HHS Public Access

Author manuscript

*J Med Genet*. Author manuscript; available in PMC 2016 January 30.

Published in final edited form as:

*J Med Genet*. 2015 December ; 52(12): 823–829. doi:10.1136/jmedgenet-2015-103389.

# CHARACTERIZING THE SPECTRUM OF AUTOSOMAL RECESSIVE HEREDITARY HEARING LOSS IN IRAN

**Christina M Sloan-Heggen**[1,2], **Mojgan Babanejad**[3], **Maryam Beheshtian**[3], **Allen C Simpson**[1], **Kevin T Booth**[1], **Fariba Ardalani**[3], **Kathy L Frees**[1], **Marzieh Mohseni**[3], **Reza Mozafari**[3], **Zohreh Mehrjoo**[3], **Leila Jamali**[3], **Saeideh Vaziri**[3], **Tara Akhtarkhavari**[3], **Niloofar Bazazzadegan**[3], **Nooshin Nikzat**[3], **Sanaz Arzhangi**[3], **Farahnaz Sabbagh**[4], **Hasan Otukesh**[5], **Seyed Morteza Seifati**[6], **Hossein Khodaei**[6], **Maryam Taghdiri**[7], **Nicole C Meyer**[1], **Ahmad Daneshi**[8], **Mohammad Farhadi**[8], **Kimia Kahrizi**[3], **Richard JH Smith**[1,2,9], **Hela Azaiez**[1], and **Hossein Najmabadi**[3]

[1]Molecular Otolaryngology & Renal Research Labs, Department of Otolaryngology-Head and Neck Surgery, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA

[2]Department of Molecular Physiology & Biophysics, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA

[3]Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

[4]Kerman Welfare Institution, Kerman, Iran

[5]Hazrat –e – Ali Asghar Educational & Treatment Center, Iran University of Medical Sciences, Tehran, Iran

[6]Meybod Genetics Research Center, Yazd, Iran

[7]Shiraz Welfare Institution, Shiraz, Iran

[8]Head and Neck Surgery Department and Research Center, Iran University of Medical Sciences, Tehran, Iran

[9]Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA 52242, USA

Correspondence to: Richard J.H. Smith, Molecular Otolaryngology and Renal Research Laboratories, 200 Hawkins Drive, Iowa City, IA 52242, USA, richard-smith@uiowa.edu; Hela Azaiez, Molecular Otolaryngology and Renal Research Laboratories, 200 Hawkins Drive, Iowa City, IA 52242, USA, hela-azaiez@uiowa.edu; Hossein Najmabadi, Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, hnajm12@yahoo.com.

CMSH, MB, and MB contributed equally to this work and share first authorship

ACS and KTB contributed equally to this work

AH, NH, and RJHS contributed equally to this work and share senior authorship

**Contributors** Concept and Design of the study: CMSH, RJHS, HN, HA, KK. Performed experiments: CMSH ACS, KTB, KLF, NCM, MB, MB, FA, MM, RM, ZM, LJ, SV, TA, NB, NN, SA, FS, HO, MS, HK, MT. Analyzed data: CMSH, ACS, KTB, RJHS, HN, HA. Provided material: HN, MF, AD. Wrote manuscript: CMSH, RJHS, HA

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** University of Social Welfare and Rehabilitation Science ethics committee and the Institute Review Board of the University of Iowa.

**Provenance and peer review** Not commissioned; externally peer reviewed.

## Abstract

**Background—**Countries with culturally accepted consanguinity provide a unique resource for the study of rare recessively inherited genetic diseases. Although hereditary hearing loss (HHL) is not uncommon, it is genetically heterogeneous, with over 85 genes causally implicated in non-syndromic hearing loss (NSHL). This heterogeneity makes many gene-specific types of NSHL exceedingly rare. We sought to define the spectrum of autosomal recessive HHL in Iran by investigating both common and rarely diagnosed deafness-causing genes.

**Design—**Using a custom targeted genomic enrichment (TGE) panel we simultaneously interrogating all known genetic causes of NSHL in a cohort of 302 *GJB2*-negative Iranian families.

**Results—**We established a genetic diagnosis for 67% of probands and their families, with over half of all diagnoses attributable to variants in five genes: *SLC26A4*, *MYO15A*, *MYO7A*, *CDH23*, and *PCDH15*. As a reflection of the power of consanguinity mapping, 26 genes were identified as causative for NSHL in the Iranian population for the first time. In total, 179 deafness-causing variants were identified in 40 genes in 201 probands, including 110 novel single nucleotide or small insertion-deletion variants and 3 novel copy number variations. Several variants represent founder mutations.

**Conclusion—**This study attests to the power of TGE and massively parallel sequencing (TGE +MPS) as a diagnostic tool for the evaluation of hearing loss in Iran, and expands on our understanding of the genetics of HHL in this country. Families negative for variants in the genes represented on this panel represent an excellent cohort for novel gene discovery.

### Keywords

Deafness; High-Throughput Nucleotide Sequencing; Iran; Genetic Testing; Founder Effect

## INTRODUCTION

The relationship between consanguinity and rare autosomal recessive disorders has long been recognized.[1–3] This association reflects the increased co-efficient of inbreeding that raises the likelihood of homozygosity for pathogenic variants in cultures allowing and encouraging intermarriage between relatives.[4] For example, in Iran the coefficient of inbreeding is 0.0185,[2] making this population extremely valuable for the identification of novel genes associated with recessive disorders like hereditary hearing loss (HHL). As compared to Western countries where the frequency of congenital hearing loss ranges from 1 to 2 per 1000,[5] in Iran it reaches 1 in 166,[6] making it the second most frequent disability following intellectual impairment.[7]

Numerous non-syndromic hearing loss (NSHL) genes have been discovered using Iranian families,[8–12] and multiple studies have shed light on its genetic etiology within this population (reviewed in [6]). However, consanguinity is a double-edged sword – while novel gene discovery is facilitated, genetic testing is more challenging as the likelihood of a high impact single genetic cause of hearing loss is decreased. For example, in Iran the prevalence of *GJB2*-related deafness is relatively low. It accounts for about 22% of

autosomal recessive severe-to-profound non-syndromic hearing loss (ARNSHL) in the Azerbaijan province of northwest Iran, but falls to about 8% in the southeast Iranian provinces of Sistan and Baluchestan.[13] In contrast, in multiple countries with a low co-efficient of inbreeding, variants in *GJB2*-related deafness account for about 50% of congenital severe-to-profound ARNSHL.[14 15]

The diagnostic challenge imposed by consanguinity can be met by using targeted genomic enrichment with massively parallel sequencing (TGE+MPS) to screen simultaneously all genes implicated in NSHL. This approach is temporally and financially sound and has been used in several studies focused on Middle Eastern populations.[16–18] To date, however, the full spectrum of genetic deafness has not been investigated in this manner.

In this study, we used a custom-designed targeted genomic enrichment (TGE) panel,[19 20] to target all NSHL-causing genes as well as select syndromic forms of hearing loss in a cohort of 302 Iranian probands. We demonstrate the efficiency of TGE+MPS as a diagnostic tool with a high probability of diagnosis and expand our understanding of the genetics of HHL in Iran. We also define a cohort enriched for families very likely segregating novel genetic causes of ARNSHL.

## MATERIALS AND METHODS

### Patients

Families with apparent autosomal recessive hearing loss were recruited through the Genetics Research Center at the University of Social Welfare and Rehabilitation Sciences, in Tehran, Iran. A detailed clinical examination was completed, measuring hearing thresholds by pure-tone audiometry at 250, 500, 1000, 2000, 4000, and 8000 Hz following standard protocols.[21] Most hearing-impaired persons had NSHL, but if Usher syndrome was suspected, funduscopy was performed, and if Pendred syndrome was considered, thyroid function testing and temporal bone imaging were completed. Blood was obtained from consenting participants and DNA was extracted following standard salting-out protocols.[22] This study was approved by the ethical committee of University of Social Welfare and Rehabilitation Science in Tehran, Iran and the Institute Review Board of the University of Iowa.

### Library preparation, sequencing and bioinformatic analysis

Prior to TGE+MPS, Sanger sequencing was used to identify participants segregating causative variants in *GJB2*. If *GJB2* screening was negative, TGE+MPS was completed as described[20] for a single proband from each family, using 1.5–3 μg of high-quality genomic DNA. Liquid-handling automation equipment (PerkinElmer, Waltham MA) was used to prepare the libraries. We used a TGE panel (OtoSCOPE v5) that targets 89 deafness-associated genes (see online supplementary table S1). All enriched libraries were sequenced on the Illumina HiSeq 2000 (Illumina, Inc., San Diego, CA) using 100 bp paired-end reads. Data analysis was performed on a local installation of Galaxy using the Burrows-Wheeler Alignment (BWA) for read mapping to the reference genome (hg19, NCBI Build 37), Picard for removal of duplicate reads, and GATK for local re-alignment and variant calling. Variant filtering was based on quality/coverage depth (QD ≥5) and minor allele frequency

(MAF<0.02) as reported in the 1000 Genomes Project Database and the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project Exome Variant Server (EVS). Variants meeting these metrics were further filtered based on coding effect (non-synonymous, indels and splice-site variants), and annotated for conservation (GERP and PhyloP) and pathogenicity (PolyPhen2, SIFT, MutationTaster and LRT). We also considered annotations from the Deafness Variation Database (deafnessvariationdatabase.org), an in-house curated, open-access database of all variants in the deafness TGE panel. Copy number analysis was included and completed as described.[20 23 24] Briefly, a custom R script provided mean-depth analysis following normalization of all samples' overall coverage to that within the entire batch using a sliding-window method. We utilized the 2011-07-01 version of the tool with default settings to make automated CNV calls. All CNV calls were then curated manually.

### Variant interpretation and segregation analysis

Manual variant interpretation was undertaken with a hierarchical scheme. Variants previously reported as pathogenic by the Deafness Variation Database were considered first. Variants were considered likely pathogenic if they met the following characteristics: 1) Low MAF (<0.005); and 2) Predicted truncating (nonsense, splice-site, and indels). Missense variants were also considered if the pathogenicity score was high (predicted deleterious by at least 4 of 6 tools, listed above). In variants meeting these criteria, Sanger validation and cosegregation in multiplex families was completed.

### Haplotype construction

Haplotypes were constructed using 14 exonic SNPs and one causative SNP spanning 36 and 719 kb of sequence flanking the putative founder mutations in *MYO15A* and *PCDH15*, respectively, and 9 exonic SNPs and one causative SNP spanning 57 kb within *SLC26A4*. Defined haplotypes were compared across families and against ethnically matched controls. SNPs selected for haplotype reconstruction had varying MAFs (0.000–0.9999).

## RESULTS

### Ascertained families and clinical description

Three-hundred-and-two (302) *GJB2*-negative multiplex families segregating apparent autosomal recessive NSHL were recruited across Iran (figure 1, see online supplementary table S2). Two notable exceptions were a single family presenting with sensorineural hearing loss, optic atrophy, and ataxia (later diagnosed with Charcot Marie Tooth syndrome) and a family with cochlear aplasia and atrial and ventricular septal defects (genetically undiagnosed). Twelve different ethnicities were represented, although 167 individuals (55%) were of Fars ethnicity. 241 families (80%) reported consanguinity. Hearing loss was severe-to-profound in 85% of probands (257), moderate-to-severe in 14%, and mild-to-moderate in 1%. It was prelingual in onset in 86% of probands, with the phenotype most commonly being non-syndromic (82%, 247 probands), however in-depth clinical evaluation identified syndromic features in 8% of probands, with Usher syndrome, Pendred syndrome, Charcot Marie Tooth syndrome, Chudley-McCullough syndrome, and Jervell and Lange-Nielsen syndrome all represented.

### Targeted genomic enrichment and massively parallel sequencing

The proband from each family underwent TGE+MPS (see online supplementary table S1). Sequencing generated an average of 7.883 (± 2.004) million reads per sample, with an average target coverage of 578× (±246). Greater than 99.3% (± 0.6%) of the panel was covered at 20× (see online supplementary table S3). An average of 7 variants per sample met all quality and functional filtering criteria.

### Genetic diagnosis

Amongst the cohort of 302 *GJB2*-negative probands, we identified the underlying genetic cause in 201, providing a diagnostic rate of 67% (see online supplementary table S4). Had the cohort not been pre-screened for *GJB2,* this figure would have increased to greater than 72%.[25] The impact of reported consanguinity on diagnostic rate was insignificant, (66% versus 67% for non-consanguineous families), likely reflecting the overall high co-efficient of inbreeding as reflected by the fact that within all positively diagnosed families, 89% were homozygous for the identified deafness-causing variant (figure 2).

### Genetic etiology of hearing loss in Iran

Over half of all diagnoses (52%) in this *GJB2*-negative cohort were attributable to variants in five genes: *SLC26A4*, *MYO15A*, *MYO7A*, *CDH23*, and *PCDH15* at 18%, 14%, 8%, 7%, and 5%, respectively (see online supplementary figure S1). In the remaining families, causal variants were identified in 35 different genes (table 1). This report is the first to implicate 26 of these genes as causal of hearing loss in the Iranian population.

The number of unique deafness-causing variants was 179, of which 66 have previously been reported as pathogenic. Of the 113 novel variants we considered pathogenic based on MAF, conservation, and *in silico* pathogenicity prediction, Sanger sequencing and segregation analysis have confirmed 106 (see online supplementary table S4). A total of 398 deafness-causing alleles, including 1 mitochondrial and 3 X-linked, were identified in 201 probands.

Only 19 probands were compound heterozygotes for deafness-causing variants; the majority, 178 were homozygous for the identified deafness-causing variant. Of this number, 153 (86%) reported consanguinity. Interesting, and also consistent with a high co-efficient of inbreeding, 6 of 19 (32%) probands with compound heterozygosity for causative variants were born to consanguineous parents. These findings indicate that a high-coefficient of inbreeding combined with geographical isolation may lead to enrichment of pathogenic variants in consanguineous families. The distribution between non-truncating and truncating variants was similar: missense variants accounted for 48% (192) of causative alleles, with nonsense 18% (69), splice-site 14% (56) and indels 17% (69) accounting for the remainder (figure 2).

Copy number variations (CNVs) constituted 3% of the causative variants (6 homozygous variants; 12 alleles) (figure 2, figure 3). Of the homozygous CNVs, 3 have been reported (a *OTOA* whole gene deletion, a *STRC* gene-to-pseudogene conversion, and a *SLC26A4* multi-exon deletion)[23 26 27] and 3 are novel (a homozygous deletion of 19 exons in *USH2A*, a homozygous deletion of exon 6 of *DFNB59*, and a homozygous duplication of 4 exons in

*TMPRSS3*, potentially leading to a frameshift—premature truncation) (see online supplementary table S4). In addition, a 35-bp, medium-sized deletion within exon 2 of *ILDR1* was identified through CNV analysis.

Variant type varied significantly across genes (chi square p <0.0001) (figure 4). Missense variants represented 75%, 74%, 60%, 43%, and 0% of diagnoses involving *CDH23*, *SLC26A4, MYO7A, MYO15A,* and *PCDH15,* respectively. Nonsense variants were causative in 72%, 27%, 12%, 7%, and 0% of diagnoses in *PCDH15, MYO7A, MYO15A, CDH23*, and *SLC26A4*.

### Causative variants observed in multiple families and possible founder effects

Thirty-one causative variants were diagnosed in at least two families (see online supplementary table S5). For 19 (61%) of these variants all families with the variant were of the same ethnicity. The most frequently identified variant, *PCDH15* p.Gln1576Stop was identified in 4 families, 3 of whom are of the Fars ethnicity (figure 5, see online supplementary figure S2). All individuals homozygous for this variant segregated the same haplotype. A similar analysis of the *MYO15A* and *SLC26A4* supported founder haplotypes for p.Tyr1392Stop and p.Leu445Trp (figure 5).

## DISCUSSION

Although deafness is the second most frequent disability in Iran, in this study we show that in spite of tremendous heterogeneity, variants in only five genes (*SLC26A4*, *MYO15A*, *MYO7A*, *CDH23*, and *PCDH15*) account for over half of all genetic diagnoses (table 1, see online supplementary figure S1). In addition to NSHL, we also identified several syndromic forms of hearing loss, including Usher syndromes types 1 and 2, Pendred syndrome, Jervell and Lange-Nielsen syndrome, Chudley-McCullough syndrome, and Charcot-Marie-Tooth Disease (see online supplementary table S4). Early diagnosis of Usher and Jervell and Lange-Nielsen syndromes are especially important due to the potential for medical intervention[28–30] and therefore are important inclusions in the genetic evaluation of presumed NSHL.

Previous gene-specific studies causally implicated 14 genes with NSHL in the Iranian population: *GJB2*, *SLC26A4*, *MYO15A*, *TECTA*, *DFNB59*, *TMC1*, *LRTOMT*, *ILDR1*, *COL11A2*, *LOXHD1*, *MARVELD2*, *MYO7A*, *OTOF, RDX* (reviewed in [31]), and *PDZD7*. [32] The most comprehensive previous study was completed on 144 *GJB2*-negative subjects using linkage analysis and Sanger sequencing, which identified variants in 10 different genes (*MYO15A, SLC26A4, ILDR1, TECTA, TMC1, DFNB59, LRTOMT, OTOF, MARVELD2*, and *MYO7A*) with a diagnosis rate of 22.9%.[33] This study showed similar diagnostic rates to ours, with notable exceptions for *SLC26A4* and *MYO7A* (see online supplementary table S6), with diagnostic rates of 0.7% versus 5% and 4.9% versus 12.3%, respectively. Direct comparison between studies is difficult, however, based on fundamental differences in study design. Had our study focused on the previously implicated14 genes, the number of positive diagnoses would have dropped from 201 to 113 (59%), decreasing the overall diagnostic rate to 39% of 302 probands. This difference emphasizes the need for a comprehensive genetic testing strategy in this population.

Using TGE+MPS, we identified differing patterns of causal variants within the five most commonly implicated genes (figure 4). *SLC26A4* causative variants were predominantly missense (74%), but conversely *PCDH15* variants were predominantly non-sense (72%). *MYO15A, CDH23*, and *MYO7A* all exhibited unique patterns of causative variant types. In-depth analysis of these gene-specific patterns will provide a better understanding of their pathophysiological mechanisms.

There were also founder mutations such as p.Gln1576Stop in *PCDH15*, p.Tyr1392Stop in *MYO15A,* and p.Leu445Trp in *SLC26A4* (figure 5, see online supplementary figure S2). Although *PCDH15* p.Gln1576Stop is novel, the *MYO15A* p.Tyr1392Stop has been reported in the Pakistani population[34] and the *SLC26A4* p.Leu445Trp mutation has been identified in Dutch, Tunisian, Brazilian, and French populations.[35–38] As expected, most variants found across families displayed phenotypic similarities, with one notable exception, *MYO7A* NM_000260:c.1344-2A>G. This splice variant was identified in two families of Fars ethnicity. In one family the phenotype was bilateral severe-to-profound NSHL, while in the other, the phenotype was Usher syndrome. This difference is potentially interesting, as it suggests the involvement of modifiers genes in these phenotypes.

Other phenotypic differences emerged within the *PCDH15* variant spectrum. The six unique nonsense, splice-site, and indel variants that result in a premature stop at or before amino acid 996 caused Usher syndrome, while variants later in the protein caused a non-syndromic phenotype. This observation is in contrast to earlier studies suggesting that all truncating variants of *PCDH15* are associated with Usher syndrome.[39]

CNVs accounted for only 3% of diagnoses and therefore did not play a dominant role in spite of their significance in other populations.[23] For example, while CNVs in *STRC* account for about 5.5% of hearing loss in outbred populations,[23 40] they accounted for just 0.5% of diagnoses in this Iranian cohort (figure 2). This difference may reflect an ascertainment bias for probands with severe-to-profound hearing loss (85% of the Iranian cohort) (figure 1, figure 3, see online supplementary table S2).

The outcomes of this study are important to the global scientific community studying hereditary hearing loss in two ways. First, with respect to the families diagnosed with ARNSHL, 93 identified novel variants have been submitted to the Deafness Variation Database (deafnessvariationdatabase.org), creating a feed-forward loop for improving variant annotation. Additionally, as a result of this study, 12 variants have been reclassified as ‘pathogenic’ from a previous classification of ‘variant of unknown significance’ (VUS) or ‘likely pathogenic’. All variant reclassifications will require continued study of the Iranian population to ensure that they are not just ethnically enriched non-pathogenic variants.

Second, in 101 families, we failed to identify plausible disease-causing variants. These negative results might reflect familial locus heterogeneity[41] or non-coding disease-causing variants. For example, it has been estimated that only ~85% of genetic disorders are due to changes in coding regions.[42] As such, the 32 families identified as carriers of a single known deafness-causing variant constitute a valuable cohort to screen for pathogenic variants in regulatory domains. However, many of these ‘negative’ families may segregate

deafness-causing variants in novel genes. As such, they represent a pre-selected familial cohort ideally suited for novel deafness-gene discovery.

## CONCLUSIONS

This study is the largest to date to investigate the genetic etiology of hearing loss in an Iranian population. We demonstrate the efficiency of TGE+MPS as a diagnostic clinical tool, and document the continued value of comprehensive studies in inbred populations to enrich our understanding of phenotype-genotype correlations, inform our ability to classify variants, and expedite our discovery of novel deafness-causing genes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We are grateful to families included within this study for their time and generosity of personal data. We thank Ms. Jalalvand for subject recruitment and DNA extraction.

**Funding** This work was supported by the Iran National Science Foundation, Iran's National Elites Foundation, T32 GM007337 to the University of Iowa MSTP, and by NIDCD RO1s DC003544, DC002842 and DC012049 to RJHS.

## References

1. Bittles A. Consanguinity and its relevance to clinical genetics. Clinical genetics. 2001; 60(2):89–98. [PubMed: 11553039]
2. Saadat M, Ansari-Lari M, Farhud DD. Consanguineous marriage in Iran. Annals of human biology. 2004; 31(2):263–269. [published Online First: Epub Date]|. [PubMed: 15204368]
3. Garrod AE. The incidence of alkaptonuria a study in chemical individuality. Lancet. 1902; 2:1616–1620.
4. Zlotogora J, Hujerat Y, Barges S, et al. The fate of 12 recessive mutations in a single village. Annals of human genetics. 2007; 71(Pt 2):202–208. [published Online First: Epub Date]|. [PubMed: 17331080]
5. Fortnum HM, Summerfield AQ, Marshall DH, et al. Prevalence of permanent childhood hearing impairment in the United Kingdom and implications for universal neonatal hearing screening: questionnaire based ascertainment study. Bmj. 2001; 323(7312):536–540. [PubMed: 11546698]
6. Mahdieh N, Rabbani B, Wiley S, et al. Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. Journal of human genetics. 2010; 55(10):639–648. [published Online First: Epub Date]|. [PubMed: 20739942]
7. Najmabadi H, Motazacker MM, Garshasbi M, et al. Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci. Human genetics. 2007; 121(1):43–48. [published Online First: Epub Date]|. [PubMed: 17120046]
8. Grillet N, Schwander M, Hildebrand MS, et al. Mutations in LOXHD1, an evolutionarily conserved stereociliary protein, disrupt hair cell function in mice and cause progressive hearing loss in humans. American journal of human genetics. 2009; 85(3):328–337. [published Online First: Epub Date]|. [PubMed: 19732867]
9. Delmaghani S, Aghaie A, Michalski N, et al. Defect in the gene encoding the EAR/EPTP domain-containing protein TSPEAR causes DFNB98 profound deafness. Human molecular genetics. 2012; 21(17):3835–3844. [published Online First: Epub Date]|. [PubMed: 22678063]

10. Taghizadeh SH, Kazeminezhad SR, Sefidgar SA, et al. Investigation of LRTOMT gene (locus DFNB63) mutations in Iranian patients with autosomal recessive non-syndromic hearing loss. International journal of molecular and cellular medicine. 2013; 2(1):41–45. [PubMed: 24551789]
11. Borck G, Ur Rehman A, Lee K, et al. Loss-of-function mutations of ILDR1 cause autosomal-recessive hearing impairment DFNB42. American journal of human genetics. 2011; 88(2):127–137. [published Online First: Epub Date]|. [PubMed: 21255762]
12. Chen W, Kahrizi K, Meyer NC, et al. Mutation of COL11A2 causes autosomal recessive non-syndromic hearing loss at the DFNB53 locus. Journal of medical genetics. 2005; 42(10):e61. [published Online First: Epub Date]|. [PubMed: 16033917]
13. Bazazzadegan N, Nikzat N, Fattahi Z, et al. The spectrum of GJB2 mutations in the Iranian population with non-syndromic hearing loss--a twelve year study. International journal of pediatric otorhinolaryngology. 2012; 76(8):1164–1174. [published Online First: Epub Date]|. [PubMed: 22695344]
14. Denoyelle F, Marlin S, Weil D, et al. Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: implications for genetic counselling. Lancet. 1999; 353(9161):1298–1303. [published Online First: Epub Date]|. [PubMed: 10218527]
15. Kenneson A, Van Naarden Braun K, Boyle C. GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: a HuGE review. Genetics in medicine : official journal of the American College of Medical Genetics. 2002; 4(4):258–274. [published Online First: Epub Date]|. [PubMed: 12172392]
16. Brownstein Z, Friedman LM, Shahin H, et al. Targeted genomic capture and massively parallel sequencing to identify genes for hereditary hearing loss in Middle Eastern families. Genome biology. 2011; 12(9):R89. [published Online First: Epub Date]|. [PubMed: 21917145]
17. Shahzad M, Sivakumaran TA, Qaiser TA, et al. Genetic analysis through OtoSeq of Pakistani families segregating prelingual hearing loss. Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery. 2013; 149(3):478–487. [published Online First: Epub Date]|. [PubMed: 23770805]
18. Duman D, Sirmaci A, Cengiz FB, et al. Screening of 38 genes identifies mutations in 62% of families with nonsyndromic deafness in Turkey. Genetic testing and molecular biomarkers. 2011; 15(1–2):29–33. [published Online First: Epub Date]|. [PubMed: 21117948]
19. Shearer AE, Black-Ziegelbein EA, Hildebrand MS, et al. Advancing genetic testing for deafness with genomic technology. Journal of medical genetics. 2013; 50(9):627–634. [published Online First: Epub Date]|. [PubMed: 23804846]
20. Shearer AE, DeLuca AP, Hildebrand MS, et al. Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(49):21104–21109. [published Online First: Epub Date]|. [PubMed: 21078986]
21. Smith, RJH.; Shearer, AE.; Hildebrand, MS., et al. Deafness and Hereditary Hearing Loss Overview. In: Pagon, RA.; Adam, MP.; Ardinger, HH., et al., editors. GeneReviews(R). Seattle (WA): 1993.
22. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids research. 1988; 16(3):1215. [PubMed: 3344216]
23. Shearer AE, Kolbe DL, Azaiez H, et al. Copy number variants are a common cause of non-syndromic hearing loss. Genome medicine. 2014; 6(5):37. [published Online First: Epub Date]|. [PubMed: 24963352]
24. Nord AS, Lee M, King MC, et al. Accurate and exact CNV identification from targeted high-throughput sequence data. BMC genomics. 2011; 12:184. [published Online First: Epub Date]|. [PubMed: 21486468]
25. Najmabadi H, Nishimura C, Kahrizi K, et al. GJB2 mutations: passage through Iran. American journal of medical genetics Part A. 2005; 133A(2):132–137. [published Online First: Epub Date]|. [PubMed: 15666300]
26. Shahin H, Walsh T, Rayyan AA, et al. Five novel loci for inherited hearing loss mapped by SNP-based homozygosity profiles in Palestinian families. European journal of human genetics : EJHG. 2010; 18(4):407–413. [published Online First: Epub Date]|. [PubMed: 19888295]

27. Anwar S, Riazuddin S, Ahmed ZM, et al. SLC26A4 mutation spectrum associated with DFNB4 deafness and Pendred's syndrome in Pakistanis. Journal of human genetics. 2009; 54(5):266–270. [published Online First: Epub Date]|. [PubMed: 19287372]

28. Goldenberg I, Moss AJ, Zareba W, et al. Clinical course and risk stratification of patients affected with the Jervell and Lange-Nielsen syndrome. Journal of cardiovascular electrophysiology. 2006; 17(11):1161–1168. [published Online First: Epub Date]|. [PubMed: 16911578]

29. Damen GW, Pennings RJ, Snik AF, et al. Quality of life and cochlear implantation in Usher syndrome type I. The Laryngoscope. 2006; 116(5):723–728. [published Online First: Epub Date]|. [PubMed: 16652078]

30. Berson EL. Nutrition and retinal degenerations. International ophthalmology clinics. 2000; 40(4): 93–111. [PubMed: 11064860]

31. Najmabadi H, Kahrizi K. Genetics of non-syndromic hearing loss in the Middle East. International journal of pediatric otorhinolaryngology. 2014; 78(12):2026–2036. [published Online First: Epub Date]|. [PubMed: 25281338]

32. Booth KTAH, Kahrizi K, Simpson AC, Tollefson WTA, Sloan CM, Meyer NC, Schnieders MJ, Najmabadi H, Smith RJH. PDZD7 and Hearing Loss: More Than Just a Modifier. Genetics in Medicine. In press.

33. Babanejad M, Fattahi Z, Bazazzadegan N, et al. A comprehensive study to determine heterogeneity of autosomal recessive nonsyndromic hearing loss in Iran. American journal of medical genetics Part A. 2012; 158A(10):2485–2492. [published Online First: Epub Date]|. [PubMed: 22903915]

34. Nal N, Ahmed ZM, Erkal E, et al. Mutational spectrum of MYO15A: the large N-terminal extension of myosin XVA is required for hearing. Human mutation. 2007; 28(10):1014–1019. [published Online First: Epub Date]|. [PubMed: 17546645]

35. Van Hauwe P, Everett LA, Coucke P, et al. Two frequent missense mutations in Pendred syndrome. Human molecular genetics. 1998; 7(7):1099–1104. [PubMed: 9618166]

36. Rebeh IB, Yoshimi N, Hadj-Kacem H, et al. Two missense mutations in SLC26A4 gene: a molecular and functional study. Clinical genetics. 2010; 78(1):74–80. [published Online First: Epub Date]|. [PubMed: 20128824]

37. de Moraes VC, dos Santos NZ, Ramos PZ, et al. Molecular analysis of SLC26A4 gene in patients with nonsyndromic hearing loss and EVA: identification of two novel mutations in Brazilian patients. International journal of pediatric otorhinolaryngology. 2013; 77(3):410–413. [published Online First: Epub Date]|. [PubMed: 23273637]

38. Ladsous M, Vlaeminck-Guillem V, Dumur V, et al. Analysis of the thyroid phenotype in 42 patients with Pendred syndrome and nonsyndromic enlargement of the vestibular aqueduct. Thyroid : official journal of the American Thyroid Association. 2014; 24(4):639–648. [published Online First: Epub Date]|. [PubMed: 24224479]

39. Ahmed ZM, Riazuddin S, Aye S, et al. Gene structure and mutant alleles of PCDH15: nonsyndromic deafness DFNB23 and type 1 Usher syndrome. Human genetics. 2008; 124(3):215–223. [published Online First: Epub Date]|. [PubMed: 18719945]

40. Francey LJ, Conlin LK, Kadesch HE, et al. Genome-wide SNP genotyping identifies the Stereocilin (STRC) gene as a major contributor to pediatric bilateral sensorineural hearing impairment. American journal of medical genetics Part A. 2012; 158A(2):298–308. [published Online First: Epub Date]|. [PubMed: 22147502]

41. Rehman AU, Santos-Cortez RL, Drummond MC, et al. Challenges and solutions for gene identification in the presence of familial locus heterogeneity. European journal of human genetics : EJHG. 2014 [published Online First: Epub Date]|.

42. Ng SB, Turner EH, Robertson PD, et al. Targeted capture and massively parallel sequencing of 12 human exomes. Nature. 2009; 461(7261):272–276. [published Online First: Epub Date]|. [PubMed: 19684571]

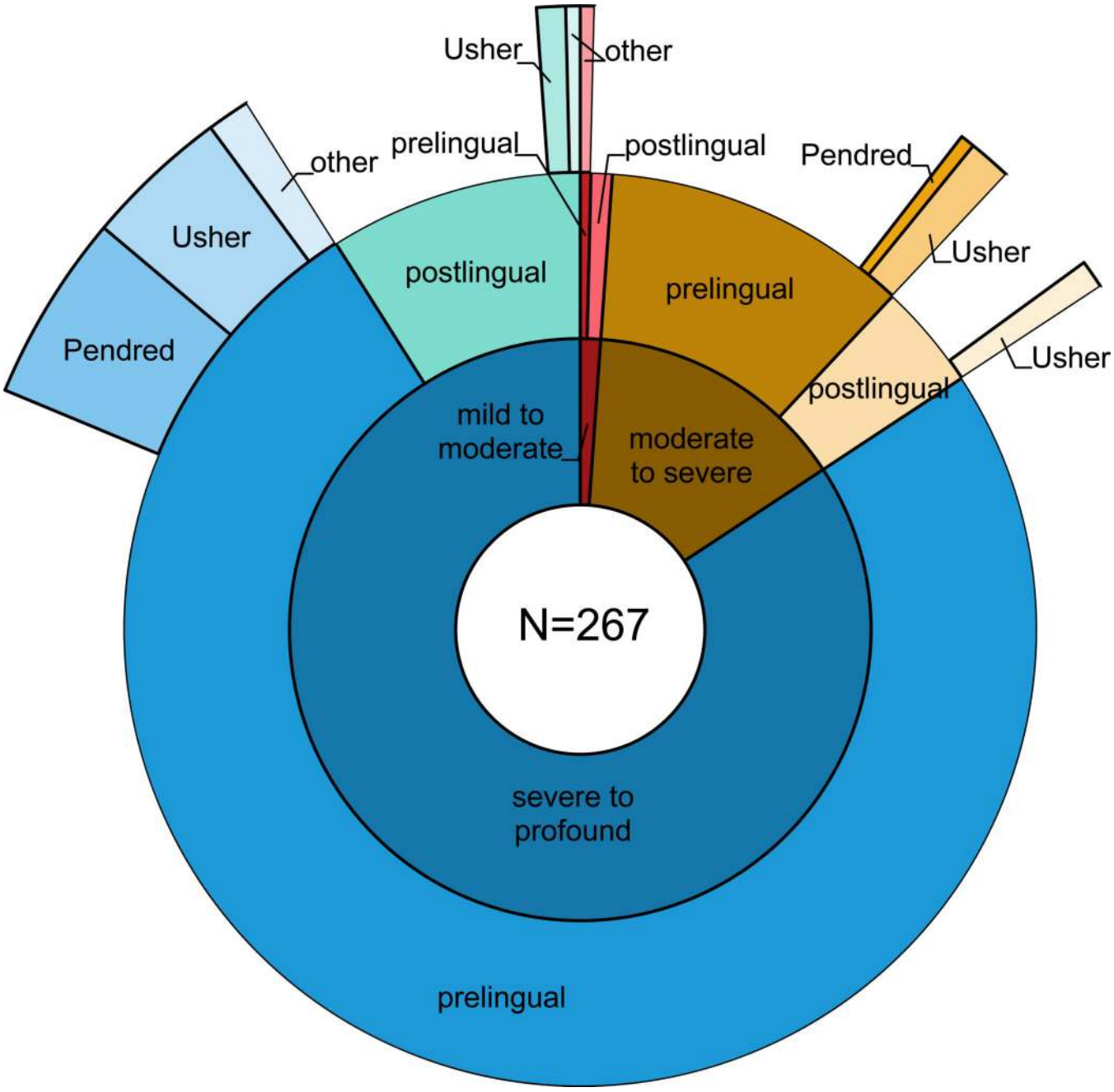


**Figure 1. Phenotypic characteristics of Iranian individuals with hearing loss**

Amongst the 302 Iranian probands with hearing loss 267 had complete phenotypic data available. The inner ring indicates the severity of hearing loss, separated by color (red, yellow, and blue for mild to moderate, moderate to severe, and severe to profound, respectively); the middle ring indicates the onset, prelingual or postlingual; and the outer ring denotes the presence of additional phenotypes, Pendred syndrome (Pendred), Usher syndrome (Usher), or another suspected syndrome (other). White segments in the outer ring represent individuals with non-syndromic hearing loss.

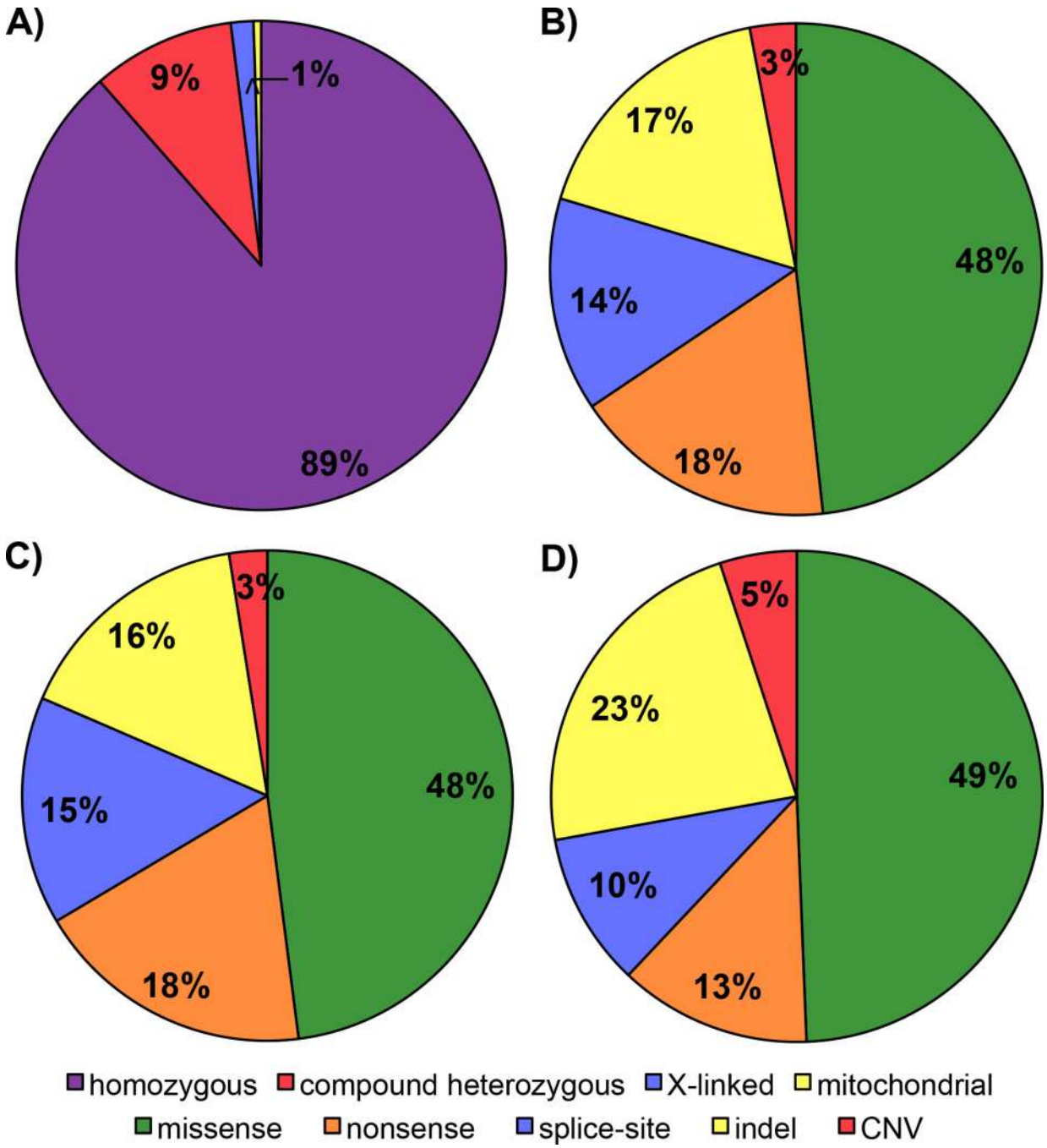


**Figure 2. Proportions of causative mutation types in 201 Iranian families**

A. Proportional identification of homozygous versus compound heterozygous, X-linked, or mitochondrial variants within 201 diagnosed families. B. Causative mutation type as a portion of all causative alleles: missense, nonsense, splice-site, indel, and CNV's. X-linked autosomal recessive, mitochondrial, and heterozygous variants are each assigned a count of one, while homozygous calls are counted as two, one for each allele (n=398). Causative variant type varied very slightly in individuals from consanguineous (C) or non-consanguineous (D) families, n=319 and 79 alleles, respectively.

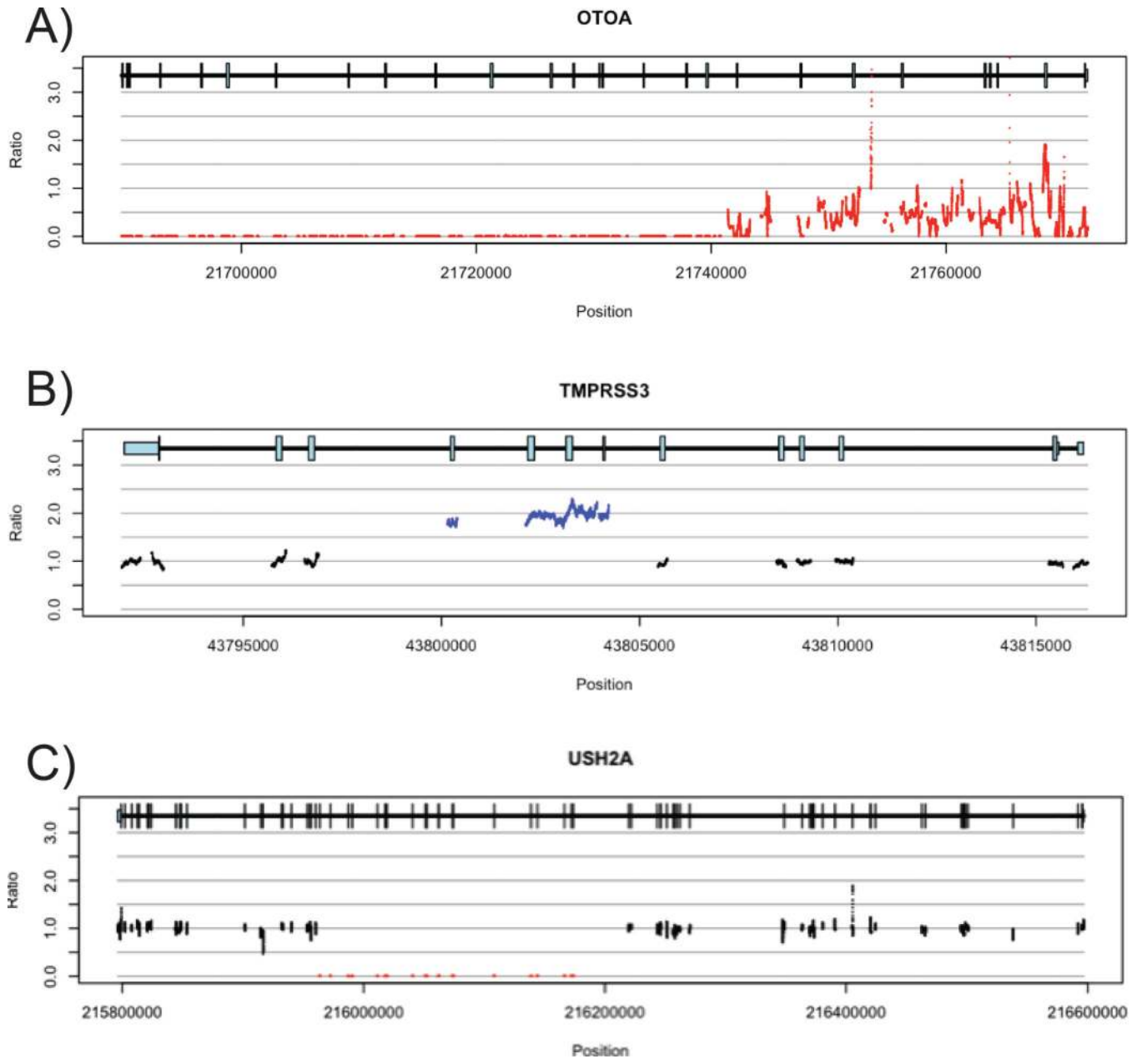


**Figure 3. Selected representative CNVs identified within a population of 302 Iranians**

Homozygous whole gene deletion of *OTOA* gene (A), homozygous duplication of exons 7–10 of *TMPRSS3* gene (B), and partial deletion of *USH2A* are shown below gene diagrams, indicating the structure of each gene and its exons. X-axis indicates chromosomal position. Y-axis shows the number of reads, represented as a ratio; 1.0 indicates the normal level of 2 alleles (black lines), 0.5 indicates a heterozygous deletion, 0 implies a homozygous deletion (both indicated by red lines), 1.5 indicates a heterozygous duplication and 2.0 corresponds to homozygous duplication (both indicated by blue lines).

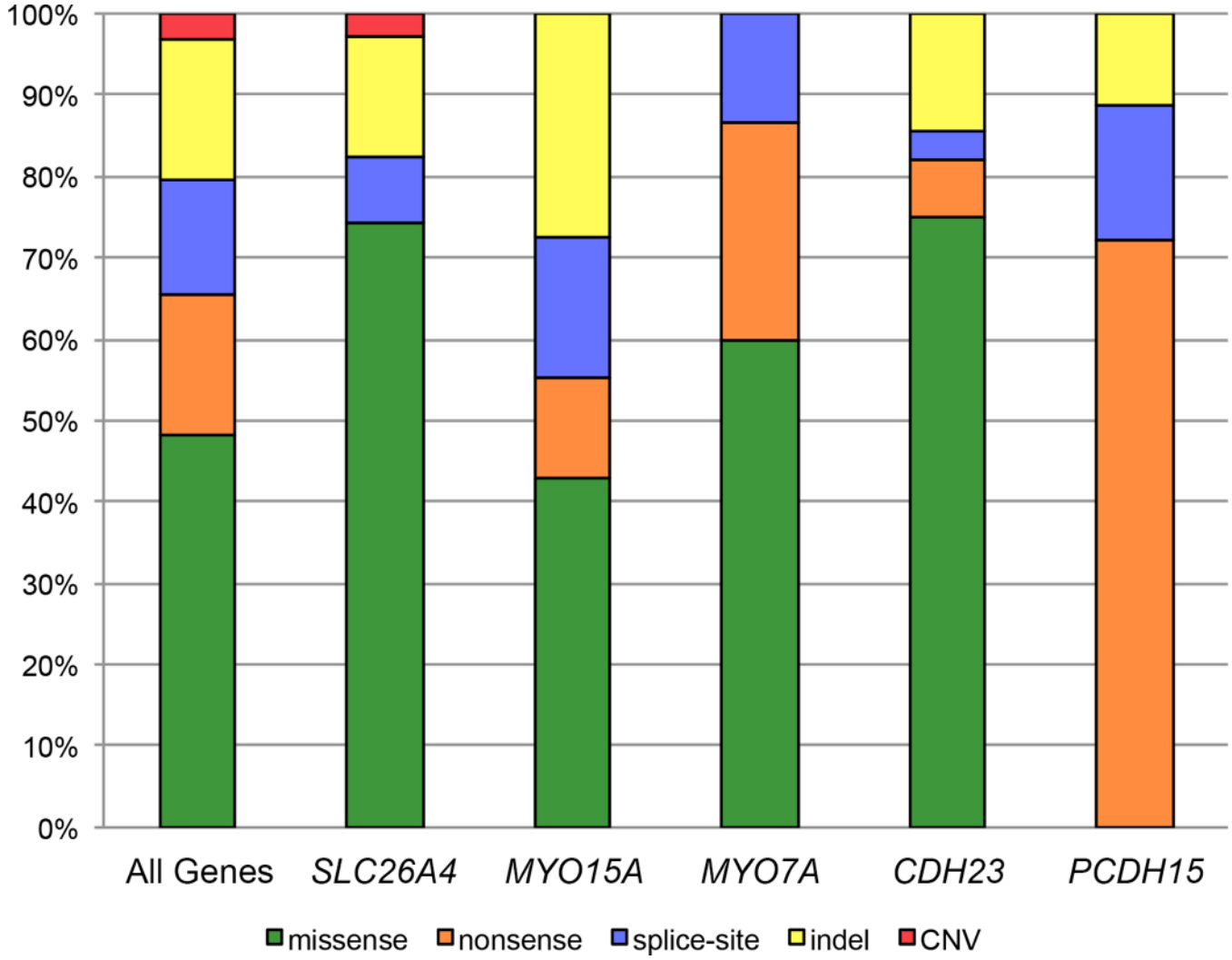


**Figure 4. Commonly diagnosed genes in Iranian families exhibit unique patterns for different mutation types**

Mutation types: missense, nonsense, splice-site, indel, and CNV's are displayed based upon allele count for all diagnosed patients, *SLC26A4, MYO15A, MYO7A*, *CDH23,* and *PCHD15* with an n = 398, 74, 58, 30, 28, and 18, respectively.

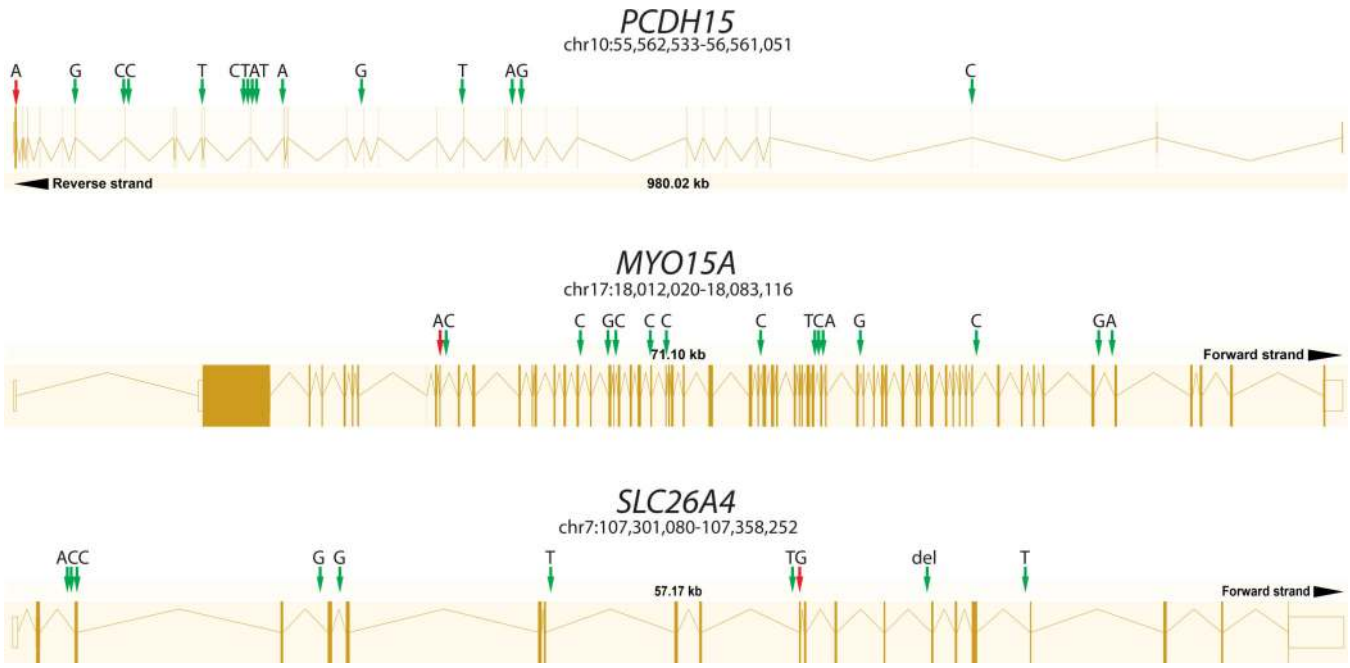


**Figure 5. Founder effects are associated with particular *PCDH15, MYO15A,* and *SLC26A4* variants**

Genotype and location for 14, 14, or 9 SNPs (green arrows) surrounding the causative variants in individuals segregating *PCDH15* p.Gln1576Stop (A), *MYO15A* p. Tyr1392Stop (B), or *SLC26A4* p.Leu445Trp (C) (red arrows) used to construct haplotypes.

## Table 1

Genetic etiology of hereditary hearing loss in 302 Iranian probands

| Gene | Number of diagnoses | % of diagnoses | % of cohort |
|---|---|---|---|
| *SLC26A4* | 37 | 18.4% | 12.3% |
| *MYO15A* | 29 | 14.4% | 9.6% |
| *MYO7A* | 15 | 7.5% | 5.0% |
| ***CDH23**** | 14 | 7.0% | 4.6% |
| ***PCDH15*** | 9 | 4.5% | 3.0% |
| ***USH2A*** | 7 | 3.5% | 2.3% |
| ***OTOA*** | 6 | 3.0% | 2.0% |
| *ILDR1* | | | |
| *TMC1* | | | |
| *DFNB59* | | | |
| ***CABP2*** | 5 | 2.5% | 1.7% |
| ***TMPRSS3*** | | | |
| ***GIPC3*** | 4 | 2.0% | 1.3% |
| *LRTOMT* | | | |
| *TECTA* | | | |
| ***ADGRV1*** | 3 | 1.5% | 1.0% |
| ***LHFPL5*** | | | |
| ***MYO6*** | | | |
| *OTOF* | | | |
| ***PTPRQ*** | | | |
| ***USH1C*** | | | |
| ***CIB2*** | 2 | 1.0% | 0.7% |
| *COL11A2* | | | |
| ***DFNB31*** | | | |
| *MARVELD2* | | | |
| ***POU3F4*** | | | |
| *RDX* | | | |
| ***TMIE*** | | | |
| ***ESPN*** | 1 | 0.5% | 0.3% |
| ***GPSM2*** | | | |
| ***GRXCR1*** | | | |
| ***KCNQ1*** | | | |
| *LOXHD1* | | | |
| ***MTRNR1*** | | | |
| ***OTOGL*** | | | |
| *PDZD7* | | | |

| Gene | Number of diagnoses | % of diagnoses | % of cohort |
|---|---|---|---|
| ***PRPS1*** | | | |
| ***STRC*** | | | |
| ***TRIOBP*** | | | |
| ***WFS1*** | | | |

*names shown in bold indicate that this study is the first to report variants in this gene as causative in an Iranian population.