Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Comprehensive analysis via exome sequencing uncovers genetic etiology in autosomal recessive nonsyndromic deafness in a large multiethnic cohort

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Purpose: Autosomal recessive nonsyndromic deafness (ARNSD) is characterized by a high degree of genetic heterogeneity, with reported mutations in 58 different genes. This study was designed to detect deafness-causing variants in a multiethnic cohort with ARNSD by using whole-exome sequencing (WES).

Methods: After excluding mutations in the most common gene, *GJB2*, we performed WES in 160 multiplex families with ARNSD from Turkey, Iran, Mexico, Ecuador, and Puerto Rico to screen for mutations in all known ARNSD genes.

Results: We detected ARNSD-causing variants in 90 (56%) families, 54% of which had not been previously reported. Identified mutations were located in 31 known ARNSD genes. The most common genes with mutations were *MYO15A* (13%), *MYO7A* (11%), *SLC26A4* (10%), *TMPRSS3* (9%), *TMC1* (8%), *ILDR1* (6%), and *CDH23* (4%). Nine mutations were detected in multiple families with shared haplotypes, suggesting founder effects.

Conclusion: We report on a large multiethnic cohort with ARNSD in which comprehensive analysis of all known ARNSD genes identifies causative DNA variants in 56% of the families. In the remaining families, WES allows us to search for causative variants in novel genes, thus improving our ability to explain the underlying etiology in more families.

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Key Words: autosomal recessive; deafness; exome; next-generation sequencing

INTRODUCTION

Deafness is a global public health concern that affects 1 to 3 per 1,000 newborns.[1] In more than half of the cases of congenital or prelingual deafness, the cause is genetic, and most demonstrate an autosomal recessive inheritance pattern.[1] Mutations in 58 different genes have been reported to cause autosomal recessive nonsyndromic deafness (ARNSD) (http://hereditaryhearingloss.org).

Except for one relatively common gene, *GJB2* (MIM 121011), most reported mutations are present in only a single or a small number of families.[2] Whole-exome sequencing (WES) allows resequencing of nearly all exons of the protein-coding genes in the genome.[3] A growing number of research and clinical diagnostic laboratories are successfully using WES for gene/variant identification because of its comprehensive analysis advantages.[4,5] In this study, we

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present the results of WES in a large multiethnic cohort consisting of 160 families with ARNSD who were negative for *GJB2* mutations.

MATERIALS AND METHODS

Statement of ethics

This study was approved by the University of Miami Institutional Review Board (USA), the Ankara University Medical School Ethics Committee (Turkey), the Growth and Development Research Ethics Committee (Iran), the Bioethics Committee of FFAA (HE-1) in Quito (Ecuador), and the Ethics Committee of National Institute of Rehabilitation (Mexico). A signed informed-consent form was obtained from each participant or, in the case of a minor, from the parents.

Subjects

We included 160 families with at least two members with nonsyndromic sensorineural hearing loss with a pedigree structure suggestive of autosomal recessive inheritance (affected siblings born to unaffected parents with or without parental consanguinity); *GJB2* mutations were negative. Hearing loss was congenital or prelingual-onset with a severity ranging from mild to profound. The study comprised 101 families from Turkey, 54 from Iran, 2 from Mexico, 2 from Ecuador, and 1 from Puerto Rico. Sensorineural hearing loss was diagnosed via standard audiometry in a soundproof room according to standard clinical practice. Clinical evaluation of all affected individuals by a geneticist and an otolaryngologist included a thorough physical examination, otoscopy, and ophthalmoscopy. Tandem walking and the Romberg test were used for initial vestibular evaluation, with more detailed tests if needed based on symptoms and findings. Laboratory investigation included, but was not limited to, an electrocardiogram, urinalysis, and, when available, a high-resolution computed tomography (CT) scan of the temporal bone or magnetic resonance imaging (MRI) to identify inner ear anomalies. DNA was extracted from peripheral leukocytes of each member of the family by standard protocols.

Whole-exome sequencing

Agilent SureSelect Human All Exon 50 Mb versions 3, 4, and 5 (Agilent Technologies Santa Clara, CA) were used for in-solution enrichment of coding exons and flanking intronic sequences following the manufacturer's standard protocol. The enriched DNA samples were subjected to standard sample preparation for the HiSeq 2000 instrument (Illumina San Diego, CA). The Illumina CASAVA v1.8 pipeline was used to produce 99-bp sequence reads. The Burrows-Wheeler Aligner[6] (http://bio-bwa.sourceforge.net) was used to align sequence reads to the human reference genome (hg19), and variants were called using the GATK (https://www.broadinstitute.org/gatk/) software package.[7] All single-nucleotide variants (SNVs) and insertion/deletions (INDELs) were submitted to

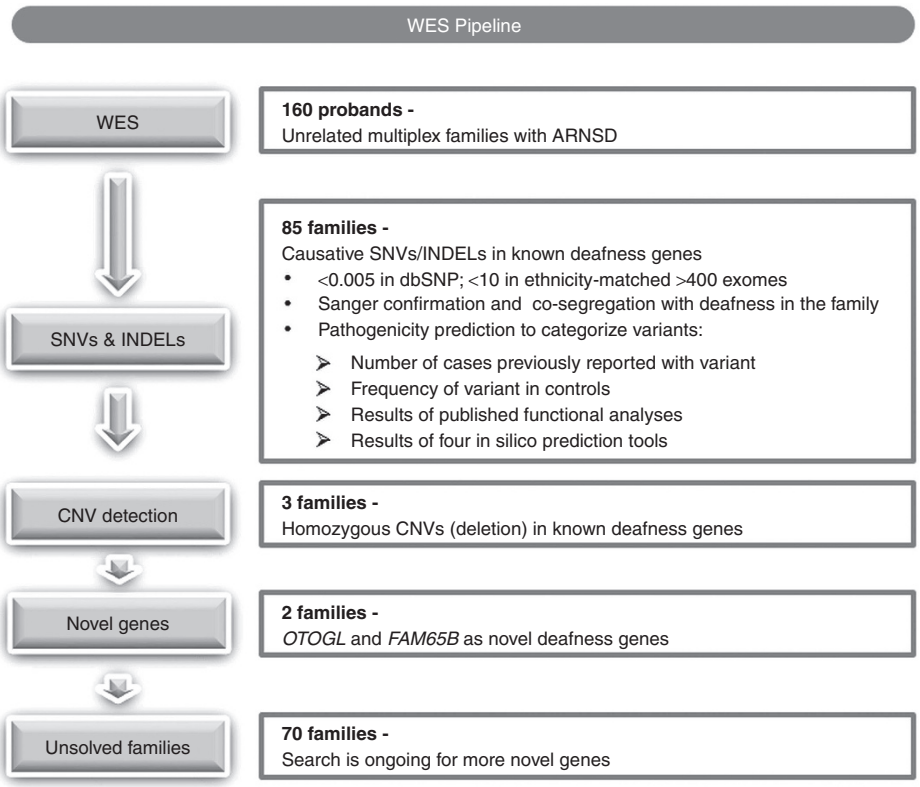

Figure 1 Overall workflow of our WES pipeline. CNV, copy-number variation; INDEL, insertion/deletion; SNV, single-nucleotide variant; WES, whole-exome sequencing.

Table 1 Mutations identified in known ARNSD genes

Family ID	Country of origin	Genotype	cDNA	Protein	NM transcript	Gene	Reference
543	Turkey	Homozygous	c.4441T>C	p.S1481P	NM_016239.3	*MYO15A*	Cengiz et al.[23]
724	Turkey	Homozygous	c.4652C>A	p.A1551D	NM_016239.3	*MYO15A*	Diaz-Horta et al.[4]
765	Turkey	Homozygous	c.4273C>T	p.Q1425X	NM_016239.3	*MYO15A*	Diaz-Horta et al.[4]
723	Turkey	Homozygous	c.8307_8309delGGA	p.E2770del	NM_016239.3	*MYO15A*	Novel
795	Turkey	Homozygous	c.5808_5814delCCGTGGC	p.R1937TfsX10	NM_016239.3	*MYO15A*	Cengiz et al.[23]
793	Turkey	Homozygous	c.5808_5814delCCGTGGC	p.R1937TfsX10	NM_016239.3	*MYO15A*	Cengiz et al.[23]
1209	*Puerto Rico*	*Heterozygous*	*c.7226delC*	*p.P2409QfsX8*	*NM_016239.3*	*MYO15A*	*Novel*
		Heterozygous	*c.9620G>A*	*p.R3207H*	*NM_016239.3*	*MYO15A*	*Novel*
1083	Turkey	Homozygous	c.5183T>C	p.L1728P	NM_016239.3	*MYO15A*	Novel
1332	Turkey	Homozygous	c.10361delT	p.V3454GfsX5	NM_016239.3	*MYO15A*	Novel
489	Turkey	Homozygous	c.5286_5287delTC	p.R1763AfsX45	NM_016239.3	*MYO15A*	Novel
1023	Iran	Homozygous	c.8638_8641delCCTG	p.P2880RfsX19	NM_016239.3	*MYO15A*	Novel
862	*Turkey*	*Heterozygous*	*c.7894G>T*	*p.V2632L*	*NM_016239.3*	*MYO15A*	*Novel*
		Heterozygous	*c.5133+1G>A*	*splice*	*NM_016239.3*	*MYO15A*	*Novel*
974	Iran	Homozygous	c.6487G>A	p.G2163S	NM_000260.3	*MYO7A*	Janecke et al.[24]
1391	Turkey	Homozygous	c.6487G>A	p.G2163S	NM_000260.3	*MYO7A*	Janecke et al.[24]
435	Turkey	Homozygous	c.3935T>C	p.L1312P	NM_000260.3	*MYO7A*	Novel
472	Turkey	Homozygous	c.1556G>T	p.G519V	NM_000260.3	*MYO7A*	Novel
1370	Turkey	Homozygous	c.722G>A	p.R241H	NM_000260.3	*MYO7A*	Cremers et al.[25]
432	Turkey	Homozygous	c.5362_5363delAG	p.R1788DfsX13	NM_000260.3	*MYO7A*	Novel
637	*Turkey*	*Heterozygous*	*c.5838delT*	*p.F1946LfsX24*	*NM_000260.3*	*MYO7A*	*Novel*
		Heterozygous	*c.5573T>C*	*p.L1858P*	*NM_000260.3*	*MYO7A*	*Bharadwaj et al.*[26]
996	Iran	Homozygous	c.5785C>T	p.Q1929X	NM_000260.3	*MYO7A*	Novel
1019	Iran	Homozygous	c.1708C>T	p.R570X	NM_000260.3	*MYO7A*	Yoshimura et al.[27]
1404	*Turkey*	*Heterozygous*	*c.1708C>T*	*p.R570X*	*NM_000260.3*	*MYO7A*	*Yoshimura et al.*[27]
		Heterozygous	*c.6025G>A*	*p.A2009T*	*NM_000260.3*	*MYO7A*	*Novel*
786	Turkey	Homozygous	c.1001G>T	p.G334V	NM_000441.1	*SLC26A4*	Landa et al.[28]
634	Turkey	Homozygous	c.1001G>T	p.G334V	NM_000441.1	*SLC26A4*	Landa et al.[28]
1418	Turkey	Homozygous	c.1061T>C	p.F354S	NM_000441.1	*SLC26A4*	Blons et al.[29]
238	Turkey	Homozygous	c.1226G>A	p.R409H	NM_000441.1	*SLC26A4*	Van Hauwe et al.[30]
973	Iran	Homozygous	c.1334T>G	p.L445W	NM_000441.1	*SLC26A4*	Van Hauwe et al.[30]
905	Turkey	Homozygous	c.2168A>G	p.H723R	NM_000441.1	*SLC26A4*	Van Hauwe et al.[30]
1417	*Turkey*	*Heterozygous*	*c.665G>A*	*p.G222D*	*NM_000441.1*	*SLC26A4*	*Novel*
		Heterozygous	*c.1198delT*	*p.C400VfsX32*	*NM_000441.1*	*SLC26A4*	*Novel*
1346	Turkey	Homozygous	c.919-2A>G	splice	NM_000441.1	*SLC26A4*	Coucke et al.[31]
1321	Turkey	Homozygous	c.1198delT	p.C400VfsX32	NM_000441.1	*SLC26A4*	Novel
395	Turkey	Homozygous	c.36dupC	p.F13LfsX10	NM_024022.2	*TMPRSS3*	Diaz-Horta et al.[4]
777	Turkey	Homozygous	c.913A>T	p.I305F	NM_024022.2	*TMPRSS3*	Novel
674	Turkey	Homozygous	c.271C>T	p.R91X	NM_024022.2	*TMPRSS3*	Novel
629	Turkey	Homozygous	c.399G>C	p.W133C	NM_024022.2	*TMPRSS3*	Novel
1368	Turkey	Homozygous	c.1126G>A	p.G376S	NM_024022.2	*TMPRSS3*	Novel
1410	Turkey	Homozygous	c.436G>A	p.G146S	NM_024022.2	*TMPRSS3*	Novel
910	Turkey	Homozygous	c.616G>T	p.A206S	NM_024022.2	*TMPRSS3*	Novel
633	Turkey	Homozygous	c.616G>T	p.A206S	NM_024022.2	*TMPRSS3*	Novel
52	Turkey	Homozygous	c.1589_1590CT	p.S530X	NM_138691.2	*TMC1*	Hildebrand et al.[32]
123	Turkey	Homozygous	c.1080_1084delGATCA	p.R362PfsX6	NM_138691.2	*TMC1*	Novel
662	Turkey	Homozygous	c.2050G>A	p.D684N	NM_138691.2	*TMC1*	Novel
1268	*Ecuador*	*Heterozygous*	*c.1718T>A*	*p.I573N*	*NM_138691.2*	*TMC1*	*Novel*
		Heterozygous	*c.2130-1delG*	*splice*	*NM_138691.2*	*TMC1*	*Novel*
911	Turkey	Homozygous	c.1534C>T	p.R512X	NM_138691.2	*TMC1*	Kurima et al.[33]
490	Turkey	Homozygous	c.1959C>G	p.Y653X	NM_138691.2	*TMC1*	Novel

Table 1 Continued on next page

Table 1 Continued

Family ID	Country of origin	Genotype	cDNA	Protein	NM transcript	Gene	Reference
393	*Turkey*	*Heterozygous*	*c.63+2T>A*	*splice*	*NM_138691.2*	*TMC1*	*Duman et al.*[18]
		Heterozygous	*c.236+1G>A*	*splice*	*NM_138691.2*	*TMC1*	*Duman et al.*[18]
988	Iran	Homozygous	c.3215C>A	p.A1072D	NM_022124.5	*CDH23*	Duman et al.[18]
1165	*Mexico*	*Heterozygous*	*c.2959G>A*	*p.D987N*	*NM_022124.5*	*CDH23*	*Novel*
		Heterozygous	*c.3628C>T*	*p.Q1210X*	*NM_022124.5*	*CDH23*	*Novel*
1015	Iran	Homozygous	c.5851G>A	p.D1951N	NM_022124.5	*CDH23*	Novel
1032	*Iran*	*Heterozygous*	*c.7822C>T*	*p.R2608C*	*NM_022124.5*	*CDH23*	*Novel*
		Heterozygous	*c.8120C>T*	*p.P2707L*	*NM_022124.5*	*CDH23*	*Novel*
968	Iran	Homozygous	c.820C>T	p.Q274X	NM_001199799.1	*ILDR1*	Diaz-Horta et al.[4]
800	Turkey	Homozygous	c.942C>A	p.C314X	NM_001199799.1	*ILDR1*	Novel
799	Turkey	Homozygous	c.942C>A	p.C314X	NM_001199799.1	*ILDR1*	Novel
782	Turkey	Homozygous	c.942C>A	p.C314X	NM_001199799.1	*ILDR1*	Novel
969	Iran	Homozygous	c.82delG	p.V28SfsX31	NM_001199799.1	*ILDR1*	Novel
1297	Turkey	Homozygous	c.5431A>T	p.K1811X	NM_194248.2	*OTOF*	Romanos et al.[34]
98	Turkey	Homozygous	c.5431A>T	p.K1811X	NM_194248.2	*OTOF*	Romanoset al.[34]
1398	Turkey	Homozygous	c.3679C>T	p.R1227X	NM_194248.2	*OTOF*	Novel
909	Turkey	Homozygous	c.765G>C	p.Q255H	NM_194248.2	*OTOF*	Rodríguez-Ballesteros et al.[35]
725	Turkey	Homozygous	c.3918T>G	p.C1306W	NM_033056.3	*PCDH15*	Novel
1238	Turkey	Homozygous	CNV	CNV	NM_033056.3	*PCDH15*	Novel
1044	Iran	Homozygous	c.3101G>A	p.R1034H	NM_033056.3	*PCDH15*	Novel
1369	Turkey	Homozygous	c.250C>T	p.R84W	NM_147196.2	*TMIE*	Naz et al.[36]
1354	Turkey	Homozygous	c.250C>T	p.R84W	NM_147196.2	*TMIE*	Naz et al.[36]
1402	Turkey	Homozygous	c.250C>T	p.R84W	NM_147196.2	*TMIE*	Naz et al.[36]
1239	Turkey	Homozygous	c.490-1G>T	splice	NM_016366.2	*CABP2*	Novel
1366	Turkey	Homozygous	c.1018G>T	p.E340X	NM_004452.3	*ESRRB*	Novel
1372	Turkey	Homozygous	c.1018G>T	p.E340X	NM_004452.3	*ESRRB*	Novel
794	Turkey	Homozygous	c.508C>A	p.H170N	NM_133261.2	*GIPC3*	Novel
1356	Turkey	Homozygous	c.508C>A	p.H170N	NM_133261.2	*GIPC3*	Novel
182	Turkey	Homozygous	c.4480C>T	p.R1494X	NM_144612.6	*LOXHD1*	Diaz-Horta et al.[4]
779	Turkey	Homozygous	c.2863G>T	p.E955X	NM_144612.6	*LOXHD1*	Diaz-Horta et al.[4]
303	Turkey	Homozygous	c.628A>T	p.K210X	NM_005709.3	*USH1C*	Novel
994	Iran	Homozygous	c.876+2delTA	splice	NM_005709.3	*USH1C*	Novel
661	Turkey	Homozygous	c.330T>A	p.Y110X	NM_006383.3	*CIB2*	Novel
262	Turkey	Homozygous	c.2662C>A	p.P888T	NM_080680.2	*COL11A2*	Chakchouk et al.[37]
448	Turkey	Homozygous	c.499C>T	p.R167X	NM_001042702.3	*DFNB59*	Collin et al.[38]
908	Turkey	Homozygous	c.102-1G>A	splice	NM_014722.2	*FAM65B*	Diaz-Horta et al.[17]
1289	Turkey	Homozygous	c.2956A>T	p.K986X	NM_032119.3	*GPR98*	Novel
820	Turkey	Homozygous	c.79C>T	p.R27X	NM_001080476.2	*GRXCR1*	Novel
67	Turkey	Homozygous	c.1498C>T	p.R500X	NM_001038603.2	*MARVELD2*	Riazuddin et al.[39]
1364	Turkey	Homozygous	c.1015C>T	p.R339W	NM_004999.3	*MYO6*	Yang et al.[40]
63	Turkey	Homozygous	CNV	CNV	NM_144672.3	*OTOA*	Bademci et al. [15]
338	Turkey	Homozygous	c.1430delT	p.V477EfsX25	NM_173591.3	*OTOGL*	Yariz et al.[16]
1294	Turkey	Homozygous	c.1108C>T	p.R370X	NM_002906.3	*RDX*	Novel
850	Turkey	Homozygous	CNV	CNV	NM_153700.2	*STRC*	Bademci et al.[15]
1035	Iran	Homozygous	c.5210A>G	p.Y1737C	NM_005422.2	*TECTA*	Diaz-Horta et al.[4]
7	Turkey	Homozygous	c.705_709dupCCTGC	p.R237PfsX215	NM_001128228.2	*TPRN*	Novel
23	Turkey	Homozygous	c.2335_2336delAG	p.R785SfsX50	NM_001039141.2	*TRIOBP*	Diaz-Horta et al.[4]
5	Turkey	Homozygous	c.387_388insC	p.K130QfsX5	NM_173477.2	*USH1G*	Novel

Families with compound heterozygous mutations are italicized.

CNV, copy-number variation.

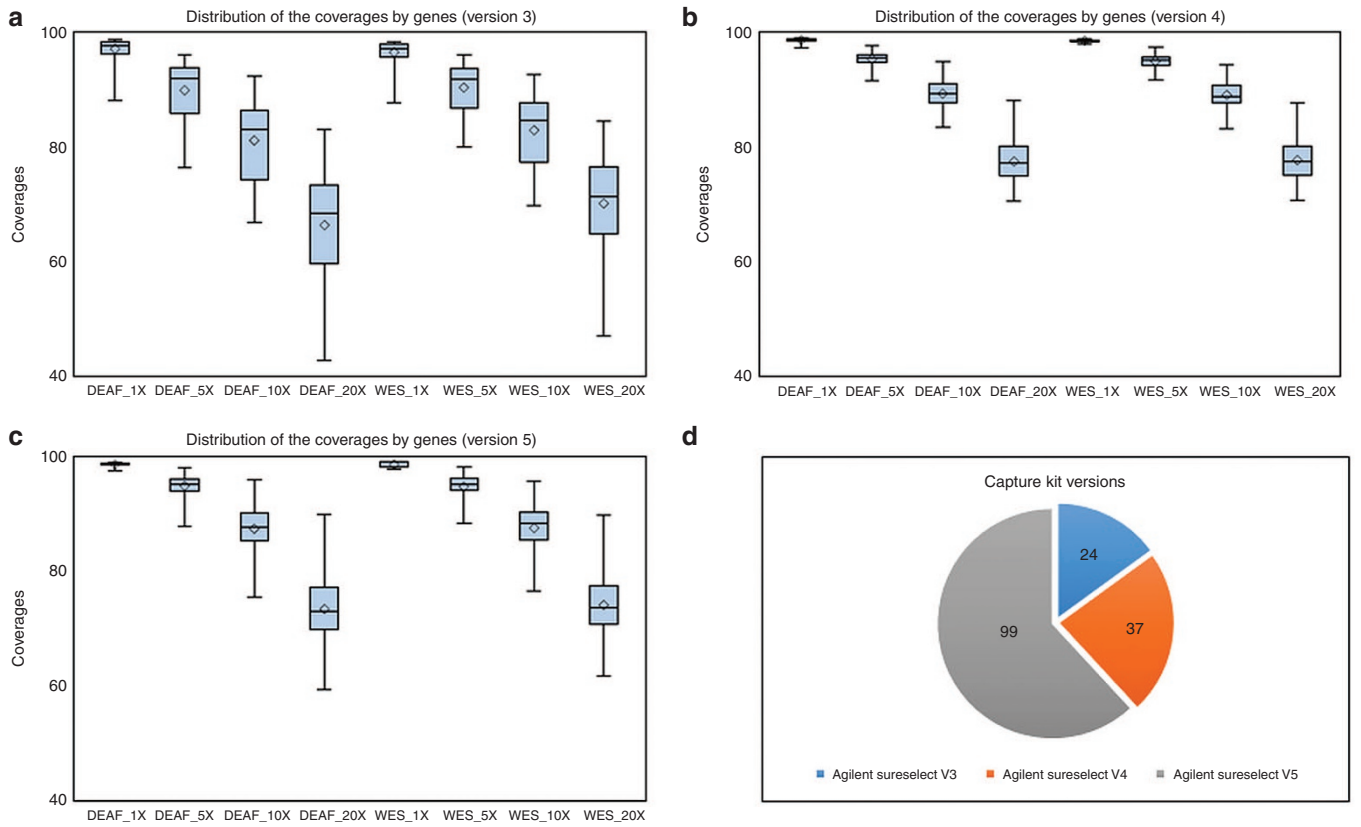

Figure 2 Capture kit versions and coverages. (**a**–**c**) Overview of coverage of 58 known ARNSD genes according to three different versions (version 3 = V3, version 4 = V4, and version 5 = V5) of the exome enrichment kit. (**d**) Numbers of samples studied with different capture kits. ARNSD, autosomal recessive nonsyndromic deafness.

Table 2 Overview of mutation detection and parental consanguinity

Countries	Number of families	Reported parental consanguinity	Number of homozygous probands (consanguineous)	Number of compound heterozygous probands (consanguineous)
Turkey	101	82	67 (59)	5 (2)
Iran	54	31	12 (10)	1 (1)
Ecuador	2	0	0	1 (0)
Mexico	2	0	0	1 (0)
Puerto Rico	1	0	0	1 (0)

SeattleSeq137 for further characterization and annotation. Sanger sequencing was used for confirmation and segregation of the variants in each family.

Bioinformatics analysis

We analyzed WES data using our in-house tool (https://genomics.med.miami.edu). Our workflow is shown in Figure 1. The analysis started with quality control (QC) checks, including the coverage and average read depth of targeted regions, numbers of variants in different categories, and quality scores. All variants were annotated and categorized into known and novel variants. As previously recommended, we filtered variants based on minor allele frequency of <0.005 in dbSNP141.[8] We also filtered out variants that are present in >10 samples in our internal database of >3,000 exomes from European, Asian, and American ancestries that includes Turkish, Iranian, Mexican, Ecuadorian, and Puerto Rican samples (Figure 1). Autosomal recessive inheritance with both homozygous and compound heterozygous inheritance models and a genotype quality (GQ) score >35 for the variant quality were chosen. Missense, nonsense, splice site, in-frame INDEL, and frame-shift INDELs in the known ARNSD genes (**Supplementary Data** online) were selected. Missense variants that remained after these filters were later analyzed for presence in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk) and for having a pathogenic prediction score at least in two of the following tools: PolyPhen2,[9] SIFT,[10] MutationAssessor,[11] and MutationTaster.[12] Finally, we used CoNIFER[13] (Copy Number Inference From Exome Reads) and XHMM[14]

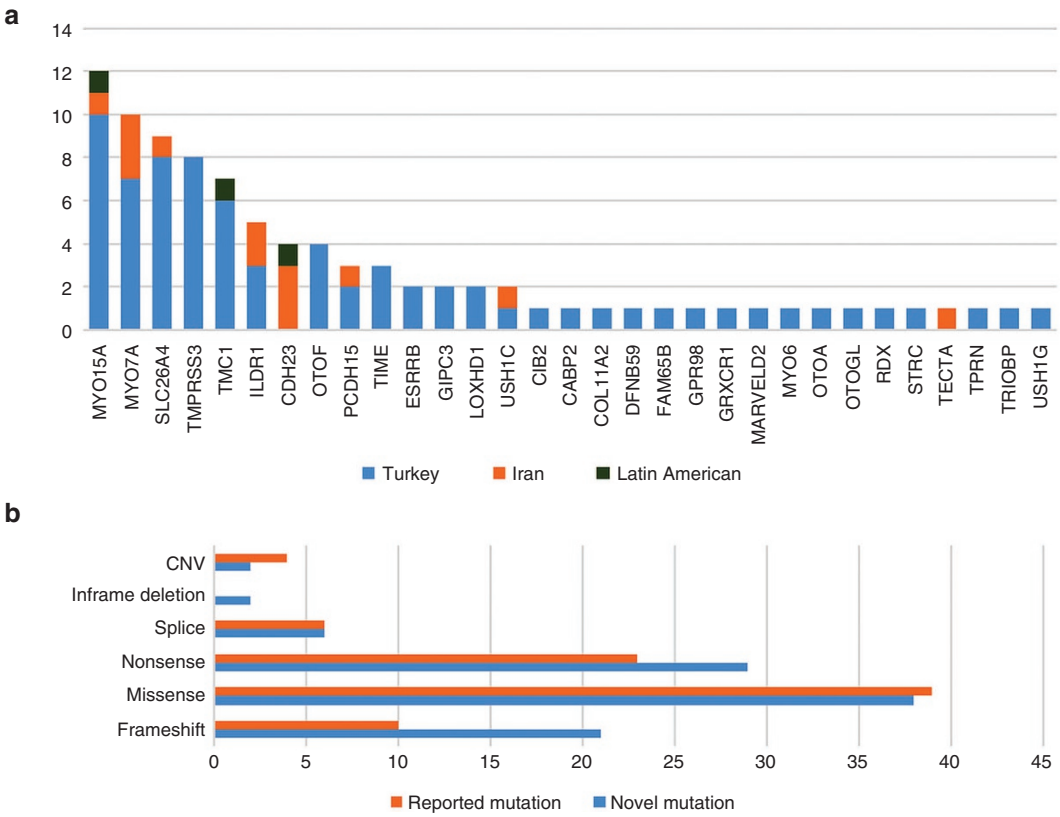

Figure 3 Distribution of causative DNA variants in known ARNSD genes according to the (**a**) family origin and (**b**) variant categories. ARNSD, autosomal recessive nonsyndromic deafness; CNV, copy-number variation.

(eXome-Hidden Markov Model) to detect copy-number variations (CNVs).[15] After this filtering, only variants cosegregated with the phenotype in the entire family were considered pathogenic.

RESULTS

On average, each exome had 99, 95, and 88% of mappable bases of the Gencode-defined exome represented by coverage of 1X, 5X, and 10X reads, respectively. Average coverage of the mappable bases for the 58 known ARNSD genes (exons and the first and last 20 bps of introns) were 99, 95, and 87% for the 1X, 5X, and 10X reads, respectively.

We detected pathogenic or likely pathogenic variants that can explain ARNSD in 90 (56%) families. All identified variants cosegregated with deafness as an autosomal recessive trait. Fifty-four percent of the mutations were not previously reported in the Human Gene Mutation Database. Mutations were identified in 31 ARNSD genes. The genes with mutations identified in at least three families are *MYO15A* (MIM 602666) (13%), *MYO7A* (MIM 276903) (11%), *SLC26A4* (MIM 605646) (10%), *TMPRSS3* (MIM 605551) (9%), *TMC1* (MIM 606706) (8%), *ILDR1* (MIM 609739) (6%), *CDH23* (MIM 605516) (4%), *OTOF* (MIM 603681) (4%), *PCDH15* (MIM 605514) (3%), and *TMIE* (MIM 607723) (3%). During the course of this study we reported mutations in *OTOGL* (MIM 614925) and *FAM65B* (MIM 611410) as novel causes of ARNSD[16,17] (**Figure 1**; **Table 1**).

DISCUSSION

Identifying causative variants in ARNSD is challenging because of the following: (i) the extreme genetic heterogeneity of ARNSD; (ii) the presence of different categories of genetic variants such as SNVs, INDELs, and CNVs; (iii) the presence of a high proportion of nonrecurrent mutations; and (iv) the variability in mutation frequencies in individual ARNSD genes across ethnicities.[18] Consequently, we performed a comprehensive analysis to detect pathogenic SNVs, INDELs, and CNVs in the ARNSD genes.

Targeted resequencing allows identification of mutations in the interested gene sets. Recent studies pioneered by Shearer et al.[8,19] have shown the effectiveness of the targeted resequencing of deafness genes. Advantages of the targeted resequencing over WES are having better coverage with higher depth and significantly lower costs, which is suitable for clinical diagnostic laboratories. However, a main limitation of the targeted sequencing is the need for revalidation of the panel after adding each new gene. By contrast, many laboratories around the world offer WES as a diagnostic tool requiring validation only when a new WES version is introduced. Our analysis using three different versions of an exome capture kit during the 4-year period shows that the depth of coverage of WES has improved to reliably identify most mutations in known ARNSD genes (**Figure 2**; **Supplementary Tables S1** and **S4** online). Recently developed WES approaches provide more coverage for genes that are known to cause Mendelian disease. They are

expected to cover deafness genes more efficiently. In addition, adding in baits to improve coverage over poorly covered regions may be considered if better coverage is desired. It was recently shown via targeted sequencing that CNVs are a common cause of deafness.[20] Although CNV analysis of the WES data is still being optimized for clinical usage, we integrated two currently available tools—XHMM and CoNIFER—into our WES analysis pipeline and identified large *OTOA* (MIM 607038), *STRC* (MIM 606440), and *PCDH15* (exon 27–28) homozygous deletions in our cohort, supporting a significant role of CNVs in deafness etiology.

In this study, after excluding *GJB2* mutations we detected pathogenic variants in the known ARNSD genes in 56% of the families. The advantage of this study is having large multiplex autosomal recessive families (including affected and unaffected children) who can be tested for cosegregation of all variants. While we identified more novel variants than those reported in **Table 1** through WES, only those variants cosegregated in the family with deafness were considered pathogenic. Similarly heterozygous variants did not explain the phenotype because they did not cosegregate with deafness and were not included. WES facilitates the cataloging of mutations in different populations. Population characteristics such as the rate of consanguineous marriages may affect the distribution of deafness mutations in different populations. As expected, the vast majority of Turkish and Iranian probands from consanguineous marriages are homozygous for the pathogenic variants (**Table 2**). However, there is a marked difference between the rates of solved families in Turkey (73%) versus Iran (24%) (**Figure 3**). As shown in **Figure 3**, the distribution of genes is also different between the two countries. In our study, the top five genes explain 39 of 101 families (39%) in Turkey, whereas they explained only 10 of 54 families (19%) in Iran. Moreover, our analysis of the WES data in the unsolved Iranian families shows that there are no common mutations in genes that are not known to be deafness genes (data not shown). Unless there are common mutations in regions that are not well covered by WES, our data suggest that many rare genes are responsible for the majority of hereditary deafness in the Iranian cohort. It is likely that there are undetected rare variants specific to certain ethnicities in Iran.[21] Another advantage of WES is that surveying of mutations for founder effects is possible. We detected *TMIE* c.250C>T (p.R84W) in three unrelated Turkish families that all shared a flanking haplotype as noted previously.[22] Furthermore, *MYO15A*, *MYO7A*, *SLC26A4*, *TMPRSS3*, *ILDR1*, *OTOF*, *ESRRB* (MIM 602167), and *GIPC3* (MIM 608792) genes had recurrent mutations with shared haplotypes, indicating founder effects (**Supplementary Table S2** online).

There is no correlation between the size of transcript and number of mutant alleles (**Supplementary Table S3** online). There may be some deafness genes that are more prone to having mutations. Founder effects appear to play a role because some small genes such as *TMIE*, *ESRRB*, and *GIPC3* ranked high in mutation frequency because of founder mutations. Some discrepancy between the size of a gene and number of mutations can be explained by the fact that only certain mutations cause nonsyndromic deafness for some genes. For instance, *CDH23*, *PCDH15*, and *MYO7A* are big genes, but many mutations in those genes cause Usher syndrome (MIM 276900) instead of ARNSD. An interesting example is *TMC1*, which ranks 20th based on size but 5th in mutation frequency. Nonsyndromic deafness is the only phenotype caused by *TMC1* mutations, and none of the *TMC1* mutations is recurrent in our cohort. These may suggest that *TMC1* is relatively more prone to have de novo mutations or that it is a highly conserved gene and its variants are rarely tolerated.

In conclusion, WES is an effective tool for identifying pathogenic SNVs, INDELs, and CNVs simultaneously in ARNSD genes and provides further analysis of the families without mutations for novel gene discovery. Identification of two novel ARNSD genes[16,17] during the course of this study testifies to its power.

SUPPLEMENTARY MATERIAL

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

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DISCLOSURE

The authors declare no conflict of interest.

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