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A novel mutation in *POU3F4* in a Chinese family with X-linked non-syndromic hearing loss

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Abstract

Objective: Based on the clinical manifestations of a hearing loss patient, the *POU3F4* gene was tested for diagnosis of etiology.
Methods: A comprehensive physical examination was performed on the proband to exclude abnormalities of other organs, and detailed audiological testing and temporal bone CT scan were also performed. Genomic DNA was extracted using the proband's peripheral blood leukocytes. Polymerase chain reactions (PCR) were performed in the coding sequence of the *POU3F4* gene. Direct DNA sequencing was subsequently applied to screen the entire coding region of the *POU3F4* gene.
Results: The proband had severe sensorineural hearing loss. Temporal CT showed bilateral cochlear incomplete partition, vestibule dysplasia, internal auditory canal fundus expansion, and cochlear interlink with the internal auditory canal fundus. A novel mutation (c.530C > A (p.S177X)) in the *POU3F4* gene was found in this patient, creating an new stop codon and was predicted to result in a truncated protein lacking normal *POU3F4* transcription factor function.
Conclusion: Through analysis of the *POU3F4* gene and clinical manifestations in the patient, we conclude that a novel mutation may have resulted in a premature stop codon, contributing to the mutation of *POU3F4* gene.

Keywords: *POU3F4*; DFNX2; New mutation

1. Introduction

Non-syndromic hearing impairment (NSHI) is a common defect in human, and most cases of NSHI are attributable to genetic factors (Morton and Nance, 2006). The inheritance patterns of NSHI include autosomal dominant (22%), autosomal recessive (77%), X-linked and Y-linked (~1%), and mitochondrial inheritances (~1%) (Morton, 2002; Petersen et al., 2008). While most genetic NSHI is caused by mutations in autosomal genes, X-linked deafness account for approximately 1%–2% of cases of hereditary hearing loss. To date, 4 genes have been implicated in X-linked NSHI, including the *POU3F4* (de Kok et al., 1995a), *COL4A6* (Rost et al., 2014), *PRPS1* (Liu et al., 2010) and *SMPX* (Huebner et al., 2011).

X-linked deafness type 2 (DFNX2) is found in ~50% of all families carrying X-linked non-syndromic hearing loss (Petersen et al., 2008). Clinical characteristics of DFNX2 in affected males include partial hypoplasia of cochlea, enlarged internal acoustic canal and a characteristic stapes gusher upon surgery and stapes fixation (Cremers et al., 2002; de KoK et al., 1995b). Anatomical anomalies of the temporal bone revealed by computer-assisted tomography (CT) include dilatation of the lateral end of the internal acoustic canal, abnormally wide communication between the internal acoustic canal and inner ear compartment, and, sometimes, partial hypoplasia of the cochlea (Phelps et al., 1991). As a result of the widening of the internal acoustic canal, cerebrospinal fluid

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can enter the vestibule, which leads to the reported "gusher" phenomenon, described as fluid gushing out upon removal of the stapes footplate during corrective surgery (Cremers et al., 2008). Female carriers of a mutation in the DFNX2 show little or no hearing loss (Petersen et al., 2008).

DFNX2 is associated with mutation in the *POU3F4* gene (de Kok et al., 1995a). Human *POU3F4* is located on chromosome Xq21.1, and has only one exon (1491 bp) with an open reading frame (ORF) length of 1083 bp, coding 361 amino acids. *POU3F4* is a transcription factor and belongs to a superfamily of POU domain transcription factor. This domain consists of a POU-specific domain (containing 76–78 amino acids) and a POU homeodomain (containing 60 amino acids) (Andersen and Rosenfeld, 2001). POU superfamily genes are very important for organ formation and cell differentiation, and the *POU3F4* is closely associated with inner ear development (de Kok et al., 1995a). Mutation of *POU3F4* can cause hearing loss, with clinical findings in audiology and on temporal bone CT scan, and during stapes surgeries. Patients with *POU3F4* mutations can display conductive, mix or sensorineural deafness.

In this paper, we report a novel mutation in the *POU3F4* gene identified in a Chinese family carrying X-linked hereditary hearing loss and its associated clinical characteristics in affected family members.

2. Materials and methods

2.1. Subject

The proband came from Hainan province – a 9 years old boy, with a weight of 20 kg and height of 123 cm. The boy was born with hearing loss. This study was approved by the Chinese PLA General Hospital Research Ethics Committee and informed consent was obtained from the proband's parents.

2.2. Phenotype

The medical history of the proband was obtained using a questionnaire that covered the degree, age of onset, progression and symmetry of hearing loss, as well as pathological changes in the ear, infection, ototoxicity, noise exposure, and other relevant clinical manifestations to understand the otologic manifestations and to exclude any history of other diseases and environmental factors. The proband underwent a number of clinical tests including physical examinations, hearing tests, chest X-rays, brain MRI, and temporal bone CT scans. Hearing test included acoustic immittance, pure tone audiometry and auditory brainstem response (ABR).

2.3. Genetic analyses

For genetic analyses, peripheral blood was collected from the proband and his parents. All genomic DNAs were extracted using a blood DNA extraction kit following the protocol provided by the manufacturer (TianGen, Beijing, China). The coding region of *POU3F4* was amplified for direct sequencing using three sets of primers: 1) forward primer 5′-ACTTCCTGCTTGGGTCTCATTG-3′ and reverse primer 5′-GGAGTGATCCTGGCAATGGT-3′, 2) forward primer 5′-GGCACCGAACCCGTCTATC-3′ and reverse primer 5′-TCCCCTGGCGGAGTCAT-3′, 3) forward primer 5′-TTGGAGAAGGAAGTGGTGCG-3′ and reverse primer 5′-CCCAGCTTGGACTGCTTAATGTA-3′. PCR amplification was performed in a total volume of 20 μL, including 2 μL of 10 × buffer, 0.5 μL of deoxynucleotide triphosphates (2.5 mmol/L), 0.5 μL of primer L (10 μmol/L), 0.5 μL of primer R (10 μmol/L), 1 μL of DNA, 0.2 μL of Taq polymerase, and 15.3 μL of water. The PCR reaction began with incubation at 95 °C for 5 min, followed by 30 cycles of denaturation for 45 s at 95 °C, annealing for 45 s at 55 °C, and extension for 30 s at 72 °C, and a final 5 min extension at 72 °C. PCR products were resolved by gel electrophoresis to confirm product amplification. Bidirectional sequences of amplified fragments were determined using an automated DNA sequencer (ABI 3700XL Genetic Analyzer) and Bigdye terminator v3.1 cycle sequencing kits (Applied Biosystems, Foster City, CA, USA). Nucleotide alteration was identified by sequence alignment with the *POU3F4* Genbank sequence (Genbank ID: NP_000298.3) using the Genetool software. Mutations in the common deafness genes *GJB2*, *SLC26A4* and mtDNA *12S rRNA* were ruling out by sequencing the proband.

3. Results

3.1. Clinical manifestations in the proband

The questionnaire answers revealed that the proband was diagnosed with severe hearing loss when he was born, and his parents were normal, denying a family history of hearing loss. Acoustic immittance showed type A tympanograms in both ears and static compliances of 0.49 ml and 0.40 ml for left and right ear, respectively. Acoustic reflex was absent at 0.5, 1, 2 and 4 kHz. Auditory brainstem responses were absent for both ears at 100 dB nHL. Pure-tone thresholds in the proband showed profound sensorineural hearing loss without conductive component (Fig. 1). Temporal bone CT scans showed bilateral cochlear incomplete partition, vestibule dysplasia, internal auditory canal fundus dilation, and cochlear interlink with the internal auditory canal fundus (Fig. 2), highly consistent with features of DNFX2.

3.2. Mutation screening of POU3F4

Mutation screening of *POU3F4* revealed a hemizygotic C > A transversion at nucleotide 530 in the proband. This mutation led to a stop codon at amino acid 177 out of 361 amino acids, p.S177X. C/A heterozygotes at nucleotide position 530 were detected in the proband's mother. Sequence variations in the family are shown in Fig. 3. The mutated genotype was consistently cosegregated with the deafness phenotype in the family. This mutation was confirmed to be a novel mutation after searching relevant databases and literatures.

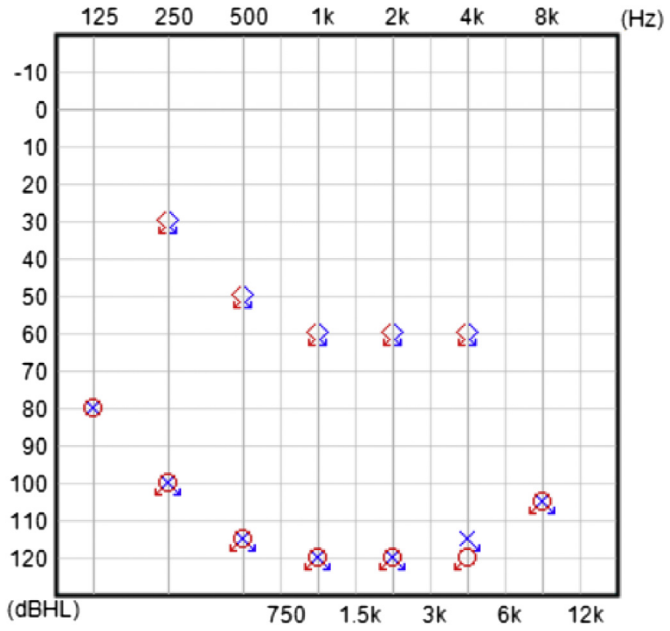

Fig. 1. Audiogram of the proband.

4. Discussion

We identified a novel mutation in the *POU3F4* gene in a Chinese family displaying X-linked inheritance of hearing loss. This mutation results in a codon TCG change to the terminator codon TAG at amino acid 177, which leads to production of truncated proteins.

POU3F4 belongs to a superfamily of POU domain transcription factors. The superfamily contains a typical structure – a POU-specific domain and a POU homeodomain, both of which are helix-turn-helix structural motifs that influence DNA binding and specificity. The *POU3F4* protein contains 361 amino acids, including the POU-specific domain with a length of 67 amino acids (from Lys194 to Asp260), a linker of 15 residues (from Ser261 to Gln275), and a POU homeodomain with a length of 60 amino acids (from Gly276 to

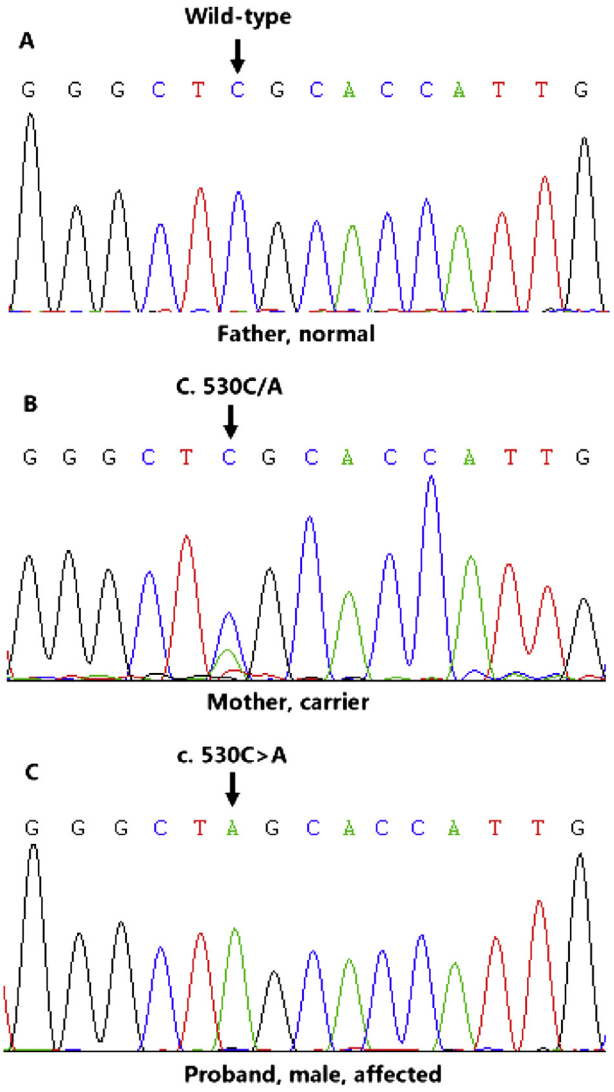

Fig. 3. Sequence variations in the family. (A) Wild type found in the father. (B) Heterozygous c.530C > A mutation found in the mother. (C) Hemizygotic c.530C > A mutation detected in the male proband.

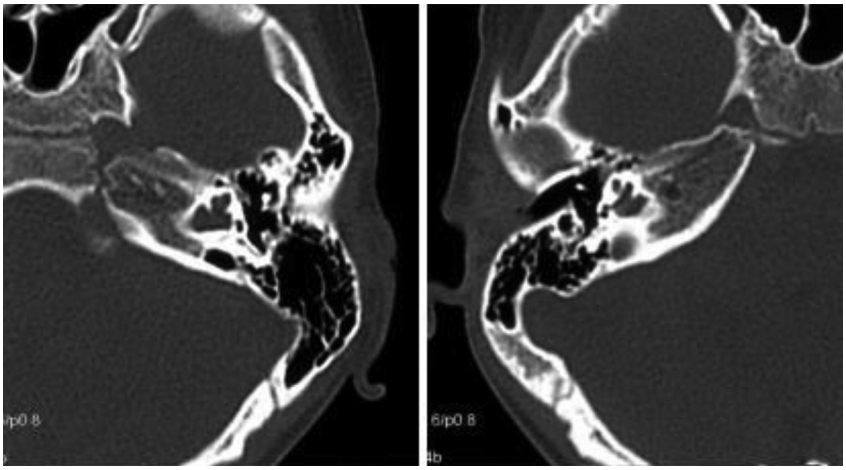

Fig. 2. Temporal bone CT scan of the proband.

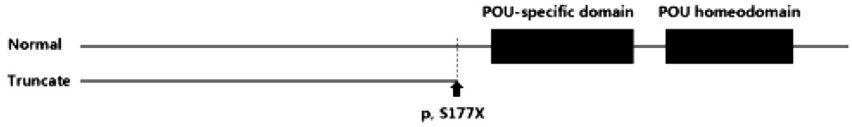

Fig. 4. The full-length and predicted truncated forms of human *POU3F4* protein.

Arg335) (Mathis et al., 1992). Crystallographic studies have revealed that the POU-specific domain and the POU homeodomain contain 4 and 3 α-helices, respectively (Klemm et al., 1994).

As shown in this study, the mutation at the position of 177 leads to the production of truncated proteins, which do not contain POU-specific domain or POU homeodomain (Fig. 4). We speculate that the truncated protein has lost the function of *POU3F4*. The proband in this study showed sensorineural hearing loss with bilateral cochlear incomplete partition, vestibule dysplasia, internal auditory canal fundus dilation and cochlear interlink with the internal auditory canal fundus, all consistent with mutation in *POU3F4*. We speculate that the c.530C > A mutation in *POU3F4* produces truncated proteins without the function of POU transcription factor, which leads to the phenotype seen in the proband.

To date, dozens of pathological mutations have been found in the *POU3F4* gene, including mainly missense, insertion and deletion mutations. Most of these mutations occur in the POU-specific domain and POU homeodomain, and only a few in upstream of the POU structure (Table 1). This indicates that POU-specific domain and POU homeodomain are important for *POU3F4*. Patients with *POU3F4* mutations demonstrate mainly mixed deafness or sensorineural hearing loss. In this study, we found sensorineural hearing loss in a patient with the novel mutation. Our finding expands the mutational spectrum of human *POU3F4*.

The *POU3F4* gene is the first cloned hereditary non-syndromic hearing loss gene by De Kok et al in 1995 (de Kok et al., 1995a)

In summary, we report the clinical and genetic characteristics of a Chinese with X-linked non-syndromic sensorineural deafness. DNA sequencing of the *POU3F4* gene revealed a novel nucleotide variation, c.530C > A (p.S177X), adding an additional mutation in DFNX2.

Table 1
Overview of *POU3F4* mutations described in DFNX2, including the mutation in the present study.

Mutation	Position of mutation	Feature of deafness	References
S177X	U	SNHL	This report
W67X	U	SNHL	Cremers et al. (2000)
Q79X	U	SNHL	Parzefall et al. (2013)
S98X	U	Mixed	Marlin et al. (2009)
W114X	U	Mixed	Waryah et al. (2011)
A116fs	U	Mixed	Lee et al. (2009a)
G128fs	U	SNHL	Lee et al. (2009b)
Q136X	U	Mixed	Waryah et al. (2011)
R167X	U	SNHL	Stankovic et al. (2010)
F201/K202del	S	Mixed	Hagiwara et al. (1998)
K202fs	S	SNHL	de Kok et al. (1995a)
L208fs	S	SNHL	Lee et al. (2009b)
T211M	S	NA	Choi et al. (2013)
R215fs	S	Mixed	de Kok et al. (1995a)
G216E	S	SNHL	Li et al. (2010)
S228L	S	SNHL	Vore et al. (2005)
E229R	S	NA	Choi et al. (2013)
T230I	S	Mixed	Friedman et al. (1997)
I285R fsX43	H	NA	Parzefall et al. (2013)
E286fs	H	Mixed	Cremers et al. (2000)
S288Q fsX37	H	Mixed	Bitner-Glindzicz et al. (1995)
L298fs	H	Mixed	de Kok et al. (1995a)
P303S	H	Mixed	Cremers et al. (2000)
I308N	H	Mixed	Marlin et al. (2009)
S309P	H	SNHL	Wang et al. (2006)
S310del	H	Mixed	Lee et al. (2009b)
A312V	H	SNHL	Bitner-Glindzicz et al. (1995)
L317F fsX12	H	NA	Choi et al. (2013)
L317W	H	Mixed	de Kok et al. (1995a)
R323G	H	Mixed	de Kok et al. (1997)
W325R	H	SNHL	Schild et al. (2011)
N328T	H	Mixed	Cremers et al. (2000)
R329G	H	Mixed	Friedman et al. (1997)
R329P	H	Mixed	Lee et al. (2009b)
R330S	H	SNHL	de Kok et al. (1995a)
K334E	H	Mixed	de Kok et al. (1995a)
T354E fx115	L	NA	Choi et al. (2013)
X362R extX113	L	NA	Choi et al. (2013)

*H, S, U and L indicate POU homeodomain, POU-specific domain, upstream and downstream of the POU-domains, respectively; SNHL, sensorineural hearing loss; Mixed, mixed hearing loss; NA, not available; fs, frameshift.

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