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REVIEW

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Autosomal recessive non-syndromic hearing loss genes in Pakistan during the previous three decades

Madiha Shadab¹  | Ansar Ahmed Abbasi¹  | Ahsan Ejaz^{2,3}  | Afif Ben-Mahmoud⁴  |
Vijay Gupta⁴  | Hyung-Goo Kim^{4,5}  | Barbara Vona^{6,7} 

¹Department of Zoology, Mirpur University of Science and Technology, Mirpur, Pakistan

²Department of Physics, University of Kotli Azad Jammu and Kashmir, Kotli, Pakistan

³School of Nuclear Science and Technology, Lanzhou University, Lanzhou, China

⁴Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Doha, Qatar

⁵College of Health & Life Sciences, Hamad Bin Khalifa University (HBKU), Doha, Qatar

⁶Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany

⁷Institute for Auditory Neuroscience and Inner Ear Lab, University Medical Center Göttingen, Göttingen, Germany

Correspondence

Barbara Vona, Institute of Human Genetics, University Medical Center Göttingen, 37073 Göttingen, Germany.
Email: barbara.vona@med.uni-goettingen.de

Madiha Shadab, Department of Zoology, Mirpur University of Science and Technology, Mirpur, 10250 Pakistan.
Email: madiha.shadab@hotmail.com

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Abstract

Hearing loss is a clinically and genetically heterogeneous disorder, with over 148 genes and 170 loci associated with its pathogenesis. The spectrum and frequency of causal variants vary across different genetic ancestries and are more prevalent in populations that practice consanguineous marriages. Pakistan has a rich history of autosomal recessive gene discovery related to non-syndromic hearing loss. Since the first linkage analysis with a Pakistani family that led to the mapping of the DFNB1 locus on chromosome 13, 51 genes associated with this disorder have been identified in this population. Among these, 13 of the most prevalent genes, namely *CDH23*, *CIB2*, *CLDN14*, *GJB2*, *HGF*, *MARVELD2*, *MYO7A*, *MYO15A*, *MSRB3*, *OTOF*, *SLC26A4*, *TMC1* and *TPRSS3*, account for more than half of all cases of profound hearing loss, while the prevalence of other genes is less than 2% individually. In this review, we discuss the most common autosomal recessive non-syndromic hearing loss genes in Pakistani individuals as well as the genetic mapping and sequencing approaches used to discover them. Furthermore, we identified enriched gene ontology terms and common pathways involved in these 51 autosomal recessive non-syndromic hearing loss genes to gain a better understanding of the underlying mechanisms. Establishing a molecular understanding of the disorder may aid in reducing its future prevalence by enabling timely diagnostics and genetic counselling, leading to more effective clinical management and treatments of hearing loss.

KEYWORDS

genetic counselling, genetic epidemiology, genotype, non-syndromic hearing loss, Pakistani population, phenotype

1 | INTRODUCTION

Pakistan, home to a population of 232 million according to the February 2023 United Nations census, ranks as the fifth most populous country in the world. The country's population is dispersed across multiple provinces, each with unique ethnic and linguistic diversity backgrounds. Consanguineous marriage is prevalent in most communities, making Pakistan one of the countries with the highest consanguinity rates globally. Consequently, homozygous pathogenic alleles steadily accumulate, leading to an ultimate increase in the prevalence of autosomal recessive disorders such as congenital hearing loss (HL). Out of the estimated 14.5 million Pakistanis living with HL, about half have a genetic aetiology.¹ According to the World Health Organization, more than 1.5 billion people around the world are impacted by HL, and roughly 28% of them, or 430 million individuals, have a disabling level of HL. This is concerning because the number of people with disabling HL is projected to exceed 700 million by 2050 (<https://www.who.int/health-topics/hearing-loss>).

Although individuals from the Pakistani community represent a minority of patients tested within the Asian population,² their contributions have yielded valuable insights into the molecular genetics and biology of hearing and deafness that hold relevance across diverse global genetic backgrounds. While this review does not aim to provide an exhaustive catalogue of all the variants identified in the Pakistani population, its goal is to highlight the exciting discoveries that have enriched our current understanding of the genetic epidemiology of HL. Although many genes have been implicated in HL (with many more yet to be discovered) in the Pakistani population, a dozen or so genes appear to play a predominant role in its genetic makeup. In this review, we provide a brief overview of the clinical heterogeneity and history of HL gene discovery, analyse gene ontology (GO) terms and pathways associated with HL and expand upon the key genes that explain a significant proportion of HL in the Pakistani population.

2 | CHARACTERISTICS AND CLINICAL CLASSIFICATIONS FOR HEARING LOSS

HL, the permanent loss of hearing ability, encompasses several key characteristics.³ Firstly, the anatomical site of the cochlear defect is used to distinguish between conductive or sensorineural HL (SNHL), with the latter being most prevalent.⁴ Conductive HL occurs due to abnormalities in the outer or middle ear that hinder sound conduction, while SNHL results from damage to the inner ear or auditory nerve. When both conductive and sensorineural components are present, mixed HL ensues. Additionally, abnormalities or malfunctions beyond the cochlea, such as in the eighth cranial nerve, auditory brainstem, or cortex, lead to central auditory impairment.⁵ The second characteristic pertains to the age of onset relative to speech development.³ Pre-lingual HL manifests before the critical period for speech development, including congenital onset or HL already present at birth. Post-lingual HL, on the other hand, includes individuals

with onset following the critical period for speech acquisition, spanning from childhood to advanced age and includes age-related HL.³ The third feature relates to the degree of HL and the associated hearing thresholds used to define severity levels, which include mild (hearing threshold 20–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–90 dB) and profound (90 dB).³ HL can affect low (<1000 Hz), mid (1000–2000 Hz) or high (>2000 Hz) frequencies. Classifying HL based on these factors is crucial for determining the most appropriate treatment and management strategies for each individual. The fourth attribute of HL concerns the presence or absence of progression, which is determined by serial audiometry. Although most late-onset HL typically follows a progressive course, the majority of individuals with congenital HL have a non-progressive form. HL can affect one or both ears, referred to as unilateral or bilateral, respectively. Asymmetric HL is another possibility where both ears are affected to differing degrees.⁶ The sixth feature is whether HL is part of a syndrome that involves concomitant abnormalities in additional organ systems or is the sole presenting feature, as seen with non-syndromic HL (NSHL), which is observed in the vast majority of congenital cases.³

Inheritance patterns are another defining characteristic of HL. Most hereditary HL exhibits an autosomal recessive inheritance, accounting for around 75% of congenital cases,⁷ while roughly 18%, 1%–3% and 1% of NSHL are caused by autosomal dominant, X-linked and mitochondrial forms, respectively.⁸ Those with autosomal recessive non-syndromic hearing loss (ARNSHL) inherit either a single heterozygous variant from each asymptomatic parent, as observed for homozygous variants, or receive two distinct variants in the same gene, one from each parent, known as compound heterozygous variants. If both parents carry a single disease allele on the same gene, their offspring have a 25% chance of inheriting two disease alleles to develop HL, a 50% chance of inheriting one disease allele to be an asymptomatic carrier and a 25% chance of inheriting no disease alleles and being unaffected.

3 | METHODS USED FOR IDENTIFICATION OF ARNSHL GENES

Over the past three decades, there has been significant progress in identifying genes and loci associated with HL in Pakistani families (see Table 1 and Figure 1), using a range of evolving technologies. One method used to locate Mendelian disease loci involves classic parametric linkage analysis, which utilizes genetic STRP markers or SNP maps along with information about mode of inheritance, penetrance and allele frequencies.⁹ This approach is based on the principle that chromosomal regions near genetic variants segregate together during meiosis. By analysing nearby markers, a genomic locus carrying a causative mutation may be statistically linked to the disease. The strength of the linkage evidence (or lack thereof) is assessed using the estimation of a logarithm of the odds (LOD) score. A LOD score greater than 3.0 is considered significant for linkage.¹⁰ Once one or more potentially significant linked intervals have been

TABLE 1 Fifty-one ARNSHL genes were found mutated in the Pakistani population in the last 25 years listed in chronological order.

Gene symbol	DFN locus	Chr position	Gene name	MIM	Discovery method	Onset and severity	Reference
GJB2	DFNB1	13q12.11	Gap junction protein beta 2	220290	LA	Prelingual, severe to profound	[13]
MYO15A	DFNB3	17q11.2	Myosin XVA	600316	NGS	Congenital, severe to profound	[14]
CLDN14	DFNB29	21q22.1	Claudin 14	614035	LA	Congenital, profound	[15]
TMIE	DFNB6	3p21.31	Transmembrane inner ear	600971	LA	Prelingual, severe to profound	[16]
TMC1	DFNB7/11	9q21.13	Transmembrane channel like 1	600974	LA	Prelingual, severe to profound, and vestibular dysfunction	[17]
USH1C	DFNB18	11p15.1	Greb1-like retinoic acid receptor coactivator	602092	LA	Prelingual, moderate to severe	[18]
PCDH15	DFNB23	10q21.1	Protocadherin-related 15	609533	LA	Prelingual, profound	[19]
TECTA	DFNB21	11q23.3	Tectorin alpha	603629	LA	Prelingual, moderate to severe	[20]
SLC26A4	DFNB4	7q22.3	Solute carrier family 26, member 4	600791	HM, LA	Prelingual, severe to profound	[21]
MYO6	DFNB37	6q14.1	Myosin VI	607821	LA	Congenital, profound	[22]
TMPRSS3	DFNB8/10	21q22.3	Transmembrane serine protease 3	614861	LA	Congenital, profound	[23]
ESPN	DFNB36	1p36.3	Espin	609006	HM, LA	Congenital, profound, with or without vestibular dysfunction	[24]
WHRN	DFNB31	9q32	Cask-interacting protein	607084	LA	Congenital, profound	[25]
TRIOBP	DFNB28	22q13.1	TRIO and F-actin-binding protein	609823	LA	Congenital, profound	[26]
MARVELD2	DFNB49	5q13.2	MARVEL domain containing 2	610153	LA	Prelingual, Moderate to severe	[27]
LHFPL5	DFNB67	6p21.31	LHFPL tetraspan subfamily member 5	610265	LA, HM	Prelingual, severe to profound	[28]
RDX	DFNB24	11q22.3	Radixin	611022	LA	Pre-lingual, profound	[29]
ESRRB	DFNB35	14q24.3	Oestrogen-related receptor beta	608565	LA	Prelingual, severe to profound	[30]
LRTOMT	DFNB63	11q13.4	Leucine-rich transmembrane and O-methyltransferase domain containing	611451	LA	Congenital, profound	[31]
OTOF	DFNB9	2p23.3	Otoferlin	601071	LA	Prelingual, severe-to-profound	[32]
HGF	DFNB39	7q21.11	Hepatocyte growth factor	608265	LA	Prelingual, severe to profound	[33]
BSND	DFNB73	1q32.3	Barttin CLCNK type accessory subunit beta	602522	LA	Prelingual, profound	[34]
TPRN	DFNB79	9q34.3	Taperin	613307	NGS / LA	Prelingual, severe to profound	[35]
GRXCR1	DFNB25	4p13	Glutaredoxin and cysteine-rich domain containing 1	615837	HM, LA	Congenital, moderate to profound	[36]
MSRB3	DFNB74	12q14.3	Methionine sulfoxide reductase B3	613718	LA	Prelingual, severe to profound	[37]
GIPC3	DFNB15/72/95	19p13.3	GIPC PDZ domain containing family member 3	601869	LA	Prelingual, mild to profound	[38]
ILDR1	DFNB42	3q13.33	Immunoglobulin-like domain containing receptor 1	609646	LA	Prelingual, profound	[39]
PJVK	DFNB59	2q31.2	Pejvakin	610220	LA, HM	Prelingual, profound	[40]
CIB2	DFNB48	15q25.1	Calcium- and integrin-binding family member 2	609439	LA		[41]

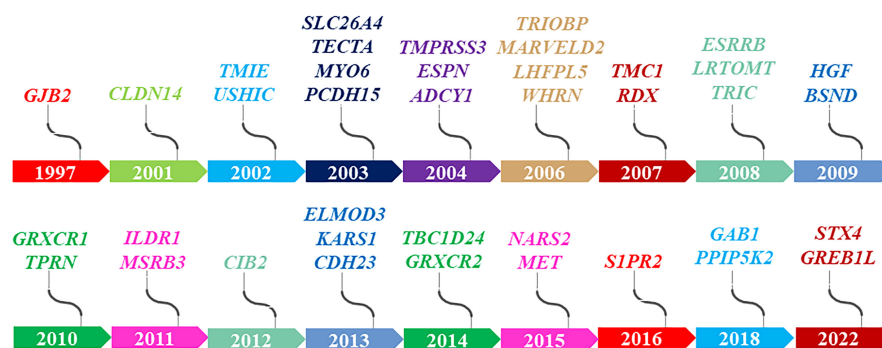
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TABLE 1 (Continued)

Gene symbol	DFN locus	Chr position	Gene name	MIM	Discovery method	Onset and severity	Reference
CDH23	DFNB12	17q12	Cadherin 23	601386	ES	Congenital, Profound	[42]
KARS1	DFNB89	16q23.1	Lysyl-tRNA synthetase 1	613916	LA, ES	Prelingual, profound	[43]
OTOA	DFNB22	16p13.1-q11.2	Otoancorin	607039	HM	Congenital, moderate to severe	[44]
ELMOD3	DFNB88	2p11.2	ELMO domain containing 3	615429	ES, HM	Prelingual, severe to profound	[45]
GRXCR2	DFNB101	5q32	Glutaredoxin and cysteine-rich domain containing 2	615837	LA, HM, ES	Congenital, severe to profound	[46]
TBC1D24	DFNB86	16p13.3	TBC1 domain family member 24	614617	ES, LA	Prelingual, profound	[47]
ADCY1	DFNB44	7p12.3	Adenylate cyclase 1	610154	LA	Congenital, Severe to profound	[48]
NARS2	DFNB94	11q14.1	Asparaginyl-tRNA synthetase 2, mitochondrial	618434	ES, LA	Prelingual severe to profound	[49]
MET	DFNB97	7q31.2	MET proto-oncogene, receptor tyrosine kinase	164860	LA, HM, ES	Congenital, severe	[50]
S1PR2	DFNB68	19p13.2	Sphingosine-1-phosphate receptor 2	608565	ES, LA, HM	Congenital, profound	[51]
EPS8L2	DFNB102	11p15.5	EPS8 like 2	617637	ES, GS	Post lingual, severe	[52]
PDZD7	DFNB57	10q24.31	PDZ domain containing 7	618003	ES	Congenital, severe to profound	[53]
PPIP5K2	DFNB100	5q21.1	Diphosphoinositol pentakisphosphate kinase 2	618422	ES, HM, LA	Prelingual, severe to profound	[54]
GAB1	DFNB26	4q31.21	GRB2-associated binding protein 1	605429	LA, ES	Congenital, severe to profound	[55]
TMEM132E	DFNB99	17q12	Transmembrane protein 132E	618481	ES	Prelingual, profound	[56]
LOXHD1	DFNB77	18q21.1	Lipoxigenase homology PLAT domains 1	613079	ES	Congenital, profound	[57]
CABP2	DFNB93	11q13.2	Calcium-binding protein 2	614899	ES	Prelingual, moderate to severe	[58]
SERPINB6	DFNB91	6p25.2	Serpin family B member 6	613453	ES	Congenital, profound	[59]
PTPRQ	DFNB84	12q21.31	Protein tyrosine phosphatase receptor type Q	613391	ES, LA	Prelingual, severe to profound	[59]
SIX5	ND	19q13.32	SIX homeobox 5	610896	ES	Congenital and post-lingual, severe to profound	[60]
STX4	ND	16p11.2	Syntaxin 4	186591	ES, HM, LA	Congenital, profound	[61]
GREB1L	ND	18q11.1-q11.2	GREB1 Like Retinoic Acid Receptor Coactivator	619274	ES	Congenital, profound	[62]

Abbreviations: ES, exome sequencing; GS, genome sequencing; HM, homozygosity mapping; LA, linkage analysis; ND, not defined; NGS, next-generation sequencing.

FIGURE 1 Timeline of 39 ARNSHL genes identified by collaborating with the Pakistani population over the past 25 years.



identified through linkage analysis, the next step involves candidate gene sequencing of all genes in the region. Thirty-nine ARNSHL genes have been identified in the Pakistani population with the support of linkage analysis. Homozygosity mapping is considered the best method for uncovering chromosomal regions that may harbour a causal variant in consanguineous families.¹¹ This method involves examining genotypes from multiple affected and unaffected family members to identify the number and size of runs of homozygosity. This information can be used to narrow down the genomic regions that are likely to contain the disease-causing variant. In genetically heterogeneous disorders, homozygosity mapping can reduce the need to sequence various candidate genes and instead focus on specific genomic regions.¹² Twelve ARNSHL genes have been identified by using homozygosity mapping.

Gene discovery has been further revolutionized by advances in exome and genome sequencing. Exome sequencing targets the protein-coding portion of genes, representing only about 1% (30Mb) of the genome but capturing 85% of the disease-causing mutations in Mendelian disorders.⁶³ Consequently, sequencing the entire exome has become a powerful tool for identifying disease-associated variants, including monogenic disorders like HL as well as predisposing variants in common diseases. This has led to faster identification of such variants.⁶⁴ Genome sequencing provides a comprehensive view of an individual's entire genome, including both coding and non-coding regions, allowing for the identification of both rare and common variants associated with HL. In recent years, genome sequencing has enabled the discovery of new HL genes, particularly when variants reside in non-coding regions that were previously overlooked.⁶⁵ Using exome and genome sequencing, 22 gene variants in novel NSHL-associated genes in the Pakistani population have been identified. Some of these 22 genes (12 genes) were also identified using other techniques described above such as linkage analysis and homozygosity mapping (as mentioned in Table 1). Moreover, it has facilitated the identification of pathogenic variants in known genes and enables the assessment of structural variations, such as copy number variations and structural rearrangements that can contribute to HL. As sequencing technologies continue to advance and become more cost-effective, genome sequencing is likely to become an increasingly valuable tool for HL gene discovery and expedite the development of effective therapies.

In the Pakistani population, variants in a total of 51 genes have been implicated in ARNSHL through the approaches described

above (Table 1). Owing to the unique genetic background and high consanguinity rate of the Pakistani population, 39 of these 51 genes have been discovered between 1997 and 2022 (Figure 1), underscoring the significant contribution of the Pakistani population to unravelling the genetics of HL.

Although next-generation sequencing-based molecular tests are still in their infancy, they have shown clinical value for single-gene diseases. The full potential of exome and genome sequencing will enrich genomic medicine beyond rare single-gene disorders. Further developments in next-generation sequencing technologies and bioinformatics tools will enhance data analysis and clinical extraction. Recent studies justify the effort and expense of incorporating these innovative ideas into molecular diagnostics practice. While advances in next-generation sequencing have sped up the detection of disease-causing variants, they cannot overcome universal limitations faced in Mendelian disorders. Exploration of the genetics of ARNSHL is optimal with large pedigrees that ideally support gene mapping approaches and next-generation sequencing. Furthermore, identification of variants in compound heterozygosity is critical for validating genetic findings. Variants with limited evidence to conclusively assign likely pathogenic or likely benign classifications remain a great challenge in genomic medicine in general. Variable expressivity can affect interpretation as well as outcomes, as not everyone who harbours particular variants will develop the disease due to possible incomplete penetrance. It is critical to use these technologies in collaboration with various patient populations, genetic counsellors, and medical geneticists. This collaborative endeavour will be crucial for increasing our understanding of the genetic basis of HL and discovering novel therapies to treat it.

4 | GENE ONTOLOGIES AND PATHWAYS OF RECURRENT ARNSHL GENES IN PAKISTAN

By conducting molecular genetic studies on consanguineous pedigrees, researchers have been able to identify deleterious alleles in 51 out of the 68 genes linked to ARNSHL, thanks in part to participation of Pakistani families with HL.⁶⁶ Notably, among the 51 autosomal recessive genes we describe, nine genes (*GJB2*, *TECTA*, *MYO6*, *ESPN*, *TMC1*, *TPRN*, *ELMOD3*, *TBC1D24* and *PTPRQ*) have

been associated with an autosomal-dominant inheritance pattern as well. While the identification of 51 genes associated with HL may seem daunting, researchers can simplify the complexity by examining the established metabolic and cell signalling pathways through which these genes interact in various organs or by identifying enriched ontology clusters. One of the fundamental presumptions of network analysis and ontology clustering is that there are functional connections between genes whose malfunction leads to disease manifestation. Understanding biological systems, disease states and the mechanisms by which drugs affect them relies on identifying these common pathways and ontology terms. Therefore, by recognizing the shared biological pathways involved in HL and other disorders, researchers can gain insights into the underlying causes of these diseases and develop effective therapeutic interventions.

To gain further insights into the functional roles of the 51 ARNSHL-associated genes, gene enrichment analysis and MCODE algorithm were employed using Metascape.⁶⁷ The annotation, enrichment and genes used to perform the Metascape analysis are shown in Table S1. Enrichment analysis using Metascape identified four areas of enriched protein clusters where proteins are closely related based on similar pathways involved. The first cluster of genes was involved in sensory perception of sound (27 genes, GO:0007605), the second cluster in sensory processing of sound by cochlear inner hair cells (IHCs) (19 genes, R-HSA-9662360),

the third cluster in actin cytoskeleton organization (7 genes, GO:0030036), and the fourth cluster in retina homeostasis (6 genes, GO:0001895) (Figure 2). To facilitate the visualization of the results, we created a pie chart using Microsoft Excel software to illustrate the count of genes belonging to each category of enriched GO terms.

Protein clustering also revealed that the 51 genes associated with ARNSHL regulate several common biological pathways. These pathways include cell-cell junction organization (5 genes, GO:0045216), cellular response to chemical stress (5 genes, GO:0062197) and regulation of plasma membrane-bounded cell projection organization (5 genes, GO:0120035). Other biological processes involved were monoatomic cation homeostasis (4 genes, GO:0055080), endocytosis (4 genes, GO:0006897), cellular response to hormone stimulus (4 genes, GO:0032870), endothelial cell development (3 genes, GO:0001885) and MET activates PTPN11 (3 genes, R-HSA-8865999) (Figure 2). 'MET activates PTPN11' refers to a signalling pathway that involves the interaction of two proteins of MET and PTPN11. This pathway has been implicated in a variety of cellular processes and diseases, including cancer and developmental disorders.

Overall, our bioinformatics analysis highlights the intricate role of ARNSHL genes in various cellular signalling pathways and biological processes. These findings have important implications for understanding the underlying mechanisms of ARNSHL and for the

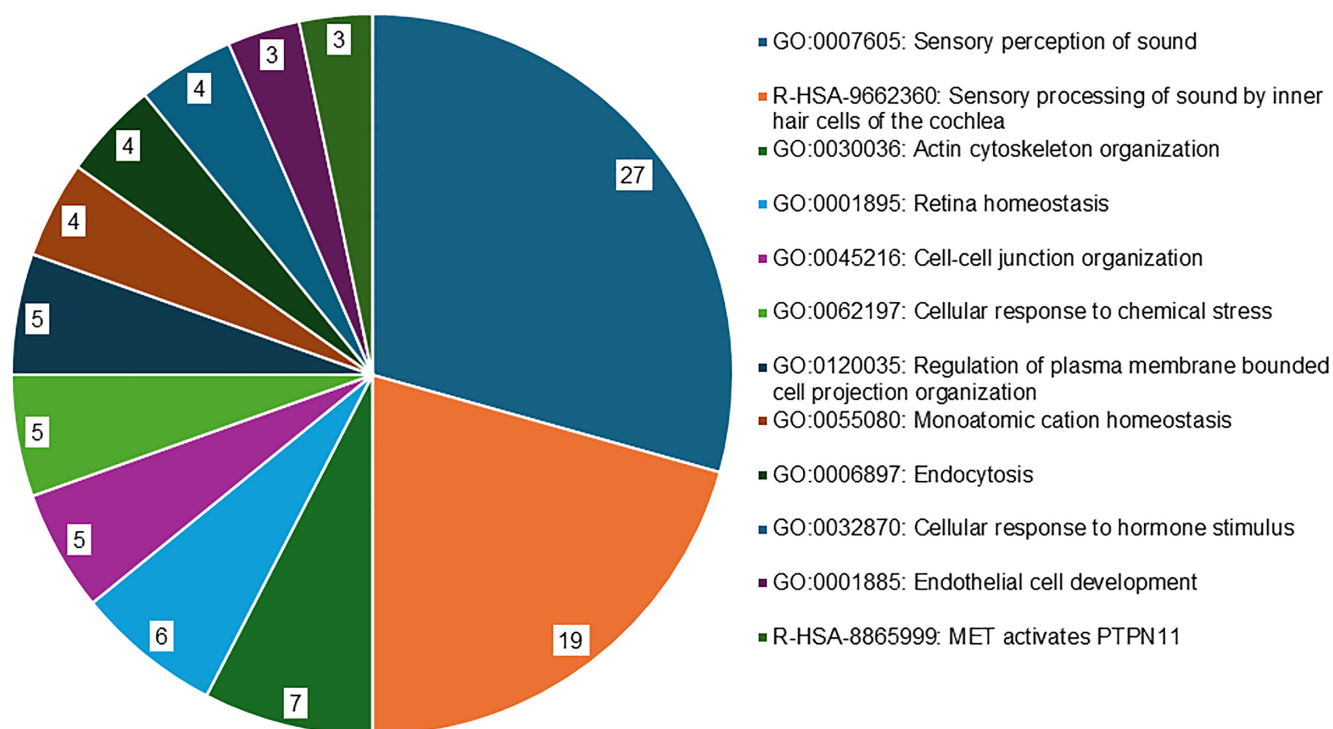


FIGURE 2 Pie chart showing the results of Metascape analysis of enriched gene ontology (GO) clusters. The chart displays the top 12 clusters and their representative enriched terms (one per cluster). The 'Count' refers to the number of genes in our list of 51 genes associated with ARNSHL that are included in the given ontology term. To classify the genes associated with ARNSHL according to their function, we performed pathway and process enrichment and network analysis using Metascape (<http://metascape.org>). Using this approach, we identified the enrichment of GO terms and genes related to various biological pathways.

development of targeted therapies aimed at restoring normal function to disrupted pathways.

5 | RECURRENT GENES AND VARIANTS ASSOCIATED WITH HEARING LOSS IN THE PAKISTANI POPULATION

Despite the extensive genetic heterogeneity in ARNSHL, a few genes account for over half of the cases. One large cohort study of Pakistani patients identified variants in six genes (*GJB2*, *HGF*, *MYO7A*, *SLC26A4*, *CIB2* and *TMC1*) that collectively explained up to 57% of recessively inherited severe to profound HL.⁶⁶ Another cohort study found pathogenic or possibly deleterious mutations in *GJB2*, *MYO7A*, *CDH23* and *MYO15A* in 13 out of 21 (61.9%) consanguineous Pakistani families with HL.⁶⁸ This part of the review aims to shed light on the most prevalent genes and variants in the Pakistani population. By providing valuable insights into the molecular genetics and epidemiology of ARNSHL, this information can be utilized to develop effective genetic screening and counselling programs for individuals and families affected by this condition and also develop personalized treatment strategies for the affected individuals.

5.1 | *CDH23*

CDH23 (DFNB12, USH1D) encodes cadherin 23, a crucial component of the extracellular filaments responsible for formation and function of the mechanosensory hair bundles of the inner ear.⁶⁹ The hair bundle consists of stereocilia, hair-like projections that respond to vibration of the basilar membrane and stimulate hair cells by depolarization.⁷⁰ *CDH23* is expressed not only in the cochlear hair cells but also in the vestibular hair cells and the photoreceptor layer of the retina.⁷¹ In fact, *CDH23* is one of the most highly expressed genes in the vestibular hair cells, where it plays a crucial role in maintaining their mechanical and electrophysiological properties. Different isoforms of *CDH23* have tissue-specific functions. Biallelic variants disrupting *CDH23* cause ARNSHL (DFNB12) and Usher syndrome type I (USH1D), characterized by congenital SNHL, vestibular dysfunction and early onset retinitis pigmentosa.⁷² Although *CDH23*-associated NSHL has traditionally been associated with congenital onset and profound severity, recent studies have revealed a broad phenotypic spectrum that includes individuals with biallelic variants presenting HL in the second to seventh decade of life, expanding the understanding of DFNB12.⁷³ As one of the most common genetic causes of HL, many *CDH23* pathogenic alleles have been identified in affected individuals of Pakistani ancestry, contributing to up to 5% of NSHL and USH1D¹ due to numerous pathogenic alleles of *CDH23*.⁶⁶ The c.2968G>A, p.(Asp990Asn) and c.6133G>A, p.(Asp2045Asn) alleles are major causes of *CDH23*-associated deafness in Pakistan but the most commonly occurring variant of these genes is c.6050-9G>A (splicing).^{66,74} At least 28 additional *CDH23* variants have

been identified in different ethnic groups in Pakistan. However, so far no experimental evidence shows an impact on protein function.^{66,74,75} A comprehensive understanding of disease mechanisms in *CDH23*-associated disorders may significantly impact the diagnosis and management of HL and deaf-blindness worldwide.

5.2 | *CIB2*

Calcium- and integrin-binding protein 2 (*CIB2*) is a crucial component of the mechano-transduction (MET) process in cochlear hair cells, demonstrated by the complete abolishment of MET currents upon genetic disruption.^{76,77} MET is the process by which sound vibrations are converted into electrical signals that the brain can interpret as sound. *CIB2* is an elongation factor-hand domain-containing protein that binds Ca²⁺ ions and has been associated with various functions, including integrin signalling in platelets and skeletal muscle and autophagy, indicating its considerable functional flexibility.⁷⁸ Although its clinical presentation seems to be limited to hearing deficits, typically manifesting as bilateral, profound, pre-lingual HL, *CIB2* has been linked to a wide range of functions beyond hearing physiology. A brief association with Usher syndrome was eventually refuted.^{41,79} A large Pakistani cohort study has estimated the prevalence of *CIB2*-associated HL as 8.6%.⁶⁶ The homozygous recurrent c.272T>C, p.Phe91Ser (NM_006383.3) variant is the major cause of NSHL in the Pakistani community and has been identified in 81 families.⁴¹ Two additional rare variants have been identified in affected Pakistani individuals: c.192G>C, p.Glu64Asp and c.297C>G, p.Cys99Trp.⁴¹

5.3 | *CLDN14*

CLDN14, also known as DFNB29, encodes the claudin-14 protein, an essential membrane protein forming tight junctions in the inner ear.¹⁵ These tight junctions are crucial for compartmentalizing the endolymphatic and perilymphatic fluid compartments, maintaining cell polarity, and regulating intercellular permeability to solutes, ions, and water.⁸⁰ *CLDN14* is expressed in the inner and outer hair cells, supporting cells, and Reissner's membrane.⁸¹ It plays a crucial role in maintaining ion homeostasis and calcium levels in the endolymph and perilymph fluids, which is essential for the MET process of cochlear hair cells.⁸² MET is initiated by the opening of cation channels near the tip of the stereocilia and requires ion homeostasis to maintain ion gradients for preservation of the endocochlear potential.⁸⁰ While the endocochlear potential in *Cldn14*-null mice has been determined as normal, rapid degeneration of outer hair cells and progressive slower degeneration of IHCs is thought to be due to the compromised tight junction barrier.⁸³ In Pakistani families, seven different variants of *CLDN14* are responsible for 2%–3.3% of cases of profound deafness or moderate to severe HL^{66,84} with the most prevalent variant being c.254T>A, p.Val85Asp (NM_001146079.2) identified in 21 families.⁸⁵ It has been shown that a founder effect contributes to the

recurrence of this variant.⁸⁵ A modifier of *CLDN14*-associated HL has been proposed but remains uncharacterized. The discovery of *CLDN14*'s role in regulating intercellular permeability in the inner ear highlights the importance of genetic testing for *CLDN14* in individuals with HL in the moderate range.

5.4 | *GJB2*

Gap junctions play a crucial role in maintaining cochlear potassium homeostasis, which is essential for hearing. *GJB2* encodes connexin 26 and is one of the most common causes of HL worldwide.⁸⁶ Connexin 26 oligomerizes to form hexameric hemichannels called 'connexons' in the plasma membrane. The connexons of neighbouring cells come together to form gap junctions, which facilitate intercellular communication.⁸⁷ Gap junctions can be homomeric or heteromeric, depending on whether they are made up of one or many different connexin proteins. This affects their ability to selectively permeate certain molecules and ions.⁸⁸ Gap junctions allow ions, nutrients and signalling molecules with molecular weights up to 1200Da to pass between cells.⁸⁹ This intercellular communication is critical for the normal growth, function, and repair of the sensory epithelia of the inner ear. These processes that are disrupted through genetic mutation result in eventual cell death and HL. In the Pakistani population, the prevalence of *GJB2*-associated HL ranges from 6.1% to 53%⁹⁰ and it is the most common cause of NSHL in South Asia.⁶⁶ Patients with biallelic pathogenic variants typically have congenital-onset HL, ranging from mild to profound severity, depending on the variant, and it can be progressive in roughly half of patients.⁸⁶ Many pathogenic variants have been identified in *GJB2*, with the most common ones in the Pakistani population being c.231G>A, p.Trp77Ter, c.71G>A, p.Trp24Ter and c.35delG, p.Gly12ValfsTer2, (NM_004004.5)^{1,7,68,91} Among these variants, individuals homozygous for the c.35delG variant exhibit extreme phenotypic heterogeneity worldwide, ranging from mild HL to profound deafness. A founder effect has been attributed to its high prevalence. The *GJB2* c.35delG variant causes a premature stop codon in exon 2 out of 2 total exons, leading to a loss of protein function.⁹² In Pakistan, except for one variant associated with moderate-to-severe HL, all others are associated with severe to profound deafness.⁶⁶ To date, 29 pathogenic variants of *GJB2* have been identified in the Pakistani population.⁶⁶

5.5 | *HGF*

HGF (DFNB39) encodes hepatocyte growth factor (HGF), which plays a crucial role in various biological processes, including cell growth, survival, differentiation and branching morphogenesis, with implications for neuronal survival and differentiation.⁹³ *HGF* dosage in the inner ear must be precisely calibrated for normal hearing. Transgenic mouse models of *Hgf* overexpression and deficiency present deafness resulting from the failure of neural crest cell migration to the intermediate cell layer of the stria vascularis, which causes

thinning, reducing the endocochlear potential and hair cell loss.^{33,93} While *HGF*-associated HL is relatively prevalent in the Pakistani population, explaining 6–8% of severe to profound pre-lingual HL, it is rarely implicated in the diagnosis of individuals with HL in other populations.⁶⁶ In the Pakistani population, three *HGF* variants have been identified, including a synonymous variant that affects splicing and two deletions in a highly conserved region of intron 4, which is part of the 3' untranslated region of a short *HGF* isoform.⁷⁵ The most common variant in the Pakistani population is c.482+1986_1988delTGA (NM_000601.6) in intron 4.³³ Mice with the 10bp deletion corresponding to c.482+1991_2000delGATGATGAAA in human intron 4 developed defects in the stria vascularis due to failure of neural crest cell migration during development, resulting in a significantly reduced endocochlear potential.⁹³ *HGF* activates the MET receptor-mediated signalling pathway and mediates diverse downstream pathways, including those involved in the epithelial-mesenchymal transition and the development of neural crest-derived lineages. Overall, deleterious variants in *HGF* can result in impaired cell migration and the development of neural crest-derived structures in the inner ear, leading to a reduced endocochlear potential and HL.

5.6 | *MARVELD2*

MARVELD2 (DFNB49) encodes a member of the marvel protein family called tricellulin that is concentrated at the tight junctions, forming part of the continuous intercellular barrier between epithelial cells. Tight junctions are multi-protein complexes preventing leakage of solutes and water, acting as a seal between epithelial cells.⁹⁴ Tricellular tight junctions are present in epithelial cells between supporting and hair cells, cochlear supporting cells and marginal cells of the stria vascularis.²⁷ Although *MARVELD2* is ubiquitously expressed in epithelial junctions, only the inner ear appears to be affected by the genetic disruption, suggesting that genetic compensation may be present in other organ systems that are absent in the inner ear.²⁷ Biallelic variants in the Occludin-ELL domain of *MARVELD2* have been shown to cause HL in multiple Pakistani families.⁹⁵ These variants explain between 1.5% and 2.4% of all HL in Pakistan to a moderate to profound degree. The most recurrent variant of *MARVELD2* is c.1331+2T>C (NM_001038603.3), and at least seven additional rare disease-associated variants have also been reported in the Pakistani population.¹ Tricellulin is essential for maintaining the integrity and stability of epithelial cells and their junctions.²⁷ The occluding-ELL domain of *MARVELD2* may be necessary for the maintenance of tricellular junctions and the proper functioning of the inner ear.⁹⁶ Deleterious variants in tricellulin lead to tight junction disorganization, resulting in severe to profound sensorineural HL.²⁷

5.7 | *MYO7A*

MYO7A (DFNB2) encodes an unconventional myosin that plays a crucial role in maintaining the mechanical stability of the hair

bundle. The hair bundle is the sensory structure located on the surface of hair cells in the inner ear, acting as a mechanotransducer that transforms sound waves or orientation information into electrical signals, which the brain interprets (Houdusse & Titus, 2021).⁹⁷ This is achieved through the transportation of extracellular stereocilia links along actin filaments⁹⁸ at the upper tip-link density and ankle link region of the stereocilia by MYO7A, which is essential for the cohesion of the hair bundle.⁹⁹ MYO7A co-localizes with several other proteins at the upper tip-link density and ankle link region of the stereocilia, including CDH23, USH1C, USH1G, ADGRV1 and USH2A, which are integral for the proper functioning of the hair bundle (Houdusse & Titus, 2021).⁹⁷ Moreover, independent of molecular trafficking, MYO7A exerts force at the upper tip-link density region and tensions the MET complex, further highlighting its important role in hearing.¹⁰⁰ In addition to its role in the inner ear, MYO7A is also expressed in retinal pigment epithelial cells where it is required for functional RPE65, a key protein in the retinoid cycle.¹⁰¹ MYO7A is also associated with Usher syndrome type I, an autosomal recessive condition characterized by deafness, vestibular impairment and retinitis pigmentosa. Biallelic variants in MYO7A account for 29% to 50% of all USH1 cases globally, making it the most common cause of this condition.¹⁰² Moreover, at least 11% of moderate to severe pre-lingual SNHL in the Pakistani population is due to MYO7A disruption⁴² with at least 59 variants reported.⁶⁶ Among these variants, c.397dupC, p.His133ProfsTer7 and c.470G>A, p.Ser157Asn (NM_000260.3) are the most prevalent in the Pakistani population.⁶⁸ These variants exhibit founder effects that are not seen in other populations.⁶⁸

5.8 | MYO15A

Myosin XVa, a product of MYO15A, belongs to the unconventional myosin superfamily and is essential for the elongation of stereocilia in cochlear sensory and vestibular hair cells. The growth of the hair bundle is facilitated by the transportation of whirlin and Eps8 to the tip of stereocilia to form the stereocilia tip complex by Myosin XVa, which can help in the conversion of microvilli into fully mature stereocilia.¹⁰³ MYO15A is involved in the regulation of actin and the transportation of elongation complexes at the distal stereocilia tip as well as other cargoes for actin.⁹⁹ Notably, the motor and tail domains of myosin XVa have been identified as crucial for normal auditory structure and function.¹⁰⁴ In Pakistani families, at least 49 MYO15A variants have been linked to 5–13% of progressive severe to profound bilateral SNHL.⁶⁶ The majority of the variants in this gene have been found in affected individuals from one to three Pakistani families, except for two variants, c.6589C>T, p.Gln2197Ter and c.8158G>C, p.Asp2720His (NM_016239.3) found in deaf members of four families each.⁵⁹ MYO15A variants significantly impact the motor domain, leading to dysfunction causing shorter stereocilia with an ectopic staircase structure, a condition associated with severe deafness.¹⁰⁵

5.9 | MSRB3

MSRB3 (DFNB74) encodes methionine sulfoxide reductase B3 (MSRB3) that plays a critical role in repairing oxidatively damaged proteins by catalysing the stereo-specific reduction of methionine-R-sulfoxides to methionine.³⁷ *MsrB3* localizes to the base of the stereocilia on the apical surface of hair cells.¹⁰⁶ Studies on the *MsrB3*^{-/-} mouse model have shown that MSRB3 is essential for the maturation and/or maintenance of stereociliary bundles since these mice exhibit progressive degeneration of the stereociliary bundles and apoptosis of hair cells.¹⁰⁶ Moreover, genetic disruption of *MsrB3* is more likely to be due to degeneration rather than abnormal development, since the hair cells of KO mice develop normally and have functional mechanotransduction channels until at least P3.¹⁰⁶ The c.265T>G, p.Cys89Gly and c.55C>T, p.Arg19Ter (NM_001031679.2) MSRB3 variants are associated with deafness in six and two unrelated DFNB74 Pakistani families, respectively.³⁷ A homozygous missense variant c.20T>G, p.Leu7Arg was identified in 2014 in one Pakistani family, and a homozygous splice variant, c.412-1G>A was described in another Pakistani family in 2019.⁶⁶ These variants are associated with severe to profound HL. Among the four variants reported in individuals with genetic ancestry from Pakistan so far, p.Cys89Gly is the most recurrent variant. This MSRB3 cysteine residue, conserved in orthologs from yeast to humans, is involved in structural zinc binding. In vitro, this non-synonymous substitution (p.Cys89Gly) reduced zinc binding and MSRB3 enzymatic activity.³⁷

5.10 | OTOF

OTOF (DFNB9) encodes otoferlin, a protein that is essential for normal hearing. Otoferlin plays a crucial role in various functions of synaptic signalling, including sensing of pre-synaptic Ca²⁺ for exocytosis following IHC depolarization, priming and replenishment of the synaptic vesicles to maintain fast neurotransmitter release and coupling of exocytosis-endocytosis.^{107,108} Most individuals exhibit profound pre-lingual deafness due to biallelic pathogenic or likely pathogenic OTOF variants. The affected individuals show isolated failure in synaptic transmission, and their otoacoustic emissions are usually initially unaffected, with healthy outer hair cells, particularly in younger individuals. The HL resulting from OTOF-associated deficits is commonly known as auditory synaptopathy.^{109,110} OTOF variants account for 3.1%–4% of pre-lingual moderate or profound HL in Pakistan. The first variant described, a nonsense variant c.4491T>A, p.Tyr1497Ter (NM_001287489.2) in OTOF, was identified in four independent Lebanese families by a candidate gene approach.¹¹¹ Since then, at least 23 OTOF variants associated with moderate-to-severe or profound HL have been identified in hearing-impaired individuals from Pakistan.^{32,84} Among these, one of the most common is the c.2122C>T, p.Arg708Ter⁶⁶ variant.¹¹² This variant has been

detected in both homozygous and compound heterozygous states in individuals with prelingual NSHL or auditory neuropathy in a large population cohort.³²

5.11 | SLC26A4

SLC26A4, also known as pendrin, is a protein that belongs to the solute carrier family 26 and functions as an anion exchanger, transporting negatively charged ions such as chloride, iodide and bicarbonate across cell membranes. This protein is crucial for the development of the cochlea and vestibular duct's bony snail shape structure.¹¹³ Variants in *SLC26A4* (DFNB4) have been linked to both ARNSHL and Pendred syndrome.¹¹⁴ While individuals with isolated HL may experience bilateral, profound SNHL, those with Pendred syndrome may have concomitant HL, enlarged vestibular aqueduct and abnormal iodine organification.¹¹⁵ Approximately 12.4% of HL cases in the Pakistani community can be attributed to *SLC26A4*.⁶⁶ While the majority of the identified variants are described as non-syndromic, especially in young individuals, the presence of Pendred syndrome may be frequently overlooked. *SLC26A4* has several recurring variations. In the majority of affected individuals, HL is associated with three variants: c.269C>T, p.(Ser90Leu), c.716T>A, p.(Val239Asp), and c.1337A>G, p.(Gln446Arg) (NM_000441.1). A founder effect has been shown for their recurrence.^{84,116}

5.12 | TMC1

Transmembrane channel-like protein isoform-1 (*TMC1*) plays a crucial role in the auditory system as it forms an ion-conducting pore of the MET channel in auditory hair cells.¹¹⁷ Over the past 5 years, *TMC1* has emerged as a leading contender for the MET channel in auditory hair cells of the inner ear. Hair cells convert acoustic and vestibular stimuli into electrical responses through the activation of MET.¹¹⁸ *TMC1* is thought to have a six-transmembrane domain structure similar to several other ion-channel subunits and is transported to the tips of the stereocilia in the sensory hair bundle where the MET channel is located.¹¹⁹ *TMC1* variants associated with human deafness result in loss of typical MET currents and hair cell senescence, causing cell death. Valuable insights into the pathogenesis of *TMC1*-associated deafness have been gained from studies of mutant mice.¹²⁰ Variants in *TMC1* cause both dominant (DFNA36) and recessive (DFNB7/11) forms of NSHL. Both progressive postlingual and pre-lingual profound HL have been associated with *TMC1* variants. In Pakistan, at least 32 different *TMC1* variants account for 6.4% of recessively inherited HL cases.⁶⁶ The most frequently occurring *TMC1* variant in the Pakistani population is c.100C>T, p.Arg34Ter (NM_138691.2), which has been reported in two studies.^{84,121} This common pathogenic nonsense variant is a likely founder mutation in North African and Middle Eastern populations.^{7,17}

5.13 | TMPRSS3

Genetic mutation of type II transmembrane serine protease (*TMPRSS3*) causes variable HL. *TMPRSS3* has four functional domains that include an N-terminal transmembrane domain, a low-density lipoprotein receptor A domain, a scavenger receptor cysteine-rich domain and a C-terminal serine protease domain.¹²² *TMPRSS3* is expressed in various components of the developing inner ear, such as the stria vascularis, spiral ganglion neurons,¹²³ IHCs and cochlear aqueduct of the foetal cochlea and is critical for their normal development and maintenance.¹²⁴ Additionally, cytoplasmic domains of *TMPRSS3* raise the possibility that they may participate in intracellular signal transduction.¹²² Biallelic variants in *TMPRSS3* are known for causing different types of HL with variable onset. DFNB10 is characterized by congenital or childhood-onset bilateral profound HL,¹²⁵ whereas DFNB8 is associated with a milder postlingual progressive HL,¹²⁶ both of which are caused by variants in *TMPRSS3* (DFNB8/10). In Pakistan, *TMPRSS3* variants are associated with stable, moderate-to-severe or profound HL and contribute up to 4% of the prevalence of HL.⁶⁶ Other frequently occurring variants include c.323-6G>A (splicing) and c.1219T>C, p.Cys407Arg.⁸⁴ The recurring homozygous c.1219T>C, p.Cys407Arg (NM_024022.2) variant has been identified in 20 Pakistani families.¹²⁷ Functional studies show that the variant protein has defective protease activity compared to the wild-type (Lee et al., 2003) as well as a failure to undergo proteolytic cleavage and activate the epithelial sodium channel (ENaC) in vitro.^{124,128} In silico analysis also supports that this missense variant has a negative effect on protein structure/function. There are 14 other rare variants in this gene that contribute to NSHL in the Pakistani population.

6 | CONCLUSION

This review explores the clinical and genetic complexities of HL, defining and describing key characteristics, terminology and genetics. After 25 years of engaging the Pakistani population, researchers have uncovered variants in 51 genes and made significant progress in understanding the most frequently implicated ARNSHL genes. Of these, 39 genes causally related to HL were discovered through the use of gene mapping methodologies and sequencing strategies in consanguineous Pakistani families. This aggregated knowledge has established 13 being the most commonly involved in the molecular diagnosis of Pakistani patients (*CDH23*, *CIB2*, *CLDN14*, *GJB2*, *HGF*, *MARVELD2*, *MYO7A*, *MYO15A*, *MSRB3*, *OTOF*, *SLC26A4*, *TMC1* and *TMPRSS3*). We detailed their purpose and highlighted important variants from the Pakistani community's standpoint. For a detailed molecular understanding of these ARNSHL genes, we also categorized enriched GO terms and shared pathways using Metascape.

Engaging the Pakistani community has been fundamental in advancing gene discovery. The high prevalence of consanguinity and congenital HL, a predominantly recessive trait, has provided

fundamental insights into the genes and variants underlying HL over several decades. This has amplified the global knowledge base, providing valuable information for the selection of therapeutic targets and improving genetic diagnoses. However, HL continues to impose a significant burden on affected individuals, necessitating the discovery of new strategies for more precise diagnosis, alleviation, and treatment. Much work remains to achieve a comprehensive understanding of all genes and variants causing HL.

AUTHOR CONTRIBUTIONS

Madiha Shadab: Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Ansar Ahmed Abbasi:** Methodology (equal); project administration (equal); supervision (equal); writing – review and editing (equal). **Ahsan Ejaz:** Data curation (equal); resources (equal). **Afif Ben-Mahmoud:** Methodology (equal); writing – review and editing (equal). **Vijay Gupta:** Formal analysis (equal); writing – review and editing (equal). **Hyung-Goo Kim:** Funding acquisition (equal); methodology (equal); supervision (equal); visualization (equal); writing – review and editing (equal). **Barbara Vona:** Conceptualization (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal).

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The authors confirm that there are no conflicts of interest.

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ORCID

Madiha Shadab  <https://orcid.org/0009-0009-6043-6811>

Ansar Ahmed Abbasi  <https://orcid.org/0000-0002-8140-0756>

Ahsan Ejaz  <https://orcid.org/0000-0002-9679-6327>

Afif Ben-Mahmoud  <https://orcid.org/0000-0003-3520-8015>

Vijay Gupta  <https://orcid.org/0000-0002-3613-6378>

Hyung-Goo Kim  <https://orcid.org/0000-0003-4497-4686>

Barbara Vona  <https://orcid.org/0000-0002-6719-3447>

REFERENCES

- Naz S. Molecular genetic landscape of hereditary hearing loss in Pakistan. *Hum Genet.* 2022;141(3–4):633–648. doi:[10.1007/s00439-021-02320-0](https://doi.org/10.1007/s00439-021-02320-0)
- Rouse SL, Florentine MM, Taketa E, Chan DK. Racial and ethnic disparities in genetic testing for hearing loss: a systematic review and synthesis. *Hum Genet.* 2022;141(3–4):485–494. doi:[10.1007/s00439-021-02335-7](https://doi.org/10.1007/s00439-021-02335-7)
- Hoefsloot LH, Feenstra I, Kunst HPM, Kremer H. Genotype phenotype correlations for hearing impairment: approaches to management: genotype phenotype correlations for hearing impairment. *Clin Genet.* 2014;85(6):514–523. doi:[10.1111/cge.12339](https://doi.org/10.1111/cge.12339)
- Stelma F, Bhutta MF. Non-syndromic hereditary sensorineural hearing loss: review of the genes involved. *J Laryngol Otol.* 2014;128(1):13–21. doi:[10.1017/S0022215113003265](https://doi.org/10.1017/S0022215113003265)
- Sanders RD, Gillig PM. Cranial nerve VIII: hearing and vestibular functions. *Psychiatry.* 2010;7(3):17–22.
- Cai Y, Zhao F, Chen Y, et al. The effect of symmetrical and asymmetrical hearing impairment on music quality perception. *Eur Arch Otorhinolaryngol.* 2016;273(9):2451–2459. doi:[10.1007/s00405-015-3838-8](https://doi.org/10.1007/s00405-015-3838-8)
- Shafique S, Siddiqi S, Schraders M, et al. Genetic spectrum of autosomal recessive non-syndromic hearing loss in Pakistani families. *PLoS ONE.* 2014;9(6):e100146. doi:[10.1371/journal.pone.0100146](https://doi.org/10.1371/journal.pone.0100146)
- Bayazit YA, Yilmaz M. An overview of hereditary hearing loss. *ORL J Otorhinolaryngol Relat Spec.* 2006;68(2):57–63. doi:[10.1159/000091090](https://doi.org/10.1159/000091090)
- Vink JM, Boomsma DI. Gene finding strategies. *Biol Psychol.* 2002;61(1–2):53–71. doi:[10.1016/S0301-0511\(02\)00052-2](https://doi.org/10.1016/S0301-0511(02)00052-2)
- Génin E, Martinez M, Clerget-Darpoux F. Posterior probability of linkage and maximal lod score. *Ann Hum Genet.* 1995;59(1):123–132. doi:[10.1111/j.1469-1809.1995.tb01610.x](https://doi.org/10.1111/j.1469-1809.1995.tb01610.x)
- Lander ES, Botstein D. Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. *Science.* 1987;236(4808):1567–1570. doi:[10.1126/science.2884728](https://doi.org/10.1126/science.2884728)
- Alkuraya FS. Homozygosity mapping: one more tool in the clinical geneticist's toolbox. *Genet Med.* 2010;12(4):236–239. doi:[10.1097/GIM.0b013e3181ceb95d](https://doi.org/10.1097/GIM.0b013e3181ceb95d)
- Kelsell DP, Dunlop J, Stevens HP, et al. Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. *Nature.* 1997;387(6628):80–83. doi:[10.1038/387080a0](https://doi.org/10.1038/387080a0)
- Liburd N, Ghosh M, Riazuddin S, et al. Novel mutations of MYO15A associated with profound deafness in consanguineous families and moderately severe hearing loss in a patient with Smith-Magenis syndrome. *Hum Genet.* 2001;109(5):535–541. doi:[10.1007/s004390100604](https://doi.org/10.1007/s004390100604)
- Wilcox ER, Burton QL, Naz S, et al. Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. *Cell.* 2001;104(1):165–172. doi:[10.1016/S0092-8674\(01\)00200-8](https://doi.org/10.1016/S0092-8674(01)00200-8)
- Naz S, Giguere CM, Kohrman DC, et al. Mutations in a novel gene, TMIE, are associated with hearing loss linked to the DFNB6 locus. *Am J Hum Genet.* 2002;71(3):632–636. doi:[10.1086/342193](https://doi.org/10.1086/342193)
- Kurima K, Peters LM, Yang Y, et al. Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. *Nat Genet.* 2002;30(3):277–284. doi:[10.1038/ng842](https://doi.org/10.1038/ng842)
- Ahmed ZM, Smith TN, Riazuddin S, et al. Nonsyndromic recessive deafness DFNB18 and usher syndrome type IC are allelic mutations of USH1C. *Hum Genet.* 2002;110(6):527–531. doi:[10.1007/s00439-002-0732-4](https://doi.org/10.1007/s00439-002-0732-4)
- Ahmed ZM, Riazuddin S, Ahmad J, et al. PCDH15 is expressed in the neurosensory epithelium of the eye and ear and mutant alleles are responsible for both USH1F and DFNB23. *Hum Mol Genet.* 2003b;12(24):3215–3223. doi:[10.1093/hmg/ddg358](https://doi.org/10.1093/hmg/ddg358)

20. Naz S. Distinctive audiometric profile associated with DFNB21 alleles of TECTA. *J Med Genet.* 2003;40(5):360-363. doi:[10.1136/jmg.40.5.360](https://doi.org/10.1136/jmg.40.5.360)
21. Park H-J. Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: global implications for the epidemiology of deafness. *J Med Genet.* 2003;40(4):242-248. doi:[10.1136/jmg.40.4.242](https://doi.org/10.1136/jmg.40.4.242)
22. Ahmed ZM, Morell RJ, Riazuddin S, et al. Mutations of MYO6 are associated with recessive deafness, DFNB37. *Am J Hum Genet.* 2003a;72(5):1315-1322. doi:[10.1086/375122](https://doi.org/10.1086/375122)
23. Ahmed ZM, Cindy Li X, Powell SD, et al. Characterization of a new full length TMPRSS3 isoform and identification of mutant alleles responsible for nonsyndromic recessive deafness in Newfoundland and Pakistan. *BMC Med Genet.* 2004;5(1):24. doi:[10.1186/1471-2350-5-24](https://doi.org/10.1186/1471-2350-5-24)
24. Naz S. Mutations of ESPN cause autosomal recessive deafness and vestibular dysfunction. *J Med Genet.* 2004;41(8):591-595. doi:[10.1136/jmg.2004.018523](https://doi.org/10.1136/jmg.2004.018523)
25. Petersen M, Willems P. Non-syndromic, autosomal-recessive deafness: autosomal-recessive deafness. *Clin Genet.* 2006;69(5):371-392. doi:[10.1111/j.1399-0004.2006.00613.x](https://doi.org/10.1111/j.1399-0004.2006.00613.x)
26. Riazuddin S, Khan SN, Ahmed ZM, et al. Mutations in TRIOBP, which encodes a putative cytoskeletal-organizing protein, are associated with nonsyndromic recessive deafness. *Am J Hum Genet.* 2006b;78(1):137-143. doi:[10.1086/499164](https://doi.org/10.1086/499164)
27. Riazuddin S, Ahmed ZM, Fanning AS, et al. Tricellulin is a tight-junction protein necessary for hearing. *Am J Hum Genet.* 2006a;79(6):1040-1051. doi:[10.1086/510022](https://doi.org/10.1086/510022)
28. Shabbir MI. Mutations of human TMHS cause recessively inherited non-syndromic hearing loss. *J Med Genet.* 2006;43(8):634-640. doi:[10.1136/jmg.2005.039834](https://doi.org/10.1136/jmg.2005.039834)
29. Khan SY, Riazuddin S, Tariq M, et al. Autosomal recessive nonsyndromic deafness locus DFNB63 at chromosome 11q13.2-q13.3. *Hum Genet.* 2007;120(6):789-793. doi:[10.1007/s00439-006-0275-1](https://doi.org/10.1007/s00439-006-0275-1)
30. Collin RWJ, Kalay E, Tariq M, et al. Mutations of ESRRB encoding estrogen-related receptor Beta cause autosomal-recessive nonsyndromic hearing impairment DFNB35. *Am J Hum Genet.* 2008;82(1):125-138. doi:[10.1016/j.ajhg.2007.09.008](https://doi.org/10.1016/j.ajhg.2007.09.008)
31. Ahmed ZM, Masmoudi S, Kalay E, et al. Mutations of LRTOMT, a fusion gene with alternative reading frames, cause nonsyndromic deafness in humans. *Nature Genetics.* 2008;40(11):1335-1340. <https://doi.org/10.1038/ng.245>
32. Choi B, Ahmed Z, Riazuddin S, et al. Identities and frequencies of mutations of the otoferlin gene (OTOF) causing DFNB9 deafness in Pakistan. *Clin Genet.* 2009;75(3):237-243. doi:[10.1111/j.1399-0004.2008.01128.x](https://doi.org/10.1111/j.1399-0004.2008.01128.x)
33. Schultz JM, Khan SN, Ahmed ZM, et al. Noncoding mutations of HGF are associated with nonsyndromic hearing loss, DFNB39. *Am J Hum Genet.* 2009;85(1):25-39. doi:[10.1016/j.ajhg.2009.06.003](https://doi.org/10.1016/j.ajhg.2009.06.003)
34. Riazuddin S, Anwar S, Fischer M, et al. Molecular basis of DFNB73: mutations of BSND can cause nonsyndromic deafness or Bartter syndrome. *Am J Hum Genet.* 2009;85(2):273-280. doi:[10.1016/j.ajhg.2009.07.003](https://doi.org/10.1016/j.ajhg.2009.07.003)
35. Rehman AU, Morell RJ, Belyantseva IA, et al. Targeted Capture and Next-Generation Sequencing Identifies C9orf75, Encoding Taperin, as the Mutated Gene in Nonsyndromic Deafness DFNB79. *The American Journal of Human Genetics.* 2010;86(3):378-388. <https://doi.org/10.1016/j.ajhg.2010.01.030>
36. Schraders M, Lee K, Oostrik J, et al. Homozygosity mapping reveals mutations of GRXCR1 as a cause of autosomal-recessive nonsyndromic hearing impairment. *Am J Hum Genet.* 2010;86(2):138-147. doi:[10.1016/j.ajhg.2009.12.017](https://doi.org/10.1016/j.ajhg.2009.12.017)
37. Ahmed ZM, Yousaf R, Lee BC, et al. Functional null mutations of MSRB3 encoding methionine sulfoxide reductase are associated with human deafness DFNB74. *Am J Hum Genet.* 2011;88(1):19-29. doi:[10.1016/j.ajhg.2010.11.010](https://doi.org/10.1016/j.ajhg.2010.11.010)
38. Rehman AU, Gul K, Morell RJ, et al. Mutations of GIPC3 cause nonsyndromic hearing loss DFNB72 but not DFNB81 that also maps to chromosome 19p. *Hum Genet.* 2011;130(6):759-765. doi:[10.1007/s00439-011-1018-5](https://doi.org/10.1007/s00439-011-1018-5)
39. Borck G, Rehman AU, Lee K, et al. Loss-of-function mutations of ILDR1 cause autosomal-recessive hearing impairment DFNB42. *Am J Hum Genet.* 2011;88(2):127-137. doi:[10.1016/j.ajhg.2010.12.011](https://doi.org/10.1016/j.ajhg.2010.12.011)
40. Mujtaba G, Bukhari I, Fatima A, Naz S. A p.C343S missense mutation in PJVK causes progressive hearing loss. *Gene.* 2012;504(1):98-101. doi:[10.1016/j.gene.2012.05.013](https://doi.org/10.1016/j.gene.2012.05.013)
41. Riazuddin S, Belyantseva IA, Giese APJ, et al. Alterations of the CIB2 calcium- and integrin-binding protein cause usher syndrome type 1J and nonsyndromic deafness DFNB48. *Nat Genet.* 2012;44(11):1265-1271. doi:[10.1038/ng.2426](https://doi.org/10.1038/ng.2426)
42. Shahzad M, Sivakumaran TA, Qaiser TA, et al. Genetic analysis through OtoSeq of Pakistani families segregating Prelingual hearing loss. *Otolaryngol Head Neck Surg.* 2013;149(3):478-487. doi:[10.1177/0194599813493075](https://doi.org/10.1177/0194599813493075)
43. Santos-Cortez RLP, Lee K, Azeem Z, et al. Mutations in KARS, encoding Lysyl-tRNA Synthetase, cause autosomal-recessive nonsyndromic hearing impairment DFNB89. *Am J Hum Genet.* 2013;93(1):132-140. doi:[10.1016/j.ajhg.2013.05.018](https://doi.org/10.1016/j.ajhg.2013.05.018)
44. Lee K, Chiu I, Santos-Cortez R, et al. Novel OTOA mutations cause autosomal recessive non-syndromic hearing impairment in Pakistani families: Letter to the Editor. *Clinical Genetics.* 2013;84(3):294-296. <https://doi.org/10.1111/cge.12047>
45. Jaworek TJ, Richard EM, Ivanova AA, et al. An alteration in ELMOD3, an Arl2 GTPase-activating protein, is associated with hearing impairment in humans. *PLoS Genet.* 2013;9(9):e1003774. doi:[10.1371/journal.pgen.1003774](https://doi.org/10.1371/journal.pgen.1003774)
46. Imtiaz A, Kohrman DC, Naz S. A frameshift mutation in GRXCR2 causes recessively inherited hearing loss. *Hum Mutat.* 2014;35(5):618-624. doi:[10.1002/humu.22545](https://doi.org/10.1002/humu.22545)
47. Rehman AU, Santos-Cortez RLP, Morell RJ, et al. Mutations in TBC1D24, a gene associated with epilepsy, also cause nonsyndromic deafness DFNB86. *Am J Hum Genet.* 2014;94(1):144-152. doi:[10.1016/j.ajhg.2013.12.004](https://doi.org/10.1016/j.ajhg.2013.12.004)
48. Santos-Cortez RLP, Lee K, Giese AP, et al. Adenylate cyclase 1 (ADCY1) mutations cause recessive hearing impairment in humans and defects in hair cell function and hearing in zebrafish. *Hum Mol Genet.* 2014;23(12):3289-3298. doi:[10.1093/hmg/ddu042](https://doi.org/10.1093/hmg/ddu042)
49. Simon M, Richard EM, Wang X, et al. Mutations of human NARS2, encoding the mitochondrial Asparaginyl-tRNA Synthetase, cause nonsyndromic deafness and Leigh syndrome. *PLoS Genet.* 2015;11(3):e1005097. doi:[10.1371/journal.pgen.1005097](https://doi.org/10.1371/journal.pgen.1005097)
50. Mujtaba G, Schultz JM, Imtiaz A, Morell RJ, Friedman TB, Naz S. A mutation of MET, encoding hepatocyte growth factor receptor, is associated with human DFNB97 hearing loss. *J Med Genet.* 2015;52(8):548-552. doi:[10.1136/jmedgenet-2015-103023](https://doi.org/10.1136/jmedgenet-2015-103023)
51. Santos-Cortez RLP, Faridi R, Rehman AU, et al. Autosomal-recessive hearing impairment due to rare missense variants within S1PR2. *Am J Hum Genet.* 2016;98(2):331-338. doi:[10.1016/j.ajhg.2015.12.004](https://doi.org/10.1016/j.ajhg.2015.12.004)
52. Wang R, Han S, Khan A, Zhang X. Molecular analysis of twelve Pakistani families with nonsyndromic or syndromic hearing loss. *Genet Test Mol Biomarkers.* 2017a;21(5):316-321. doi:[10.1089/gtmb.2016.0328](https://doi.org/10.1089/gtmb.2016.0328)
53. Le Quesne Stabej P, James C, Ocaka L, et al. An example of the utility of genomic analysis for fast and accurate clinical diagnosis of complex rare phenotypes. *Orphanet J Rare Dis.* 2017;12(1):24. doi:[10.1186/s13023-017-0582-8](https://doi.org/10.1186/s13023-017-0582-8)
54. Yousaf R, Gu C, Ahmed ZM, et al. Mutations in Diphosphoinositol-Pentakisphosphate kinase PPIP5K2 are associated with hearing

- loss in human and mouse. *PLoS Genet.* 2018b;14(3):e1007297. doi:[10.1371/journal.pgen.1007297](https://doi.org/10.1371/journal.pgen.1007297)
55. Yousaf R, Ahmed ZM, Giese APJ, et al. Modifier variant of METTL13 suppresses human GAB1-associated profound deafness. *J Clin Invest.* 2018a;128(4):1509-1522. doi:[10.1172/JCI97350](https://doi.org/10.1172/JCI97350)
 56. Liaqat K, Hussain S, Bilal M, et al. Further evidence of involvement of TMEM132E in autosomal recessive nonsyndromic hearing impairment. *J Hum Genet.* 2020;65(2):187-192. doi:[10.1038/s10038-019-0691-4](https://doi.org/10.1038/s10038-019-0691-4)
 57. Zhou Y, Tariq M, He S, Abdullah U, Zhang J, Baig SM. Whole exome sequencing identified mutations causing hearing loss in five consanguineous Pakistani families. *BMC Med Genet.* 2020;21(1):151. doi:[10.1186/s12881-020-01087-x](https://doi.org/10.1186/s12881-020-01087-x)
 58. Park HR, Kanwal S, Lim SO, Nam DE, Choi YJ, Chung KW. Homozygous mutations in Pakistani consanguineous families with prelingual nonsyndromic hearing loss. *Mol Biol Rep.* 2020;47(12):9979-9985. doi:[10.1007/s11033-020-06037-7](https://doi.org/10.1007/s11033-020-06037-7)
 59. Mahmood U, Bukhari SA, Ali M, Ahmed ZM, Riazuddin S. Identification of hearing loss-associated variants of PTPRQ, MYO15A, and SERPINB6 in Pakistani families. *Biomed Res Int.* 2021;2021:1-6. doi:[10.1155/2021/5584788](https://doi.org/10.1155/2021/5584788)
 60. Kakar MU, Akram M, Zubair Mehboob M, et al. Identification of homozygous missense variant in SIX5 gene underlying recessive non-syndromic hearing impairment. *PLoS ONE.* 2022;17(6):e0268078. doi:[10.1371/journal.pone.0268078](https://doi.org/10.1371/journal.pone.0268078)
 61. Schrauwen I, Ghaffar A, Bharadwaj T, et al. Syntaxin 4 is essential for hearing in human and zebrafish. *Hum Mol Genet.* 2022;ddac257:1184-1192. doi:[10.1093/hmg/ddac257](https://doi.org/10.1093/hmg/ddac257)
 62. Adadey SM, Aboagye ET, Esoh K, et al. A novel autosomal dominant GREB1L variant associated with non-syndromic hearing impairment in Ghana. *BMC Med Genet.* 2022;15(1):237. doi:[10.1186/s12920-022-01391-w](https://doi.org/10.1186/s12920-022-01391-w)
 63. Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. *J Hum Genet.* 2014;59(1):5-15. doi:[10.1038/jhg.2013.114](https://doi.org/10.1038/jhg.2013.114)
 64. Vona B, Nanda I, Hofrichter MAH, Shehata-Dieler W, Haaf T. Non-syndromic hearing loss gene identification: a brief history and glimpse into the future. *Mol Cell Probes.* 2015;29(5):260-270. doi:[10.1016/j.mcp.2015.03.008](https://doi.org/10.1016/j.mcp.2015.03.008)
 65. Avraham KB, Khalaily L, Noy Y, Kamal L, Koffler-Brill T, Taiber S. The noncoding genome and hearing loss. *Hum Genet.* 2022;141(3-4):323-333. doi:[10.1007/s00439-021-02359-z](https://doi.org/10.1007/s00439-021-02359-z)
 66. Richard EM, Santos-Cortez RLP, Faridi R, et al. Global genetic insight contributed by consanguineous Pakistani families segregating hearing loss. *Hum Mutat.* 2019;40(1):53-72. doi:[10.1002/humu.23666](https://doi.org/10.1002/humu.23666)
 67. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun.* 2019;10(1):1523. doi:[10.1038/s41467-019-09234-6](https://doi.org/10.1038/s41467-019-09234-6)
 68. Doll J, Vona B, Schnapp L, et al. Genetic Spectrum of syndromic and non-syndromic hearing loss in Pakistani families. *Genes.* 2020;11(11):1329. doi:[10.3390/genes11111329](https://doi.org/10.3390/genes11111329)
 69. Müller U. Cadherins and mechanotransduction by hair cells. *Curr Opin Cell Biol.* 2008;20(5):557-566. doi:[10.1016/j.ceb.2008.06.004](https://doi.org/10.1016/j.ceb.2008.06.004)
 70. Ramzan K, Al-Numair NS, Al-Ageel S, et al. Identification of novel CDH23 variants causing moderate to profound progressive non-syndromic hearing loss. *Genes.* 2020;11(12):1474. doi:[10.3390/genes11121474](https://doi.org/10.3390/genes11121474)
 71. Lagziel A, Overlack N, Bernstein SL, Morell RJ, Wolfrum U, Friedman TB. Expression of cadherin 23 isoforms is not conserved: implications for a mouse model of usher syndrome type 1D. *Mol Vis.* 2009;15:1843-1857.
 72. Bolz H, von Brederlow B, Ramírez A, et al. Mutation of CDH23, encoding a new member of the cadherin gene family, causes usher syndrome type 1D. *Nat Genet.* 2001;27(1):108-112. doi:[10.1038/83667](https://doi.org/10.1038/83667)
 73. Usami S-I, Isaka Y, Miyagawa M, Nishio S-Y. Variants in CDH23 cause a broad spectrum of hearing loss: from non-syndromic to syndromic hearing loss as well as from congenital to age-related hearing loss. *Hum Genet.* 2022;141(3-4):903-914. doi:[10.1007/s00439-022-02431-2](https://doi.org/10.1007/s00439-022-02431-2)
 74. Bork JM, Peters LM, Riazuddin S, et al. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. *Am J Hum Genet.* 2001;68(1):26-37. doi:[10.1086/316954](https://doi.org/10.1086/316954)
 75. Schultz JM, Bhatti R, Madeo AC, et al. Allelic hierarchy of CDH23 mutations causing non-syndromic deafness DFNB12 or usher syndrome USH1D in compound heterozygotes. *J Med Genet.* 2011;48(11):767-775. doi:[10.1136/jmedgenet-2011-100262](https://doi.org/10.1136/jmedgenet-2011-100262)
 76. Michel V, Booth KT, Patni P, et al. CIB2, defective in isolated deafness, is key for auditory hair cell mechanotransduction and survival. *EMBO Mol Med.* 2017;9(12):1711-1731. doi:[10.15252/emmm.201708087](https://doi.org/10.15252/emmm.201708087)
 77. Wang Y, Li J, Yao X, et al. Loss of CIB2 causes profound hearing loss and abolishes Mechano-electrical transduction in mice. *Front Mol Neurosci.* 2017b;10:401. doi:[10.3389/fnmol.2017.00401](https://doi.org/10.3389/fnmol.2017.00401)
 78. Dal Cortivo G, Dell'Orco D. Calcium- and integrin-binding protein 2 (CIB2) in physiology and disease: bright and dark sides. *Int J Mol Sci.* 2022;23(7):3552. doi:[10.3390/ijms23073552](https://doi.org/10.3390/ijms23073552)
 79. Booth KT, Kahrizi K, Babanejad M, et al. Variants in CIB2 cause DFNB48 and not USH1J. *Clin Genet.* 2018;93(4):812-821. doi:[10.1111/cge.13170](https://doi.org/10.1111/cge.13170)
 80. Kitano T, Kitajiri S-I, Nishio S-Y, Usami S-I. Detailed clinical features of deafness caused by a Claudin-14 variant. *Int J Mol Sci.* 2019;20(18):4579. doi:[10.3390/ijms20184579](https://doi.org/10.3390/ijms20184579)
 81. Nishio S-Y, Hattori M, Moteki H, et al. Gene expression profiles of the cochlea and vestibular endorgans: localization and function of genes causing deafness. *Ann Otol Rhinol Laryngol.* 2015;124(Suppl 1):6S-48S. doi:[10.1177/0003489415575549](https://doi.org/10.1177/0003489415575549)
 82. Kawashima Y, Géléoc GSG, Kurima K, et al. Mechanotransduction in mouse inner ear hair cells requires transmembrane channel-like genes. *J Clin Invest.* 2011;121(12):4796-4809. doi:[10.1172/JCI60405](https://doi.org/10.1172/JCI60405)
 83. Ben-Yosef T, Belyantseva IA, Saunders TL, et al. Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. *Hum Mol Genet.* 2003;12(16):2049-2061. doi:[10.1093/hmg/ddg210](https://doi.org/10.1093/hmg/ddg210)
 84. Naz S, Imtiaz A, Mujtaba G, et al. Genetic causes of moderate to severe hearing loss point to modifiers: genetic causes of hearing loss point to modifiers. *Clin Genet.* 2017;91(4):589-598. doi:[10.1111/cge.12856](https://doi.org/10.1111/cge.12856)
 85. Bashir R, Fatima A, Naz S. Mutations in CLDN14 are associated with different hearing thresholds. *J Hum Genet.* 2010;55(11):767-770. doi:[10.1038/jhg.2010.104](https://doi.org/10.1038/jhg.2010.104)
 86. Kenna MA, Feldman HA, Neault MW, et al. Audiologic phenotype and progression in GJB2 (Connexin 26) hearing loss. *Arch Otolaryngol Head Neck Surg.* 2010;136(1):81-87. doi:[10.1001/archoto.2009.202](https://doi.org/10.1001/archoto.2009.202)
 87. Denoyelle F, Lina-Granade G, Plauchu H, et al. Connexin 26 gene linked to a dominant deafness. *Nature.* 1998;393(6683):319-320. doi:[10.1038/30639](https://doi.org/10.1038/30639)
 88. Anselmi F, Hernandez VH, Crispino G, et al. ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca^{2+} signals across the inner ear. *Proc Natl Acad Sci.* 2008;105(48):18770-18775. doi:[10.1073/pnas.0800793105](https://doi.org/10.1073/pnas.0800793105)
 89. Bruzzone R, Giaume C, eds. *Connexin Methods and Protocols*. Humana Press; 2001.

90. Santos RLP, Wajid M, Pham TL, et al. Low prevalence of Connexin 26 (GJB2) variants in Pakistani families with autosomal recessive non-syndromic hearing impairment. *Clin Genet*. 2005;67(1):61-68. doi:[10.1111/j.1399-0004.2005.00379.x](https://doi.org/10.1111/j.1399-0004.2005.00379.x)
91. Kausar N, Haque A, Masoud MS, et al. Disease-associated variants of gap junction Beta 2 protein (GJB2) in the deaf population of southern Punjab of Pakistan. *PLoS ONE*. 2021;16(10):e0259083. doi:[10.1371/journal.pone.0259083](https://doi.org/10.1371/journal.pone.0259083)
92. Sloan-Heggen CM, Bierer AO, Shearer AE, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum Genet*. 2016;135(4):441-450. doi:[10.1007/s00439-016-1648-8](https://doi.org/10.1007/s00439-016-1648-8)
93. Morell RJ, Olszewski R, Tona R, et al. Noncoding microdeletion in mouse Hgf disrupts neural crest migration into the Stria Vascularis, reduces the Endocochlear potential, and suggests the neuropathology for human nonsyndromic deafness DFNB39. *J Neurosci Off J Soc Neurosci*. 2020;40(15):2976-2992. doi:[10.1523/JNEUROSCI.2278-19.2020](https://doi.org/10.1523/JNEUROSCI.2278-19.2020)
94. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. *Cold Spring Harb Perspect Biol*. 2009;1(2):a002584. doi:[10.1101/cshperspect.a002584](https://doi.org/10.1101/cshperspect.a002584)
95. Nayak G, Varga L, Trincot C, et al. Molecular genetics of MARVELD2 and clinical phenotype in Pakistani and Slovak families segregating DFNB49 hearing loss. *Hum Genet*. 2015;134(4):423-437. doi:[10.1007/s00439-015-1532-y](https://doi.org/10.1007/s00439-015-1532-y)
96. Nayak G, Lee SI, Yousaf R, et al. Tricellulin deficiency affects tight junction architecture and cochlear hair cells. *J Clin Invest*. 2013;123(9):4036-4049. doi:[10.1172/JCI69031](https://doi.org/10.1172/JCI69031)
97. Houdusse A, Titus MA. The many roles of myosins in filopodia, microvilli and stereocilia. *Current Biology: CB*. 2021;31(10):R586-R602. <https://doi.org/10.1016/j.cub.2021.04.005>
98. Sweeney HL, Holzbaur ELF. Motor proteins. *Cold Spring Harb Perspect Biol*. 2018;10(5):a021931. doi:[10.1101/cshperspect.a021931](https://doi.org/10.1101/cshperspect.a021931)
99. Moreland ZG, Bird JE. Myosin motors in sensory hair bundle assembly. *Curr Opin Cell Biol*. 2022;79:102132. doi:[10.1016/j.ceb.2022.102132](https://doi.org/10.1016/j.ceb.2022.102132)
100. Li S, Mecca A, Kim J, et al. Myosin-VIIa is expressed in multiple isoforms and essential for tensioning the hair cell mechanotransduction complex. *Nat Commun*. 2020;11(1):2066. doi:[10.1038/s41467-020-15936-z](https://doi.org/10.1038/s41467-020-15936-z)
101. Lopes VS, Gibbs D, Libby RT, et al. The usher 1B protein, MYO7A, is required for normal localization and function of the visual retinoid cycle enzyme, RPE65. *Hum Mol Genet*. 2011;20(13):2560-2570. doi:[10.1093/hmg/ddr155](https://doi.org/10.1093/hmg/ddr155)
102. Jaijo T, Aller E, Oltra S, et al. Mutation profile of the MYO7A gene in Spanish patients with usher syndrome type I. *Hum Mutat*. 2006;27(3):290-291. doi:[10.1002/humu.9404](https://doi.org/10.1002/humu.9404)
103. Belyantseva IA, Boger ET, Naz S, et al. Myosin-XVa is required for tip localization of whirlin and differential elongation of hair-cell stereocilia. *Nat Cell Biol*. 2005;7(2):148-156. doi:[10.1038/ncb1219](https://doi.org/10.1038/ncb1219)
104. Ballesteros A, Yadav M, Cui R, Kurima K, Kachar B. Selective binding and transport of protocadherin 15 isoforms by stereocilia unconventional myosins in a heterologous expression system. *Sci Rep*. 2022;12(1):13764. doi:[10.1038/s41598-022-17757-0](https://doi.org/10.1038/s41598-022-17757-0)
105. Zhang J, Guan J, Wang H, et al. Genotype-phenotype correlation analysis of MYO15A variants in autosomal recessive non-syndromic hearing loss. *BMC Med Genet*. 2019;20(1):60. doi:[10.1186/s12881-019-0790-2](https://doi.org/10.1186/s12881-019-0790-2)
106. Kwon T-J, Cho H-J, Kim U-K, et al. Methionine sulfoxide reductase B3 deficiency causes hearing loss due to stereocilia degeneration and apoptotic cell death in cochlear hair cells. *Hum Mol Genet*. 2014;23(6):1591-1601. doi:[10.1093/hmg/ddt549](https://doi.org/10.1093/hmg/ddt549)
107. Pangrsic T, Lasarow L, Reuter K, et al. Hearing requires otoferlin-dependent efficient replenishment of synaptic vesicles in hair cells. *Nat Neurosci*. 2010;13(7):869-876. doi:[10.1038/nn.2578](https://doi.org/10.1038/nn.2578)
108. Vogl C, Cooper BH, Neef J, et al. Unconventional molecular regulation of synaptic vesicle replenishment in cochlear inner hair cells. *J Cell Sci*. 2015;128(4):638-644. doi:[10.1242/jcs.162099](https://doi.org/10.1242/jcs.162099)
109. Moser T, Starr A. Auditory neuropathy—neural and synaptic mechanisms. *Nat Rev Neurol*. 2016;12(3):135-149. doi:[10.1038/nrneurol.2016.10](https://doi.org/10.1038/nrneurol.2016.10)
110. Vona B, Rad A, Reisinger E. The many faces of DFNB9: relating OTOF variants to hearing impairment. *Genes*. 2020;11(12):1411. doi:[10.3390/genes11121411](https://doi.org/10.3390/genes11121411)
111. Yasunaga S, Grati M, Cohen-Salmon M, et al. A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a non-syndromic form of deafness. *Nat Genet*. 1999;21(4):363-369. doi:[10.1038/7693](https://doi.org/10.1038/7693)
112. Rodríguez-Ballesteros M, del Castillo FJ, Martín Y, et al. Auditory neuropathy in patients carrying mutations in the otoferlin gene (OTOF). *Hum Mutat*. 2003;22(6):451-456.
113. Ito T, Choi BY, King KA, et al. SLC26A4 genotypes and phenotypes associated with enlargement of the vestibular aqueduct. *Cell Physiol Biochem*. 2011;28(3):545-552. doi:[10.1159/000335119](https://doi.org/10.1159/000335119)
114. Albert S, Blons H, Jonard L, et al. SLC26A4 gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations. *Eur J Hum Genet*. 2006;14(6):773-779. doi:[10.1038/sj.ejhg.5201611](https://doi.org/10.1038/sj.ejhg.5201611)
115. Garabet Diramerian L, Ejaz S. Pendred Syndrome. *StatPearls*. StatPearls Publishing; 2022. <http://www.ncbi.nlm.nih.gov/books/NBK549839/>
116. Anwar S, Riazuddin S, Ahmed ZM, et al. SLC26A4 mutation spectrum associated with DFNB4 deafness and Pendred's syndrome in Pakistanis. *J Hum Genet*. 2009;54(5):266-270. doi:[10.1038/jhg.2009.21](https://doi.org/10.1038/jhg.2009.21)
117. Fettiplace R, Furness DN, Beurg M. The conductance and organization of the TMC1-containing mechanotransducer channel complex in auditory hair cells. *Proc Natl Acad Sci*. 2022;119(41):e2210849119. doi:[10.1073/pnas.2210849119](https://doi.org/10.1073/pnas.2210849119)
118. Basu A, Lagier S, Vologodskaya M, Fabella BA, Hudspeth A. Direct mechanical stimulation of tip links in hair cells through DNA tethers. *eLife*. 2016;5:e16041. doi:[10.7554/eLife.16041](https://doi.org/10.7554/eLife.16041)
119. Pan B, Akyuz N, Liu X-P, et al. TMC1 forms the pore of Mechanosensory transduction channels in vertebrate inner ear hair cells. *Neuron*. 2018;99(4):736-753.e6. doi:[10.1016/j.neuron.2018.07.033](https://doi.org/10.1016/j.neuron.2018.07.033)
120. Nakanishi H, Kurima K, Kawashima Y, Griffith AJ. Mutations of TMC1 cause deafness by disrupting mechanoelectrical transduction. *Auris Nasus Larynx*. 2014;41(5):399-408. doi:[10.1016/j.anl.2014.04.001](https://doi.org/10.1016/j.anl.2014.04.001)
121. Kitajiri S, McNamara R, Makishima T, et al. Identities, frequencies and origins of TMC1 mutations causing DFNB7/B11 deafness in Pakistan. *Clin Genet*. 2007;72(6):546-550. doi:[10.1111/j.1399-0004.2007.00895.x](https://doi.org/10.1111/j.1399-0004.2007.00895.x)
122. Liu W, Löwenheim H, Santi PA, Glueckert R, Schrott-Fischer A, Rask-Andersen H. Expression of trans-membrane serine protease 3 (TMPS3) in the human organ of Corti. *Cell Tissue Res*. 2018;372(3):445-456. doi:[10.1007/s00441-018-2793-2](https://doi.org/10.1007/s00441-018-2793-2)
123. Chen Y-S, Cabrera E, Tucker BJ, et al. TMPS3 expression is limited in spiral ganglion neurons: implication for successful cochlear implantation. *J Med Genet*. 2022;59(12):1219-1226. doi:[10.1136/jmg-2022-108654](https://doi.org/10.1136/jmg-2022-108654)
124. Guipponi M. The transmembrane serine protease (TMPS3) mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) in vitro. *Hum Mol Genet*. 2002;11(23):2829-2836. doi:[10.1093/hmg/11.23.2829](https://doi.org/10.1093/hmg/11.23.2829)

125. Molina L, Fasquelle L, Nouvian R, et al. Tmprss3 loss of function impairs cochlear inner hair cell Kcnma1 channel membrane expression. *Hum Mol Genet.* 2013;22(7):1289-1299. doi:[10.1093/hmg/dds532](https://doi.org/10.1093/hmg/dds532)
126. Weegerink NJD, Schraders M, Oostrik J, et al. Genotype-phenotype correlation in DFNB8/10 families with TMPRSS3 mutations. *J Assoc Res Otolaryngol.* 2011;12(6):753-766. doi:[10.1007/s10162-011-0282-3](https://doi.org/10.1007/s10162-011-0282-3)
127. Lee K, Khan S, Islam A, et al. Novel TMPRSS3 variants in Pakistani families with autosomal recessive non-syndromic hearing impairment. *Clin Genet.* 2012;82(1):56-63. doi:[10.1111/j.1399-0004.2011.01695.x](https://doi.org/10.1111/j.1399-0004.2011.01695.x)
128. Lee SJ, Lee S, Han JH, et al. Structural analysis of pathogenic TMPRSS3 variants and their cochlear implantation outcomes of sensorineural hearing loss. *Gene.* 2023;865:147335. <https://doi.org/10.1016/j.gene.2023.147335>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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