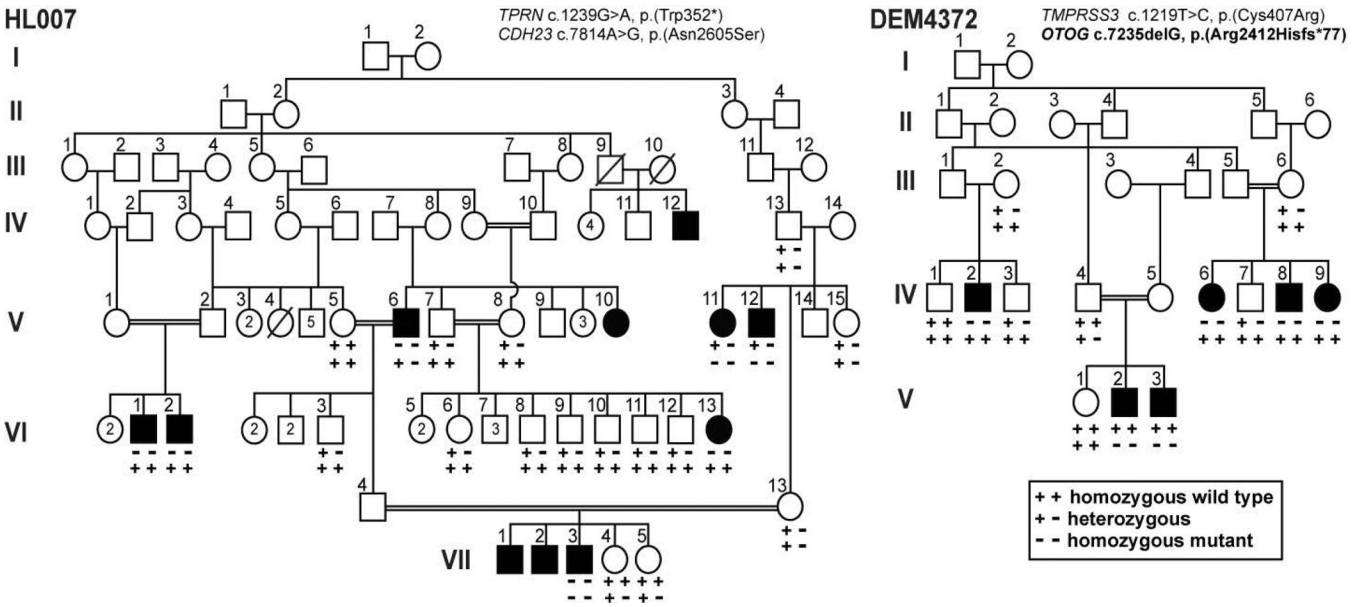

Figure 2. Pakistani families co-segregating autosomal recessive HL due to multiple homozygous variants.

Families HL007 and DEM4372 are examples in which there is intra-familial locus heterogeneity of variants in genes known to cause HL. Circles indicate females, squares indicate males. Black-filled symbols represent affected individuals. Double lines indicate consanguineous marriages. In family HL007, homozygous variants in two different genes *TPRN* and *CDH23* co-segregate with HL. Affected individuals are homozygous for either the *TPRN* (V:6, VI:1, VI:2, VI:13) or the *CDH23* variant (V:11, V:12), except for affected individual VII:3 who is double homozygous. In family DEM4372, variants in two different genes *TMPRSS3* and *OTOG* co-segregate with HL in different branches of this family. Novel mutations from this study are in bold font.

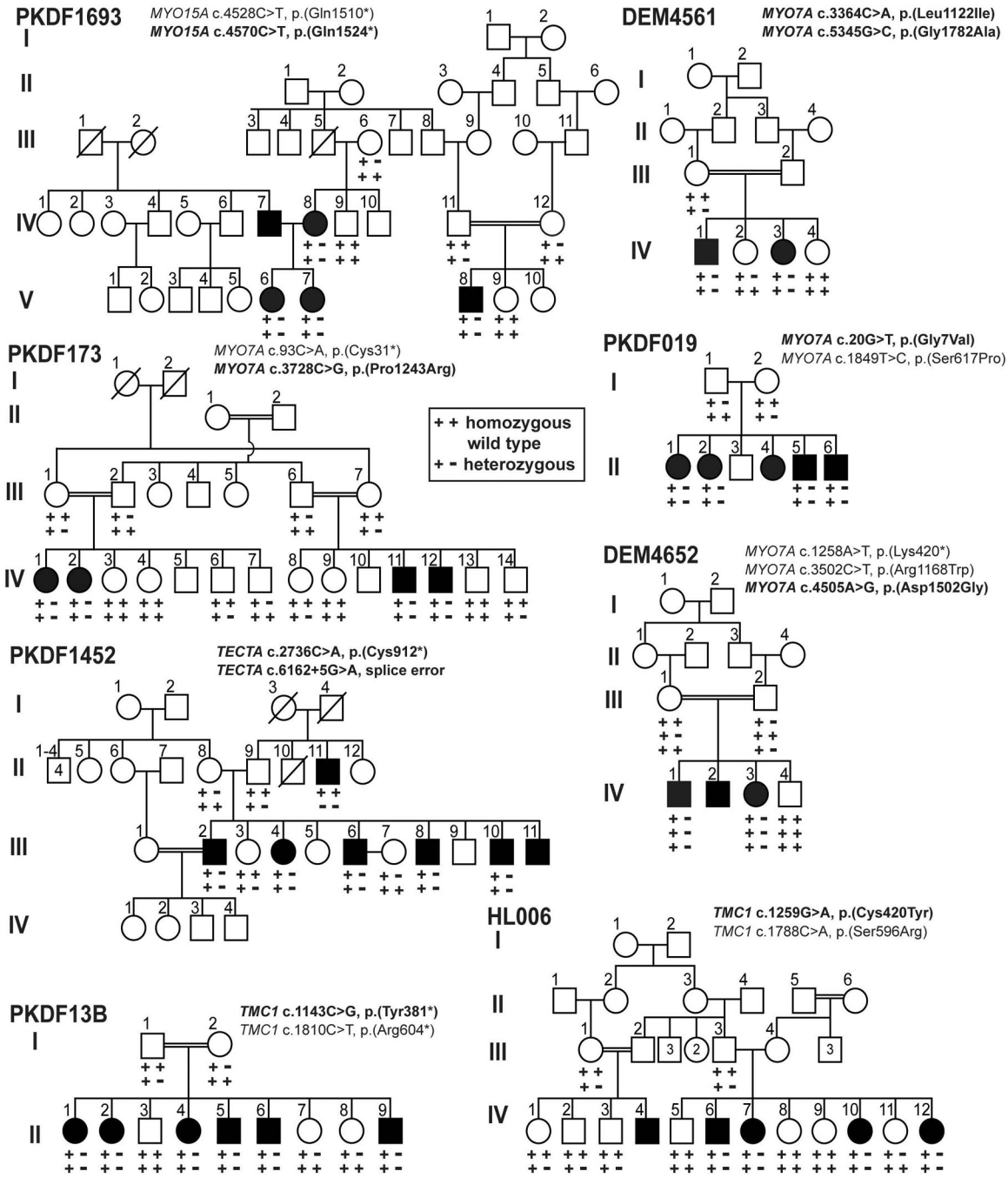

Figure 3. Consanguineous Pakistani families with compound heterozygous variants of reported HL genes.

Eight pedigrees segregating HL due to compound heterozygous variants in *MYO7A* (PKDF173, DEM4561, DEM4652, PKDF019), *TMC1* (HL006, PKDF13B), *MYO15A* (PKDF1693) and *TECTA* (PKDF1452) genes. In family DEM4652, the normal hearing father III:2 is heterozygous for two different variants of *MYO7A* suggesting that the newly identified c.4505A>G variant might be non-pathogenic. Novel mutations from this study are in bold font.

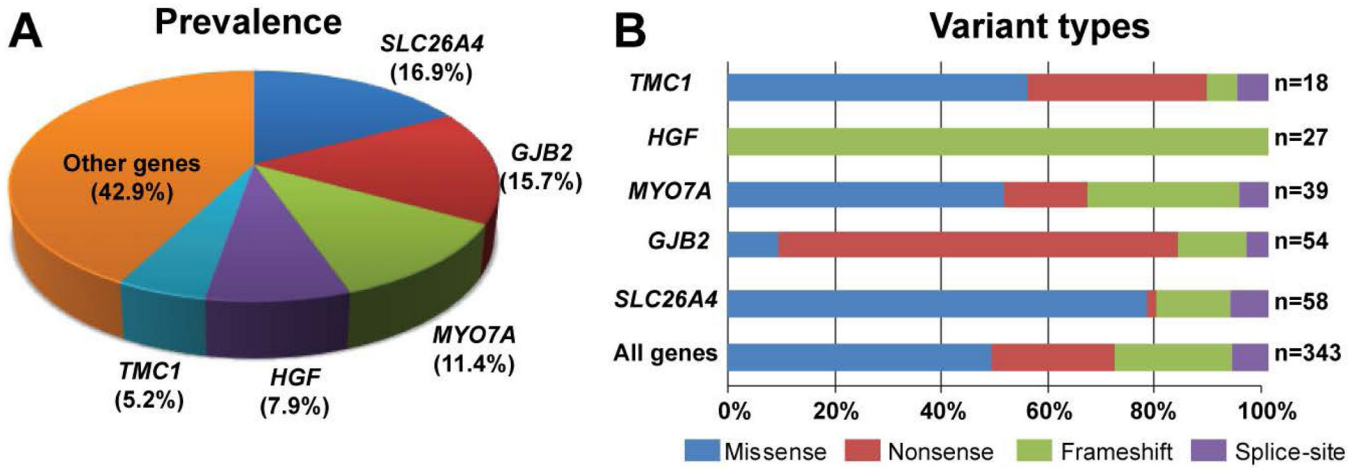

Figure 4. Distribution of variants in the five most prevalent genes associated with nonsyndromic recessively inherited HL in Pakistani families.

(A) Percentage of identified alleles by gene. (B) Percentage of variants by type and occurrence of identified variants. The percentages are based on the total number of variants identified in this study.

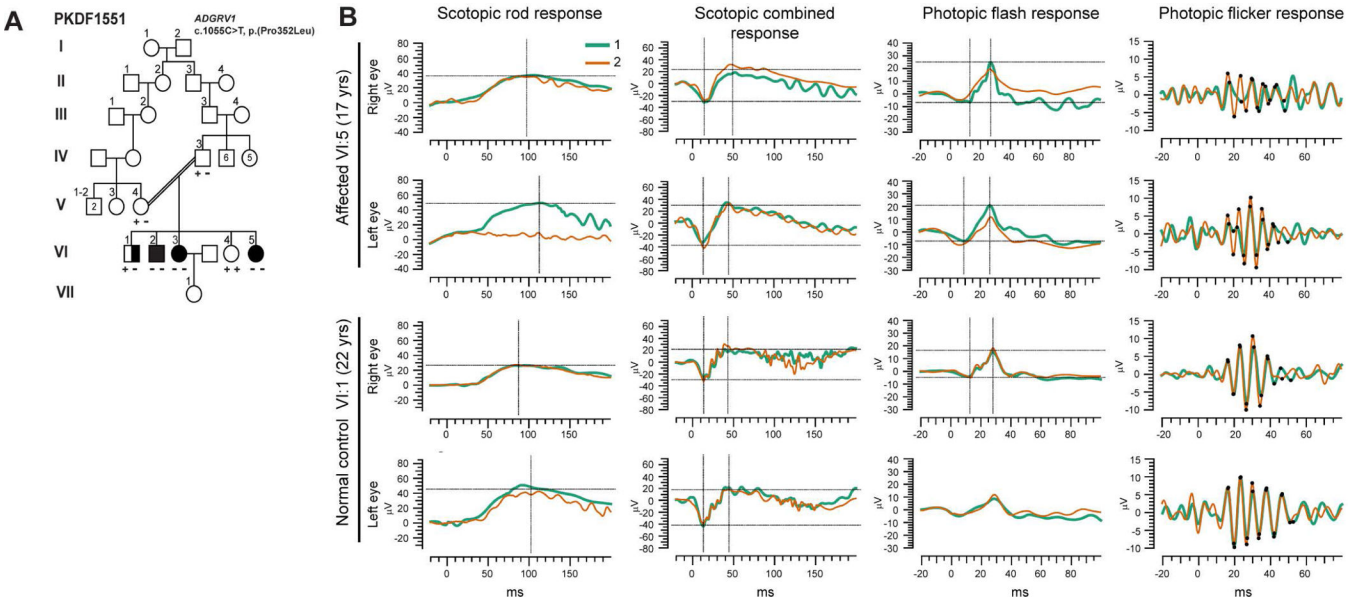

Figure 5. A new variant of *ADGRV1* associated with non-syndromic hearing loss in Pakistani family PKDF1551.

(A) Pedigree of family PKDF1551 segregating HL due to a missense mutation (c.1055C>T, p.(Pro352Leu)) in *ADGRV1* (also referred to *VLGR1*, MIM# 602851, at the *USH2C* locus). The filled symbols represent affected individuals, and a double horizontal line connecting parents represents a consanguineous marriage. Half-filled symbol represents an individual with potential middle ear infection and/or carrying another variant responsible for his hearing impairment. Exome sequencing was performed on a DNA sample from individual VI:5. (B) Full-field electroretinograms of affected (VI:5, 17 years old) and non-affected individuals (VI:1, 22 years old) revealed no significant difference in a- and b-waves amplitudes among the two subjects, suggesting normal visual function. To show reproducibility, two responses (annotated 1 and 2, green and orange respectively) are superimposed per test and individual. The first crosshair indicates the a-wave while the second crosshair shows the b-wave. No differences in amplitude nor latency for waves a and b were detected in any of the performed tests.

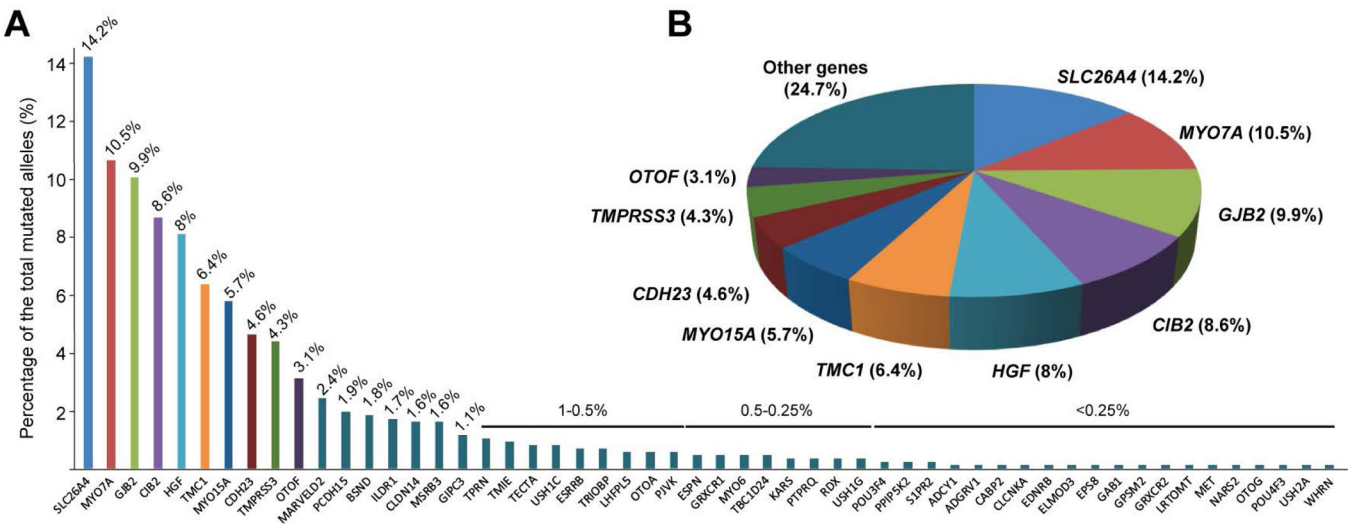

Figure 6. Distribution of variants in the Pakistani population from 62 studies on hearing loss.

(A) Percentage of identified variants by gene. (B) Distribution of the 10 most prevalent genes in our collaborative studies. The percentages are based on the total number of alleles identified in our collaborative studies.

Table 1.

Novel and reported variants in this study of genes known to be associated with autosomal recessive hearing loss.

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
PKDF1551	*ADGRV1*	**c.1055C>T**	**p.(Pro352Leu)**	This study	-	-	2.5E-05
DEM4304, DEM4384, DEM4558, DEM4150, DEM4576, DEM4592, DEM4593, PKDF970b, PKDF1673, PKSR20	*BSND*	c.35T>C	p.(Ile12Thr)	Riazuddin et al., 2009	-	-	2E-05
DEM4545	*CABP2*	c.637+1G>T	Splice error	Schrauwen et al., 2012	-	0.0008	0.001
PKDF1767	*CDH23*†	c.2866G>A	p.(Glu956Lys)	Miyagawa, Naito, Nishio, Kamatani, & Usami, 2013	-	-	-
PKDF055	*CDH23*†	c.2968G>A	p.(Asp990Asn)	Bork et al., 2001	-	-	-
DEM4556	*CDH23*†	c.3481C>T	p.(Arg1161*)	Roux et al., 2006	-	-	-
DEM4504	*CDH23*†	c.3880C>T	p.(Gln1294*)	Bork et al., 2001	-	-	-
DEM4324	*CDH23*†	c.4892C>T	p.(Ala1631Val)	Shearer et al., 2013	0/188	0.0010	0.0009
DEM4108	*CDH23*†	c.5149T>C	p.(Cys1717Arg)	Sloan-Heggen et al., 2015	0/188	-	8.E-06
PKDF055	*CDH23*†	c.6049+1G>A	Splice error	Bork et al., 2001	-	-	-
DEM4363	*CDH23*†	c.6050–9G>A	Splice error	von Brederlow et al., 2002	-	-	-
LUAB14	*CDH23*†	**c.6083A>C**	**p.(Asp2028Ala)**	This study	0/100	-	-
DEM4112, PKDF1781	*CDH23*†	c.6133G>A	p.(Asp2045Asn)	Bork et al., 2001	-	-	8E-06
DEM4544	*CDH23*†	**c.6202A>C**	**p.(Thr2068Pro)**	This study	0/202	-	8E-06
DEM4710	*CDH23*†	c.6604G>A	p.(Asp2202Asn)	Bork et al., 2001	-	-	8E-06
HL007	*CDH23*†	c.7814A>G	p.(Asn2605Ser)	Naz et al., 2017	0/125	-	-
DEM4491	*CDH23*†	c.7987_7989delTTC	p.(Phe2664del)	Schultz et al., 2011	0/188	-	-
DEM4305	*CDH23*†	**c.8351_8352insCGAT**	**p.(Leu2785Aspfs*43)**	This study	0/284	-	-
DEM4042, DEM4053, DEM4630, DEM4722, DEM4749, DEM4785, DEM4789, DEM4791, DEM4793,	*CIB2*†	c.272T>C	p.(Phe91Ser)	Riazuddin et al., 2012	-	-	5E-05

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
DEM4868, DEM4870, PKDF1093, PKDF1285, PKDF520, PKDF781							
PKZA13	*CIB2*†	c.297C>G	p.(Cys99Trp)	Riazuddin et al., 2012	-	-	-
DEM4312	*CLCNKA*	**c.1985G>T**	**p.(Gly662Val)**	This study	0/198	-	-
DEM4101, DEM4210, PKDF958, PKDF1660	*CLDN14*†	c.254T>A	p.(Val85Asp)	Lee, Ansar, et al., 2012	-	-	8E-06
DEM4005	*EDNRB*	c.553G>A	p.(Val185Met)	Puffenberger et al., 1994	-	-	0.0001
DEM4134	*EPS8*	**c.205–8A>G**	**Splice error**	This study	-	-	-
PKDF1550	*ESPN*†	**c.2496_2496delC**	**p.(Tyr832*)**	This study	-	-	8.4E-06
DEM4704	*ESRRB*†	c.536G>A	p.(Arg179His)	Miyagawa et al., 2013	-	-	2E-05
PKDF969, PKDF1442, PKDF1573	*GIPC3*†	c.662C>T	p.(Thr221Ile)	Rehman et al., 2011	-	-	4E-05
DEM4551B, DEM4582	*GJB2*	c.−23+1G>A	Splice error	Denoyelle et al., 1997	-	-	-
DEM4347, DEM4648, DEM4659, DEM4352A, DEM4871, PKSR8a, PKDF1621	*GJB2*	c.35delG	p.(Gly12Valfs*2)	Zelante et al., 1997	-	0.002	0.006
DEM4307, DEM4339, DEM4346, DEM4351, DEM4409, DEM4550, DEM4573, DEM4582, DEM4583, DEM4656, DEM4717, DEM4438A, DEM4866, DEM4981, PKDF021, PKDF936, PKDF939, PKDF1368, PKDF1553, PKDF1655, PKDF1760	*GJB2*	c.71G>A	p.(Trp24*)	Kelsell et al., 1997; Santos, Wajid, Pham, et al., 2005	-	0.0004	0.0006
PKDF1666	*GJB2*	c.223C>T	p.(Arg75Trp)	Richard et al., 1998	-	-	-
DEM4528, DEM4537	*GJB2*	c.224G>A	p.(Arg75Gln)	Uyguner et al., 2002	-	-	-
DEM4015A, DEM4143, DEM4255, DEM4262, DEM4479, DEM4660, DEM4670, DEM4696, DEM4737, DEM4769, DEM4770, DEM4972, PKDF936, PKDF1588, PKDF1633, PKDF1665, PKDF1675, PKDF1790	*GJB2*	c.231G>A	p.(Trp77*)	Santos, Wajid, Pham, et al., 2005	-	-	0.0001
DEM4614	*GJB2*	**c.355G>T**	**p.(Glu119*)**	This study	0/454	-	7E-05
PKDF1694, PKDF1764	*GJB2*	c.457G>A	p.(Val153Ile)	Marlin et al., 2001	-	-	-
DEM4431, DEM4653	*GPSM2*	**c.138C>A**	**p.(Phe46Leu)**	This study	0/328	-	8E-06
PKDF1679	*GRXCR1*†	c.229C>T	p.(Gln77*)	Schraders et al., 2010	-	-	-

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
DEM4153	*GRXCR1*†	**c.655G>A**	**p.(Glu219Lys)**	This study	0/114	-	-
DEM4494, DEM4521, DEM4522, DEM4562, DEM4564, DEM4586, DEM4622, DEM4628, DEM4642, DEM4665, DEM4701, DEM4865, DEM4861, DEM4880, DEM4925, DEM4943, PKDF970a, PKDF1017, PKDF1331, PKDF1579, PKDF1604, PKDF1637, PKDF1668, PKDF1754, PKDF1787, PKDF1791	*HGF*†	c.482 +1986_88delTGA		Schultz et al., 2009	-	-	-
PKDF1775	*HGF*†	c.482 +1991_2000delGATGATGAAA		Schultz et al., 2009	-	-	-
PKDF1651	*ILDR1*	c.3G>A	p.(Met1Ile)	Borck et al., 2011	-	-	-
DEM4175	*ILDR1*	**c.900delG**	**p.(Thr301Profs*20)**	This study	0/188	-	-
PKDF1455	*ILDR1*	**c.1005_1005delG**	**p.(Glu335Aspfs*30))**	This study	-	-	-
DEM4353	*ILDR1*	**c.1029_1030delTT**	**p.(Trp344Glyfs*17)**	This study	0/282	-	-
PKDF1428	*ILDR1*	**c.1384C>T**	**p.(Arg462*)**	This study	-	-	6E-05
PKDF1311, PKDF1549	*LHFPL5*†	c.250delC	p.(Leu84*)	Shabbir et al., 2006	-	-	-
PKDF1178	*LHFPL5*†	**c.452 G>T**	**p.(Gly151Val)**	This study	-	-	8.00E-06
PKDF1662	*MARVELD2*†	c.1138C>T	p.(Gln380*)	Sloan-Heggen et al., 2015	-	-	-
DEM4204	*MARVELD2*†	**c.1223_1224insA**	**p.(Glu408Argfs*4)**	This study	0/280	-	-
DEM4393, DEM4533, PKDF1639, PKDF1641	*MARVELD2*†	c.1295+2T>C	Splice error	Riazuddin et al., 2006	-	-	4E-05
PKDF1111, PKDF1489, PKDF1606, PKDF1610, PKDF1727	*MSRB3*†	c.265T>G	p.(Cys89Gly)	Ahmed et al., 2011	-	-	2E-05
PKDF1789	*MSRB3*†	**c.412–1G>A**	**Splice error**	This study	-	-	8.2E-06
PKDF019A	*MYO7A*	**c.20G>T**	**p.(Gly7Val)**	This study	0/170	-	1E-05
PKDF173	*MYO7A*	c.93C>A	p.(Cys31*)	Weston et al., 1996	-	0.017	5E-05
DEM4493	*MYO7A*	**c.247C>A**	**p.(Arg83Ser)**	This study	0/112	-	-
PKDF312	*MYO7A*	c.397dupC	p.(His133Profs*7)	Riazuddin et al., 2008	-	-	0.0006
PKDF1465, PKDF1490	*MYO7A*	c.496delG	p.(Glu166Argfs*5)	Riazuddin et al., 2008	-	-	-
DEM4141, DEM4299, DEM4365	*MYO7A*	c.722G>A	p.(Arg241His)	Cremers et al., 2007	-	0.0002	2E-05

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
PKDF370	*MYO7A*	c.977T>A	p.(Leu326Gln)	Riazuddin et al., 2008	-	-	-
PKDF537, PKDF1786	*MYO7A*	c.1183C>T	p.(Arg395Cys)	Shahzad et al., 2013	0/176	-	-
DEM4190, DEM4652	*MYO7A*	c.1258A>T	p.(Lys420*)	Le Quesne Stabej et al., 2012	-	-	4E-05
DEM4331	*MYO7A*	c.1591C>T	p.(Gln531*)	Riazuddin et al., 2008	-	-	8E-06
PKDF019A	*MYO7A*	c.1849T>C	p.(Ser617Pro)	Carss et al., 2017	0/160	-	5E-05
PKDF721	*MYO7A*	**c.2339delG**	**p.(Gly780Valfs*10)**	This study	0/154	-	-
PKDF312	*MYO7A*	c.3136dupC	p.(Leu1046Profs*9)	Riazuddin et al., 2008	-	-	-
DEM4561	*MYO7A*	**c.3364C>A**	**p.(Leu1122Ile)**	This study	-	-	-
DEM4281, DEM4692, PKDF628	*MYO7A*	c.3502C>T	p.(Arg1168Trp)	Le Guedard-Mereuze et al., 2010	-	0.0002	0.0001
PKDF1484	*MYO7A*	c.3508G>A	p.(Glu1170Lys)	Riazuddin et al., 2008	-	-	-
DEM4052A	*MYO7A*	**c.3590T>C**	**p.(Leu1197Pro)**	This study	0/282	-	-
PKDF173	*MYO7A*	**c.3728C>G**	**p.(Pro1243Arg)**	This study	0/164	-	-
DEM4652	*MYO7A*	**c.4505A>G**	**p.(Asp1502Gly)**	This study	0/454	-	2E-05
DEM4182, DEM4190, DEM4281, DEM4513, DEM4692, DEM4694	*MYO7A*	c.4838delA	p.(Asp1613Valfs*32)	Riazuddin et al., 2008	-	-	-
DEM4433	*MYO7A*	**c.5326+3A>G**	**Splice error**	This study	0/118	-	-
DEM4561	*MYO7A*	**c.5345G>C**	**p.(Gly1782Ala)**	This study	-	-	4E-05
DEM4206	*MYO7A*	c.5434G>A	p.(Glu1812Lys)	Roux et al., 2006	-	-	-
DEM4327	*MYO7A*	**c.5522C>G**	**p.(Thr1841Arg)**	This study	0/178	-	-
DEM4725	*MYO7A*	c.5660C>T	p.(Pro1887Leu)	Ben-Salem et al., 2014; Bharadwaj, Kasztejna, Huq, Berson, & Dryja, 2000	-	-	2E-05
PKDF974	*MYO7A*	**c.5856+5G>C**	**Splice error**	This study	0/148	-	-
PKDF1382	*MYO15A*	c.2456C>A	p.(Ser819*)	Naz et al., 2017	-	-	-
PKDF1657	*MYO15A*	**c.3843C>T**	**p.(Arg1169*)**	This study	-	-	8E-06
PKDF1693	*MYO15A*	c.4528C>T	p.(Gln1510*)	Rehman, et al., 2016	-	-	8E-06
PKDF1693	*MYO15A*	**c.4570C>T**	**p.(Gln1524*)**	This study	-	-	-
PKDF1780	*MYO15A*	**c.3505C>T**	**p.(Gln1669*)**	This study	-	-	-
PKDF016	*MYO15A*	c.8224+3A>G	**Splice error**	Rehman et al., 2016	-	-	-

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
DEM4725	*OTOA*	c.1148C>T	p.(Ala383Val)	EVS variant	-	-	8E-04
PKDF889	*OTOA*	c.1352G>A	p.(Gly451Asp)	Lee et al., 2013	-	-	8E-06
DEM4362, DEM4366	*OTOF*	**c.1550T>C**	**p.(Leu517Pro)**	This study	0/188	-	-
PKDF1723	*OTOF*	**c.1904T>A**	**p.(Val635Asp)**	This study	-	-	-
PKDF1535	*OTOF*	c.2122C>T	p.(Arg708*)	Choi et al., 2009	-	-	-
DEM4078	*OTOF*	c.2508C>A	p.(Tyr836*)	Choi et al., 2009	-	-	0.0006
DEM4512	*OTOF*	c.2896G>A	p.(Glu966Lys)	Choi et al., 2009	-	-	8E-06
DEM4548	*OTOF*	c.2965_2967delTTC	p.(Phe989del)	Naz et al., 2017	0/188	-	-
DEM4756	*OTOF*	**c.3376dupA**	**p.(Ile1126Asnfs*5**	This study	-	-	-
PKDF1336	*OTOF*	**c.3514C>T**	**p.(Arg1172Trp)**	This study	-	0.0002	2.7E-05
PKDF1450	*OTOF*	**c.4799+1G>A**	**Splice error**	This study	-	-	8.24e-06
PKDF1066a	*OTOF*	**c.5714G>T**	**p.(Gly1905Val)**	This study	0/168	-	
DEM4372B	*OTOG*	**c.7235delG**	**p.(Arg2412Hisfs*77)**	This study	1/326	-	0.0009
PKDF627	*PCDH15*†	**deletion of exons 14 and 15**		This study	0/100	-	-
PKDF1440	*PCDH15*†	c.400C>G	p.(Arg134Gly)	Ahmed et al., 2003	-	-	-
PKDF1499	*PCDH15*†	**c.788C>A**	**p.(Pro263Gln)**	This study	-	-	-
DEM4711	*PCDH15*†	c.1737C>A	p.(Tyr584*)	Jaijo et al., 2006	-	-	-
DEM4040	*PJVK*	**c.158C>G**	**p.(Ser53*)**	This study	0/284	-	-
DEM4529	*PJVK*	**c.162_172del**	**p.(Pro55Glufs*23)**	This study	0/454	-	-
DEM4752	*PJVK*	**c.406C>T**	**p.(Arg136*)**	This study	-	-	-
PKDF880	*PJVK*	**c.908_910delACA**	**p.(Asn303del)**	This study	0/168	-	-
PKDF908	*POU3F4*	**c.478C>T**	**p.(Gln160*)**	This study	0/71	-	-
PKDF1595	*POU4F3*	**c.374C>T**	**p.(Pro125Leu)**	This study	-	-	-
DEM4437	*PTPRQ*	**c.5158_5159delAT**	**p.(Ile1720Glnfs*7)**	This study	0/180	-	-
DEM4123	*PTPRQ*	**c.6739–1G>A**	**Splice error**	This study	0/172	-	-
DEM4465	*SLC26A4*	**c.42delC**	**p.(Glu15Serfs*51)**	This study	0/454	-	-
DEM4149B, HL005	*SLC26A4*	c.71G>T	p.(Arg24Leu)	Anwar et al., 2009	-	-	0.0003

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
DEM4549	*SLC26A4*	**c.154A>T**	**p.(Lys52*)**	This study	0/454	-	-
DEM4511	*SLC26A4*	**c.317C>T**	**p.(Ala106Val)**	This study	0/454	-	-
DEM4014A	*SLC26A4*	**c.413T>A**	**p.(Val138Asp)**	Chen, Wang, Sun, & Jiang, 2012			
DEM4436	*SLC26A4*	c.416G>T	p.(Gly139Val)	Anwar et al., 2009	-	-	4E-05
DEM4092, DEM4149B, DEM4152, DEM4220, DEM4241, DEM4271, DEM4290, DEM4292, DEM4293, DEM4295, DEM4296, DEM4429, DEM4482, DEM4483, DEM4523, DEM4575, DEM4726, DEM4766, DEM4192, DEM4291, DEM4481, DEM4982, PKDF1161, PKDF1664	*SLC26A4*	c.716T>A	p.(Val239Asp)	Park et al., 2003	-	0.0002	0.0003
DEM4186, DEM4454, DEM4455	*SLC26A4*	c.919–2A>G	Splice error	Coucke et al., 1999	-	-	0.0003
DEM4336	*SLC26A4*	c.1001G>T	p.(Gly334Val)	Kahrizi et al., 2009	-	-	8E-06
DEM4109	*SLC26A4*	c.1198delT	p.(Cys400Valfs*32)	Everett et al., 1997	-	-	2.00E-05
PKDF384b	*SLC26A4*	c.1226G>A	p.(Arg409His)	Van Hauwe et al., 1998	-	-	0.0001
PKDF128, PKDF384b, PKDF910	*SLC26A4*	c.1226G>C	p.(Arg409Pro)	Park et al., 2003	-	-	-
DEM4283, PKDF384b	*SLC26A4*	c.1229C>T	p.(Thr410Met)	Coucke et al., 1999	-	0.0002	0.0002
DEM4355	*SLC26A4*	c.1238A>G	p.(Gln413Arg)	Ji et al., 2009	-	-	8E-06
DEM4200	*SLC26A4*	**c.1264–3C>G**	**Splice error**	This study	0/454	-	-
DEM4004, DEM4094A, DEM4220, DEM4276, DEM4574, DEM4695, DEM4702, DEM4966	*SLC26A4*	c.1337A>G	p.(Gln446Arg)	Reardon, CF, Trembath, Jan, & Phelps, 2000	-	-	
DEM4286, DEM4287, DEM4294, DEM4392, DEM4425, DEM4553	*SLC26A4*	c.2106delG	p.(Lys702Asnfs*19)	Yazdanpanahi et al., 2012	-	-	8E-06
PKDF1283	*TECTA*	**c.840_841insT**	**p.(Val281Cysfs*11)**	This study	0/162	-	-
HL04	*TECTA*	**c.1247_1248delGG**	**p.(Gly416Aspfs*24)**	This study	0/100	-	-
DEM4242A	*TECTA*	**c.1774G>A**	**p.(Val592Met)**	This study	0/132	-	-
PKDF1452	*TECTA*	**c.2736C>A**	**p.(Cys912*)**	This study	-	-	-
PKDF1452	*TECTA*	**c.6162+5G>A**	**Splice error**	This study			
DEM4367, PKDF1584	*TMC1*†	c.100C>T	p.(Arg34*)	Kurima et al., 2002	-	-	-
PKSR39a	*TMC1*†	c.596A>T	p.(Asn199Ile)	Imtiaz et al., 2016	0/148	-	8E-06

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
DEM4451	*TMC1*†	**c.945G>A**	**p.(Trp315*)**	This study	0/454	-	-
DEM4396	*TMC1*†	c.1114G>A	p.(Val372Met)	Santos, Wajid, Khan, et al., 2005	-	-	7E-05
PKSR13b	*TMC1*†	**c.1143C>G**	**p.(Tyr381*)**	This study	0/166	-	-
DEM4147	*TMC1*†	**c.1209G>A**	**p.(Trp403*)**	This study	-	-	-
DEM4132	*TMC1*†	**c.1224+2T>C**	**Splice error**	This study	0/112	-	-
HL006	*TMC1*†	**c.1259G>A**	**p.(Cys420Tyr)**	This study	0/125	-	-
PKDF025	*TMC1*†	c.1333C>T	p.(Arg445Cys)	Sirmaci et al., 2009	-	-	-
DEM4325	*TMC1*†	c.1363T>C	p.(Tyr454His)	NSHI	-	-	-
PKDF1449	*TMC1*†	c.1543C>T	p.(Cys515Arg)	Kitajiri et al., 2007	-	-	-
DEM4350	*TMC1*†	**c.1728C>G**	**p.(Asn576Lys)**	This study	0/454	-	-
PKDF1617	*TMC1*†	**c. 1753_1754insA**	**p.(Asn407Lysfs*2)**	This study	-	-	-
HL006	*TMC1*†	c.1788C>A	p.(Ser596Arg)	Imtiaz et al., 2016	0/125	-	-
PKSR13b	*TMC1*†	c.1810C>T	p.(Arg604*)	Sirmaci et al., 2009	8E-06	-	-
DEM4370A, DEM4724	*TMC1*†	c.2035G>A	p.(Glu679Lys)	Santos, Wajid, Khan, et al., 2005	-	-	-
DEM4486	*TMIE*†	c.241C>T	p.(Arg81Cys)	Santos et al., 2006	-	-	-
DEM4687	*TMIE*†	c.250C>T	p.(Arg84Trp)	Naz et al., 2002	-	-	2E-05
DEM4020, PKDF1053	*TMPRSS3*†	c.271C>T	p.(Arg91*)	Bademci et al., 2016	-	-	2E-05
DEM4003	*TMPRSS3*†	c.323–6G>A	Splice error	Scott et al., 2001	-	-	6E-05
PKSR31	*TMPRSS3*†	c.581G>T	p.(Cys194Phe)	Ben-Yosef et al., 2001	-	-	-
DEM4106, DEM4124	*TMPRSS3*†	c.767C>T	p.(Ala256Val)	Lee, Khan, et al., 2012	-	-	-
PKDF765	*TMPRSS3*†	**c.783–1G>T**	**Splice error**	This study	0/130	-	

Family	Gene	Variant	Protein change	Reference	Allele frequency		
					Pakistan	1000G	ExAC
DEM4046, DEM4202, DEM4372A, DEM4560, LUAB13, HL003, PKDF1057, PKDF1330, PKDF1634	*TMPRSS3*†	c.1219T>C	p.(Cys407Arg)	Ben-Yosef et al., 2001	-	-	5E-05
HL007, PKDF1756, PKDF1757, PKDF1758	*TPRN*†	c.1056G>A	p.(Trp352*)	Rehman et al., 2010	-	-	-
DEM4492	*TRIOBP*†	**c.3460_3461delCT**	**p.(Leu1154Alafs*29)**	This study	0/454	-	-
PKDF1430	*TRIOBP*†	**c.3634_3646delCTGATCCCCCAAG**	**p.(Leu1212Cysfs*22)**	This study	-	-	-
DEM4707	*USH1C*	c.238dup	p.(Arg80Profs*69)	Bitner-Glindzicz et al., 2000	-	-	0.0003
DEM4335B	*USH1C*	c.463C>T	p.(Arg155*)	Ebermann et al., 2007	-	-	8E-06
DEM4675	*USH1G*	**c.511G>T**	**p.(Glu171*)**	This study	-	0.0002	2E-05
DEM4268	*USH1G*	**c.812delC**	**p.(Pro271Argfs*52)**	This study	0/454	-	-
DEM4689	*USH2A*	**c.3661C>T**	**p.(Gln1221*)**	This study	0/134	-	8E-06
DEM4171	*WHRN*	**c.2388_2389delCG**	**p.(Asn796Lysfs*46)**	This study	0/284		

Novel mutations from this study are in bold font.

(†) indicates genes associated with non-syndromic autosomal recessive hearing loss that were discovered through studies in Pakistani pedigrees.

Pak: Pakistani controls, 1000G: 1000 Genomes Project database, ExAc: Exome Aggregation Consortium database.

(-) indicates that the variant is absent from the respective database.