Original Article

A *POU3F4* Mutation Causes Nonsyndromic Hearing Loss in a Chinese X-linked Recessive Family

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Abstract

Background: The molecular genetic research showed the association between X-linked hearing loss and mutations in *POU3F4*. This research aimed to identify a *POU3F4* mutation in a nonsyndromic X-linked recessive hearing loss family.

Methods: A series of clinical evaluations including medical history, otologic examinations, family history, audiologic testing, and a high-resolution computed tomography scan were performed for each patient. Bidirectional sequencing was carried out for all polymerase chain reaction products of the samples. Moreover, 834 controls with normal hearing were also tested.

Results: The pedigree showed X-linkage recessive inheritance pattern, and pathogenic mutation (c.499C>T) was identified in the proband and his family member, which led to a premature termination prior to the entire POU domains. This mutation co-segregated with hearing loss in this family. No mutation of *POU3F4* gene was found in 834 controls.

Conclusions: A nonsense mutation is identified in a family displaying the pedigree consistent with X-linked recessive pattern in *POU3F4* gene. In addition, we may provide molecular diagnosis and genetic counseling for this family.

Key words: c.499C>T; Nonsyndromic Hearing Loss; *POU3F4*; X-linked

Introduction

Hearing loss is one of the common sensory disorders in humans, affecting 1 in 1000 newborns. More than one half of it may attribute to genetic factors. Among them, it showed that hearing loss is transmitted with a heredity form concurrent with autosomal recessive (75%), dominant (20%), X-linked (3%), and others (2%). So far, five loci (DFNX1, DFNX2, DFNX3, DFNX4, and DFNX6) and four genes (*PRPS1*, *POU3F4*, *SMPX*, and *COL4A6*) have been found for X-linked hearing impairment.[1] The clinical features of DFNX3 comprise profound mixed hearing loss and vestibular problems in males.[2]

The molecular genetic research showed the association between X-linked hearing loss and mutations in *POU3F4*.[3] This gene belongs to a superfamily of POU domain transcription factors (transcription factor 4 of the POU domain, Class III), which encodes transcription factor with bipartite DNA-binding domains, a 75-amino acid POU-specific domain that is linked by 17 amino acids to a 63-amino acid homeobox domain. Both subdomains contain helix-turn-helix motifs that directly associate with the two components of bipartite DNA-binding sites. Animal models also showed that the expression of *Brn-4* (*POU3F4* synonyms) was highly associated with remodeling of the otic capsule.[4]

There are 52 variants reported in the Human Gene Mutation Database, including intragenic mutations, complete or partial deletions, duplications, inversions, and other chromosomal deletions.[5] To identify the mutation in the *POU3F4* gene, in this study, we performed the analysis in a Chinese nonsyndromic X-linked hereditary hearing loss family.

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Methods

Clinical evaluation

The proband, a 10-year-old boy, presented to the otolaryngology clinic with genetic severe deafness and speech delay. His family history was obtained by his mother. He was subjected to detailed clinical evaluations including medical history, family history, otologic examinations, audiologic testing (pure-tone audiometry, tympanometry, acoustic reflex, auditory brainstem response, and 40 Hz auditory events-related potential), and high-resolution axial computed tomography (CT) scan of temporal bones. This research was approved by the collectors' Institutional Review Board of the Ethics Committee at Chinese People's Liberation Army General Hospital.

Molecular genetic analysis

After getting informed consent from all participants and parents of persons <18 years, genomic DNA was extracted from peripheral blood leukocytes of the proband (V1) and other members of family (IV1, IV2, IV3, IV4, IV5, IV6, III1) using standard procedures. Moreover, 834 controls with normal hearing were also tested. The coding region of *POU3F4* was amplified by polymerase chain reaction (PCR) with two pairs of primers. The first pair primers were a forward primer (5′-TAACCCGTGCTAGCGTCTTT-3′) and a reverse primer (5′-GAACCTGCAGATGGTGGTCT-3′) and the second pair primers were a forward primer (5′-CAACCTCTGATGAGTTGGAACA-3′) and a reverse primer (5′-AAAGGAAGAGATGGAAGGGAAG-3′). The PCR procedure was as follows: 95°C for 5 min, followed by 32 cycles of denaturation at 94°C for 45 s; annealing at 56°C for 30 s, extension at 72°C for 30 s, and a final elongation at 72°C for 7 min. All the PCR products were purified and subjected to sequencing in both directions using a DNA sequencer (ABI 3730).

Results

Clinical investigations

The five-generation pedigree presented with an X-linkage recessive inheritance pattern of hearing loss. Otomicroscopical examination showed intact tympanic membranes and no middle ear effusion in proband (V1). Moreover, pure tone audiometry indicated bilateral severe to profound deafness, but bone hearing thresholds were normal at 250 Hz. Further, high-resolution axial CT scan revealed a dilatation of the lateral end of the internal auditory meatus and a deficit or absence in the basal turn of the cochlea [Figure 1].

Identification of the mutation in *POU3F4* gene

Variant analysis of the *POU3F4* was performed for the radiologic results. Sequence screening of all the family members showed a nonsense mutation at nucleotide position c.499C>T in the proband (V1) and his uncle with hearing loss phenotype (IV3). The proband's mother (IV1), one aunt (IV2), and grandmother (III1) carried the c.499C>T mutation without hearing loss phenotype. The proband's other two aunts (IV4, IV5) and another uncle (IV6) showed normal hearing without any mutation. No mutation of *POU3F4* gene was found in 834 controls. The location of *POU3F4* gene on the X chromosome and the variations found on this gene were shown in Figure 2.

Discussion

In recent years, studies have performed on *POU3F4* gene mutations in different countries. Until now, mutations in this gene have been identified containing missense mutations, in-frame deletions, and truncating mutations [Table 1].

This is the first study to identify a nonsense mutation c.499C>T in *POU3F4* gene in Asians. So far, the most identified mutations of *POU3F4* gene are located in nucleotide poison-encoding POU domain.[4,5,9,18] The

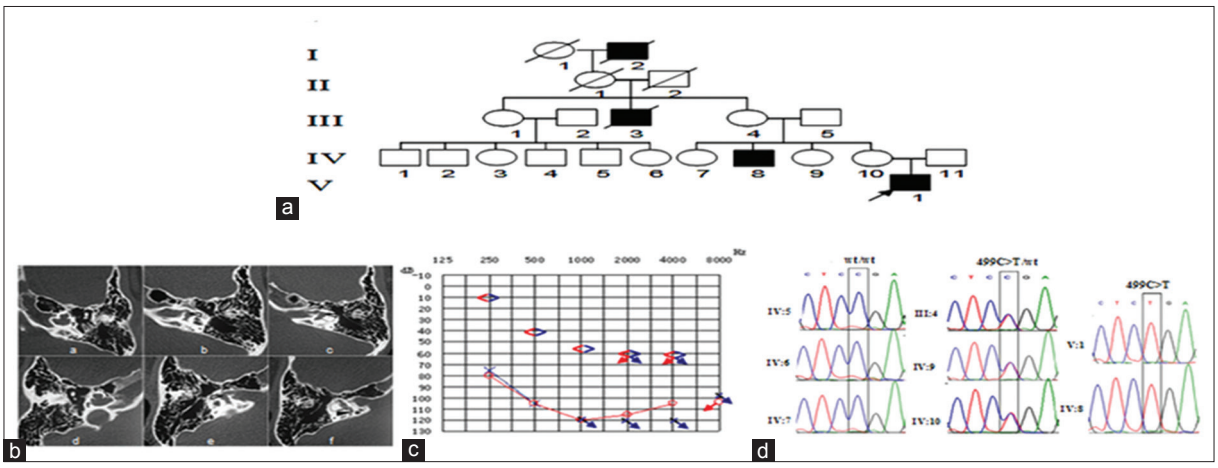

Figure 1: The clinical features and genetic testing results of the proband and the family member. (a) Pedigree of the family. The incidence pattern of the pedigree shows X-linked hereditary; (b) The imaging tests of the proband. The high-resolution axial computed tomography reveals a dilatation of lateral end of the internal auditory meatus and a deficit or absence in the basal turn of the cochlea; (c) Audiograms of the proband. The pure tone audiometry shows belated severe to profound hearing loss, but bone hearing thresholds was normal (10 dB) at 250 Hz; (d) Sequencing chromatograms of POU3F4. The molecular testing result of proband and so family members.

c.499C>T mutation changes an arginine (CGA) to a stop code (ATG), which leads to a premature termination prior to the entire POU domains in amino acid level.[17] This mutation co-segregates with hearing loss in this family. Male members carried c.499C>T mutation. However, females with this mutation are normal hearing. Therefore, we consider that this mutation may be the cause of disease etiology of the pedigree. By molecular testing, we provided definitive diagnosis and genetic counseling for this family and further enriched pathogenic mutation spectrum of *POU3F4* gene.

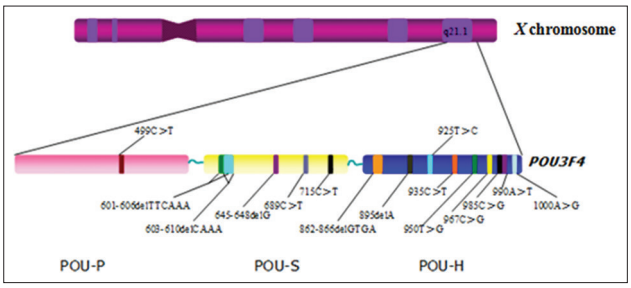

Figure 2: The location of *POU3F4* gene on the X chromosome and the variations found on this gene.

Pedigree analysis is an important approach to search the underlying cause of hearing loss. However, more patients with hearing loss were sporadic and there is a lack of characteristic phenotype in clinical practice. Hence, using all the available phenotypic clues to direct molecular testing would be cost-effective. The clinical features of CT images of the temporal bones are now well recognized through reported cases.[11,21,23,24] Besides the characteristics of CT images, audiological phenotype of DFNX3 is defined as profound deafness with or without a conductive element.[7,14,19,25] The conductive component in

Table 1: The genotype distribution of *POU3F4* gene mutations

Year	Nucleotide	Protein	Authors	Reference
1988	c.715C>T	p.S228L	Brunner *et al*.	[6]
1995	c.604A>T	p.K202X	De Kok *et al*.	[3]
1995	c.607_610del4	–	De Kok *et al*.	[3]
1995	c.648del1	–	De Kok *et al*.	[3]
1995	c.862_865del4	–	Bitner-Glindzicz *et al*.	[7]
1995	c.896del1	–	De Kok *et al*.	[3]
1995	c.935C>T	p.A312V	Bitner-Glindzicz *et al*.	[7]
1995	c.950T>G	p.L317W	De Kok *et al*.	[3]
1995	c.1000A>G	p.K334E	De Kok *et al*.	[3]
1997	c.689C>T	p.T230I	Friedman *et al*.	[8]
1997	c.985C>G	p.R329G	Friedman *et al*.	[8]
1997	c.990A>T	p.R330S	De Kok *et al*.	[9]
1998	c.601_606del6	–	Hagiwara *et al*.	[10]
2000	c.200G>A	p.W67X	Cremers *et al*.	[11]
2000	c.907C>T	p.P303S	Cremers *et al*.	[11]
2000	c.967C>G	p.R323G	Cremers *et al*.	[11]
2000	c.983A>C	p.N328T	Cremers *et al*.	[11]
2005	c.683C>T	p.S228L	Vore *et al*.	[12]
2006	c.925T>C	p.S309P	Wang *et al*.	[13]
2009	c.293C>A	p.S98X	Marlin *et al*.	[14]
2009	c.346del1	–	Lee *et al*.	[15]
2009	c.383del1	–	Lee *et al*.	[16]
2009	c.623T>A	p.L208X	Lee *et al*.	[16]
2009	c.923T>A	p.I308N	Marlin *et al*.	[14]
2009	c.927_929del3	–	Lee *et al*.	[15]
2009	c.986G>C	p.R329P	Lee *et al*.	[15]
2010	c.499C>T	p.R167X	Stankovic *et al*.	[17]
2010	c.647G>A	p.G216E	Li *et al*.	[18]
2011	c.341G>A	p.W114	Waryah *et al*.	[19]
2011	c.406C>T	p.Q136	Waryah *et al*.	[19]
2011	c.973T>A	p.W325R	Schild *et al*.	[20]
2013	c.235C>T	p.Q79X	Parzefall *et al*.	[21]
2013	c.632C>T	p.T211M	Choi *et al*.	[22]
2013	c.686A>G	p.Q229R	Choi *et al*.	[22]
2013	c.853_854delAT	–	Parzefall *et al*.	[21]
2013	c.950dupT	–	Choi *et al*.	[22]
2013	c.1060delA	–	Choi *et al*.	[22]
2013	c.1084T>C	p.X362R	Choi *et al*.	[22]

the audiogram could also be concerned about because of an atypical communication between an internal auditory canal and the inner ear leading to a pathologic third window which deteriorates air-conducted thresholds and increases bone-conducted thresholds. The supposed improvement in bone conduction sensitivity could be concealed by a true sensorineural deafness following the disorder.[15,26]

The proband had a profound deafness but displayed normal bone conductive hearing at 250 Hz. Based on other authors' records and our experience, it was revealed that patients with this type of audiogram of low-frequency air–bone gap increased suspicion for inner malformation. Subsequent axial CT images of the temporal bones showed the defects of the bony labyrinth in proband such as a symmetric bulbous dilatation of the internal auditory canals in both ears and partial separation of the cochlea with the fundus of internal auditory canals, which was consistent with DFNX3. It was the above clinical information that guided us to reveal the causative mutation of this family. Last but not the least, DFNX3 has already been diagnosed in molecular level, so *POU3F4* gene should be screened in the outpatient persons who have the clinical phenotypes of DFNX3.[27-29] Our study also has some limitations. On one hand, more pedigree and sporadic people should be recruited. On the other hand, new and rapid technique should be adopted to detect the patients.

In conclusion, a nonsense mutation is identified in a Chinese family displaying the pedigree consistent with X-linked recessive pattern in *POU3F4* gene. Furthermore, we may provide molecular diagnosis and genetic counseling for this family.

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Conflicts of interest

There are no conflicts of interest.

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