Research Article

Identification of TMPRSS3 as a Significant Contributor to Autosomal Recessive Hearing Loss in the Chinese Population

Xue Gao,1,2 Sha-Sha Huang,1 Yong-Yi Yuan,1 Jin-Cao Xu,2 Ping Gu,3 Dan Bai,4 Dong-Yang Kang,1 Ming-Yu Han,1 Guo-Jian Wang,1 Mei-Guang Zhang,2 Jia Li,2 and Pu Dai1

1Department of Otolaryngology, Head and Neck Surgery, PLA General Hospital, No. 28, Fuxing Road, Beijing 100853, China
2Department of Otolaryngology, The General Hospital of the PLA Rocket Force, No. 16, XinWai Da Jie, Beijing 100088, China
3Department of Otorhinolaryngology, Shenzhen Children’s Hospital, No. 7019, Yitian Road, Shenzhen 518026, China
4Department of Otolaryngology, Xi’an Medical College, No. 1, XinWang Road, Wei yang qu, Xi’an 710021, China

Correspondence should be addressed to Pu Dai; daipu301@vip.sina.com

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Hereditary hearing loss is characterized by a high degree of genetic heterogeneity. Mutations in the TMPRSS3 (transmembrane protease, serine 3) gene cause prelingual (DFNB10) or postlingual (DFNB8) deafness. In our previous study, three pathogenic mutations in TMPRSS3 were identified in one Chinese family. To evaluate the importance of TMPRSS3 mutations in recessive deafness among the Chinese, we screened 150 autosomal recessive nonsyndromic hearing loss (ARNSHL) families and identified 6 that carried seven causative TMPRSS3 mutations, including five novel mutations (c.809T>A, c.1151T>G, c.1204G>A, c.1244T>C, and c.1250G>A) and two previously reported mutations (c.323-6G>A and c.916G>A). Each of the five novel mutations was classified as severe, by both age of onset and severity of hearing loss. Together with our previous study, six families were found to share one pathogenic mutation (c.916G>A, p.Ala306Thr). To determine whether this mutation arose from a common ancestor, we analyzed six short tandem repeat (STR) markers spanning the TMPRSS3 gene. In four families, we observed linkage disequilibrium between p.Ala306Thr and STR markers. Our results indicate that mutations in TMPRSS3 account for about 4.6% (7/151) of Chinese ARNSHL cases lacking mutations in SLC26A4 or GJB2 and that the recurrent TMPRSS3 mutation p.Ala306Thr is likely to be a founder mutation.

1. Introduction

Hearing impairment is a very common sensory disorder, affecting 1 of 500–650 newborns [1, 2]. Genetic factors contribute to approximately 60% of congenital sensorineural hearing loss cases. Nonsyndromic hearing loss (NSHL), in which hearing impairment is the only obvious clinical abnormality, accounts for 70% of genetic cases. To date, more than 200 genetic loci have been mapped, and 100 deafness genes have been identified (http://hereditaryhearingloss.org/). Autosomal recessive nonsyndromic hearing loss (ARNSHL) is the most common type and accounts for ~80% of cases of inherited hearing loss.

Individuals with mutations in TMPRSS3 (transmembrane protease, serine 3) present with two different phenotypes: DFNB10-associated hearing impairment that is prelingual (OMIM 605511) and DFNB8-associated hearing impairment that is typically late onset and postlingual (OMIM 601072). TMPRSS3 mutations can be divided into mild or severe, and the hearing phenotype is dependent upon the combination of two TMPRSS3 mutations. The combination of two severe mutations leads to prelingual, profound hearing loss, whereas severe mutations in combination with mild mutations lead to a milder phenotype with postlingual-onset hearing loss [3, 4]. The genetic load of TMPRSS3 in ARNSHL varies with ethnicity but is commonly a responsible gene in several populations. The frequency of TMPRSS3 mutations in childhood ARNSHL cases was 12% (3/25) in Turkish families negative for GJB2 mutations [5]; 13.1% (5/38) in Slovenian ARNSHL patients negative for...
GJB2, GJB6, and mitochondrial A1555G mutations [6]; and 0.45% (2/448) in a European population with childhood deafness negative for the GJB2 35delG mutation [7]. The frequency is approximately 1.8% (8/449) in the Pakistani population [8], 5% (2/39) in Tunisian families affected by profound ARNSHL [9], 2.5% (1/40) in a Korean ARNSHL study [10], and 5.9% (3/51) in a Korean ARNSHL population negative for the GJB2 mutation [11]. In some populations, GJB2 mutations were not excluded and only congenital and profound hearing loss cases were involved. In these populations, TMRRSS3 mutations might still be a significant cause of deafness.

Although several causative mutations in TMRRSS3 have been identified, little is known about the contribution of this gene to ARNSHL in the Chinese population. In a prior study, we performed targeted next-generation sequencing of 129 known deafness genes in one Chinese ARNSHL family (FH1523) and identified 3 TMRRSS3 mutations (c.36delC, c.316C>T, and c.916G>A) [12]. In this study, we screened the TMRRSS3 coding region in 150 ARNSHL families previously shown to lack mutations in GJB2 or SLC26A4 genes. In seven families, we identified two known and five novel mutations in TMRRSS3. A previously reported mutation (c.916G>A, p.Ala306Thr) is identified as founder mutation in four families. Our results suggest that mutations in TMRRSS3 are a relatively common cause for ARNSHL in the Chinese population.

### 2. Materials and Methods

#### 2.1. Ethics Statement

This study was approved by the Chinese PLA General Hospital Research Ethics Committee (Beijing, China). Fully informed written consent for participation and for publication of clinical data was obtained from each subject or from the guardians of subjects < 18 years old (yo).

#### 2.2. Clinical Data

DNA samples from the Departments of Otolaryngology and of Head and Neck Surgery, PLA General Hospital, were analyzed. The 150 affected patients originated from 150 families presenting with ARNSHL, in whom previous screening had found no mutations within the GJB2 or SLC26A4 genes. Computed tomography of the temporal bone was performed on the index patients of each family. A physical examination, otoscopy, and pure-tone audiometric examination (at frequencies from 125 to 8000 Hz) were performed to establish the diagnosis of sensorineural hearing loss. The hearing loss range was described based on pure-tone audiometry (PTA) parameters: low frequency, 125–500 Hz; mid frequency, 1–2 kHz; and high frequency, 4–8 kHz. Prelingual and postlingual hearing loss were classified by the onset age of prominent hearing loss. Prelingual hearing loss is present before speech develops and usually begins before age 3, whereas postlingual hearing loss occurs after the development of normal speech [13]. Evaluation of vestibular function included evaluation of the vestibuloocular reflex using electroneystagmography with computer analysis and saccadic, smooth-pursuit, and horizontal optokinetic nystagmus responses. Vestibular stimulation comprised rotatory and caloric tests.

#### 2.3. DNA Preparation

All of the genomic DNA was extracted from peripheral blood using a blood DNA extraction kit, according to the manufacturer's instructions (TianGen, Beijing, China).

#### 2.4. Mutational Detection and Analysis of TMRRSS3

All of the 13 exons and 100 bp of exon-intron boundaries of TMRRSS3 (NM_024022.2) were screened via Sanger sequencing of DNA from 150 index patients of ARNSHL families. Primer sequences are available upon request. To identify pathogenic mutations, cosegregation analyses were performed with the family members and with an in-house database of 481 Chinese controls with normal hearing.

#### 2.5. Multiple Sequence Alignment

Multiple sequence alignment was performed for the five novel mutations identified, using a HomoloGene package with default settings and the sequences NP_001243246.1 (H. sapiens), XP_001105841.2 (M. mulatta), XP_001137100.3 (P. troglodytes), XP_001179855.1 (B. taurus), XP_853682.3 (C. lupus), NP_001157248.1 (M. musculus), NP_001101089.1 (R. norvegicus), XP_425558.3 (G. gallus), and XP_001340422.5 (D. rerio) (http://www.ncbi.nlm.nih.gov/homologene?cmd=Retrieve&dopt=MultipleAlignment&list_uids=56985).

#### 2.6. Haplotype Analysis

DNA samples (23) from six families were haplotyped using six STR markers (D21S266, D21S1260, D21S2092, D21S1225, D21S1411, and D21S1890) within a 1 Mb region surrounding TMRRSS3 Ala306, as previously described (Table 1) [11]. Haplotype analysis was performed by direct sequencing.

### 3. Results

#### 3.1. Mutation Analysis of TMRRSS3

In our previous study of one Chinese ARNSHL family (FH1523), three disease-segregating mutations in TMRRSS3 (c.916G>A, p.Ala306Thr; c.316C>T, p.Arg106Cys; and c.36delC, p.Pro126fs) were identified and described [12]. In this study, we performed direct sequencing of TMRRSS3 in probands of another 150 Chinese ARNSHL families negative for GJB2 and SLC26A4 mutations. Among these, we identified six families carrying seven causative TMRRSS3 mutations, including five novel mutations (c.809T>A, p.Ile270Asn; c.1151T>G, p.Met384Arg; c.1204G>A, p.Gly402Arg; c.1244T>G, p.Leu413Ser; and c.1250G>A, p.Gly417Glu) and two previously reported

| STR marker | Distance to A306 |
|------------|-----------------|
| D21S266    | 1,117,653       |
| D21S1260   | 1,006,184       |
| D21S266    | 312,954         |
| Ala306     | 0               |
| D21S1260   | 97,528          |
| D21S266    | 358,798         |

Table 1: Distance of STR marker to TMRRSS3 Ala306.
pathogenic mutations (c.916G>A, p.Ala306Thr and c.323-6G>A) (Figure 1) [4, 8, 14]. The five novel mutations were all located within the catalytic serine protease domain. Analysis using Polyphen-2 software predicted them to be damaging, and they were also identified as deleterious by analysis using SIFT. These amino acid substitutions occurred in an evolutionarily conserved region (Figure 2). They had not been reported in previous studies, were not present in the ExAC database (http://exac.broadinstitute.org/), and were not seen in the 481 Chinese controls with normal hearing. Combining the results of this and our previous study, the frequency of TMPRSS3 mutations found in Chinese ARNSHL families was 4.6% (7/151). The most prevalent mutation was c.916G>A (p.Ala306Thr) at 2% (6/302), accounting for 47% of all TMPRSS3 mutations.

3.2. Haplotype Analysis. To determine whether the c.916G>A (p.Ala306Thr) mutation found in families FH1523, 6932, 8082, 8961, 6519, and 10706 was derived from a common founder, we performed linkage analysis using 6 STR markers in 23 DNA samples (Figure 1, (A)). In four families, we observed linkage disequilibrium between p.Ala306Thr and one STR marker (D21S1260) within a 97 kb interval, while in two of the four families, we found linkage disequilibrium between p.Ala306Thr and two STR markers (D21S1260 and D21S266) within a 359 kb interval, suggesting that p.Ala306Thr probably arose from a common founder, as was previously reported in a Korean population [11].

3.3. Clinical Characteristics and Genotype-Phenotype Correlations. Table 2 summarizes clinical characteristics of 14 patients from seven ARNSHL families with TMPRSS3 mutations including onset age, audiogram configuration, progression of hearing loss, and vestibular symptoms. Age at onset ranged from newborn to 40 years, although the majority of patients showed evidence of deafness in childhood, at 3 to 6 years of age. None of the patients had vestibular symptoms. Examining the types of hearing loss identified some correlations between genotype and phenotype (Figure 1B and C)). Hearing loss in 10706-II:1 (male/8 yo) and 10706-II:2 (female/3 yo) (Figure 1) was diagnosed at 2 yo, and their hearing loss progressed rapidly with age. An audiogram of 10706-II:1 at 8 yo was flat with an average PTA of 90-decibel hearing level (dB HL), whereas that of 10706-II:2 at 3 yo showed a ski-slope loss with an average PTA of 85 dB HL. 10706-II:2 underwent cochlear implantation at age 3, and her language ability improved after surgery. The proband and his affected sister were compound heterozygotes for c.916G>A (p.Ala306Thr) and one novel mutation c.1250G>A (p.Gly417Glu). In family 6519 (Figure 1), c.916G>A (p.Ala306Thr) was detected in patient I:1 in the heterozygous state. The patient’s mother with normal hearing was also heterozygous for the mutation. No other candidate mutation in the coding region of TMPRSS3 was detected. Hearing loss in 6519-II:1 (Figure 1, male/3 yo) was initially detected at age 3, with a ski-slope audiogram and a normal threshold at 250 Hz; the average threshold at 500 Hz to 4 kHz was 55 dB HL. We speculated that a gene copy number variation might exist, that a mutation may lie within a noncoding region, or that other deafness genes were responsible. Hearing loss in 6932-II:1 (Figure 1, female/17 yo) was initially detected by the age of 9 yo with a ski-slope audiogram. The threshold at 125 and 250 Hz was 10 dB HL and that at 2 Hz to 8 kHz was greater than 100 dB HL. The patient complained of slowly progressing hearing loss with age. She underwent cochlear implantation at age 14, and her language ability improved after surgery. Patient 6932-II:1 was a compound heterozygote for c.916G>A (p.Ala306Thr) and c.323-6G>A; both mutations were previously reported. The splice site mutation c.323-6G>A was reported to be pathogenic in Dutch and Pakistani patients [3, 8]. However, there was inconsistency in this classification for the c.323-6G>A (p.Cys107fs) mutation. In
A mutation was (relatively) severe and homozygous mutation results in prelingual (DFNB10) hearing impairment. However, a homozygous c.323-6G>A mutation was described by Veske et al. (1996) to be the underlying cause of postlingual (DFNB8) hearing impairment. In this study, considering the hearing phenotype of 6932-II:1, we classified c.323-6G>A as a mild mutation.

Figure 2: Conservation analysis of TMPRSS3 mutations and genomic structure of TMPRSS3 based on the open reading frame (NM_024022.2). Protein alignment showing conservation of residues TMPRSS3 Ile270, Met384, Gly402, Leu415, and Gly417 across nine species. All mutations occurred at evolutionarily conserved amino acids or areas (in red box) in trypsin-like serine protease domain. TM: transmembrane domain; LDLRA: LDL receptor-like domain; SRCR: scavenger receptor cysteine-rich domain; serine protease: trypsin-like serine protease domain.

Table 2: Clinical features and TMPRSS3 mutation combinations of affected family members identified in the present study and our previous study.

| Family number | Mutation 1 | Mutation 2 | Patient number | Phenotype | Age of onset | Progression | Vertigo |
|---------------|------------|------------|----------------|-----------|--------------|-------------|---------|
| FH1523        | c.36delC   | c.916G>A   | 1              | Downsloping audiogram configuration with impairment of the low frequencies except 125 Hz at 3 years of age | Newborn  | Yes | No |
|               | (p.Pro12fs)| (p.Ala306Thr) |               |           |              |             |         |
|               | c.316C>T   | c.916G>A   | 5              | Downsloping audiogram configuration with normal threshold of the low frequencies at 50 years of age | 20–30 yo | Yes | No |
|               | (p.Arg106Cys)| (p.Ala306Thr) |               |           |              |             |         |
| 6519          | —          | c.916G>A   | 1              | Moderate slope audiogram configuration with normal threshold of 250 Hz at 3 years of age | 3 yo | Yes | No |
| 6932          | c.323-6G>A | c.916G>A   | 1              | Downsloping audiogram configuration with normal hearing threshold of 125 Hz and 256 Hz at 14 years of age | 9 yo | Yes | No |
| 8082          | c.809T>A   | c.916G>A   | 1              | Downsloping audiogram configuration with impairment of the low frequencies at a very young age | 3 yo | Yes | No |
|               | (p.Ile270Asn)| (p.Ala306Thr) |               |           |              |             |         |
| 10706         | c.1250G>A  | c.916G>A   | 2              | Flat audiogram configuration with thresholds of about 90 dB at 8 years of age | 2 yo | Yes | No |
|               | (p.Gly417Glu)| (p.Ala306Thr) |               |           |              |             |         |
| 8961          | c.1204G>A  | c.916G>A   | 1              | Flat audiogram configuration with average PTA is are over 90 dB at 6 yo | 2 yo | Yes | No |
|               | (p.Gly402Arg)| (p.Ala306Thr) |               |           |              |             |         |
| 234           | c.1151T>G  | c.1244T>C   | 2              | Downsloping audiogram configuration with impairment of the low frequencies at 35 yo | 3 yo | Yes | No |
|               | (p.Met384Arg)| (p.Leu415Ser) |               |           |              |             |         |
Hearing loss in 8082-II:1 (Figure 1, female/5yo) was initially detected at 5yo with a ski-slope audiogram. Her parents had noticed occasional poor hearing since she was 3yo. At age 5, she showed severe hearing loss at 1–8 kHz and moderate hearing loss at 250–500 Hz, with a normal threshold at 125 Hz. Thereafter, the hearing loss showed slow progression, with annual threshold deteriorations of 5–8 dB HL at 500 Hz and 2.5–5 dB HL at 1–8 kHz, according to a recent audiogram at 7yo. She is now wearing hearing aids. Patient 8082-II:1 was a compound heterozygote for c.916G>A (p.Ala306Thr) and the novel mutation c.809T>A (p.Ile270Asn). The novel missense mutation c.809T>A is located within exon 9 and causes an amino acid substitution from isoleucine to asparagine at position 270, which is close to the active site histidine (H257), and therefore affects the activity of the enzyme. Hearing loss of 8891-II:1 (Figure 1, male/6yo) was initially detected by the age of 2, with the auditory brainstem response (ABR) threshold for both ears significantly elevated to 97 dB HL. According to the parent’s description, hearing loss progressed slowly. A recent audiogram at 6yo was flat, and the average PTA was more than 90 dB HL. Patient 8961-II:1 was a compound heterozygote for c.916G>A (p.Ala306Thr) and the novel mutation c.1204G>A (p.Gly402Arg). Hearing loss in M234-II:1 (female/35yo) and M234-II:3 (male/27yo) (Figure 1) was detected at 3yo, and their hearing loss progressed rapidly with advancing age. An audiogram of M234-II:3 at 27yo was flat, and the average PTA was more than 90 dB HL, while that of M234-II:1 at 35yo was a ski-slope, with an average PTA of 75 dB HL. The two patients carried two of the novel mutations as compound heterozygotes: c.1151T>G (p.Met384Arg) and c.1244T>C (p.Leu415Ser). Family FH1523 has previously been described [12]. According to the onset and severity of hearing loss, the five novel missense mutations were classified as severe mutations, while c.323-6G>A was probably a mild mutation.

4. Discussion

The function of TMPRSS3 is very important to the auditory system; it has also been identified as a tumor-associated gene that is overexpressed in pancreatic, ovarian, and breast tumors [15–17]. In 2001, Scott et al. showed that TMPRSS3 was mutated in nonsyndromic autosomal recessive deafness (DFNB8/10) and is associated with both congenital and childhood-onset forms [14]. TMPRSS3 contains a transmembrane domain, a low-density lipoprotein receptor class A domain, a scavenger receptor cysteine-rich domain, and a trypsin-like serine protease domain (NP_001243246.1). TMPRSS3 is expressed in spiral ganglion neurons, inner hair cells, supporting cells, and stria vascularis of the rat cochlea [18]. It plays an important role in activating the ENaC sodium channel, which is regulated by serine protease activity [19], and maintains a low Na⁺ concentration in the endolymph of the inner ear [3, 18, 20].

The typical ski-slope audiogram configuration in ARNSHL is suggestive of TMPRSS3 involvement, with a hearing phenotype and inheritance similar to those of SLC26A4. Our results indicate that TMPRSS3 mutations account for about 4.6% (7/151) of ARNSHL in Chinese patients negative for GJB2 and SLC26A4 mutations, an incidence similar to that seen in Korean and Tunisian populations [9, 11]. It has been reported that mutations in different domains of TMPRSS3 result in various hearing impairment phenotypes, likely due to the distinct influence on protease activity of different mutations [3, 5, 7–11, 14, 18, 21–29]. Lee et al. proposed that disruption of the proteolytic activity of TMPRSS3 is tightly correlated with the pathogenesis of hearing loss and predicted that mutations in the SRCR and LDLRA domains affect the proper folding or assembly of the catalytic domain or alter protease substrate recognition and binding [3].

TMPRSS3 mutations can be classified as mild or severe, and the hearing phenotype is dependent on the combination of the two TMPRSS3 mutant alleles. Compound heterozygosity for a mild and severe mutation leads to postlingual hearing loss (DFNB8), whereas the combination of two severe mutations leads to profound hearing impairment with prelingual onset (DFNB10) [4]. Apparently, genotype-phenotype correlations can be drawn based on the position or the truncating/nontruncating nature of the TMPRSS3 mutations.

Our study expanded the mutation spectrum of TMPRSS3. Table 3 summarizes the type, position, origin, and mutation classification of the 39 TMPRSS3 mutations reported to date, which are associated with ARNSHL in more than 15 ethnic groups worldwide. Eleven mutations were truncating and were predicted to lead to a prematurely terminated protein product or to nonsense-mediated decay of the mRNA, while another 28 TMPRSS3 mutations were missense mutations leading to single amino acid substitutions. Almost all of the mutations were predicted to disrupt the proteolytic activity of the protein. The hearing impairment in these families was prelingual or postlingual, mostly with a typical ski-slope audiogram configuration. Consistent with previous reports, the TMPRSS3 mutations identified in this study were associated with progressive hearing loss with considerable variability in the age of onset and degree of severity and this variability in hearing phenotype was both interfamilial and intrafamilial. The mutation summary data (Table 3) shows that not only protein-truncating mutations (frameshift, stop codon, and splice site mutations) but also missense mutations, particularly those located within the catalytic serine protease domain or close to the active site, have severe effects.

We observed that four combinations of TMPRSS3 mutations resulted in prelingual, profound hearing impairment: c.1250G>A (p.Gly417Glu) and c.916G>A (p.Ala306Thr), c.1204G>A (p.Gly402Arg) and c.916G>A (p.Ala306Thr), c.809T>A (p.Ile270Asn) and c.916G>A (p.Ala306Thr), and c.1151T>G (p.Met384Arg) and c.1244T>C (p.Leu415Ser). The combination of c.323-6G>A and c.916G>A (p.Ala306Thr) was manifested by postlingual, milder hearing impairment. Our data suggest that the five novel missense mutations identified in this study have relatively severe effects. TMPRSS3 c.916G>A (p.Ala306Thr), which was identified in five families from this study and one family from a previous study, is a pathogenic mutation in German, Dutch,
| Mutation | Protein change | Exon | Domain | Origin | Mutation classification | Reference |
|---------|----------------|------|--------|--------|-------------------------|-----------|
| c.323-6G>A | p.Ile270Asn | E9 | Serine protease | Chinese | Severe | Present study |
| c.809T>A | p.Ala306Thr | E9 | Serine protease | Korean | Severe | Present study |
| c.1151T>G | p.Met384Arg | E9 | Serine protease | Chinese | Severe | Present study |
| c.607C>T | p.Arg216Cys | E8 | SRCR | Pakistani | Severe | Miyagawa et al., 2013 |
| c.595G>A | p.Val199Met | E6 | SRCR | Dutch | Severe | Weegerink et al., 2011 |
| c.782+8insT | p.Arg216Cys | E8 | SRCR | Turkish | Severe | Elbracht et al., 2007 |
| c.413C>G | p.Ala138Glu | E5 | SRCR | British | Mild | Weegerink et al., 2011 |
| c.753G>C | p.Cys194Phe | E6 | SRCR | Palestinian | Severe | Ahmed et al., 2004 |
| c.646C>T | p.Arg216Cys | E8 | SRCR | German | Mild | Elbracht et al., 2007 |
| c.726C>G | p.Arg216Leu | E8 | SRCR | Turkish | Severe | Wattenhofer et al., 2005 |
| c.743C>T | p.Thr248Met | E8 | SRCR | Korean | Mild | Chung et al., 2014 |
| c.726C>G | p.Arg216Leu | E8 | SRCR | Turkish | Severe | Scott et al., 2001 |
| c.703G>C | p.Trp251Cys | E8 | SRCR | Tunisian | Severe | Masmoudi et al., 2001 |
| c.595G>A | p.Val199Met | E6 | SRCR | Dutch | Severe | Weegerink et al., 2011 |
| c.607C>T | p.Arg216Cys | E8 | SRCR | Turkish | Severe | Elbracht et al., 2007 |
| c.413C>G | p.Ala138Glu | E5 | SRCR | British | Mild | Weegerink et al., 2011 |
| c.753G>C | p.Cys194Phe | E6 | SRCR | Palestinian | Severe | Ahmed et al., 2004 |
| c.595G>A | p.Val199Met | E6 | SRCR | Dutch | Severe | Weegerink et al., 2011 |
| c.607C>T | p.Arg216Cys | E8 | SRCR | Turkish | Severe | Elbracht et al., 2007 |
| c.413C>G | p.Ala138Glu | E5 | SRCR | British | Mild | Weegerink et al., 2011 |
| c.753G>C | p.Cys194Phe | E6 | SRCR | Palestinian | Severe | Ahmed et al., 2004 |
| c.595G>A | p.Val199Met | E6 | SRCR | Dutch | Severe | Weegerink et al., 2011 |
| c.607C>T | p.Arg216Cys | E8 | SRCR | Turkish | Severe | Elbracht et al., 2007 |
and Korean deaf patients [4, 10, 11, 21], indicating that this mutation is the main contributor to the DFNB8/DFNB10 phenotype in many ethnicities. The proposal by Chung et al. that p.Ala306Thr could be a "founder mutation" was supported by the observation of linked haplotypes of STR markers segregating with hearing loss in two families [11]. Our haplotype analysis of six families showed linkage disequilibrium in four of them. Therefore, we propose that TMPRSS3 c.916G>A (p.Ala306Thr) is likely to be a founder mutation in the Chinese population. Combining the data from this study and our previous work, we determined that mutations in TMPRSS3 are a pathogenic cause of deafness in 7 of 151 (4.6%) Chinese families with ARNSHL. To the best of our knowledge, this is the first study to investigate the etiological contribution of TMPRSS3 to deafness in a Chinese population.

This study had two minor limitations. First, there are no precise criteria for classifying mutations as severe or mild; therefore, we drew our own conclusions based only on the age of onset and severity of hearing loss. Second, we did not test for copy number variants in the samples; this remains an area for a future study.

In summary, combined with our previous study, we have described the clinical and genetic characteristics of seven Chinese families with ARNSHL carrying causative TMPRSS3 mutations, resulting in the TMPRSS3 mutation spectrum to be reported in a Chinese ARNSHL population for the first time. This should have an important impact on clinical patient management, genetic counseling, molecular diagnosis, and the development of advanced therapeutic strategies.

Additional Points

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see http://www.textcheck.com/certificate/33mfci.

Disclosure

Xue Gao, Sha-Sha Huang, and Yong-Yi Yuan are listed as co-first authors. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Xue Gao, Sha-Sha Huang, and Pu Dai conceived the study, participated in its design, and drafted the manuscript. Yong-Yi Yuan, Jin-Cao Xu, Mei-Guang Zhang, and Jia Li participated in the data analysis. Guo-Jian Wang, Ping Gu, Dan Bai, Dong-Yang Kang, and Ming-Yu Han participated in the collection of clinical data and blood samples. All authors read and approved the final manuscript. Xue Gao, Sha-Sha Huang, and Yong-Yi Yuan are listed as co-first authors.

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