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When transcripts matter: delineating between non-syndromic hearing loss DFNB32 and hearing impairment infertile male syndrome (HIIMS)

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Abstract

Mutations in the *CDC14A* (Cell Division-Cycle 14A) gene, which encodes a conserved dual-specificity protein tyrosine phosphatase, have been identified as a cause of autosomal recessive non-syndromic hearing loss (DFNB32) and hearing impairment infertility male syndrome (HIIMS).

We used next-generation sequencing to screen six deaf probands from six families segregating sensorineural moderate to profound hearing loss. Data analysis and variant prioritization were completed using a custom bioinformatics pipeline.

We identified three homozygous loss of function variants (p.Arg345Ter, p.Arg376Ter, and p.Ala451Thrfs*43) in the *CDC14A* gene, segregating with deafness in each family. Of the six families, four segregated the p.Arg376Ter mutation, one family segregated the p.Arg345Ter

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mutation and one family segregated a novel frameshift (p.Ala451Thrfs*43) mutation. In-depth phenotyping of affected individuals ruled out secondary syndromic findings.

This study implicates the p.Arg376Ter mutation might be as a founder mutation in the Iranian population. It also provides the first semen analysis for deaf males carrying mutations in exon 11 of *CDC14A* and reveals a genotype-phenotype correlation that delineates between DFNB32 and HIIMS. The clinical results from affected males suggest the NM_033313.2 transcript alone is sufficient for proper male fertility, but not for proper auditory function. We conclude that DFNB32 is a distinct phenotypic entity in males.

Keywords

CDC14A gene; p.Arg376Ter mutation; ARNSHL; sperm immobility; Iranian; Founder mutation

Introduction

Many molecules are fundamental for the development, function, and maintenance of the auditory and reproductive systems. Although these systems differ in their structure, cellular components, and physiological processes, they share many molecular components. Defects in these shared components may impair both hearing and fertility.

Dual-specificity phosphatases (DUSPs) are a large group of phosphatases that are indispensable regulators of a large host of functions in many tissues, including the inner ear and the testis. They are classified broadly by their ability to dephosphorylate tyrosine and serine/threonine residues and subgrouped based on their targets (1). One such DUSP, CDC14A, belongs to a family of proteins that function in various pathways including centrosome duplication cycle and cytokinesis, DNA damage response, Cdk1 activity control, cell adhesion, and migration, transcription regulation, and primary cilia dynamics (2–13). CDC14A has also been shown as an integral protein required in the mammalian auditory system for proper hearing and in the testes for spermatogenesis (14, 15).

Mutations in the *CDC14A* gene (OMIM #603504) are responsible for autosomal recessive non-syndromic hearing loss (ARNSHL) at the DFNB32 locus and hearing impairment infertile male syndrome (HIIMS) (OMIM #608653)(14, 15). To date, 11 mutations have been identified; six are linked to HIIMS, and 5 are linked to DFNB32. The phenotypic spectrum seen in *CDC14A* patients in these reports suggested a sex and genotype-phenotype correlation. Females with bi-allelic mutations exhibit only NSHL. Males with bi-allelic mutations that result in complete loss of CDC14A phosphatase function exhibit sperm abnormalities and infertility. In contrast males with residual CDC14A function exhibit hearing loss without infertility (14–16).

In this study, we characterize the phenotypic spectrum in an Iranian cohort with autosomal recessive non-syndromic hearing loss (ARNSHL) due to loss of function variants in the *CDC14A* gene. Here we report six Iranian families segregating deafness due to mutations in *CDC14A*. In-depth phenotyping of affected males in these families pinpoints a clear genotype-phenotype correlation between DFNB32 and HIIMS. We also report a novel

variant p.Ala451Thrfs*43 further confirm the pathogenicity of both the p.Arg345Ter and p.Arg376Ter.

Materials and Methods

Subjects

Six Iranian families (L1096, L692, GL10179, GL9988, L1347, and GL9307100) segregating apparent ARNSHL were ascertained for this study. Affected individuals underwent clinical examination and pure tone audiometry to determine air conduction thresholds at 0.125, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 kHz. After obtaining written informed consent whole blood samples were collected from all family members and genomic DNA was extracted. This protocol has been approved by the ethics committee of the University of Social Welfare and Rehabilitation Sciences for Medical Research, Tehran, Iran.

Next-generation sequencing (NGS)

Exome sequencing (ES) for families L1096, L692, L1347, and GL 9307100 was performed in the GRC NGS core facility, using the SureSelect XT V6 Human All Exon kit (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's protocol. After library quantification and pooling, samples were sequenced on an Illumina NextSeq 500 System (Illumina, San Diego, CA, USA) using the Illumina V3 High Throughput kit.

For probands in families GL10179 and GL9988 library preparation, sequencing and bioinformatics analysis were performed using the OtoSCOPE® V8 platform (152 genes) (Agilent Technologies, Santa Clara, CA, USA) in the Molecular Otolaryngology and Renal Research Laboratories (MORL; University of Iowa, Iowa City, IA, USA), as described (17–19).

Bioinformatics Analysis

Raw sequence files were aligned to the reference human genome (UCSC hg19) using the Burrows-Wheeler Aligner (BWA)(20). Variant detection and functional annotation of genetic variants from high-throughput sequencing data were performed with the Genome Analysis Toolkit (GATK)(21) and ANNOVAR(22) software, respectively. Additionally, variants were annotated with minor allele frequencies (MAF) from the 1000 Genomes (23), ExAC (24) gnomAD (25), and the Iranome (26). Variants were first filtered for quality (depth >10 and quality score >30) followed by minor allele frequency (MAF) (<2%). Variants were then prioritized based on variant-type (missense, nonsense, indel or splice-site), followed by *in-silico* prediction for conservation (GERP and PhyloP), and predicted deleteriousness (SIFT, PolyPhen2, and the (CADD) (27–30).

Segregation Analysis

Segregation analysis of candidate variants was completed by Sanger sequencing on an ABI 3130 Sequencer (Foster City, CA, USA). All sequencing chromatograms were compared to published cDNA sequences for *CDC14A* (NM_033313.2), and nucleotide changes were detected using CodonCode Aligner.

Results

Subjects and Clinical Evaluations

Families L1096, L692, and GL9307100, are three families that originate from the Lorestan Province in Southwest Iran. In each family, affected and unaffected individuals were born at term with unremarkable pregnancies and neonatal periods. Family GL10179 originates from Hamadan Province (central Iran). All four families report consanguinity (Figure 1A–D). In each family hearing impairment was reported to have occurred between ages 1 and 3 years. Affected individuals in each family underwent a comprehensive clinical evaluation and pure tone audiometry.

In family L1096, affected individual II.2 showed slightly asymmetric hearing loss (Figure 1E), with a moderate-to-severe downsloping audioprofile in the left ear, and a less severe loss in the mid-frequencies in the right. He and his brother (II.3) both opted to perform a sperm analysis at ages 30 and 31 years old, respectively (Table 1). Individual II.2 results showed: a sperm count of 20×10^6/mL, 96% abnormal morphology, 87% amorphous head shape, and 40% immotile sperm. Individual II.3 analysis revealed a sperm count: 13×10^6/mL, 97% abnormal morphology, 78% amorphous head shape, and 38% immotile sperm (Table 1).

At age 16, 17, and 31 years, the three affected individuals in family L692 had bi-lateral profound hearing loss (Figure 1E). The male proband (II.1) opted not to perform a sperm analysis. Affected individuals in family GL9307100 have severe to profound hearing loss. Affected male II.4 underwent a semen analysis which showed normal sperm count (69×10^6/mL) with 32% abnormal morphology and 26% immotile sperm (Table 1). Affected individuals in family GL10179 are reported to have moderate to severe non-progressive sensorineural hearing loss. A semen analysis could not be performed on individual III.1 due to his age during the time of the study.

The first cousin, healthy parents of two affected and three unaffected individuals in family GL9988 (Figure 2A), originate from the Qazvin Province (central Iran). Affected individuals report postlingual and progressive hearing loss that was first noticed when they started school. At age 24 years, sperm analysis from proband II.2 revealed a normal sperm count (111×10^6/mL) with 40% abnormal morphology and 40% immotile sperm (Table 1).

Family L1347 is from the Semnan Province (north-central Iran) and reports consanguinity. The two affected females (II.1 and II.3) reported prelingual severe-to-profound hearing loss (Figure 2B).

Variant Identification and Segregation Analysis

Probands from families L1096, L692, GL9307100 all underwent Exome Sequencing. Applying the filtering strategy applied described above, and further filtering based on recessive inheritance identified each proband was homozygous for the previously reported mutation c.1126C>T (p.Arg376Ter) in *CDC14A*. The proband from family GL10179 underwent OtoSCOPE® testing, which showed the proband was homozygous for c.1126C>T (p.Arg376Ter) mutation (Table 2). Segregation analysis showed that this variant

also segregated with the hearing-loss phenotype in each family (Figure 1A–D). The frequency of this variant is 0.0004% on gnomAD and 0.06 % on Iranome (Table 2). The proband from GL9988 was also screened using OtoSCOPE®. In this case, the previously reported pathogenic variant c.1033C>T (p.Arg345Ter) was identified in *CDC14A*. The frequency of this variant is 0.0004% on gnomAD and is absent on Iranome (Table 2). Segregation analysis revealed that the affected sister was also homozygous for this mutation (Figure 2A). Finally, the proband from family L1347 underwent Exome Sequencing, which identified a novel homozygous frameshift c.1351_1352del (p.Ala451Thrfs*43) in *CDC14A* (Figure 2B). This variant is absent from gnomAD and from 800 healthy Iranian samples (Table 2).

Discussion

We used targeted genomic enrichment and massively parallel sequencing to implicate *CDC14A* as the causal gene underlying the ARSNHL in six families. We identified two previously reported nonsense mutations (p.Arg345Ter and Arg376Ter) and a novel frameshift mutation p.Ala451Thrfs*43. All affected individuals in each family are homozygous for the variants (Figure 1A–D and Figure 2A–B). In two families, the variants segregated in multiple generations (Figure 1A and Figure 1D). The identification of the p.Ala451Thrfs*43 mutation brings the total number of *CDC14A* reported mutations to twelve (Table 2).

The *CDC14A* gene encodes a dual-specificity phosphatase (31, 32). In humans, *CDC14A* has six alternatively spliced transcripts encoding six isoforms ranging in size from 330–623 amino acids (Figure 2C). Currently, the expression pattern of each transcript has yet to be elucidated. Although widely expressed in many tissues, *CDC14A* is essential for the proper function of the auditory and reproductive systems (14, 15). Extensive phenotyping studies of the patho-consequences of loss of CDC14A and its phosphatase activity have been performed in several murine mutant models (15).

In the murine auditory system, CDC14A localizes to the microtubule networks of the hair cells (the kinocilia and basal bodies) but also concentrated at the tips of the stereocilia(15). Loss of CDC14A and its phosphatase activity resulted in profound deafness with an age-related cochlear deterioration. Male mice homozygous or compound heterozygous for two defective alleles of CDC14A displayed infertility. In contrast to the auditory system, CDC14A seems to play a critical role in developing and maintaining the structural and morphological integrity of the seminiferous tubules. Consequently, these mice also had abnormal sperm and were not fertile. Female mice with loss of CDC14A activity displayed only hearing loss (15). These phenotypes mimic the phenotypes seen in male and female patients with the loss of CDC14A.

In families L1096, L692, GL9307100, and GL10179, we identified the previously reported p.Arg376Ter mutation, which segregated as homozygous in all affected individuals. This mutation is expected to cause five out of the six transcripts of CDC14A to undergo nonsense mRNA decay (NMD)(33). Transcript NM_033313.2 is expected to translate a shortened CDC14A peptide (Figure 2C). Deaf males due to the p.Arg376Ter mutation are reported to

be fertile (14, 15); however, until now; no semen analysis has been available for males with this mutation. Three affected male individuals from two families opted to perform a semen analysis (Table 1). In each instance, there were no abnormalities in the cell count or the motility, and these values fell well within reference ranges (34). The two brothers of L1096 however, had a borderline percentage of healthy morphological sperm, with only 3–4% of overall sperm exhibiting normal morphology. Affected individual II.4 from family GL9307100 has a total percentage of sperm with normal morphology that falls within normal limits. Confirming his fertility is his son that is reported to be conceived naturally (Figure 1C).

Interestingly, the p.Arg376Ter mutation has only been reported in the Iranian population. These four new reports bring the total number of families reported to six with this mutation. The detection of p.Arg376Ter mutation only in Iranian families suggests this mutation is a founder mutation in the Iranian population. To further explore this possibility, we exploited the large amount of data generated by ES and compared the haplotypes between affected individuals from families L1096, L692, GL9307100 and MORL2 Iranian family published in Imtiaz et al (15). We identified these four families share a common haplotype in and around *CDC14A* (Table 3), which supports this as a founder mutation.

In family GL9988, we identified a previously reported termination mutation p.Arg345Ter in CDC14A that segregated with the deafness in the family. Affected individuals in this family were reported to have postlingual and progressive hearing loss. This clinical presentation differs from the typical congenital onset reported in other families and suggests a potential unrecognized less severe auditory phenotype associated with CDC14A-related deafness. Like the p.Arg376Ter, the p.Arg345Ter mutation also occurs in exon 11 and is expected to oblate the expression of five out of the six transcripts of *CDC14A*. While previously, this mutation was described in a Pakistani male with children, a semen analysis for individuals homozygous for this mutation has not been available. Individual II.2 of family GL9988 opted to perform a semen analysis. Results showed cell count, motility, and morphology all within normal ranges (34)(Table 1).

The clinical results from males in these five families suggest the NM_033313.2 transcript alone is sufficient for proper male fertility, but not for proper auditory function. This hints to one of the other five transcripts, or a combination of them as critical for the proper function of the auditory system.

Finally, in family L1347, we identified a novel frameshift mutation p.Ala451Thrfs*43 in CDC14A that segregated as homozygous in two deaf siblings. To date, this is the most 3’ exonic mutation described and is in the second to last exon in three of the transcripts (Figure 2C). The frameshift causes a newly created termination codon in the last exon of these transcripts and is not expected to undergo NMD. Therefore, individuals homozygous for this mutation have a fully functional NM_033313.2 transcript and truncated transcripts of NM_001319211.1, NM_001319212.1, and NM_033312.2, which are predicted to produce truncated protein products (Figure 2C). This suggests that in the inner ear the C-terminal of CDC14A is critical for proper auditory function and/or transcripts NM_003672.3 and NM_001319210.1 are transcripts required for proper auditory function (Figure 2C). In any

case, males homozygous for this mutation are expected to be fertile as transcript NM_033313.2 is not affected.

Genes that give rise to multiple phenotypes is not new for the deafness-related genes. Indeed this pleiotropy is evident in the group of genes that cause Usher Syndrome and NSHL (35–37), Stickler Syndrome (38, 39) and NSHL, and Waarensburg Syndrome and NSHL (40). In the era of precision medicine, delineating between syndromic forms and non-syndromic forms of deafness is critical for patient care and counseling. The semen analysis results in this study help provide a road map for counseling and caring for individuals with CDC14A-related deafness. Regardless of gender, the loss of CDC14A and its phosphatase activity results in hearing loss. Females, irrespective of mutation type or location do not experience infertility. For males, fertility is directly linked to the mutation and its location. Mutations that preserve the function of the NM_033313.2 transcripts are expected to result in DFNB32 deafness rather than HIIMS, whereas mutations that oblate this transcript are expected to result in HIIMS.

In summary, we identified mutations in *CDC14A* as the cause of ARNSHL in six Iranian families. In doing so, we identified a novel frameshift mutation in the 3'end of the gene and highlighted the p.Arg376Ter mutation might be a founder mutation in the Iranian population. We also report the first clinical results of a semen test from patients carrying nonsense mutations in exon 11 of *CDC14A*, illustrating males can have the DFNB32 phenotype. In doing so, we have shed further light on the importance of transcript NM_033313.2 in fertility and have highlighted the importance of the coding sequence at the 3' end of CDC14A for hearing. Finally, we established a roadmap for the clinical interpretation of variants in CDC14A, which will help with the care of patients with mutations in CDC14A.

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References

1. Patterson KI, Brummer T, O'Brien PM, Daly RJ. Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem J. 2009;418(3):475–89. [PubMed: 19228121]
2. Uddin B, Partscht P, Chen NP, Neuner A, Weiss M, Hardt R, et al. The human phosphatase CDC14A modulates primary cilium length by regulating centrosomal actin nucleation. EMBO Rep. 2019;20(1).
3. Mocciaro A, Berdougo E, Zeng K, Black E, Vagnarelli P, Earnshaw W, et al. Vertebrate cells genetically deficient for Cdc14A or Cdc14B retain DNA damage checkpoint proficiency but are impaired in DNA repair. J Cell Biol. 2010;189(4):631–9. [PubMed: 20479464]
4. Clement A, Solnica-Krezel L, Gould KL. The Cdc14B phosphatase contributes to ciliogenesis in zebrafish. Development. 2011;138(2):291–302. [PubMed: 21177342]
5. Chen NP, Uddin B, Voit R, Schiebel E. Human phosphatase CDC14A is recruited to the cell leading edge to regulate cell migration and adhesion. Proc Natl Acad Sci U S A. 2016;113(4):990–5. [PubMed: 26747605]

6. Lin H, Ha K, Lu G, Fang X, Cheng R, Zuo Q, et al. Cdc14A and Cdc14B Redundantly Regulate DNA Double-Strand Break Repair. Mol Cell Biol. 2015;35(21):3657–68. [PubMed: 26283732]
7. Ovejero S, Ayala P, Bueno A, Sacristan MP. Human Cdc14A regulates Wee1 stability by counteracting CDK-mediated phosphorylation. Mol Biol Cell. 2012;23(23):4515–25. [PubMed: 23051732]
8. Bremmer SC, Hall H, Martinez JS, Eissler CL, Hinrichsen TH, Rossie S, et al. Cdc14 phosphatases preferentially dephosphorylate a subset of cyclin-dependent kinase (Cdk) sites containing phosphoserine. J Biol Chem. 2012;287(3):1662–9. [PubMed: 22117071]
9. Vazquez-Novelle MD, Mailand N, Ovejero S, Bueno A, Sacristan MP. Human Cdc14A phosphatase modulates the G2/M transition through Cdc25A and Cdc25B. J Biol Chem. 2010;285(52):40544–53. [PubMed: 20956543]
10. Schindler K, Schultz RM. The CDC14A phosphatase regulates oocyte maturation in mouse. Cell Cycle. 2009;8(7):1090–8. [PubMed: 19270517]
11. Schindler K, Schultz RM. CDC14B acts through FZR1 (CDH1) to prevent meiotic maturation of mouse oocytes. Biol Reprod. 2009;80(4):795–803. [PubMed: 19129509]
12. Trautmann S, McCollum D. Cell cycle: new functions for Cdc14 family phosphatases. Curr Biol. 2002;12(21):R733–5. [PubMed: 12419203]
13. Bembenek J, Yu H. Regulation of the anaphase-promoting complex by the dual specificity phosphatase human Cdc14a. J Biol Chem. 2001;276(51):48237–42. [PubMed: 11598127]
14. Delmaghani S, Aghaie A, Bouyacoub Y, El Hachmi H, Bonnet C, Riahi Z, et al. Mutations in CDC14A, Encoding a Protein Phosphatase Involved in Hair Cell Ciliogenesis, Cause Autosomal-Recessive Severe to Profound Deafness. Am J Hum Genet. 2016;98(6):1266–70. [PubMed: 27259055]
15. Imtiaz A, Belyantseva IA, Beirl AJ, Fenollar-Ferrer C, Bashir R, Bukhari I, et al. CDC14A phosphatase is essential for hearing and male fertility in mouse and human. Hum Mol Genet. 2018;27(5):780–98. [PubMed: 29293958]
16. Doll J, Kolb S, Schnapp L, Rad A, Ruschendorf F, Khan I, et al. Novel Loss-of-Function Variants in CDC14A are Associated with Recessive Sensorineural Hearing Loss in Iranian and Pakistani Patients. International journal of molecular sciences. 2020;21(1).
17. Booth KT, Azaiez H, Kahrizi K, Wang D, Zhang Y, Frees K, et al. Exonic mutations and exon skipping: Lessons learned from DFNA5. Hum Mutat. 2018;39(3):433–40. [PubMed: 29266521]
18. Sloan-Heggen CM, Babanejad M, Beheshtian M, Simpson AC, Booth KT, Ardalani F, et al. Characterising the spectrum of autosomal recessive hereditary hearing loss in Iran. J Med Genet. 2015;52(12):823–9. [PubMed: 26445815]
19. Booth KT, Azaiez H, Kahrizi K, Simpson AC, Tollefson WT, Sloan CM, et al. PDZD7 and hearing loss: More than just a modifier. Am J Med Genet A. 2015;167a(12):2957–65. [PubMed: 26416264]
20. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754–60. [PubMed: 19451168]
21. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011;43(5):491–8. [PubMed: 21478889]
22. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164. [PubMed: 20601685]
23. The Genomes Project C, Auton A, Abecasis GR, Altshuler DM, Durbin RM, Abecasis GR, et al. A global reference for human genetic variation. Nature. 2015;526:68. [PubMed: 26432245]
24. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285. [PubMed: 27535533]
25. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. bioRxiv. 2019:531210.
26. Fattahi Z, Beheshtian M, Mohseni M, Poustchi H, Sellars E, Nezhadi SH, et al. Iranome: A catalog of genomic variations in the Iranian population. Hum Mutat. 2019.

27. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46(3):310–5. [PubMed: 24487276]
28. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7(4):248–9. [PubMed: 20354512]
29. Sim N-L, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Research. 2012;40(W1):W452–W7. [PubMed: 22689647]
30. Cooper GM, Stone EA, Asimenos G, Green ED, Batzoglou S, Sidow A. Distribution and intensity of constraint in mammalian genomic sequence. Genome Res. 2005;15(7):901–13. [PubMed: 15965027]
31. Gray CH, Good VM, Tonks NK, Barford D. The structure of the cell cycle protein Cdc14 reveals a proline-directed protein phosphatase. Embo j. 2003;22(14):3524–35. [PubMed: 12853468]
32. Mocciaro A, Schiebel E. Cdc14: a highly conserved family of phosphatases with non-conserved functions? J cell sci. 2010;123(17):2867–76. [PubMed: 20720150]
33. Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. Trends Biochem Sci. 1998;23(6):198–9. [PubMed: 9644970]
34. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231–45. [PubMed: 19934213]
35. Petit C Usher syndrome: from genetics to pathogenesis. Annu Rev Genomics Hum Genet. 2001;2:271–97. [PubMed: 11701652]
36. Lentz J, Keats B. Usher Syndrome Type II In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA): University of Washington, Seattle University of Washington, Seattle GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
37. Lentz J, Keats BJB. Usher Syndrome Type I In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA): University of Washington, Seattle University of Washington, Seattle GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
38. Booth KT, Askew JW, Talebizadeh Z, Huygen PLM, Eudy J, Kenyon J, et al. Splice-altering variant in COL11A1 as a cause of nonsyndromic hearing loss DFNA37. Genet Med. 2019;21(4):948–54. [PubMed: 30245514]
39. Robin NH, Moran RT, Ala-Kokko L. Stickler Syndrome In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA): University of Washington, Seattle University of Washington, Seattle GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
40. Zazo Seco C, Serrao de Castro L, van Nierop JW, Morin M, Jhangiani S, Verver EJ, et al. Allelic Mutations of KITLG, Encoding KIT Ligand, Cause Asymmetric and Unilateral Hearing Loss and Waardenburg Syndrome Type 2. Am J Hum Genet. 2015;97(5):647–60. [PubMed: 26522471]

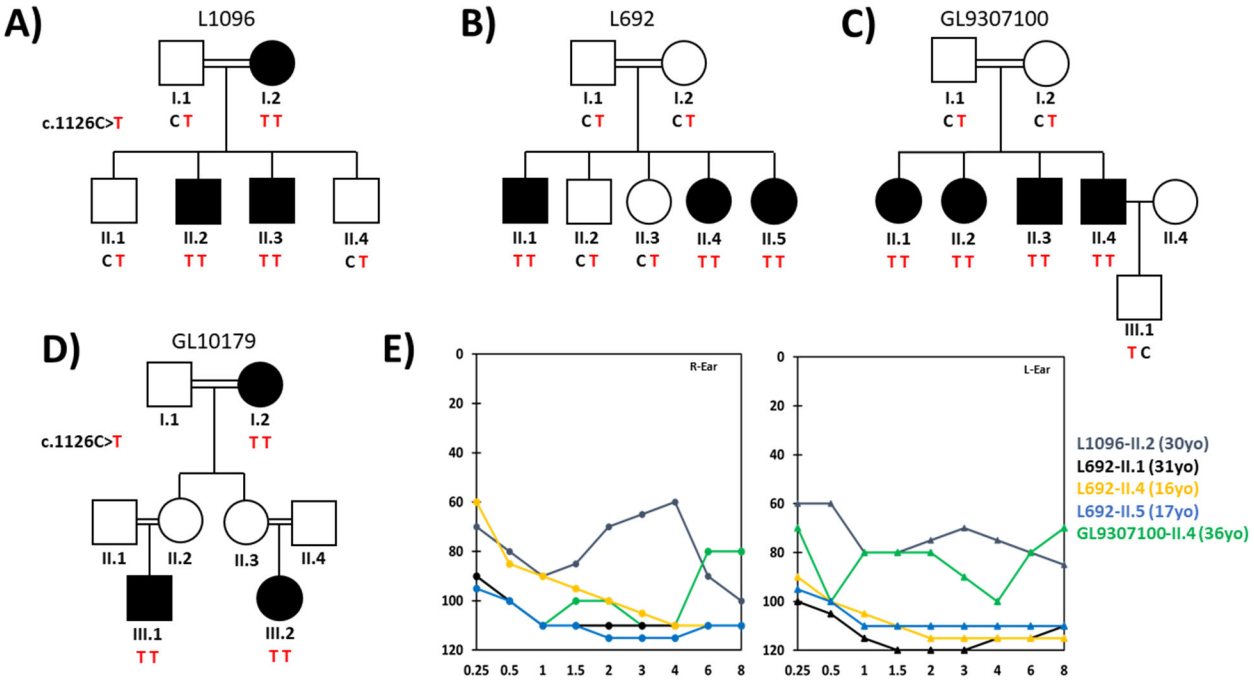

Figure 1.

Pedigrees and audiometric data from four families segregating the p.Arg376Ter mutation. A-D) Filled symbols denote affected individuals and double lines indicate consanguinity. Red and bold represents the c.1126C>T (p.Arg376Ter) mutant allele segregating with the ARNSHL. An "*" denotes the individual that underwent exome sequencing and an "**" indicates the individual that underwent OtoSCOPE testing. (E) Audiograms were obtained using pure tone audiometry with air conduction from frequencies from 250 Hz to 8,000 Hz.

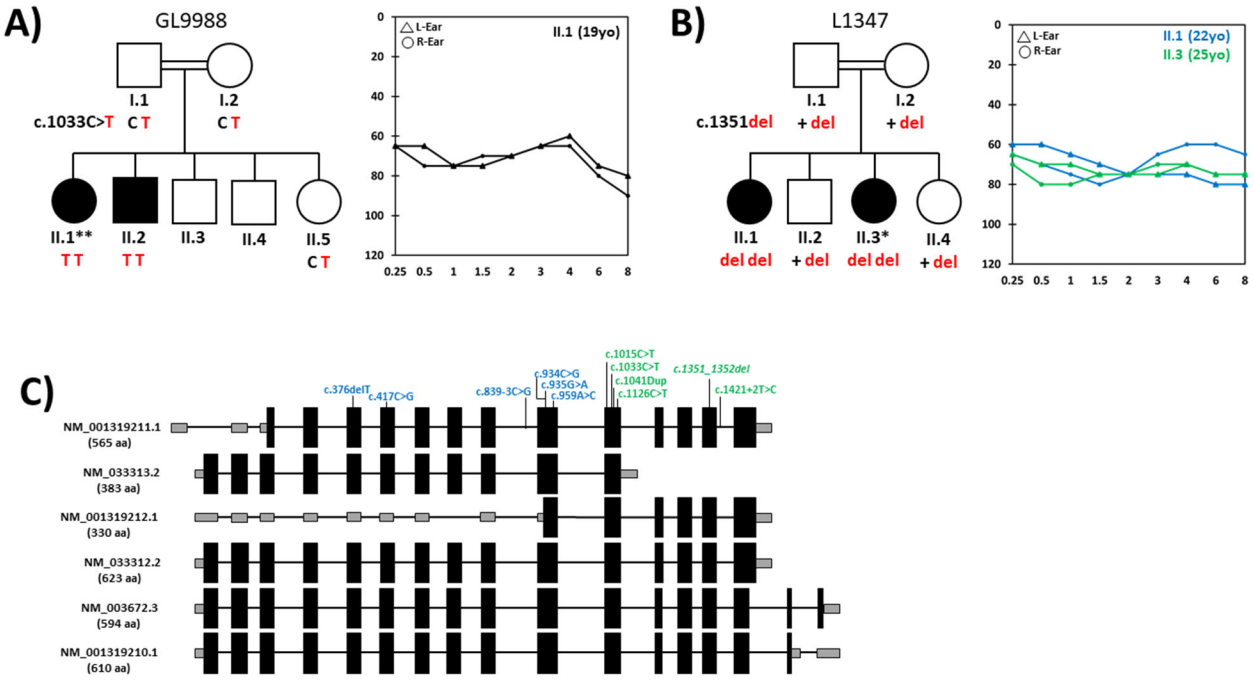

Figure 2.

Pedigrees, audiometric data and gene schematic. A-B) Filled symbols denote affected individuals and double lines indicate consanguinity. Red and bold represents the mutant allele segregating with the DFNB32 hearing loss in each family. An "*" denotes the individual that underwent exome sequencing. Audiograms were obtained using pure tone audiometry with air conduction from frequencies from 250 Hz to 8,000 Hz. (C) Gene schematic of CDC14A denoting pathogenic variants and their associated phenotypes. The grey box show non-coding exons whereas black denote protein-coding exons. Text colored blue and green indicates the phenotypes associated with pathogenic variants in CDC14A: HIIMS and DFNB32, respectively. Italics denotes the novel frameshift c.1351_1352del (p.Ala451Thrfs*43). Nucleotide numbering: the A of the ATG translation initiation site is noted as +1 using transcript NM_033312.2.

Table 1

Clinical characteristics of our six investigated families with hereditary hearing loss

	Family ID												
	L1096		**L692**		**L1347**		**GL9988**		**GL10179**			**GL9307100**	
Variant	c.1126C>T p.R376X		c.1126C>T p.R376X		c.1351_1352del p.Ala451Thrfs*43		c.1033C>T p.R345X		c.1126C>T p.R376X			c.1126C>T p.R376X	
Individual	II:2	II:3	II:1	II:4 & II:5	II:1	II:3	II:1	II:2	I:2	III:1	III:2	II:1 & II:2	II:3 & II:4[a]
Gender	M		M	F	F		F	M	F	M	M	F	M
Onset	Prelingual		Prelingual		Prelingual	Postlingual[b]			Prelingual			Prelingual	
Severity	Moderate-to-severe		Profound		Severe-to- profound		Severe		Severe		Moderate	Severe-to-profound	
Speech impairment	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N
OA/RP	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
Skin disorder	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
Cognition Impairment	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
Unbalanced gait	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL
Age at semen analysis test (years)	30	31	NP	–	–	–	–	23	–	NP	N	–	41
Cell counts (million/mL)	20	13	NA	–	–	–	–	111	–	NA	–	–	69
Immotile sperm	40%	38%	NA	–	–	–	–	40%	–	NA	–	–	26%
Sperm morphology abnormal/ amorphous	96%/87%	97%/78%	NA	–	–	–	–	40%/0%	–	NA	–	–	32%/0%

Clinical features seen in patients

M and *F* male and female, respectively, *OA/RP* optic atrophy and retinitis pigmentosa, *NL* normal, *Y, NP* and *N* yes, not possible and no, respectively

[a] The male in family GL9307100 that underwent semen analysis

[b] Progressive hearing loss

Table 2.

Reported variants in CDC14A and their corresponding phenotypes

g.DNA Position (chr1)	Exon	c.DNA	Protein	Variant Type	gnomAD (%)	Iranome	Sperm Abnormality (Y/N)	Infertile (Y/N)	Phenotype	Ethnicity	Reference
100889844:delT	5	c.376delT	p.Y126Ifs64X	Frameshift	0	0	Y	Y	HIIMS	Pakistani	Imtiaz et al. 2018
100905515:C>G	6	c.417C>G	p.Y139X	Nonsense	0. 001	0	Y	Y	HIIMS	Pakistani	
100933509:C>G	-	c.839–3C>G	p.K279fs10X	Splice-Altering	0	0	Y	Y	HIIMS	Pakistani	
100933607:C>G	10	c.934C>G	p.R312G	Missense	0	0	Y	Y	HIIMS	Iranian	
100933608:G>A	10	c.935G>A	p.R312Q	Missense	0.001	0	NA	NA	HIIMS *	Tunisian	
100933632:A>C	10	c.959A>C	p. Q320P	Missense	0.0003	0	Y	Y	HIIMS	Pakistani	
100949885C>T	11	c.1015C>T	p.R339X	Nonsense	0.0008	0	NA	NA	DFNB32	Mauritanian	Delmaghani et al 2016
100949903:C>T	11	c.1033C>T	p. R345X	Nonsense	0.0004	0	NA	N	DFNB32	Pakistani	**This study** + Imtiaz et al. 2018
100949911:dup	11	c.1041dup	p.S348Qfs*2	Frameshift	0	0	NA	NA	DFNB32	Pakistani	Doll et al. 2020
100949996:C>T	11	c.1126C>T	p.Arg376X	Nonsense	0.0004	0.06	Y	N	DFNB32	Iranian	**This study** + Delmaghani et al 2016 + Imtiaz et al. 2018
100950221_100950222del	14	c.1351_1352del	p.A451Tfs*43	Frameshift	0	0	Y	NA	DFNB32[a]	Iranian	**This study**
100950293T>C	-	c.1421+2T>C	p.V472Lfs*20	Splice-Altering	0	0	NA	NA	DFNB32	Iranian	Doll et al. 2020

HIIMS: Hearing Impairment and Infertile Male Syndrome; DFNB32: Autosomal Recessive Nonsyndromic Hearing Loss without male infertility; NA: Not Available.

"*" Presumed HIIMS.

[a]Males in this family are expected to be fertile. cDNA number from the ATG of RefSeq transcript NM_033313.2.

Table 3

Haplotype analysis in and around CDC14A for p.Arg376Ter mutation in five Iranian families

	L692	L9307100	L1096	MORL2[a]	Control[b]	gnomAD MAF (global)
rs35121076	**A**|**A**	**A**|**A**	**A**|**A**	**A**|**A**	G|**A**	5.40%
rs41285734	**C**|**C**	**C**|**C**	**C**|**C**	**C**|**C**	G|**C**	4.20%
rs11166393	**G**|**G**	**G**|**G**	**G**|**G**	**G**|**G**	A|**G**	5%
rs644835	**G**|**G**	**G**|**G**	**G**|**G**	**G**|**G**	A|**G**	11.40%
rs115696850	**A**|**A**	**A**|**A**	**A**|**A**	**A**|**A**	C|**A**	3.10%
rs876661408	**T**|**T**	**T**|**T**	**T**|**T**	**T**|**T**	C|**T**	0.00043%
rs35904273	**Del**|**Del**	**Del**|**Del**	**Del**|**Del**	**Del**|**Del**	AG|**Del**	31%

Bold indicates the nonreference allele. Red represents the p.Arg376Ter. SNPs are a ~2.5 Mb window around *CDC14A*

[a] MORL2 family published in [15]

[b] Control is an unaffected sibling carrier of the p.Arg376Ter in the pedigree MORL2