# Clinical and genetic study of twelve Chinese Han families with non-syndromic deafness

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**Research**

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# Abstract

BACKGROUND: Non-syndromic hearing loss is clinically and genetically heterogeneous. In this study, we characterized the clinical features of twelve Chinese Han deaf families in which mutations in common deafness genes GJB2 , SLC26A4 and MT-RNR1 were excluded.

RESULTS: Targeted next-generation sequencing of 147 known deafness genes was performed in probands of ten families, while whole-exome sequencing was applied in those of the rest two. Pathogenic mutations in a total of 11 rare deafness genes, OTOF , CDH23 , PCDH15 , PDZD7 , ADGRV1 , KARS , OTOG , GRXCR2 , MYO6 , GRHL2 , and POU3F4 , were identified in all 12 probands, with 17 mutations being novel. Intrafamilial co-segregation of the mutations and the deafness phenotype were confirmed by Sanger sequencing.

CONCLUSIONS: Our results expanded the mutation spectrum and genotype-phenotype correlation of non-syndromic hearing loss in Chinese Hans and also emphasized the importance of combining both next-generation sequencing and detailed auditory evaluation to achieve a more accurate diagnosis for non-syndromic hearing loss.


# Background

Non-syndromic hearing loss (NSHL) is one of the most common sensory defects in humans and is a remarkably complex and heterogeneous disease with variable phenotypes (1). Genetic components contribute significantly to the cause of hearing loss (http://hereditaryhearingloss.org), with mutations in a great variety of deafness genes being reported in the Chinese Han population (2–6). In recent years, next-generation sequencing (NGS) technology including both targeted and whole-exome sequencing has provided an easier and more cost-effective approach for identifying causative mutations (2–6). It provides crucial information for diagnosis, intervention and treatment of hearing disorders (6). In this light, we recruited a series of Chinese Han deaf families that were pre-excluded from mutations in common deafness genes *GJB2, SLC26A4* and *MT-RNR1.* Targeted NGS for known deafness genes or whole-exome sequencing (WES) were performed on the probands of each family to search for pathogenic mutations.

# Methods

*Clinical evaluation*

Patients from twelve deaf families were enrolled through the Department of Otolaryngology, Affiliated Hospital of Nantong University, Nantong, China. Comprehensive clinical evaluations, imaging examination results, audiograms, and other relevant clinical information were collected for the probands. All affected individuals were evaluated through detailed audiological evaluations as described previously (5, 6). The probands had no obvious syndromic symptoms other than the hearing loss. All subjects or their family members gave written, informed consent to participates in this study. This study was approved by the Ethics Committee of the Affiliated Hospital of Nantong University.

*Genetic Analysis*

Genomic DNA from the family members were extracted from the blood samples using the Blood DNA kit (Tiangen Biotech, China). Pre-screening of mutations in *GJB2, SLC26A4* and *MT-RNR1* was performed in all probands by Sanger sequencing. Among probands of the 12 deaf families, ten were subjected to targeted NGS of 147 deafness-related genes (Supplementary file 1) and rest two to WES. Targeted gene capturing, data processing, bioinformatic analysis, and filtering against multiple databases for SNPs were performed as previously reported (2–4). Intrafamilial co-segregate of the candidate variants and the deafness phenotype was confirmed by Sanger sequencing in all available family members.

# Results

### Clinical Manifestations

Patients in the twelve Chinese families(Supplementary file 3), aged from 11 months to 87 years, exhibited bilateral, symmetrical, sensorineural hearing loss with the variable developing course and degree of severity, ranging from stable to progressive and from mild to profound(Supplementary file 4). The age at onset of HL in these patients ranged from at birth to 44 years. Patients with X-linked deafness in Families NT-42 and NT-43, carrying mutations in *POU3F4* as subsequently revealed, showed characteristic inner ear radiological features (Supplementary file 5) compatible with incomplete partition type3 (IP3), including absent modiolus and lamina spiralis but preserved interscalar septum in a normal-sized cochlea and abnormal dilatation of the lateral end of the internal auditory canal (IAC). Through physical examination, no other abnormalities, such as retinal pigment degeneration or other optic defects, vestibular, neurologic or systemic abnormalities, were detected in any of the patients, suggesting that the hearing loss is non-syndromic.

### Genetic Findings

The 12 Chinese probands have been previously excluded for mutations in common deafness genes *GJB2, SLC26A4* and *MT-RNR1* by Sanger sequencing. To detect possible causative mutations by targeted NGS or WES, non-synonymous variants with minor allele frequencies lower than 0.005 were filtered through as previously described (2-5). Candidate causative variants were summarized in (Supplementary file 2). In eight recessive probands, bi-allelic mutations, confirmed by parental genotyping, were identified in known deafness genes *OTOF, CDH23, PCDH15, ADGRV1, PDZD7, KARS, OTOG and GRXCR2.* (n = 1 each, Table 1). In two dominant families, we identified two heterozygous variants in genes associated with dominant deafness, p.T197I in *MYO6* and p.R426X in *GRHL2*, co-segregating with the hearing impairment (Table 1). Consistent with X-linked recessive inheritance pattern, in Family NT-42 and NT-43, we identified two hemizygous candidate mutations p.C233X and p.V321G in *POU3F4*, respectively (Table 1). Sanger sequencing in extended family members confirmed the co-segregation of the reported mutations with the hearing phenotype (Supplementary 6). Among the 20 mutations identified in this study, 17 mutations have not been associated with deafness in previous reports (Table 1) (2, 7, 8).

Table 1. Mutations detected in twelve Chinese Han families.

| Family ID | Gene | Mutation type | Nucletide Change (Transcript version) | Aminoacid change | Phylop Score | Mutation Taster | PROVEAN (score) | SIFT (score) | Allele Frequency in controls | Novel or HGMD |
|---|---|---|---|---|---|---|---|---|---|---|
| Autosomal Recessive | | | | | | | | | | |
| NT-41 | *OTOF* | Splicing | c.4961-3C>G (NM_194248) | Splicing | - | - | - | - | - | Y |
| | *OTOF* | Missense | c.145C>T (NM_194248) | p.R49W | 8.066 | DC | -3.38 | 0.002 | 0.0095 | Y |
| | *OTOF* | Frameshift | c.1366_1367insC (NM_194248) | p.V456Afs*20 | - | - | - | - | - | N |
| | *OTOF* | Missense | c.1364A>T (NM_194248) | p.Y455F | 7.962 | DC | -2.17 | 0.238 | - | N |
| NT-44 | *CDH23* | Frameshift | c.9469_9470insGT (NM_022124) | p.E3158Vfs*58 | 4.707 | DC | - | - | - | N |
| NT-45 | *PCDH15* | Missense | c.4310C>T (NM_033056) | p.P1437L | 1.89 | DC | -0.75 | 0.081 | - | N |
| | *PCDH15* | Codon Mutation | c.5254_5280delCCTATTTCTCCTCCTTCTCCTCCTCCT (NM_033056) | p.1752_1760delPISPPSPPP | - | - | - | - | 0.0001 | N |
| NT-46 | *ADGRV1* | Missense | c.11411G>A (NM_032119) | p.R3804Q | 8.61 | DC | -3.21 | 0 | - | N |
| | *ADGRV1* | Splicing | c.13893+8T>G (NM_032119) | splicing | - | - | - | - | - | N |
| NT-47 | *PDZD7* | Nonframeshift | c.1574_1597delACCAGGAGAGGGGCCGGGCCCTGC (NM_001195263) | p.525_533delDQERGRALLinsV | - | - | - | - | - | N |
| | *PDZD7* | Missense | c.490C>T (NM_001195263) | p.R164W | 0.653 | DC | -6.05 | 0.008 | 0.00005283 | N |
| NT-48 | *KARS* | Missense | c.685T>C (NM_001130089) | p.Y229H | 0.277 | polymorphism | 0.36 | 0.593 | 0.0011 | Y |
| | *KARS* | Missense | c.403G>A (NM_001130089) | p.D135N | 3.049 | DC | -2.26 | 0.241 | - | N |
| NT-51 | *OTOG* | Missense | c.433G>A (NM_001277269) | p.G145S | 9.516 | DC | -4.95 | - | 0.0006 | N |
| | *OTOG* | Splicing | c.2117-6C>T (NM_001277269) | Splicing | - | - | - | - | - | N |
| NT-52 | *GRXCR2* | Missense | c.65A >G (NM_001080516) | p.K22R | 3.254 | DC | -2.61 | 0.006 | - | N |
| Autosomal Dominant | | | | | | | | | | |
| NT-49 | *MYO6* | Missense | c.590C> T (NM_004999) | p.T197I | 7.568 | DC | -5.84 | 0 | - | N |
| NT-50 | *GRHL2* | Nonsense | c.1276C >T | p.R426X | 1.858 | DC | - | - | - | N |
| X-linked recessive | | | | | | | | | | |
| NT-42 | *POU3F4* | Nonsense | c.699C>A (NM_000307) | p.C233X | 3.78 | DC | - | - | - | N |
| NT-43 | *POU3F4* | Missense | c.962T>G (NM_000307) | p.V321G | 6.105 | DC | -6.95 | 0 | - | N |

# Discussion

In this study, we performed a detailed clinical and genetic characterization of 12 Chinese Han families affected by autosomal recessive, autosomal dominant and X-linked NSHL. For family NT–41, our audiological assessments revealed that proband NT–41-Ⅱ:2 had congenital bilateral profound sensorineural hearing loss. Characteristic of auditory neuropathy spectrum disorder (ANSD), this patient lacked auditory brainstem response (ABR) in both ears while the distortion product otoacoustic emission (DPOAE) was present. At age 1-year–2-month, the affected proband underwent left-side cochlear implantation (CI). Three years after CI, the ANSD subject is enrolled in regular school with good post-CI outcome, similar to our previous report (6). Consistent with this phenotype, bi-allelic candidate variants in *OTOF,* a gene associated with ANSD, were identified by targeted NGS. Interestingly, four different variants in *OTOF* were identified in this patient. Among them, c.4961–3C>G and p.R49W have been previously reported to be associated with non-syndromic deafness (2, 7) while c.1366_1367insC and p.Y455F were novel. c.4961–3C>G and p.R49W, however, were inherited from the same maternal allele, suggesting that only one of them should be pathogenic. Similarly, c.1366_1367insC and p.Y455F were inherited from the same paternal allele. Considering c.1366_1367insC introduces a frameshifting variant in *OTOF,* in which many similar truncating mutations have been well documented, the former is probably the true pathogenic mutation while the latter is likely a coincidental benign variant. In accordance with the report by He et al. (9), our data suggested that targeted NGS accompanied by parental genotyping provides a simple but effective step towards minimizing the false-positive results.

Two novel variants in *POU3F4,* p.C233X and p.V321G, were identified in Family NT–42 and NT–43, respectively. In family NT–42, the p.C233X mutation was identified in two male patients NT42-Ⅱ:1 and NT42-II:5. Both patients had congenital severe-to-profound sensorineural hearing loss (Supplementary file 4). Similarly, proband NT–43-III:2 also exhibited severe sensorineural deafness. Female mutation carriers in the two families had completely normal hearing. Temporal bone Computed tomography images of the three patients revealed characteristic anomalies for IP3 with an increased risk of gusher during CI surgery (Supplementary file 5). Patient NT–42-IV:1 received right-side CI at the age of one year and two months. As expected, Cerebrospinal fluid gusher was seen while no complications related to surgery were observed. Three years after CI, the patient was enrolled in regular school with good CI outcome. Patient NT–43-III:2 showed a slight progression in hearing loss after 3-years of follow-up. The patient used a hearing aid with satisfactory effect. Our study adds two novel pathogenic *POU3F4* mutations to the literature and provides guidance toward effective genetic counseling for these two families.

Mutations in *CDH23* and *PCDH15* may lead to both NSHL (DFNB12 and DFNB23, respectively) and Usher syndrome type 1 (USH1D and USH1F, respectively) characterized by both congenital hearing loss and childhood retinitis pigmentosa (5, 10, 11). Proband NT–44-Ⅱ:1 carried a homozygous c.9469_9470insGT mutation in *CDH23.* The parents of this patient, though not consanguineously married, each carried a heterozygous mutation and are likely distally related. Proband NT–44-Ⅱ:1 was 11 months old at the time of test when congenital profound sensorineural HL was diagnosed. At the age of 13 months, the patient underwent right-side CI. Followed up until four years old, the patient showed good speech and language recognition. Though no ophthalmologic abnormalities were observed, we cannot definitely rule out the possibility that this young patient may develop retinopathy later in life. In combination with our previous study (5), we identified a relatively high prevalence (4/22) of *CDH23* mutations in Chinese Han deaf patients, and our reports of these novel mutations expanded the *CDH23* mutation spectrum. For family NT–45, the two affected

siblings NT45-□:1 and NT45-□:2 carried compound heterozygous mutations p.P1437L/p.1752_1760del in *PCDH15.* The two patients, aged 39 and 49,respectively, at the time of the test, showed slowly progressive and moderate hearing loss with onset between 25 and 30 years of age. Troublesome tinnitus was also reported for both, while no ophthalmologic abnormalities were observed, supporting that mutations in *PCDH15* cause not only Usher syndrome type 1F but also DFNB23(10,11).

In family NT–46, we detected compound heterozygous mutations p.R3804Q/c.13893+8T>G in *ADGRV1,* which segregated with hearing loss in this family. Both mutations are novel. The age of two affected siblings NT–46-□:3 and NT–46-□:6 were 67 and 61, respectively. Both patients experienced moderate and slowly progressive hearing loss with onset between 30 and 35 years and suffered from tinnitus. No signs of visual or vestibular disorder were observed. Mutations in *ADGRV1* may result in Usher syndrome 2C, which is characterized by congenital moderate-to-severe hearing loss, retinal degeneration in the second decade of life or later, and normal vestibular function(12). To date, only two previous reports have associated this gene for NSHL(2, 4), but the affected patients in those reports may be too young to present signs of retinal degeneration and vestibular dysfunction. In contrast, our cases had milder and progressive HL, yet at the age of over 60 did not have any vision problems, which may further validate *ADGRV1* in association with NSHL. For Family NT–47, we identified novel compound heterozygous mutations p.525_533delDQERGRALLinsV/p.R164W in *PDZD7.* The previous report has associated *PDZD7* mutations with digenic Usher syndrome(13) and DFNB57(14, 15). To our knowledge, our study is the third report to identify *PDZD7* as a causative gene for autosomal recessive non-syndromic hearing loss (ARNSHL) in the Chinese population(14, 15). Patient NT–47-□:1 failed the newborn hearing screening by automated auditory brainstem response (AABR) and had congenital moderate sensorineural hearing loss confirmed by ABR and audio steady-state response (ASSR) at two years of age. After a 3-year follow-up, patient NT46-□:2 had no signs of visual or vestibular disorder and no obvious progression in hearing loss at the age of 5. The patient was enrolled in a regular school with hearing aids. For Family NT–48, novel compound heterozygous mutations p.Y229H and p.D135 N were identified in *KARS,* which encodes lysyl-tRNA synthetase (LysRS), as the only candidate causative variants(16). The affected Individual NT–48-□:5 was a 47 years old female with severe hearing impairment affecting primarily higher frequencies. She experienced progressive hearing loss with onset between 10 and 15 years of age. No other systemic abnormalities were detected.

We also ascertained two Chinese families with an autosomal dominant form of progressive NSHL. For Family NT–49, we identified a novel missense p.T197I mutation in*MYO6.* Mutations in *MYO6* have been associated with dominant and recessive non-syndromic hearing loss DFNA22 and DFNB37 (17, 18). The hearing loss in affected members of Family NT–49 was progressive, mid-life onset, and mild to severe, affecting high frequencies to the greatest degree. The hearing impairment gradually progressed to all frequencies later and eventually became severe in the seventh decade. For Family NT–50, A nonsense p.R426X mutation in *GHRL2* was found responsible for autosomal dominant hearing loss DFNA28. To date, only two mutations in *GRHL2* have been described (19, 20). Phenotypic characterization of Family NT–50 shows that the p.R426 X mutation in *GRHL2* resulted in progressive, bilateral hearing loss with a typical onset in middle adulthood, which was consistent with the phenotype reported for the other two DFNA28 families(19, 20). Our clinical data supported the emerging genotype-phenotype correlation for DFNA22 and DFNA28.

In this study, our targeted NGS analysis identified mutations in nine rare deafness genes in the aforementioned 10 families. In recent years, whole-exome sequencing (WES) has become a powerful tool for both new gene discovery and molecular diagnosis in hereditary hearing loss(3, 4). Here we used proband-WES approach to successfully identify novel compound heterozygous mutations p.G145S/c.2117–6C>T in *OTOG* and a homozygous p.K22R mutation in *GRXCR2* in Family NT–51 and NT–52, respectively. To our knowledge, this is the first reported *OTOG* mutation associated with hearing loss in China(21, 22). In Family NT–51, the patient had experienced progressive and steeply sloping high-frequency hearing loss without any vestibular impairment. She also reported troublesome tinnitus. *GRXCR2* mutations are rare causes of recessive deafness as there is only one report worldwide (23). The 72-year-old proband in NT–52 had a moderate sensorineural hearing loss affecting primarily high frequencies, resulting in a downsloping audiometric configuration. Her hearing loss started during her mid–40s and followed by steady and gradual progression. The proband had a less severe hearing loss as compared to the previous study(23), suggesting a variable genotype-phenotype correlation.

# Conclusion

In this report, we performed a comprehensive mutation screening by targeted NGS or WES in twelve Chinese families with NSHL. Our results revealed a number of novel or recurrent mutations in rare deafness genes and supported the heterogeneity of the genetic and phenotypic spectrum of NSHL in Chinese Hans. Our study also showed that combining NGS-based molecular diagnosis and detailed clinical evaluation can achieve a more accurate diagnosis for NSHL patients.

# Abbreviations

NSHL: Non-syndromic hearing loss; NGS: Next-generation sequencing; WES: whole-exome sequencing; IP3: Incomplete partition type3; IAC: the internal auditory canal; ANSD: auditory neuropathy spectrum disorder; ABR: auditory brainstem response; DPOAE: the distortion product otoacoustic emission; CI: cochlear implantation

# Declarations

*Competing interests*

The authors declare that they have no competing interests.

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*Authors' contributions*

LPZ conceived and designed the experiments. DW, WYH, SL, J Z, XHC, YT, JHQ, ZXW, and ZHX contributed to the gathering and interpretation of clinical data. DW and XCD performed NGS screening and bioinformatics

analyses. LPZ acted as a head surgeon for cochlear implantation performed in this study. All authors read and approved the final manuscript and collected data.

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*Data Availability Statement*

The data relating to the findings of this study are available from the corresponding author.

*Ethics approval and consent to participate*

Approval was obtained from the Ethics Committee of the Affiliated Hospital of Nantong University and written informed consent was obtained from each studied family member.

*Consent for publication*

Consent for publication of individuals' details were obtained from each subject or their family members.

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