

Novel Homozygous Mutation in PDZD7 Gene in a Family with Nonsyndromic Sensorineural Hearing Loss

Qiang Du

Department of the Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, ENT Institute and Otorhinolaryngology, Fudan University

Qin Sun

Department of the Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, ENT Institute and Otorhinolaryngology, Fudan University

Jinchao Wang

Department of Otorhinolaryngology, Huizhou Municipal Central Hospital

Weitao Li

Department of the Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, ENT Institute and Otorhinolaryngology, Fudan University

Luo Guo (✉ guoluo.gary@foxmail.com)

Department of the Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, ENT Institute and Otorhinolaryngology, Fudan University

Huawei Li

Department of the Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, ENT Institute and Otorhinolaryngology, Fudan University

Xiaodong Gu

Department of the Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, ENT Institute and Otorhinolaryngology, Fudan University

Research Article

Keywords: Novel homozygous mutation, nonsyndromic sensorineural, hearing loss

Posted Date: December 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1126711/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Hearing loss is the most common sensory neural disorder in human, and according to WHO estimation, 5.5% (466 million) people worldwide have disabling hearing loss. In this study, a Chinese family with prelingual sensorineural hearing loss was investigated. The affected individuals showed moderately-severe hearing loss at all frequencies. Using target genome enrichment and high-throughput sequencing, a homozygous mutation c.2372delC, p.S791Ffs*17 was identified in *PDZD7*. The deletion mutation lies in exon 15 of *PDZD7* and results in a frame shift followed by an early stop codon. Our study expand the mutation spectrum of *PDZD7* and strengthens the clinical importance of this gene in patients with moderately-severe hearing loss.

Introduction

Hearing loss is the most common sensory neural disorder in human, and according to WHO estimation, 5.5% (466 million) of the world population have disabling hearing loss (<https://www.who.int/health-topics/hearing-loss>). It is estimated that more than half of prelingual hearing loss were caused by genetic factors[1]. Non-syndromic hearing loss (NSHL), which no other symptoms occur, accounts for about 70% of hereditary hearing loss. While syndromic hearing loss (SHL), which associates with other symptoms, accounts for about 30% of hereditary hearing loss[2]. To date, 124 genes has been implicated for NSHL, and more than 400 SHL have been identified (<https://hereditaryhearingloss.org/>)[3]. In the hearing system, most of these pathogenic genes were located at the inner ear hair cells, a polarized epithelial cell with stereocilia bundles at the top, which translate motion to neuronal signals[4].

PDZD7 encodes a scaffold protein that express in the cortex and inner ear. Mutations in *PDZD7* have been reported to cause autosomal recessive NSHL[5, 6]. Moreover, *PDZD7* is suggested to be a contributor to digenic Usher syndrome type IIC and a modifier in patients with Usher Syndrome Type IIA[7]. *PDZD7* protein contains three PDZ domains, a proline-rich (PR) region as well as a harmonin-N like (HNL) domain. The PDZ domains enable *PDZD7* to interact with SAN, harmonin, *USH2A* and *ADGRV1*, all of which were essential components of the ankle-link complex joint the stereocilium to hair bundles[8]. To date, 28 pathogenic mutations in *PDZD7* have been identified in different regions (<https://deafnessvariationdatabase.org/gene/PDZD7>).

In this study, we present the genetic characteristics of a Chinese family with congenital sensorineural HL. The affected individuals had moderately-severe hearing loss at all frequencies. We used targeted genome enrichment (TGE) and high-throughput sequencing (HTS) to identify a novel homozygous deletion mutation in exon 15 of *PDZD7*. The deletion mutation results in a frame shift followed by an early stop codon, which leads to the loss of several functional domains and results in the pathogenic effect of hearing loss. Our study has enriched the mutation spectrum of *PDZD7*, and proved that TGE and HTS are a reliable tools for genetic testing of hereditary hearing loss for large genes such as *PDZD7*.

Materials And Methods

Subjects

Patients of this study were recruited from outpatient department of Affiliated Eye and ENT Hospital, Fudan University. All family members were evaluated by audiological tests as previously described[9]. Pure tone audiometry at frequencies of 125, 250, 500, 1000, 2000, 4000, and 8000 Hz was performed on family members above age 6. Romberg and tandem gait tests were performed to evaluate vestibular functions. Auditory brainstem response (ABR) test was performed on family members under age 6. High-resolution computed tomography (HRCT) scans of the temporal bone were obtained to exam inner ear malformations. Written informed consent was obtained from all individuals, and this study was approved by the ethics committee of the Institutional Review Board of the Eye, Ear, Nose and Throat Hospital affiliated with Fudan University (Shanghai, China). To screen common deafness mutations in *GJB2*, *SLC26A4*, and *MT-RNR1* genes, the patients were prescreened by PCR amplification and Sanger sequencing according to previous studies[9].

Targeted exome sequencing

Genomic DNA was extracted from whole blood of the participants using a genomic DNA isolation kit (Qiagen, Hilden, Germany). Paired end sequencing library was prepared using library preparation kit (New England Biolabs, Ipswich, MA, catalogue# E6040) as previously reported[9]. A human deafness gene exon enrichment kit including 168 genes was used to capture target genome intervals. High-throughput sequencing was performed on illumine Hiseq 2000 according to the manufacturer's instructions..

Bioinformatics and validation of the variants

Sequencing reads generation, bioinformatic analysis were performed as previously reported[10]. Sequencing reads were generated by the Illumina CASAVA v1.8 pipeline and aligned to the human reference genome (hg19) using the Burrows–Wheeler Aligner (BWA) program. Variants were called using GATK package v4.1.8.1. All variants were annotated and characterized using ANNOVAR software. To validate the variants, Sanger sequencing of the *PDZD7* exon 15 was performed on genomic DNA from all family members and 96 normal hearing controls. PCR and sequencing primers were designed by Primer3 online software. Sanger sequencing was performed on a 3730XL sequencer (Applied Biosystems) according to the manufacturer's instructions.

Results

Family and clinical presentations

Family D27 is a non-consanguineous Chinese family that includes three affected siblings and two normal hearing parents (Fig. 1A). The patients were 9, 7 and 2 years old during examine. Audiograms of the two older patients showed bilateral moderate to severe HL with a slightly downward slope (Fig. 1B). ABR test

of II:3 revealed bilateral HL with a threshold around 65dB (Fig. 1C). Although new-born hearing screening was not performed, the parents recalled no responses to subtle sounds at 1 to 2 years of age, suggesting a congenital phenotype. A vestibular functional test of the two older patients revealed no abnormalities. HRCT scan of the II:1 and II:2 revealed no inner ear malformations. We also performed pure tone audiometry on the parents and detected no HL features. Otoscopy and full physical examination with special attention to renal, and ophthalmological evaluations revealed no additional abnormalities.

Targeted massively parallel sequencing

Targeted high-throughput sequencing of all exons and exon–intron boundaries for 168 deafness genes was performed on the proband II:1. Sequencing yielded 2.8 million 100 bp paired-end reads. After adaptor trimming and low-quality reads filtering, paired-end fastq files were aligned to the human genome (hg19). A mean depth of 118.1 for the targeted exons was achieved, and 97.8% of the targeted genome intervals were covered by at least 10 sequencing reads. After filtering against MAFs from various databases (1000 Genome Project, gnomad v2.1.1 and in-house database), we focused on variants in the coding region and intronic variants that might affect splicing. Based on the assumption of an autosomal recessive mode of inheritance, we focused on genes with homozygous or compound heterozygous variants. A homozygous variant, c.2372delC and p.S791Ffs*17 (NM_001195263), was identified in exon 15 of *PDZD7*. This variant was not present in any of the reference databases.

Genetic analysis of the *PDZD7* mutation

Sanger sequencing of exon 15 of *PDZD7* was performed on all family members (Fig. 2A). The three affected siblings were homozygous for the *PDZD7* mutation, while their normal hearing parents were both heterozygous, which suggests that the *PDZD7* mutation co-segregated with HL in this family. The deletion mutation resulted in a frame shift followed by an early stop codon and truncated protein p.S791Ffs*17 (Fig. 2A). Moreover, the mutation was absent in 96 healthy controls screened by sanger sequencing.

Discussion

PDZD7 locates on chromosome 10q24.31 and was originally identified as an autosomal recessive NSHL gene[5, 6]. Subsequently, heterozygous mutations in *PDZD7* was identified as a modifier of retinal disease and a contributor to digenic Usher syndrome[7]. In Usher syndrome patients with biallelic *USH2A* mutations, another heterozygous *PDZD7* mutation causes earlier-onset and more severe retinal disease. Heterozygous mutations in *PDZD7* and *ADGRV1* together induces Usher Syndrome Type IIC. In this study, using HTS, we identified a novel *PDZD7* mutation (c.2372delC, p.S791Ffs*17) in a Chinese family with congenital NSHL. The frameshift mutation locates on exon 15 and produces an early stop codon and a truncated protein. Furthermore, no other mutations in the Usher syndrome genes were identified in the

proband. The affected sibling in the present study were 9, 7 and 2 years of age during examinations. Ophthalmological tests revealed no retinal abnormalities. Newborn hearing screening was not performed, but the parents recalled reduced response to small sounds and mispronunciations of the two older sisters, which indicated congenital or prelingual onset.

PDZD7 protein is a paralog of harmonin (*USH1C*) and whirlin (*WHRN*), sharing 35% and 55% similarity with harmonin and whirlin, respectively[11]. It contains three PDZ domains, a HNL domain as well as a proline-rich (PR) region. *PDZD7* expresses in the inner ear hair cell, and forms the Usher quaternary protein complex with USH2A, ADGRV1 and WHRN, which is essential for the development and organization of ankle-link complex localize at the ankle region of hair cell stereocilia[12, 13]. The first two PDZ domains of PDZD7 mediate interaction with the other Usher quaternary protein complex components USH2A, ADGRV1 and WHRN, whereas the third PDZ domain is only involved in the interaction with WHRN[12].

To date, 16 *PDZD7* mutations have been reported in literature, 6 of them are truncating variants including of the present study (Fig. 2B). Four alternative splicing isoforms of *PDZD7* have been detected, which encodes either a full-length protein or short isoforms mainly containing the first two PDZ domains[6]. The mutation identified in this study localizes in exon 15 of *PDZD7*, which is unique to the long isoform (Fig. 2B). Including our research, 3 mutations have been discovered in domains unique to the long isoform (Fig. 2B). Patients with these 3 mutations (p.S703fs, p.R781_S784del and p.S791Ffs*17) showed the same characteristic auditory phenotype, bilateral moderately severe hearing loss at all frequencies with gentle downward sloping, as patients with mutations in other parts of the gene[14, 15]. Mouse model lacking exons 2-5 of *Pdzd7*, which disrupt all isoforms, and mouse model lacking exon 14, which only disrupt the long isoform, both manifest stereocilia disorganization and MET deficits, leading to similar hearing loss phenotype. Moreover, in mouse lacking exon 14 of *Pdzd7* gene, *PDZD7* short isoforms were not detected in inner ear at the protein level. These findings suggested that *PDZD7* long isoform is indispensable for hair cell function, whereas *PDZD7* short isoform may not localize in the stereocilia and not be required for stereocilia function.

Hereditary hearing loss is a genetically and phenotypically heterozygous disorder. To date, 124 genes have been identified for NSHL, and 46 genes have been identified for the nine most common syndromic HL (<https://hereditaryhearingloss.org/>). Phenotypes of the patients vary at audiogram, age of onset, progression, vestibular complication, inner ear malformation, retinal complication, etc[16]. These heterogeneities hindered genetic diagnosis of hearing loss, and called for more complicated mutation screening strategy that take phenotype-genotype correlation into consideration. Since most of ARNSHL characterize with prelingual severe to profound HL, the relatively rare moderately-severe audiogram at all frequencies may serve as a reminder for potential causative *PDZD7* mutations.

We report a novel frameshift mutation on *PDZD7* in a Chinese family with moderately-severe HL. The mutation lies in exon 15, and is unique to the long isoform of PDZD7 protein. Our study extends the mutations spectrum of the *PDZD7* gene in Chinese population. The relatively uncommon moderately-

severe audiogram with a slightly downward slope is characteristic for *PDZD7* patients. The identification of a novel *PDZD7* mutation may be valuable for genetic consultation and functional research.

Declarations

Acknowledgements

We would like to thank our patients for agreeing to donate their personal data for our study and to have these data published.

Funding

This work was supported by the National Key R&D Program of China (Nos. 2017YFA0103900, 2016YFC0905200), National Natural Science Foundation of China (Nos. 81620108005, 81500801, 81830029).

Ethics approval and consent to participate

All procedures performed in this study involving human participants were performed in accordance with the Declaration of Helsinki. This study was approved by the Eye, Ear, Nose and Throat Hospital affiliated with Fudan University Review Board of the Office of Research Compliance through protocol 2017044. Written informed consent was obtained from all individuals, and this study was approved by the ethics committee of the Institutional Review Board of the Eye, Ear, Nose and Throat Hospital affiliated with Fudan University (Shanghai, China).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Affiliations

1. Department of the Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, ENT Institute and Otorhinolaryngology, Fudan University, No. 83, Fenyang Road, Shanghai 200031, China.

Qiang Du, Qin Sun, Xiaodong Gu, Weitao Li, Luo Guo, Huawei Li

2. Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China.

Huawei Li

3. NHC Key Laboratory of Hearing Medicine, Fudan University, Shanghai 200031, China.

Qiang Du, Qin Sun, Xiaodong Gu, Weitao Li, Luo Guo, Huawei Li

4. Shanghai Engineering Research Centre of Cochlear Implant, Shanghai 200031, China.

Huawei Li

5. The Institutes of Brain Science and the Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032, China.

Huawei Li

6. Department of Otorhinolaryngology, Huizhou Municipal Central Hospital, Huizhou 516002, Guangdong, China.

Jinchao Wang

Authors' contributions

Luo Guo and Huawei Li designed the experiment. Qiang Du, Qin Sun and Xiaodong Gu carried out the experiment. Qiang Du and Luo Guo wrote the main manuscript text and prepared figures 1-2. All authors reviewed and approved the final manuscript.

References

1. Dror AA, Avraham KB: **Hearing loss: mechanisms revealed by genetics and cell biology.** *Annu Rev Genet*2009, **43**:411-437.
2. Morton CC, Nance WE: **Newborn hearing screening—a silent revolution.** *The New England journal of medicine*2006, **354**(20):2151-2164.

3. Bolz HJ: **Hereditary Hearing Loss and Its Syndromes Third Edition.** *European Journal of Human Genetics*2016, **24**(11):1650-1650.
4. Dror AA, Avraham KB: **Hearing impairment: a panoply of genes and functions.** *Neuron*2010, **68**(2):293-308.
5. Booth KT, Azaiez H, Kahrizi K, Simpson AC, Tollefson WTA, Sloan CM, Meyer NC, Babanejad M, Ardalani F, Arzhang *et al*: **PDZD7 and hearing loss: More than just a modifier.** *American Journal of Medical Genetics Part A*2015, **167**(12):2957-2965.
6. Schneider E, Märker T, Daser A, Frey-Mahn G, Beyer V, Farcas R, Schneider-Rätzke B, Kohlschmidt N, Grossmann B, Bauss *et al*: **Homozygous disruption of PDZD7 by reciprocal translocation in a consanguineous family: a new member of the Usher syndrome protein interactome causing congenital hearing impairment.** *Human Molecular Genetics*2009, **18**(4):655-666.
7. Ebermann I, Phillips JB, Liebau MC, Koenekoop RK, Schermer B, Lopez I, Schäfer E, Roux A-F, Dafinger C, Bernd *et al*: **PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome.** *Journal of Clinical Investigation*2010, **120**(6):1812-1823.
8. Grati M, Shin JB, Weston MD, Green J, Bhat MA, Gillespie PG, Kachar B: **Localization of PDZD7 to the stereocilia ankle-link associates this scaffolding protein with the Usher syndrome protein network.** *J Neurosci*2012, **32**(41):14288-14293.
9. Gu X, Guo L, Ji H, Sun S, Chai R, Wang L, Li H: **Genetic testing for sporadic hearing loss using targeted massively parallel sequencing identifies 10 novel mutations.** *Clinical genetics*2015, **87**(6):588-593.
10. Gu X, Sun S, Guo L, Lu X, Mei H, Lai C, Li H: **Novel biallelic OTOGL mutations in a Chinese family with moderate non-syndromic sensorineural hearing loss.** *Int J Pediatr Otorhinolaryngol*2015, **79**(6):817-820.
11. Du H, Zou L, Ren R, Li N, Li J, Wang Y, Sun J, Yang J, Xiong W, Xu Z: **Lack of PDZD7 long isoform disrupts ankle-link complex and causes hearing loss in mice.** *The FASEB Journal*2020, **34**(1):1136-1149.
12. Chen Q, Zou J, Shen Z, Zhang W, Yang J: **Whirlin and PDZ Domain-containing 7 (PDZD7) Proteins Are Both Required to Form the Quaternary Protein Complex Associated with Usher Syndrome Type 2.** *Journal of Biological Chemistry*2014, **289**(52):36070-36088.
13. Zou J, Zheng T, Ren C, Askew C, Liu X-P, Pan B, Holt JR, Wang Y, Yang J: **Deletion of PDZD7 disrupts the Usher syndrome type 2 protein complex in cochlear hair cells and causes hearing loss in mice.** *Human Molecular Genetics*2014, **23**(9):2374-2390.
14. Vona B, Lechno S, Hofrichter MA, Hopf S, Laig AK, Haaf T, Keilmann A, Zechner U, Bartsch O: **Confirmation of PDZD7 as a Nonsyndromic Hearing Loss Gene.** *Ear Hear*2016, **37**(4):e238-246.
15. Luo H, Hassan RN, Yan J, Xie J, Du P, Hu Q, Zhu Y, Jiang W: **Novel recessive PDZD7 biallelic mutations associated with hereditary hearing loss in a Chinese pedigree.** *Gene*2019, **709**:65-74.
16. Shearer AE, Hildebrand MS, Sloan CM, Smith RJ: **Deafness in the genomics era.** *Hearing research*2011, **282**(1-2):1-9.

Figures

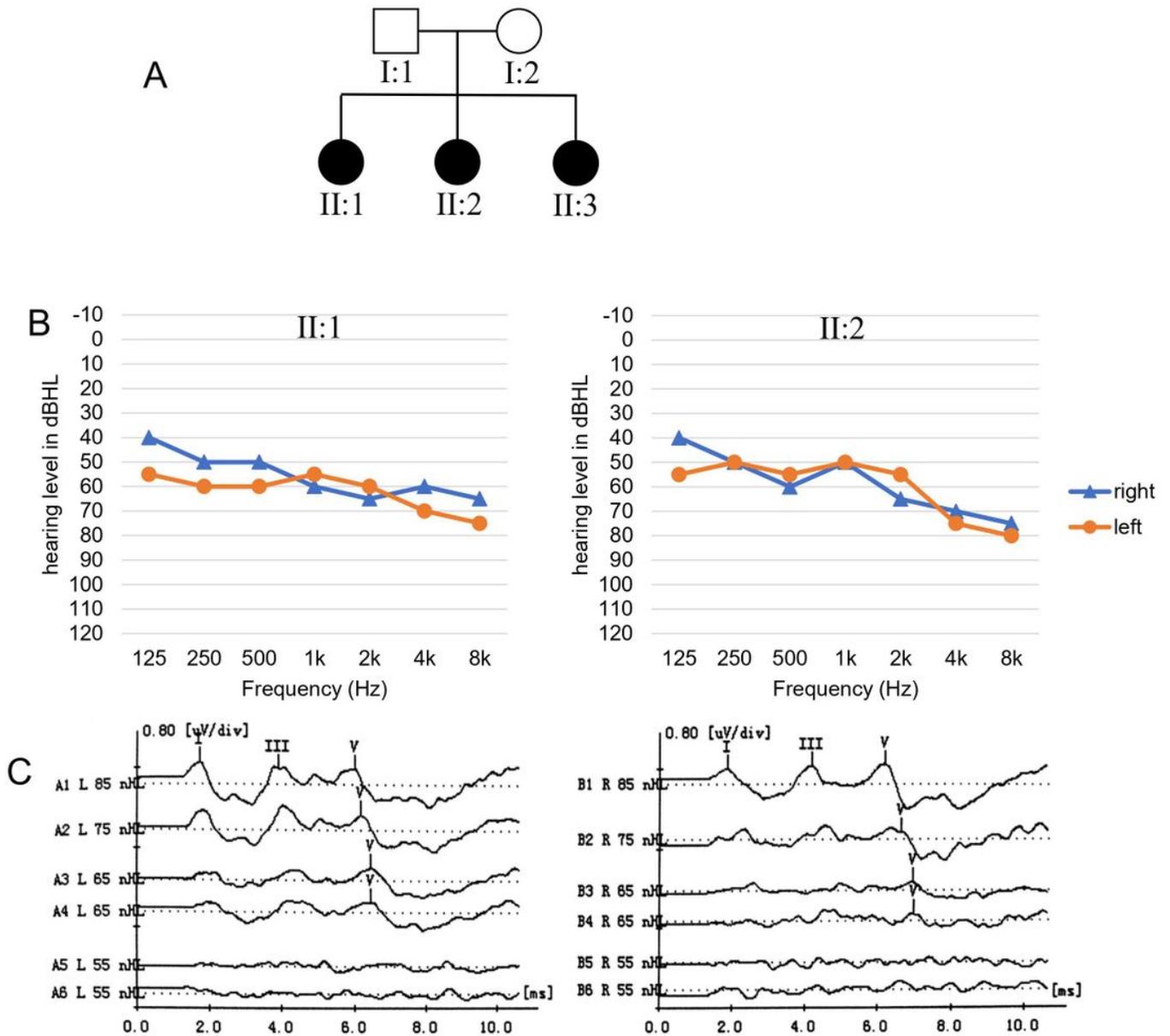


Figure 1

Pedigree and audiograms of the hearing loss family. (A) Pedigree of the family. Darkened symbols denote affected individuals. (B) Audiograms of the two affected Siblings (II:1 and II:2) in the family. (C) ABR results of II:3.

