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

Bang-qing Huang a,*, Jia-ling Zeng a, Yong-yi Yuan b, Pu Dai b

a Department of Otorhinolaryngology, Hainan Branch of PLA General Hospital, Sanya 572013, China
b Department of Otorhinolaryngology, PLA General Hospital, Beijing 100853, China

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 a 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
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 otopathological 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’-ACTTCCGTGCTGTCATGTC-3’ and reverse primer 5’-GAGGTGATCCTGGCAATGGT-3’, 2) forward primer 5’-GCCACGCAAACCTGTATC-3’ and reverse primer 5’-CTCCCTGGCAGTCAT-3’, 3) forward primer 5’-TTGGAGAAAGTGGGAGGCG-3’ and reverse primer 5’-CCCAAGCTTAGTTAATGTA-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 ruled 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 dilatation, 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.
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...
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 POU transcription factor, which leads to the phenotype seen in the proband.

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               | Binner-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                | Binner-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) |

Table 1
*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.

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

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.

Acknowledgments

These investigations were supported by Chinese National Nature Science Foundation (81230020) and grant from Ministry of Science and Technology of China (2012BA109B02) to P.D., Chinese National Nature Science Foundation (81371098), Beijing Natural Science Foundation (7132177), Beijing Nova programme (2009B34) to Y.Y.Y.

References

Andersen, B., Rosenfeld, M.G., 2001. POU domain factors in the neuroendocrine system: lessons from developmental biology provide insights into human disease. Endocr. Rev. 22 (1), 2–35.

Binner-Glindzicz, M., Turnpenny, P., Högland, P., et al., 1995. Further mutations in brain 4 (POU3F4) clarify the phenotype in the X-linked deafness, DFN3. Hum. Mol. Genet. 4 (8), 1467–1469.

Choi, B.Y., Kim, D.H., Chung, T., et al., 2013. Destabilization and mislocalization of POU3F4 by C-terminal frameshift truncation and extension mutation. Hum. Mutat. 34 (2), 309–316.

Cremers, F.P., Cremers, C.W., Ropers, H.H., 2000. The ins and outs of X-linked deafness 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.

Acknowledgments

These investigations were supported by Chinese National Nature Science Foundation (81230020) and grant from Ministry of Science and Technology of China (2012BA109B02) to P.D., Chinese National Nature Science Foundation (81371098), Beijing Natural Science Foundation (7132177), Beijing Nova programme (2009B34) to Y.Y.Y.

References

Andersen, B., Rosenfeld, M.G., 2001. POU domain factors in the neuroendocrine system: lessons from developmental biology provide insights into human disease. Endocr. Rev. 22 (1), 2–35.

Binner-Glindzicz, M., Turnpenny, P., Högland, P., et al., 1995. Further mutations in brain 4 (POU3F4) clarify the phenotype in the X-linked deafness, DFN3. Hum. Mol. Genet. 4 (8), 1467–1469.

Choi, B.Y., Kim, D.H., Chung, T., et al., 2013. Destabilization and mislocalization of POU3F4 by C-terminal frameshift truncation and extension mutation. Hum. Mutat. 34 (2), 309–316.

Cremers, F.P., Cremers, C.W., Ropers, H.H., 2000. The ins and outs of X-linked deafness type 3. Adv. Otorhinolaryngol. 56, 184–195.

Cremers, C.W., Sinik, A.F., Huygen, P.L., et al., 2002. X-linked mixed deafness syndrome with congenital fixation of the stapedial footplate and perilymphatic gusher (DFN3). Adv. Otorhinolaryngol. 61, 161–167.
Cremers, F.P., Cremers, F.R., Kremer, H., 2008. *POU3F4* and mixed deafness with temporal defect (DFN3). In: Epstein, C.J., Erickson, R.P., Wynshaw-Boris, A. (Eds.), Inborn Errors of Development. Oxford University Press, New York, pp. 1042–1047.

de Kok, Y.J., van der Maarel, S.M., Bitner-Glindzicz, M., et al., 1995a. Association between X-linked mixed deafness and mutations in the *POU3F4* domain gene. Science 267 (5198), 685–688.

de Kok, Y.J., Merkx, G.F., van der Maarel, S.M., et al., 1995b. A duplication/paracentric inversion associated with familial X-linked deafness (DFN3) suggests the presence of a regulatory element more than 400 kb upstream of the *POU3F4* gene. Hum. Mol. Genet. 4 (11), 2145–2150.

de Kok, Y.J., Cremers, C.W., Ropers, H.H., et al., 1997. The molecular basis of Friedmann, R.A., Bykhovskaya, Y., Tu, G., et al., 1997. Molecular analysis of Hagiwara, H., Tamagawa, Y., Kitamura, K., et al., 1998. A new mutation in the Huebner, A.K., Gandia, M., Frommolt, P., et al., 2011. Nonsense mutations in the *POU3F4* gene in patients with clinical and radiographic evidence of X-linked mixed deafness with perilymphatic gusher. Ann. Otol. Rhinol. Laryngol. 106 (4), 320–325.

Hagiwara, H., Tamagawa, Y., Kitamura, K., et al., 1998. A new mutation in the *POU3F4* gene in a Japanese family with X-linked mixed deafness (DFN3). Laryngoscope 108 (10), 1544–1547.

Huebner, A.K., Gandia, M., Frommolt, P., et al., 2011. Nonsense mutations in SMPX, encoding a protein responsive to physical force, result in X-chromosomal hearing loss. Am. J. Hum. Genet. 88 (5), 621–627.

Klemm, J.D., Rould, M.A., Aurora, R., et al., 1994. Crystal structure of the Oct-1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. Cell 77 (1), 21–32.

Lee, H.K., Bykhovsky, Y., Tu, G., et al., 1997. Molecular analysis of the *POU3F4* gene in patients with clinical and radiographic evidence of X-linked mixed deafness. Am. J. Hum. Genet. 68 (5), 621–627.

Parzefall, T., Shivatzki, S., Lenz, D.R., et al., 2013. Cytoplasmic mislocalization of *POU3F4* due to novel mutations leads to deafness in humans and mice. Hum. Mutat. 34 (8), 1102–1110.

Petersen, M.B., Wang, Q., Willems, P.J., 2008. Sex-linked deafness. Clin. Genet. 73 (1), 14–23.

Phelps, P.D., Reardon, W., Pembrey, M., et al., 1991. X-linked deafness, stapes gushers and a distinctive defect of the inner ear. Neuroradiology 33 (4), 326–330.

Rost, S., Bach, E., Neuner, C., et al., 2014. Novel form of X-linked nonsyndromic hearing loss with cochlear malformation caused by a mutation in the type IV collagen gene COL4A6. Eur. J. Hum. Genet. 22 (2), 208–215.

Schild, C., Prera, E., Lüblinghoff, N., et al., 2011. Novel mutation in the homeobox domain of transcription factor *POU3F4* associated with profound sensorineural hearing loss. Otol. Neurotol. 32 (4), 690–694.

Stankovic, K.M., Hennessey, A.M., Herrmann, B., et al., 2010. Cochlear implantation in children with congenital X-linked deafness due to novel mutations in *POU3F4* gene. Ann. Otol. Rhinol. Laryngol. 119 (12), 815–822.

Vore, A.P., Chang, E.H., Hoppe, J.E., et al., 2005. Deletion of and novel missense mutation in *POU3F4* in 2 families segregating X-linked nonsyndromic deafness. Arch. Otolaryngol. Head Neck Surg. 131 (12), 1057–1063.

Wang, Q.J., Li, Q.Z., Rao, S.Q., et al., 2006. A novel mutation of *POU3F4* causes congenital profound sensorineural hearing loss in a large Chinese family. Laryngoscope 116 (6), 944–950.

Waryah, A.M., Ahmed, Z.M., Bhinder, M.A., et al., 2011. Molecular and clinical studies of X-linked deafness among Pakistani families. J. Hum. Genet. 56 (7), 534–540.