HHS Public Access

Author manuscript

Genet Med. Author manuscript; available in PMC 2016 May 18.

Published in final edited form as:

Genet Med. 2016 April ; 18(4): 364–371. doi:10.1038/gim.2015.89.

Comprehensive Analysis via Exome Sequencing Uncovers Genetic Etiology in Autosomal Recessive Non-Syndromic Deafness in a Large Multiethnic Cohort

Guney Bademci, MD[1], **Joseph Foster II, BSc**[1], **Nejat Mahdieh, PhD**[2], **Mortaza Bonyadi, PhD**[3], **Duygu Duman, PhD**[4], **F.Basak Cengiz, PhD**[4], **Ibis Menendez, MD**[1], **Oscar Diaz Horta, PhD**[1], **Atefeh Shirkavand, PhD**[5], **Sirous Zeinali, PhD**[5,6], **Asli Subasioglu, MD**[7], **Suna Tokgoz-Yilmaz, PhD**[8], **Fabiola Huesca Hernandez, BSc**[9], **Maria de la Luz Arenas Sordo, MD**[9], **Juan Dominguez-Aburto, BSc**[9], **Edgar Hernandez-Zamora, PhD**[9], **Paola Montenegro, PhD**[10], **Rosario Paredes, MD**[10], **Germania Moreta, MD**[10], **Rodrigo Vinueza, BSc**[10], **Franklin Villegas, BSc**[10], **Santiago Mendoza Benitez, MD**[11], **Shengru Guo, MSc**[1], **Nazim Bozan, MD**[12], **Tulay Tos, MD**[13], **Armagan Incesulu, MD**[14], **Gonca Sennaroglu, PhD**[8], **Susan H. Blanton, PhD**[1], **Hatice Ozturkmen Akay, MD**[15], **Muzeyyen Yildirim-Baylan, MD**[16], and **Mustafa Tekin, MD**[1]

[1]Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA

[2]Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

[3]Faculty of Natural Sciences, Center of Excellence for Biodiversity, University of Tabriz, Tabriz, Iran

[4]Ankara University School of Medicine, Division of Genetics, Department of Pediatrics, Ankara, Turkey

[5]Kawsar's Human Genetic Research Center, Tehran, Iran

[6]Department of Moleculare Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

[7]Department of Medical Genetics, Izmir Katip Celebi University, Ataturk Training and Research Hospital, Izmir, Turkey

[8]Department of Audiology, Hacettepe University Health Sciences Faculty, Ankara, Turkey

[9]Genetic Service, National Institute of Rehabilitation, Mexico D.F, Mexico

[10]Molecular Genetic Lab, FF.AA. Hospital, Quito, Ecuador

[11]Audiology Department, Cuernavaca General Hospital, Morelos, Mexico

Corresponding author: Mustafa Tekin, M.D., 1501 NW 10th Avenue, BRB-610 (M-860), Miami, FL 33136, Phone: 305-243-2381, ; Email: mtekin@med.miami.edu

Author Disclosure Statement: Authors declare that there is no conflict of interest to report.

Supplementary Material:Supplementary information is available at the Genetics in Medicine website

[12]Department of Otolaryngology, Faculty of Medicine, Yuzuncu Yıl University, Van, Turkey

[13]Department of Medical Genetics, Dr. Sami Ulus Research and Training Hospital of Women's and Children's Health and Diseases, Ankara, Turkey

[14]Department of Otolaryngology, Head and Neck Surgery, Eskisehir Osmangazi University, Eskisehir, Turkey

[15]Department of Radiology, Istanbul Zeynep Kamil Maternity and Children Training and Research Hospital, Istanbul, Turkey

[16]Department of Otorhinolaryngology, Dicle University School of Medicine, Diyarbakir, Turkey

Abstract

Purpose—Autosomal recessive non-syndromic 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.

Keywords

Autosomal Recessive; Deafness; Exome; Next-Generation Sequencing

Introduction

Deafness is a global public health concern which affects 1 to 3 per 1,000 newborns.[1] In more than half of the cases with 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 non-syndromic 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 few 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, owing to its comprehensive analysis advantages.[4,5] In this study, we present the results of WES in a large multiethnic cohort consisting of 160 families with ARNSD that were negative for *GJB2* mutations.

Material and Methods

Statement of Ethics

This study was approved by the University of Miami Institutional Review Board (USA), Ankara University Medical School Ethics Committee (Turkey), Growth and Development Research Ethics Committee (Iran), 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 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) and *GJB2* mutations were negative. Hearing loss was congenital or prelingual onset with a severity ranging from mild to profound. One hundred and one families from Turkey, fifty-four from Iran, two from Mexico, two from Ecuador and one from Puerto Rico were included. Sensorineural hearing loss was diagnosed via standard audiometry in a sound-proof 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 EKG, urinalysis, and, when available, a high resolution CT scan of the temporal bone or an 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. BWA[6] 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 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 seen in Figure 1. The analysis started with 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) were selected. Missense variants that remained after these filters were later analyzed for presence in the Human Gene Mutation Database (HGMD) (www.hgmd.cf.ac.uk) and 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] (eXome-Hidden Markov Model) to detect CNVs.[15] After this filtering, only those variants co-segregated with the phenotype in the entire family was 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%, 87% for the 1X, 5X, 10X reads, respectively.

We detected pathogenic or likely pathogenic variants that can explain ARNSD in 90 (56%) families. All identified variants co-segregated with deafness as an autosomal recesive trait. 54% of the mutations were not previously reported in HGMD. 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 (1) the extreme genetic heterogeneity of ARNSD; (2) the presence of different categories of genetic variants such as SNVs, INDELs and CNVs; (3) the presence of a high proportion of non-recurrent mutations and (4) 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. have shown the effectiveness of the targeted resequencing of deafness genes.[8,19] An advantage of the targeted resequencing over WES is having better coverage with higher depth and significantly lowered costs, which is suitable for clinical diagnostic labs. However, a main limitation of the targeted sequencing is the need for revalidation of the panel after adding each new gene. In 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 four year period shows that the depth of coverage of WES has improved to reliably identify most mutations in known ARNSD genes (Figure 2) (Table S1 and Table S4). 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 a better coverage is desired. It was recently shown via targeted sequencing that CNVs are a common cause of deafness.[20] While CNV analysis of the WES data is being still 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 to in deafness etiology.

In this study after excluding *GJB2* mutations we detected pathogenic variants in the known ARNSD genes in 56% of the studied families. The advantage of this study is to have large multiplex autosomal recessive families (including affected and unaffected children) that can be tested for co-segregation of all variants. While we identified more novel variants than those reported in Table 1 through WES, only those variants co-segregated in the family with deafness were considered pathogenic. Similarly heterozygous variants didn't explain the phenotype since they did not co-segregate with deafness and were not included. WES facilitates the cataloguing 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%) vs. Iran (24%) (Figure 3). As seen in figure 3, the distribution of genes is also different between the two countries. In our study, the top five genes explain 39 out of 101 families (39%) in Turkey, while only 10 out 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 to allow surveying of mutations for founder effects. We detected *TMIE* c.250C>T (p.R84W) in three unrelated Turkish families, which 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 (Table S2).

There is no correlation between the size of transcript and number of mutant alleles (Table S3). There may be some deafness genes that are more prone to have 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*, *MYO7A* are big genes but many mutations in those genes cause Usher syndrome (MIM 276900) instead of ARNSD. An interesting example is *TMC1* that ranks the 20th based on size but the 5th for mutation frequency. Nonsyndromic deafness is the only phenotype caused by *TMC1* mutations and none of the *TMC1* mutations are recurrent in our cohort. These may suggest that *TMC1* is relatively more prone to have *de novo* mutations or it is a highly conserved gene and its variants are rarely tolerated.

In conclusion, WES is a an effective tool for identifying pathogenic SNVs, INDELs and CNVs simultaneously in ARNSD genes and provides further analysis of the unsolved families for novel gene discovery. Identification of two novel ARNSD genes[16,17] during the course of this study testifies its power.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the participating families.

This work was supported by National Institutes of Health grant R01DC009645 to M.T.

References

1. Morton CC, Nance WE. Newborn hearing screening--a silent revolution. N Engl J Med. 2006; 354:2151–2164. [PubMed: 16707752]
2. Duman D, Tekin M. Autosomal recessive nonsyndromic deafness genes: a review. Front Biosci (Landmark Ed). 2012; 17:2213–2236. [PubMed: 22652773]
3. Ng SB, Turner EH, Robertson PD, et al. Targeted capture and massively parallel sequencing of 12 human exomes. Nature. 2009; 461:272–276. [PubMed: 19684571]
4. Diaz-Horta O, Duman D, Foster J 2nd, et al. Whole-exome sequencing efficiently detects rare mutations in autosomal recessive nonsyndromic hearing loss. PLoS One. 2012; 7:e50628. [PubMed: 23226338]
5. Atik T, Bademci G, Diaz-Horta O, Blanton S, Tekin M. Whole-exome sequencing and its impact in hereditary hearing loss. Genet Res (Camb). 2015; 97:e4. [PubMed: 25825321]
6. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010; 26:589–595. [PubMed: 20080505]
7. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010; 20:1297–1303. [PubMed: 20644199]
8. Shearer AE, Eppsteiner RW, Booth KT, et al. Utilizing ethnic-specific differences in minor allele frequency to recategorize reported pathogenic deafness variants. Am J Hum Genet. 2014; 95:445–453. [PubMed: 25262649]
9. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7:248–249. [PubMed: 20354512]

10. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009; 4:1073–1081. [PubMed: 19561590]
11. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res. 2011; 39:e118. [PubMed: 21727090]
12. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014; 11:361–362. [PubMed: 24681721]
13. Krumm N, Sudmant PH, Ko A, et al. Copy number variation detection and genotyping from exome sequence data. Genome Res. 2012; 22:1525–1532. [PubMed: 22585873]
14. Fromer M, Moran JL, Chambert K, et al. Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. Am J Hum Genet. 2012; 91:597–607. [PubMed: 23040492]
15. Bademci G, Diaz-Horta O, Guo S, et al. Identification of copy number variants through whole-exome sequencing in autosomal recessive nonsyndromic hearing loss. Genet Test Mol Biomarkers. 2014; 18:658–661. [PubMed: 25062256]
16. Yariz KO, Duman D, Seco CZ, et al. Mutations in OTOGL, encoding the inner ear protein otogelin-like, cause moderate sensorineural hearing loss. Am J Hum Genet. 2012; 91:872–882. [PubMed: 23122586]
17. Diaz-Horta O, Subasioglu-Uzak A, Grati M, et al. FAM65B is a membrane-associated protein of hair cell stereocilia required for hearing. Proc Natl Acad Sci U S A. 2014; 111:9864–9868. [PubMed: 24958875]
18. Duman D, Sirmaci A, Cengiz FB, Ozdag H, Tekin M. Screening of 38 genes identifies mutations in 62% of families with nonsyndromic deafness in Turkey. Genet Test Mol Biomarkers. 2011; 15:29–33. [PubMed: 21117948]
19. Shearer AE, DeLuca AP, Hildebrand MS, et al. Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. Proc Natl Acad Sci U S A. 2010; 107:21104–21109. [PubMed: 21078986]
20. Shearer AE, Kolbe DL, Azaiez H, et al. Copy number variants are a common cause of non-syndromic hearing loss. Genome Med. 2014; 6:37. [PubMed: 24963352]
21. Mahdieh N, Rabbani B, Wiley S, Akbari MT, Zeinali S. Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. J Hum Genet. 2010; 55:639–648. [PubMed: 20739942]
22. Sirmaci A, Ozturkmen-Akay H, Erbek S, et al. A founder TMIE mutation is a frequent cause of hearing loss in southeastern Anatolia. Clin Genet. 2009; 75:562–567. [PubMed: 19438934]
23. Cengiz FB, Duman D, Sirmaci A, et al. Recurrent and private MYO15A mutations are associated with deafness in the Turkish population. Genet Test Mol Biomarkers. 2010; 14:543–550. [PubMed: 20642360]
24. Janecke AR, Meins M, Sadeghi M, et al. Twelve novel myosin VIIA mutations in 34 patients with Usher syndrome type I: confirmation of genetic heterogeneity. Hum Mutat. 1999; 13:133–140. [PubMed: 10094549]
25. Cremers FP, Kimberling WJ, Kulm M, et al. Development of a genotyping microarray for Usher syndrome. J Med Genet. 2007; 44:153–160. [PubMed: 16963483]
26. Bharadwaj AK, Kasztejna JP, Huq S, Berson EL, Dryja TP. Evaluation of the myosin VIIA gene and visual function in patients with Usher syndrome type I. Exp Eye Res. 2000; 71:173–181. [PubMed: 10930322]
27. Yoshimura H, Iwasaki S, Nishio SY, et al. Massively parallel DNA sequencing facilitates diagnosis of patients with Usher syndrome type 1. PLoS One. 2014; 9:e90688. [PubMed: 24618850]
28. Landa P, Differ AM, Rajput K, Jenkins L, Bitner-Glindzicz M. Lack of significant association between mutations of KCNJ10 or FOXI1 and SLC26A4 mutations in Pendred syndrome/enlarged vestibular aqueducts. BMC Med Genet. 2013; 14:85. [PubMed: 23965030]
29. Blons H, Feldmann D, Duval V, et al. Screening of SLC26A4 (PDS) gene in Pendred's syndrome: a large spectrum of mutations in France and phenotypic heterogeneity. Clin Genet. 2004; 66:333–340. [PubMed: 15355436]
30. Van Hauwe P, Everett LA, Coucke P, et al. Two frequent missense mutations in Pendred syndrome. Hum Mol Genet. 1998; 7:1099–1104. [PubMed: 9618166]

31. Coucke PJ, Van Hauwe P, Everett LA, et al. Identification of two different mutations in the PDS gene in an inbred family with Pendred syndrome. J Med Genet. 1999; 36:475–477. [PubMed: 10874637]
32. Hildebrand MS, Kahrizi K, Bromhead CJ, et al. Mutations in TMC1 are a common cause of DFNB7/11 hearing loss in the Iranian population. Ann Otol Rhinol Laryngol. 2010; 119:830–835. [PubMed: 21250555]
33. Kurima K, Peters LM, Yang Y, et al. Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. Nat Genet. 2002; 30:277–284. [PubMed: 11850618]
34. Romanos J, Kimura L, Favero ML, et al. Novel OTOF mutations in Brazilian patients with auditory neuropathy. J Hum Genet. 2009; 54:382–385. [PubMed: 19461658]
35. Rodriguez-Ballesteros M, Reynoso R, Olarte M, et al. A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (OTOF) in subjects with nonsyndromic hearing impairment and auditory neuropathy. Hum Mutat. 2008; 29:823–831. [PubMed: 18381613]
36. Naz S, Giguere CM, Kohrman DC, et al. Mutations in a novel gene, TMIE, are associated with hearing loss linked to the DFNB6 locus. Am J Hum Genet. 2002; 71:632–636. [PubMed: 12145746]
37. Chakchouk I, Grati M, Bademci G, et al. Novel mutations confirm that COL11A2 is responsible for autosomal recessive non-syndromic hearing loss DFNB53. Mol Genet Genomics. 2015; doi: 10.1007/s00438-015-0995-9
38. Collin RW, Kalay E, Oostrik J, et al. Involvement of DFNB59 mutations in autosomal recessive nonsyndromic hearing impairment. Hum Mutat. 2007; 28:718–723. [PubMed: 17373699]
39. Riazuddin S, Ahmed ZM, Fanning AS, et al. Tricellulin is a tight-junction protein necessary for hearing. Am J Hum Genet. 2006; 79:1040–1051. [PubMed: 17186462]
40. Yang T, Wei X, Chai Y, Li L, Wu H. Genetic etiology study of the non-syndromic deafness in Chinese Hans by targeted next-generation sequencing. Orphanet J Rare Dis. 2013; 8:85. [PubMed: 23767834]

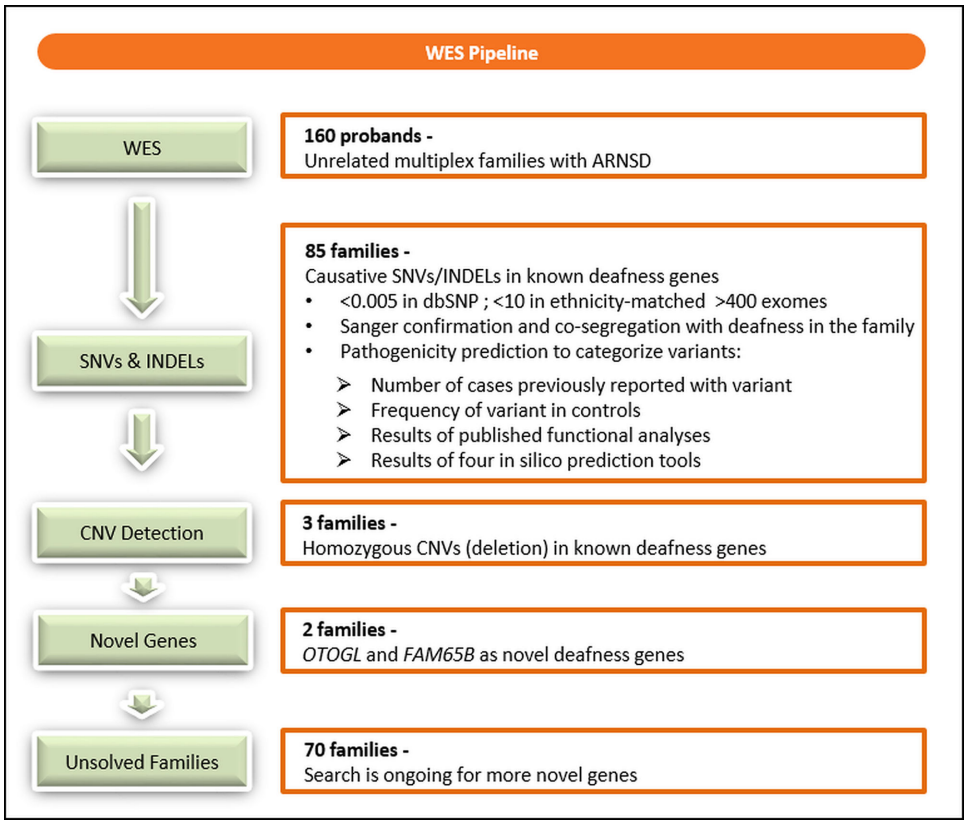

Fig.1. Overall workflow of our WES pipeline

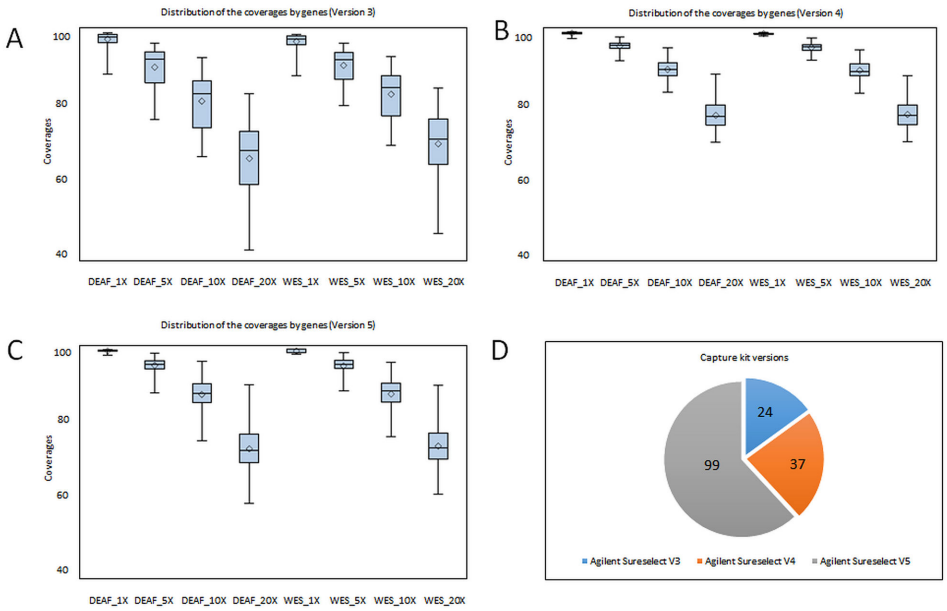

Fig.2.

Overview of coverage of 58 known ARNSD genes according to 3 different versions (Version 3=V3, Version 4=V4 and Version 5=V5) of the exome enrichment kit (A,B,C). Numbers of samples studied with different capture kits (D).

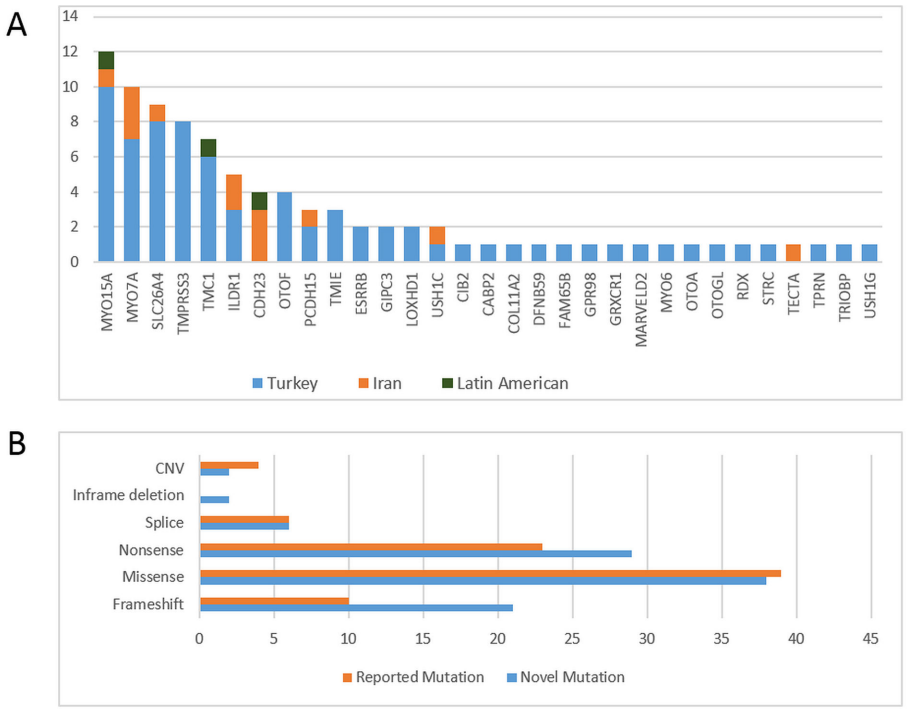

Fig.3.

Distribution of causative DNA variants in known ARNSD genes according to the family origin (A) and variant categories (B).

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 2010)[23]
724	Turkey	Homozygous	c.4652C>A	p.A1551D	NM_016239.3	MYO15A	(Diaz-Horta 2012)[4]
765	Turkey	Homozygous	c.4273C>T	p.Q1425X	NM_016239.3	MYO15A	(Diaz-Horta 2012)[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 2010)[23]
793	Turkey	Homozygous	c.5808_5814delCCGTGGC	p.R1937TfsX10	NM_016239.3	MYO15A	(Cengiz 2010)[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 1999)[24]
1391	Turkey	Homozygous	c.6487G>A	p.G2163S	NM_000260.3	MYO7A	(Janecke 1999)[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 2007)[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 2000)*[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 2014)[27]
1404	*Turkey*	*Heterozygous*	*c.1708C>T*	*p.R570X*	*NM_000260.3*	*MYO7A*	*(Yoshimura 2014)*[27]
		Heterozygous	*c.6025G>A*	*p.A2009T*	*NM_000260.3*	*MYO7A*	*Novel*

Family ID	Country of origin	Genotype	cDNA	Protein	NM Transcript	Gene	Reference
786	Turkey	Homozygous	c.1001G>T	p.G334V	NM_000441.1	SLC26A4	(Landa 2013)[28]
634	Turkey	Homozygous	c.1001G>T	p.G334V	NM_000441.1	SLC26A4	(Landa 2013)[28]
1418	Turkey	Homozygous	c.1061T>C	p.F354S	NM_000441.1	SLC26A4	(Blons 2004)[29]
238	Turkey	Homozygous	c.1226G>A	p.R409H	NM_000441.1	SLC26A4	(Van hauwe 1998)[30]
973	Iran	Homozygous	c.1334T>G	p.L445W	NM_000441.1	SLC26A4	(Van hauwe 1998)[30]
905	Turkey	Homozygous	c.2168A>G	p.H723R	NM_000441.1	SLC26A4	(Van hauwe 1998)[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 1999)[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 2012)[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 2010)[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 2002)[33]
490	Turkey	Homozygous	c.1959C>G	p.Y653X	NM_138691.2	TMC1	Novel
393	*Turkey*	*Heterozygous*	*c.63+2T>A*	*splice*	*NM_138691.2*	*TMC1*	*(Duman 2011)*[18]
		Heterozygous	*c.236+1G>A*	*splice*	*NM_138691.2*	*TMC1*	*(Duman 2011)*[18]
988	Iran	Homozygous	c.3215C>A	p.A1072D	NM_022124.5	CDH23	(Duman 2011)[18]
1165	*Mexico*	*Heterozygous*	*c.2959G>A*	*p.D987N*	*NM_022124.5*	*CDH23*	*Novel*

Family ID	Country of origin	Genotype	cDNA	Protein	NM Transcript	Gene	Reference
		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 2012)[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 2009)[34]
98	Turkey	Homozygous	c.5431A>T	p.K1811X	NM_194248.2	OTOF	(Romanos 2009)[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	(Rodriguez 2008)[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 2002)[36]
1354	Turkey	Homozygous	c.250C>T	p.R84W	NM_147196.2	TMIE	(Naz 2002)[36]
1402	Turkey	Homozygous	c.250C>T	p.R84W	NM_147196.2	TMIE	(Naz 2002)[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 2012)[4]
779	Turkey	Homozygous	c.2863G>T	p.E955X	NM_144612.6	LOXHD1	(Diaz-Horta 2012)[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

Genet Med. Author manuscript; available in PMC 2016 May 18.

Family ID	Country of origin	Genotype	cDNA	Protein	NM Transcript	Gene	Reference
262	Turkey	Homozygous	c.2662C>A	p.P888T	NM_080680.2	COL11A2	(Chakchouk 2015)[37]
448	Turkey	Homozygous	c.499C>T	p.R167X	NM_001042702.3	DFNB59	(Collin 2007)[38]
908	Turkey	Homozygous	c.102-1G>A	splice	NM_014722.2	FAM65B	(Diaz-Horta 2014)[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 2006)[39]
1364	Turkey	Homozygous	c.1015C>T	p.R339W	NM_004999.3	MYO6	(Yang 2013)[40]
63	Turkey	Homozygous	CNV	CNV	NM_144672.3	OTOA	(Bademci 2014)[15]
338	Turkey	Homozygous	c.1430delT	p.V477EfsX25	NM_173591.3	OTOGL	(Yariz 2012)[16]
1294	Turkey	Homozygous	c.1108C>T	p.R370X	NM_002906.3	RDX	Novel
850	Turkey	Homozygous	CNV	CNV	NM_153700.2	STRC	(Bademci 2014)[15]
1035	Iran	Homozygous	c.5210A>G	p.Y1737C	NM_005422.2	TECTA	(Diaz-Horta 2012)[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 2012)[4]
5	Turkey	Homozygous	c.387_388insC	p.K130QfsX5	NM_173477.2	USH1G	Novel

* Families with compound heterozygous mutations are italicized.

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)