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Recurrent variants in *OTOF* are significant contributors to prelingual nonsydromic hearing loss in Saudi patients

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Purpose: Hearing loss is more prevalent in the Saudi Arabian population than in other populations; however, the full range of genetic etiologies in this population is unknown. We report the genetic findings from 33 Saudi hearing-loss probands of tribal ancestry, with predominantly prelingual severe to profound hearing loss.

Methods: Testing was performed over the course of 2012–2016, and involved initial *GJB2* sequence and *GJB6*-D13S1830 deletion screening, with negative cases being reflexed to a next-generation sequencing panel with 70, 71, or 87 hearing-loss genes.

Results: A "positive" result was reached in 63% of probands, with two recurrent *OTOF* variants (p.Glu57* and p.Arg1792His) accountable for a third of all "positive" cases. The next most common cause was pathogenic variants in *MYO7A* and *SLC26A4*,

each responsible for three "positive" cases. Interestingly, only one "positive" diagnosis had a DFNB1-related cause, due to a homozygous *GJB6*-D13S1830 deletion, and no sequence variants in *GJB2* were detected.

Conclusion: Our findings implicate *OTOF* as a potential major contributor to hearing loss in the Saudi population, while highlighting the low contribution of *GJB2*, thus offering important considerations for clinical testing strategies for Saudi patients. Further screening of Saudi patients is needed to characterize the genetic spectrum in this population.

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Key Words: genetic testing; OTOF; prelingual profound hearing loss; Saudi Arabia

INTRODUCTION

Congenital hearing loss is one of the most common sensory disabilities affecting newborns worldwide, with prevalence estimates ranging from 0.2% to ~ 1%.[1] Genetic causes are responsible for 50% of congenital hearing loss, with more than 100 genes associated with nonsyndromic hearing loss (NSHL) and over 400 genes associated with syndromic hearing loss. The inheritance pattern of hearing loss can be autosomal recessive (AR), autosomal dominant, mitochondrial, or X-linked, depending on causative gene and variant type. A small number of genes have emerged as major contributors to hearing loss across several multicenter cohort studies, namely the *GJB2* and *STRC* genes,[2,3] which are estimated to be responsible for more than 50% of AR-NSHL, or 20–30% of all congenital hearing loss.

The prevalence of sensorineural hearing loss (SNHL) in Saudi children is high—it is estimated to be 1.5%, with 36 to 66% being hereditary in nature.[4] This higher prevalence than that in other populations may be partially attributed to increased rates of consanguinity (57–67%) in the Saudi population.[5] However, there are limited published data on the

variant spectrum in Saudi hearing-loss patients. The few studies that are available in Saudi patients report a minor contribution (3%)[6] from the DFNB1 locus (*GJB2* and *GJB6* genes). This is a significant difference from findings in Caucasian and other well-studied populations, where this locus accounts for nearly 30% of early-onset hearing loss.[1]

Currently, the variant spectrum and genes carrying a significant burden of causative variants are undefined for Saudi hearing-loss patients and previously published reports have failed to show the major contributory genes in the Saudi population. Thus, our study assesses genetic findings from a cohort of 33 Saudi patients with severe to profound prelingual hearing loss who were tested on a multigene hearing-loss gene next-generation sequencing (NGS) panel, in order to distinguish the genetic landscape of hearing loss in this population.

MATERIALS AND METHODS

Cohort description

Our study included genetic data from 33 patients and their family members (when available), who are of Saudi tribal

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ancestry from various regions of Saudi Arabia (**Figure 2**). Most of the probands had hearing loss in the severe to profound range (**Supplementary Table S1** online), and were referred to the Cochlear Implant and Middle Ear Implant Program at King Abdulaziz Medical City in Riyadh, to assess their suitability for cochlear implantation after limited or no benefit from hearing aids. As part of this assessment, a complete medical history and clinical evaluation is performed, including an audiological evaluation, temporal bone CT/MRI imaging, a full physical examination, and a three-generation family history. Cases were selected for genetic testing after referral to the genetic clinic by the in-charge otolaryngologist if a genetic etiology was suspected based on family history, clinical presentation, or absence of a clear nongenetic etiology. (**Supplementary Table S1**).

DFNB1 screening and OtoGenome NGS testing

DNA extracted from whole blood from patients was submitted for hearing-loss genetic testing to the Laboratory for Molecular Medicine (LMM) at Partners HealthCare Personalized Medicine (Cambridge, MA) over the course of 5 years (2012–2016), and involved initial DFNB1 screening followed by an expanded gene panel analysis on the OtoGenome test (**Figure 1**). DFNB1 screening included Sanger sequencing of the *GJB2* gene and testing for the common *GJB6*-D13S1830 deletion using gel-based fragment analysis, as previously described.[7] Cases where DFNB1 screening did not identify causative variants were reflexed to the OtoGenome test, which targets > 70 genes associated with NSHL or with hearing-loss syndromes (such as Usher syndrome and Pendred syndrome) whose initial or most prominent presentation is NSHL (**Supplementary Table S3**). Owing to the continual discovery of new genes, the LMM has performed validation and offered clinical testing using multiple versions of its NGS panel, with three different versions, OtoGenome-v1, OtoGenome-v2, and OtoGenome-v3, used in this study. All three tests were performed using oligonucleotide-based target capture (Agilent SureSelect, Santa Clara, CA) followed by

Illumina (San Diego, CA) sequencing of the coding regions and splice sites (±15) of target genes/loci. Sequencing for OtoGenome-v1 and OtoGenome-v2 was done using Illumina HiSeq, whereas OtoGenome-v3 utilized the Illumina MiSeq. All three versions used the same bioinformatics pipelines for postsequencing analysis, which includes alignment of sequencing reads using Burrows-Wheeler Aligner and variant calling using Genome Analysis Toolkit Unified Genotyper (Broad Institute, Cambridge, MA). Additional details on the bioinformatics pipeline used by the LMM for targeted NGS panels have been given in previous articles.[8,9]

OtoGenome-v1was used for diagnostic testing of hearing-loss patients between 2010 and 2014, and included the following 71 genes: *ACTG1, ATP6V1B1, BSND, CCDC50, CDH23, CLDN14, CLRN1, COCH, COL11A2, CRYM, DFNA5, DFNB31, DFNB59, DIAPH1, ESPN, ESRRB, EYA1, EYA4, GIPC3, GJB2, GJB3, GJB6, GPR98, GPSM2, GRHL2, GRXCR1, HGF, ILDR1, KCNE1, KCNQ1, KCNQ4, LHFPL5, LOXHD1, LRTOMT, MARVELD2, MIR183, MIR96, MSRB3, MTRNR1 (12S rRNA), MTTS1 (tRNAser(UCN)), MYH14, MYH9, MYO15A, MYO1A, MYO3A, MYO6, MYO7A, OTOA, OTOF, PCDH15, PDZD7, POU3F4, POU4F3, PRPS1, RDX, SERPINB6, SLC17A8, SLC26A4 (PDS), SLC26A5, TECTA, TIMM8A, TJP2, TMC1, TMIE, TMPRSS3, TPRN, TRIOBP, USH1C, USH1G, USH2A,* and *WFS1.*

OtoGenome-v2 included 70 genes (2014–2015) and differed from v1 in the addition of the *STRC* gene, due to the development of Sanger sequencing for follow-up regions within the *STRC* gene and the development of a copy-number variant (CNV) caller, VisCap, as described in previously published articles.[8,9] In addition, the *PDZD7* and *SLC26A5* genes were removed from this version, their removal being based on limited evidence supporting a gene-disease association with hearing loss at the time of assessment.[10]

OtoGenome-v3 included 87 genes (2015–2017) and differed from the previous version by the removal of six genes with limitations on their clinical validity or association with hearing loss (*CRYM, GJB3, MIR182, MYO1A, SLC17A8,* and
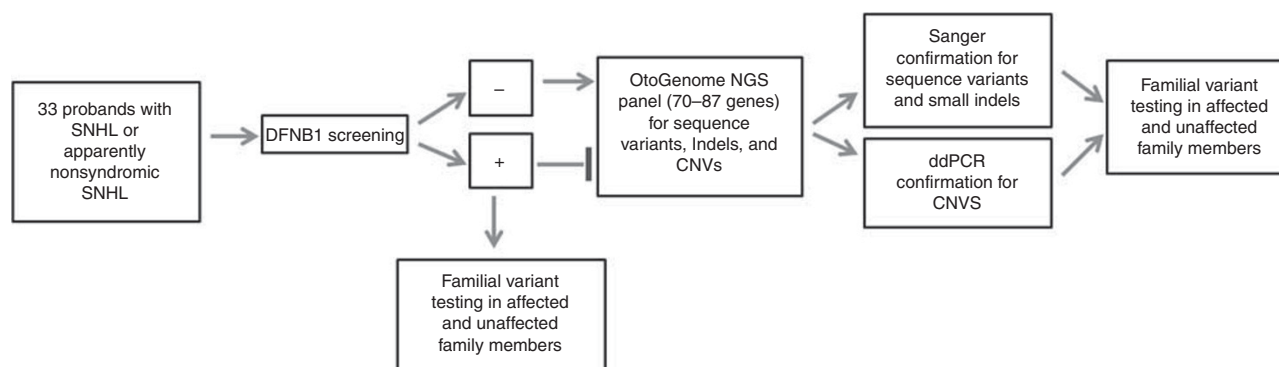

Figure 1 Schematic diagram of the genetic testing strategy used to identify causal variants in Saudi patients with prelingual sensorineural hearing loss (SNHL). CNVs, copy-number variants; ddPCR, droplet digital PCR; indels, insertions and/deletions; NGS, next-generation sequencing.

TJP2), and the addition of the following 23 genes with sufficient evidence to support a gene-disease association:[10] *CACNA1D, CATSPER2, CEACAM16, CIB2, CLPP, DIABLO, EDN3, EDNRB, HARS2, HSD17B4, KARS, LARS2, MITF, OTOG, OTOGL, P2RX2, PAX3, SIX1, SMPX, SOX10, SYNE4, TBC1D24*, and *TSPEAR*. **Supplementary Table S3** lists transcripts and exons covered in this version of the OtoGenome.

Whenever a variant was detected, we performed testing on parents as well as other affected and unaffected family members, if available, to aid in the interpretation of genetic findings (familial screening results are included in **Supplementary Table S1**). Confirmatory testing was performed using orthogonal methods: Sanger sequencing for SNVs, or droplet digital PCR for CNVs called by our CNV caller, VisCap.[6,9]

Variant interpretation

The variant interpretation and classification workflow at our laboratory has been previously described.[11] Briefly, data collected from general population data sets (such as the Exome Aggregation Consortium (ExAC)), internal or external disease databases, the literature, functional studies, and prediction tools, were used to manually classify each variant into one of five categories: pathogenic (P), likely pathogenic (LP), of uncertain significance (VUS), likely benign, or benign. The VUS category was further subdivided into three subcategories, VUS-favor pathogenic, VUS, and VUS-favor benign. The VUS subcategory is reserved for variants where there is no clear supportive evidence for or against pathogenicity or where the evidence is conflicting. On the other hand, a variant is classified as VUS-favor benign or VUS-favor pathogenic when existing evidence points to a benign or pathogenic effect as more likely but still does not reach the threshold for a likely benign or likely pathogenic classification, respectively. Variants classified as likely benign or benign are not reported in this article, but have been submitted to ClinVar (www.ncbi.nlm.nih.gov/clinvar/) along with the LMM's entire database of interpreted variation.

RESULTS

Cohort description

A total of 33 Saudi Arabian probands with mostly congenital or prelingual onset of hearing loss were initially screened for causative variants in the DFNB1 locus (*GJB2* sequencing and *GJB6*-D13S1830 deletion gel assay) followed by reflex testing on the OtoGenome NGS panel when biallelic causative variants at the DFNB1 locus were not detected (**Figure 1**). All probands were reported to have congenital or prelingual hearing loss, and the average age of testing was ~ 7 yr (range 0.5–42 yr). The severity of hearing loss was profound (absent at <90 dB) for most of the probands (29/33), and cochlear implants were used to improve hearing in 55% (18/33) of probands. Additional clinical features were noted in eight probands, including retinitis pigmentosa (one proband: F16), renal dysplasia (one proband: F15), delayed walking and attention deficit hyperactivity disorder (one proband: F11),

short stature (one proband: F17), enlarged vestibular aqueducts in two probands (F3, F5), galactosemia (one proband: F26), and osteogenesis imperfecta (one proband: F31) (**Supplementary Table S1**). All the families in this study were reportedly of Saudi tribal ancestry, originating from different regions in Saudi Arabia (**Figure 2**). The rate of parental consanguinity for this cohort was 85% (28/33), and a family history of hearing loss was reported for 76% (25/33) of probands (**Supplementary Table S1**).

Diagnostic rate and overall genetic findings

A "positive" molecular diagnosis is reached by identification of causative variants in hearing-loss genes consistent with the pattern of inheritance and clinical presentation (i.e., nonsyndromic versus syndromic, age at hearing-loss onset, and severity of hearing loss) reported for the patient. An "inconclusive" finding is reported when VUS or only heterozygous clinically significant AR variants are detected in a patient, while cases with only benign or likely benign variants in panel genes were reported as "negative." The molecular diagnosis was "positive" in 21 families, yielding a 64% (21/33) detection rate for this cohort (**Figure 3b**). For these "positive" cases, initial DFNB1 testing was negative and required reflexing to the OtoGenome NGS panel for all but one proband (F-30) who was homozygous for the *GJB6*-D13S1830 deletion. "Inconclusive" results were reported for 30% (10/33) of probands in this cohort, and two probands had "negative" reports (**Figure 3b**; **Supplementary Table S1**).

We identified 49 variants in 25 genes across our cohort, of which 19 were classified as clinically significant (**Figure 3a**; **Supplementary Table S2**). Of the 49 variants, 67% have not been previously reported in the literature on hearing-loss cases (6 P/LP, 27 VUS), and 20 of these (11 P/LP, 9 VUS) were also absent in over 200,000 alleles across racially diverse populations in the gnomAD database (http://gnomad.broad institute.org/). However, Middle Eastern and Saudi populations are not well represented in this database or in any other publicly available population database.

The majority of variants were unique to one family, and only three variants occurred in more than one family. These recurrent variants were clinically significant (p.Arg1792His in *OTOF* in four families, p.Glu57* in *OTOF* in three families, and p.Lys2078fs in *MYO7A* in two families) (**Supplementary Tables S1 and S2**). Of these, only the p.Glu57* variant has been previously reported in other hearing-loss probands by other studies.[12,13]

Genotypic and phenotypic spectrum in "positive" probands

In "positive" cases, causative variants were identified across nine different genes (**Figure 4**, **Table 1**), with three genes (*OTOF, MYO7A*, and *SLC26A4)* being responsible for 62% (13/21) of solved cases (**Figure 3**). The *OTOF* gene was by far the greatest contributor to hearing loss, responsible for 33% (7/21) of "positive" cases, owing to one of two recurrent deleterious variants in this gene (p.Arg1972His and p.Glu57*). All responsible genes in "positive" cases were
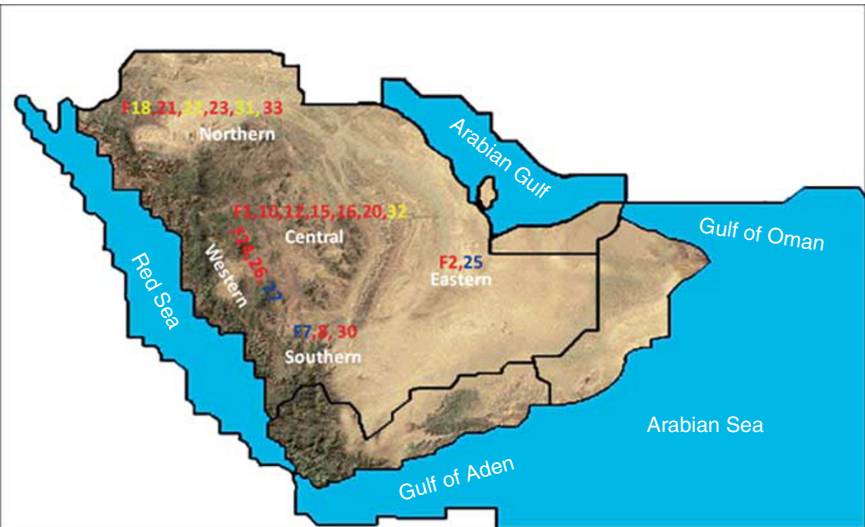
Figure 2 Regional distribution of the Saudi families with a "positive" genetic testing result in our cohort. For families with a nonrecurring variant, family IDs are shown in red near the province (in white) of their current place of residence. The family IDs of the recurrent p.Glu57* and p. Arg1792His variants are shown in dark blue and yellow, respectively.
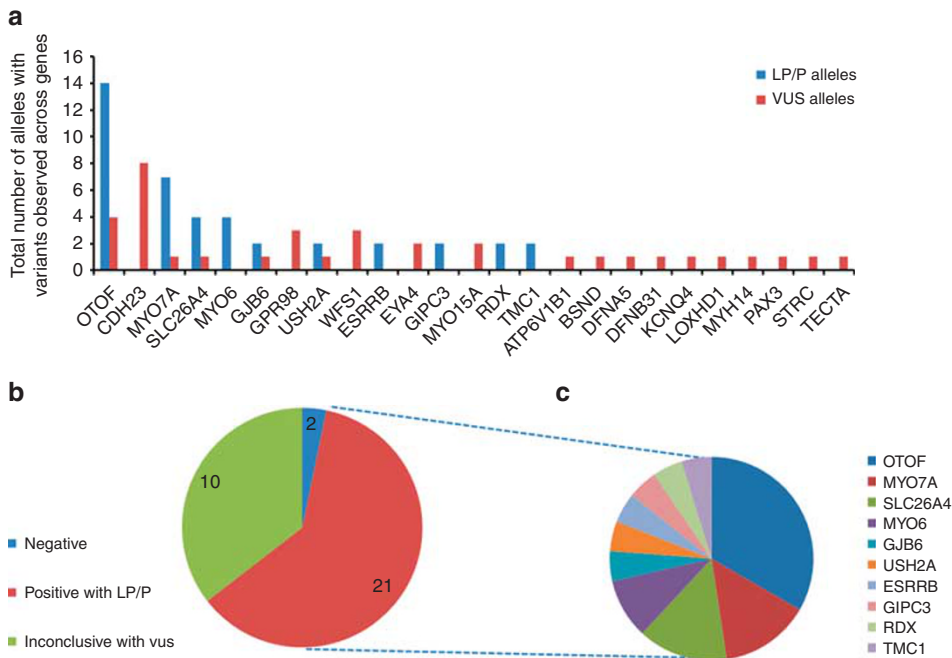

Figure 3 Diagnostic rate and distribution of variants across genes in 33 Saudi sensorineural hearing loss probands. (**a**) Counts of total variants across hearing loss genes by classification. (**b**) Diagnostic rate of DFNB1 screening/OtoGenome NGS panel testing for this cohort (**c**). Gene contribution of causative variants in "positive" probands. LP/P: likely pathogenic/pathogenic variant; VUS, variant of uncertain significance.

associated with AR hearing loss, and the causative variants were homozygous (86%, 18/21) or compound heterozygous (14%, 3/21). None of the families was found to have clinically significant variants in autosomal dominant or X-linked genes, nor did any family report a family history suggesting these patterns of inheritance for hearing loss.

In six probands, the causative variants were in genes associated with syndromic hearing loss, namely, in three probands (F-10, F-16, F-26), *MYO7A*, which is associated with Usher syndrome type I (USH1), and, also in three probands (F-2, F-20, F-33), *SLC26A4*, which can result in Pendred syndrome. Retinitis pigmentosa with abnormal
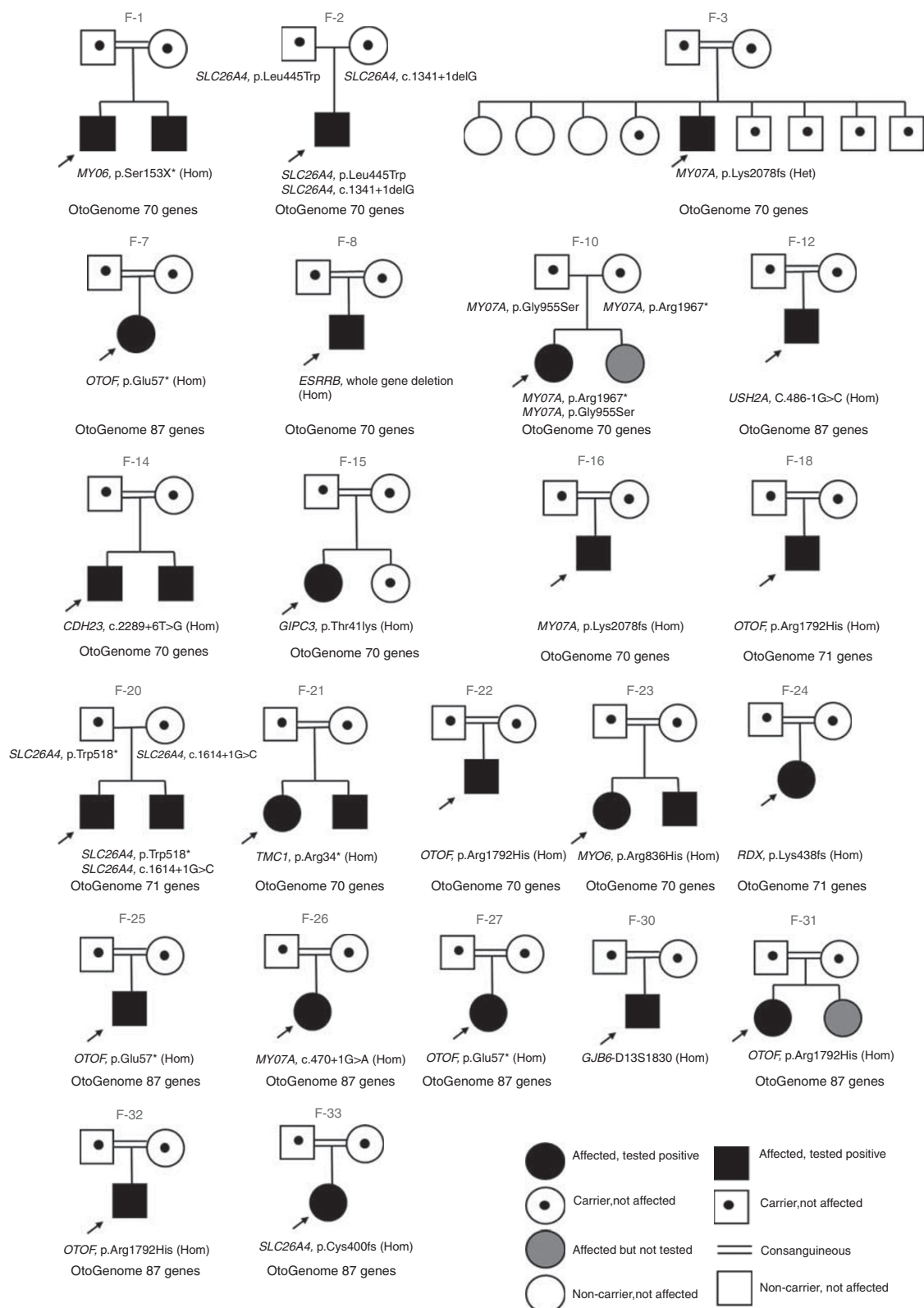
Figure 4 Pedigrees for Saudi families with clinically significant variants or suspect VUS variants (F-14). The OtoGenome panel version used for proband testing is shown below the pedigree. For Family F-30, a causative variant (*GJB6*-D13S1830) was identified on the initial DFNB1 assay. Please note that the parents in in families F-8, F-9, F-21, F-25, F-26, F-27, F-30, F-31, F-33 were not tested and represent obligate carriers (see **Supplementary Table 1** for a list of all family members who were screened for familial variants). Het, heterozygous; Hom, homozygous.

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Table 1 Causative variants in "positive" Saudi hearing-loss probands

Family ID	Gene	cDNA change	Protein change	Zygosity	Variant classification	Overall result	Ref.
F-1	MYO6	c.458C > G	p.Ser153*	Homozygous	Pathogenic	Positive	Novel
F-2	SLC26A4	c.1334T > G	p.Leu445Trp	Heterozygous	Pathogenic	Positive	27,28
		c.1341+1delG	p.?	Heterozygous	Pathogenic		29
F-7	OTOF	c.169G > T	p.Glu57*	Homozygous	Pathogenic	Positive	13
F-8	ESRRB	c.(?_1)_(1527_?)del	p?	Homozygous	Likely pathogenic	Positive	Novel
F-10	MYO7A	c.5899C > T	p.Arg1967*	Heterozygous	Pathogenic	Positive	30
		c.2863G > A	p.Gly955Ser	Heterozygous	Likely pathogenic		31
F-12	USH2A	c.486-1G > C	p?	Homozygous	Pathogenic	Positive	32
F-15	GIPC3	c.122C > A	p.Thr41Lys	Homozygous	Pathogenic	Positive	33
F-16	MYO7A	c.6231_6232insG	p.Lys2078fs	Homozygous	Pathogenic	Positive	Novel
F-18	OTOF	c.5375G > A	p.Arg1792His	Homozygous	Likely pathogenic	Positive	Novel
F-20	SLC26A4	c.1554G > A	p.Trp518*	Heterozygous	Pathogenic	Positive	34
		c.1614+1G > C	p?	Heterozygous	Pathogenic		35
F-21	TMC1	c.100C > T	p.Arg34*	Homozygous	Pathogenic	Positive	36
F-22	OTOF	c.5375G > A	p.Arg1792His	Homozygous	Likely pathogenic	Positive	Novel
F-23	MYO6	c.2507G > A	p.Arg836His	Homozygous	Likely pathogenic	Positive	Novel
F-24	RDX	c.1308delG	p.Lys438fs	Homozygous	Pathogenic	Positive	Novel
F-25	OTOF	c.169G > T	p.Glu57*	Homozygous	Pathogenic	Positive	13
F-26	MYO7A	c.470+1G > A	p?	Homozygous	Pathogenic	Positive	37
F-27	OTOF	c.169G > T	p.Glu57*	Homozygous	Pathogenic	Positive	13
F-30	DFNB1	GJB6-D13S1830	p?	Homozygous	Pathogenic	Positive	38
F-31	OTOF	c.5375G > A	p.Arg1792His	Homozygous	Likely pathogenic	Positive	Novel
F-32	OTOF	c.5375G > A	p.Arg1792His	Homozygous	Likely pathogenic	Positive	Novel
F-33	SLC26A4	c.1198delT	p.Cys400fs	Homozygous	Pathogenic	Positive	27,28,29

electroretinography, consistent with Usher syndrome, was reported for one of the MYO7A cases (F-16). The other two MYO7A cases were younger than 2 years of age at the time of testing, which is probably too early for the onset of detectable retinal abnormalities, typical in Usher-syndrome patients (**Supplementary Table S1**). For the three SLC26A4 "positive" cases, Mondini dysplasia was detected by temporal imaging, but perchlorate discharge testing was not performed; however, clinically they have no goiter, and TSH and T4 were normal at <2 years of age. This is not unusual, as it is recognized that a significant number of causative variants in SLC26A4 may result in nonsyndromic hearing loss only (DFNB4).

The OTOF gene is associated with AR auditory neuropathy (AN). Three of the seven probands with causative variants in OTOF were clinically diagnosed with AN. Although AN was not indicated by the referring otolaryngologist for the remaining four OTOF cases, all seven patients had bilateral severe or profound hearing loss, with prelingual onset in six cases, consistent with an OTOF cause for their hearing loss.

DISCUSSION

In this study, we report the genetic findings of 33 Saudi hearing-loss probands screened for causative variants across more than 70 hearing-loss genes. The majority of probands (78%) presented with prelingual severe to profound SNHL and reported a family history of hearing loss. To our knowledge, this is the first study to report on the pathogenic variant spectrum in familial hearing-loss cases from Saudi Arabia, tested on a 70+ gene NGS panel. A positive molecular diagnosis was reached for 21/33 (64%) of the Saudi probands. The genes with the largest contribution of causative variants in the "positive" cases were OTOF (33%; seven families), MYO7A (15%; three families), and SLC26A4 (15%; three families). A recent study of 25 Saudi individuals with sporadic hearing loss, using an amplicon-based targeted sequencing panel, also showed an enrichment of novel or rare biallelic OTOF variants,[12] although it was unclear whether all the variants were clinically significant. In contrast, in a large multiethnic cohort ($n = 1,119$) of predominantly Caucasian hearing-loss probands, OTOF accounted for only 2.4% of resolved cases.[14] Although our cohort size is small, our findings implicate OTOF as a major contributor to hearing loss in the Saudi population, and may account for a large portion of the hearing loss not attributable to the GJB2 gene.

Two recurrent variants in OTOF (p.Arg1792His and p.Glu57*) were responsible for hearing loss in seven Saudi families, suggesting a shared ancestry in the families sharing one of these recurrent OTOF variants. The variants were observed in 1/251,632 alleles (p.Glu57*; dbSNP rs397515591) and in 1/252,426 alleles (p.Arg1792His; dbSNP rs111033349) from non-Middle Eastern populations in the gnomeAD population database (http://gnomad.broadinstitute.org). The p.Arg1792His variant was novel in hearing-loss probands, while the p.Glu57* variant was previously reported in two

Saudi siblings and a single Libyan family with nonsyndromic SNHL.[12,13] Thus, these two variants may represent common pathogenic variants in Saudi and/or Middle Eastern cases. The presence in understudied populations of common pathogenic variants that are otherwise absent or rare in current large population databases has important implications for minor allele frequency thresholds that diagnostic laboratories use to automatically filter out benign variants.[15] Clinical laboratories base these thresholds on prevalence of disease, penetrance, and genetic heterogeneity.[15] These minor allele frequency thresholds, however, may not account for the higher prevalence of hearing loss in Saudi Arabia and common recurrent pathogenic variants unique to individuals of Saudi or Middle Eastern ancestry.

The importance of tribal affiliation and a preference for consanguineous marriage in Saudi and Middle Eastern societies are reflected in the high rate of homozygous causative variants. The only "positive" case with homozygous causative variants who did not indicate parental consanguinity (F-32) carried the recurrent p.Arg1792His variant, which would support the notion that this pathogenic allele is enriched in the Saudi population. Interestingly, most of the families with one of the two recurrent OTOF variants geographically inhabit areas along the trade and pilgrimage routes in the western regions of the Saudi Arabian peninsula (**Figure 2**), and may represent tribes with a larger presence in Saudi Arabia. Because all families in this study reported a tribal origin, other pathogenic variants identified only once in this cohort may have drifted further, with tribal migration, along the same trade and tribal migration routes, into neighboring Middle Eastern countries (Syria, Lebanon, Jordan, Palestine, Libya, and Tunisia to the north, and Yemen to the south). Screening of Saudi and Middle Eastern populations would determine the allele frequency of these variants, and identify enrichment of recurrent pathogenic variants across the region.

Of the 10 "inconclusive" probands, 7 had a family history of hearing loss. In one of the "inconclusive" families, a homozygous novel variant in CDH23 (c.2289+6T > G) segregated in two siblings (F-14) with SNHL. Splice prediction tools suggested a disruption of the nearby 5′ splice site, and the variant was absent in > 200,000 alleles in the gnomAD database; however, it was classified as VUS because of an absence of race-matched allele frequency data, which might rule out a high frequency in the Saudi population, as well as an absence of functional data to confirm an impact to splicing. This example illustrates the challenge in variant classification for several racial and ethnic populations not represented in publicly available population databases. Screening of these underrepresented populations and public access to their genetic data would improve data interpretation for patients from those populations. In addition, identifying common benign variants in Saudi and ethnically diverse populations would improve novel variant classification for all patients through improved protein domain tolerance predictions, by enriching the spectrum of benign variation across domains within genes.[16]

Besides the CDH23 VUS variants, only two other suspect variants were observed in "inconclusive" cases: a homozygous novel missense variant in GPR98 in one proband (F-19), and a MYO7A pathogenic heterozygous variant in another proband (F-3) (**Supplementary Table S1**). None of the remaining four "inconclusive" probands with family history of hearing loss had variants for which evidence suggested a causal role. In addition, the two "negative" probands were also reported to have family histories, making in all a total of six probands, or 18% of this cohort, without positive molecular diagnoses but with family histories suggestive of genetic etiology. Some of these familial cases may potentially be resolved by segregation analysis of VUS variants identified in the proband, or may harbor causative variants in noncoding regions of panel genes. Alternatively, some of these "inconclusive" or "negative" cases may have unique or previously unidentified genetic etiologies for their hearing loss.

The OTOF gene was responsible for the hearing loss in 21.2% (7/33) of the Saudi probands in this study. OTOF is associated with AR AN, a unique subtype of SNHL that is thought to be due to dyssynchrony of the neural transmission of the auditory signal. Patients with AN have an abnormal auditory brain response, but otoacoustic emissions (OAEs) are typically present, at least initially, as is a cochlear microphonic.[17] The OAEs may disappear over time.[17] In our cohort, OAEs were absent in 3 of 7 OTOF cases. A genotype–phenotype correlation for OAE loss is not suspected, since both recurrent variants are present in these cases (two cases with homozygous p.Arg1792His, one case with homozygous p.Glu57*); however, mean age at time of testing for the cases with OAE loss is higher than the mean age (6.7 yr) of the four cases with preserved OAEs (2.8 yr) (**Supplementary Table S1**).

Variable severity, ranging from mild to profound, has been reported in families with OTOF-related hearing loss;[18] however, most OTOF cases in the literature present with profound NSHL.[13,19] Estimates of the contribution of OTOF to nonsyndromic prelingual hearing loss range from 0.5% to 3.5% across multiethnic cohorts,[14,19,20] and would be expected to be enriched in severe to profound cases. This was evident in one Spanish cohort, where a recurrent pathogenic variant in OTOF was responsible for 8% of prelingual NSHL cases;[13] however, significantly lower rates of OTOF cases have not been reported in other NSHL ethnically diverse cohorts, even when considering only severe to profound cases.[14,20,21] The enrichment of OTOF causative variants in our cohort may be partially explained by the selection bias for severe to profound hearing loss, but it is significantly greater than found in any other cohort reported to date. In addition, several cohort studies identify GJB2 as the most common contributor to severe to profound hearing loss, responsible for ~ 17–50% of severe to profound cases from various ethnic populations.[14,22,23] In our cohort, no GJB2-sequencing variants were identified, and only one case was caused by the DFNB1 deletion. The low contribution of GJB2 is consistent with other studies of Saudi hearing-loss cohorts.[5,12] The SLC26A4

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and *MYO7A* genes, which were responsible for hearing loss in three families each, are also common contributors across ethnically diverse prelingual SNHL cohorts.[14,20,22] Variants in the *STRC* gene, which harbors common pathogenic large deletions in Caucasians and neighboring Middle Eastern populations,[3] were not identified. This is as expected, given that the majority of hearing loss due to variants in *STRC* is in the mild to moderate range.[3] Expanding genetic screening for Saudi hearing-loss patients with mild or moderate levels of hearing loss would further elucidate the genetic contributors in Saudi hearing, and reveal the degree of overlap in neighboring populations. The low genetic burden of *GJB2* highlights the unique genetic spectrum in Saudi hearing-loss patients, and raises the possibility that other hearing-loss genes or recurrent variants may constitute a significant burden in this population. Future analysis of these families via exome and/or genome sequencing may help identify novel hearing-loss genes or provide data to support gene–disease associations for genes not currently on clinical NGS testing panels because there is limited evidence available to establish their association with hearing loss.[10]

For SNHL, management is typically driven by the severity of the hearing loss with amplification by hearing aids in individuals with mild to moderate SNHL, and cochlear implantation in individuals with a severe or profound auditory threshold, provided that the anatomy of the inner ear appears favorable. However, it has been suggested that cochlear implants be considered in individuals with AN, who often fail to gain benefit from conventional hearing aids,[24] with several reports of successful cochlear implantation outcomes in AN patients with an *OTOF*-related cause.[25,26] This being the case, early detection of AN may be critical to maximize the verbal and cognitive benefits of cochlear implantation at an earlier age.[24] Currently, newborn screening in Saudi Arabia relies on OAE testing, which will often appear as normal in infants with *OTOF*-related hearing loss. The enrichment of *OTOF* variants in this cohort makes a strong case for mandatory auditory brain response testing as part of newborn screening programs in Saudi Arabia, as well as neighboring countries with populations having common tribal ancestry.

In conclusion, NGS gene panel testing identified *OTOF* as a major contributor to hearing loss in this Saudi cohort, while *GJB2*, commonly responsible for prelingual NSHL in Caucasians and other populations, accounts for a small percentage of Saudi cases. However, this study identified recurrent pathogenic variants that are either absent or rare across previously described multiethnic hearing-loss cohorts and show a unique genetic burden distribution. Thus, as genetic testing becomes more globally available, comprehensive NGS gene panel testing for hearing loss as a primary test, instead of being performed reflexively after a negative DFNB1 screen, may be a more efficient and cost-effective testing strategy. In addition, the presence of the remaining unresolved families in this cohort, many of which have a family history consistent with a genetic etiology, suggests that

Saudi and Middle Eastern populations, like others, have genetic etiologies that remain to be identified. The extent of genetic and allelic variation in hearing loss will become evident as more genetic data are generated for the Saudi and other global populations.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

DISCLOSURE
Several authors (A.O. H.L.R., and S.S.A.) are employed by the Laboratory for Molecular Medicine, a nonprofit fee-for-service clinical laboratory performing hereditary hearing loss testing. The other authors declare no conflict of interest.

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