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Case Report

A novel pathogenic variant c.975G > A (p.Trp325*) in the *POU3F4* gene in Yakut family (Eastern Siberia, Russia) with the X-linked deafness-2 (DFNX2)

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ABSTRACT

Here, we report a novel hemizygous transition c.975G > A (p.Trp325*) in *POU3F4* gene (Xq21) found in two deaf half-brothers from one Yakut family (Eastern Siberia, Russia) with identical inner ear abnormalities ("corkscrew" cochlea with an absence of modiolus) specific to X-linked deafness-2 (DFNX2). Comprehensive clinical evaluation (CT and MR-imaging, audiological and stabilometric examinations) of available members of this family revealed both already known (mixed progressive hearing loss) and additional (enlargement of semicircular canals and postural disorders) clinical DFNX2 features in affected males with c.975G > A (p.Trp325*). Moreover, mild enlargement of semicircular canals, postural abnormalities and different types of hearing thresholds were found in female carrier of this *POU3F4*-variant.

1. Introduction

One in 1000 newborns is affected by congenital deafness [1,2]. About half of all cases are hereditary and inherited by a recessive autosomal pattern (70–80%) or a dominant autosomal pattern (10–20%) [3]. The proportion of X-linked deafness (DFNX) is less than 1–2% [3]. One of the common forms of X-linked deafness is the perilymphatic Gusher-deafness syndrome also known as the X-linked deafness-2 (DFNX2, MIM 304400) caused by pathogenic variants in the *POU3F4* gene (MIM 300039, Xq21) [4]. DFNX2 is characterized by progressive conductive and sensorineural hearing loss and a pathognomonic temporal bone deformation that includes dilatation of the inner auditory canal and a fistulous connection between the internal auditory canal and the cochlear basal turn, resulting in a perilymphatic fluid “gusher” during stapes surgery [4–8]. The *POU3F4* gene encodes the transcription factor POU3F4 (Brain 4). Douville et al. (1994) showed that the rat homolog of *POU3F4*, called *RHS2*, is expressed during embryonic development in the brain, the neural tube, and the otic vesicle at 15.5 and 17.5 days after conception [9]. More than 70 pathogenic variants (41 missense/nonsense substitutions, 11 small deletions, 3 small insertions, 15 large deletions, one large insertion, and 3 complex variants) have been described for the *POU3F4* gene in the Human Gene Mutation Database (accessed July, 2017) [10] and 156 variants of different clinical significance in the ClinVar Database (accessed July, 2017) [11]. However, data on the clinical characteristics and the outcomes of patients with different pathogenic *POU3F4* variants causing DFNX2 are scarce for populations worldwide.

In our previous studies in the Sakha Republic (Eastern Siberia,

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Russia), we found that hearing impairment in 192 out of 393 examined patients (48.9%) was caused by the pathogenic variants in the *GJB2* gene (MIM 220290) while the causes of ~50% of early childhood deafness cases have not been determined [12]. To identify the causes of hearing loss in *GJB2*-negative patients (n = 98) we performed computed tomography examinations and revealed one Yakut family (belonging to indigenous population of the Sakha Republic) with inner ear abnormalities specific to DFNX2.

In this report, we conducted comprehensive clinical examination including computed tomography, audiological examination, magnetic resonance imaging, and stabilometric examination of four members of one Yakut family (two affected half-siblings and their non-affected parents) with X-linked recessive deafness associated with novel variant c.975G > A (p.Trp325*) in the *POU3F4* gene.

2. Methods

2.1. Computed tomography (CT)

CT-imaging of the temporal bone was performed using the 4-slice Siemens SOMATOM Sensation 4 CT scanner (Germany). The section thickness of the axial plane was set to 1 mm (InnerEarSpi software). The 2D images in native axial planes were used for the visualization of temporal bone structure.

2.2. Audiological examination (AE)

Pure tone audiometry was performed using the audiometer MAICO ST 20 (Germany) at air conduction frequencies 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 kHz and at bone conduction frequencies 0.25, 0.5, 1.0, 4.0 kHz with 5.0 dB increments. Impedance measurements were conducted using the Interacoustics AA222 (Denmark). The degree of hearing loss was evaluated at the hearing threshold in the speech frequency range 0.5, 1.0, 2.0, and 4.0 kHz: mild–20–40 dB, moderate–41–55 dB, severe–56–70 dB, profound - > 90 dB.

2.3. Magnetic resonance imaging (MRI)

The condition of the cerebellopontine angle was estimated using MRI on the Siemens Magnetom Espree (Germany) following a 3D CISS sequence with a 0.9 mm slice thickness. Axial MIP-reformation (maximum intensity projection) with a 20 mm slab was used for visualization of the volume of the cerebellopontine angle area.

2.4. Stabilometric examination (SE)

SE was performed using the Romberg test on the stabiloplatform ST-150 Biomera (Russia), in the erect position, both with open and closed eyes. Stabiloplatform is a rectangular shape unit divided into four quadrants capable of perceiving the weight force exerted on each quadrant.

2.5. POU3F4 gene coding region sequencing

The *POU3F4* gene coding region was sequenced in the DNA samples of five Yakut family members: both affected probands (II:3 and II:4), their mother (I:2), sister (II:5) and the father of proband II:3 (I:1) (Fig. 1). We also analyzed DNA samples from 68 *GJB2*-negative (without changes in the *GJB2* gene sequence) deaf males obtained from the DNA Bank of the Yakut Science Centre for Complex Medical Problems (Yakutsk, Russia).

DNA was extracted from blood leukocytes using the phenol-chloroform method. Amplification of the *POU3F4* gene coding region (exon 1) was conducted by PCR on the MJ Mini Bio-Rad (USA) thermocycler with primers presented in the Supplementary Table 1 (Appendix A). PCR products were subjected to direct sequencing using the same primers on the ABI PRISM 3130XL Applied Biosystems (USA). Obtained nucleotide sequences were analyzed by the Sequence analysis v.5.4 and the Chromas v.2.0 softwares and compared with the reference sequence of the *POU3F4* gene (Reference Sequence: NC_0000023.11).

2.6. PCR-RFLP analysis

Screening for c.975G > A (p.Trp325*) in the *POU3F4* gene was performed by PCR-RFLP analysis (restriction enzyme *Hinf*I) (Appendix A. Supplementary Table 1) in 123 DNA samples of healthy Yakut women obtained from the DNA Bank of the Yakut Science Centre of Complex Medical Problems (Yakutsk, Russia).

2.7. Ethical approval

Written informed consent was obtained from all individuals. This study was approved by the local Committee on Biomedical Ethics of the Yakut Science Centre of Complex Medical Problems (Yakutsk, Russia, Protocol No 16, April 16, 2009).

3. Case report

We revealed identical abnormalities of the inner ear in two half-brothers with DFNX2 (MIM 304400) from one Yakut family belonging to indigenous population of the Sakha Republic (Fig. 1). We observed this family during two years 2012–2014 (the ages of both brothers at examinations were 9–10 years and 11–12 years, respectively). Both brothers are the students of special boarding school for deaf children. They had a prelingual hearing loss, normal intelligence, normal physical development and no changes in the *GJB2* gene. All their close relatives (mother, father and sister) had no hearing problems. We identified a novel nucleotide substitution c.975G > A (*POU3F4* gene) in a hemizygous state in both probands (II:3 and II:4), in a heterozygous state in their mother (I:2) and sister (II:5) (she was not subjected to a detailed clinical examination) while variant c.975G > A was not found in the father of proband II:3 (Fig. 1). We performed comprehensive clinical examination including computed tomography, audiological examination, magnetic resonance imaging and stabilometric examination in both probands (II:3 and II:4), their mother (I:2), and the father of proband II:3.

The CT examination of both half-brothers demonstrated an abnormal dilatation of the internal auditory canal (IAC) as well as an abnormally wide communication between the IAC and the inner ear compartment (“corkscrew” cochlea with an absence of modiolus) (Fig. 1B). The audiological examination of both half-brothers revealed mixed (sensorineural and conductive) progressive bilateral hearing loss (Appendix B. Supplementary Fig. 1C). The acoustic impedance for both probands corresponded to the “As” type of tympanograms – an increase in acoustic impedance probably due to the increasing resistance of the cochlear endolymph (Appendix B. Supplementary Fig. 1C). MRI of the cerebellopontine angle detected bilateral enlargements of the semicircular canals in probands II:3 and II:4 (Appendix B. Supplementary Fig. 2C). Both brothers showed difficulties in maintaining the standing position and vertical instability in the Romberg test with open and closed eyes (Appendix B. Supplementary Fig. 2C). Stabilometry examination also revealed dystaxia probably caused by the enlarged semicircular canals visualized by CT and MRI (Appendix B. Supplementary Fig. 1C and Supplementary Fig. 2C). The father of proband II:3 (without c.975G > A) did not show any clinical features of DFNX2 (Appendix B. Supplementary Fig. 1A and Supplementary Fig. 2A).

The mother of both probands (I:2, heterozygous for c.975G > A) had inner auditory canal abnormalities with a cylindrical shape on the left side and a conic shape on the right side (Fig. 1B). Moreover, she presented different hearing thresholds (with the better ear – up to 20 dB at low frequencies and up to 25 dB at high frequencies) and a

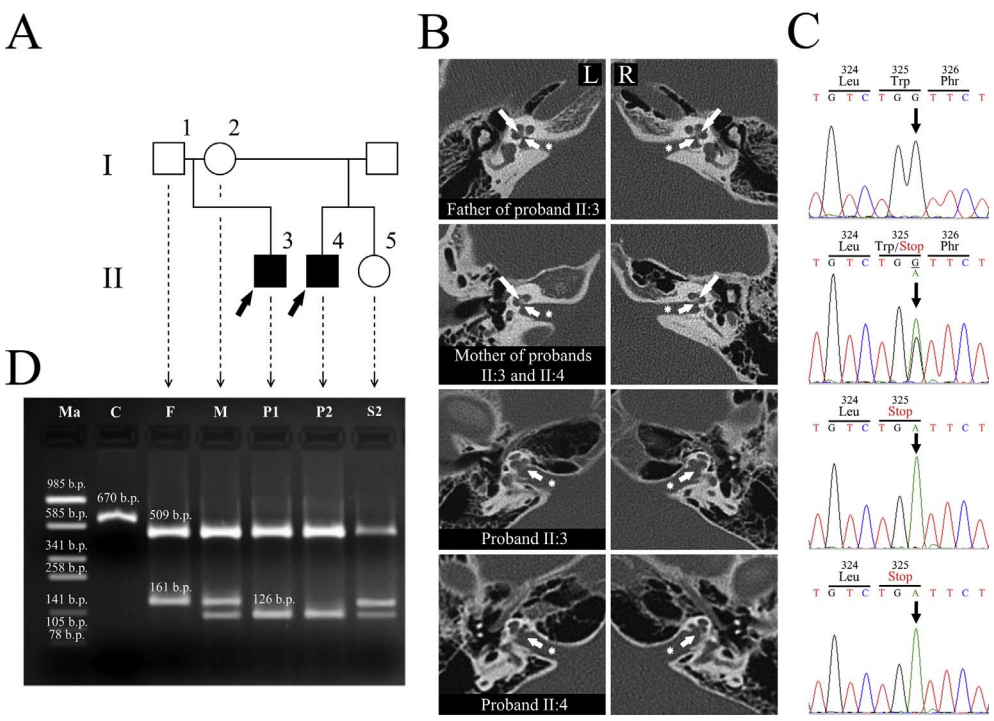

Fig. 1. The Yakut family with X-linked recessive deafness. A: Pedigree of the Yakut family. Deaf individuals are shown in black. **B:** CT images of the temporal bone in the axial plane (tomographic section thickness of 1 mm). L - left temporal bone, R - right temporal bone. Short arrow - cochlea, long arrow - modiolus, asterisk - the internal auditory canal (IAC). Abnormal dilatation of the IAC, abnormally wide communication between the IAC and inner ear compartments, and absence of a modiolus ("corkscrew" cochlea) were detected in probands II:3 and II:4. **C:** Pathogenic variant c.975G > A (p.W325*) in the *POU3F4* gene in Yakut family (top to bottom): normal hearing father of proband II:3 (*POU3F4*-genotype c.[=]; [0]); normal hearing heterozygous mother of probands II:3 and II:4 (*POU3F4*-genotype c.[975G > A]; [=]); deaf hemizygous probands II:3 and II:4 (*POU3F4*-genotype c.[975G > A]; [0]). **D:** Detection of c.975G > A (p.Trp325*) by PCR–RFLP analysis (*Hinf*I, 4% agarose gel) (see details in Appendix A. Supplementary Table 1). Ma - marker *PUC*19/*Kzo9*I; C - control sample - PCR product was not treated by *Hinf*I (fragment 670 bp); F - father of proband II:3 (fragments 509 and 161 bp); M - mother of probands II:3 and II:4 (509 bp, 161 bp, 126 bp, and invisible 35 bp); P1 and P2 - probands II:3 and II:4 (fragments 509 bp, 126 bp, and invisible 35 bp); S2 - sister of probands II:3 and II:4 (509 bp, 161 bp, 126 bp, and invisible 35 bp).

tympanogram of type “As” (Appendix B. Supplementary Fig. 1B). The proportions of her semicircular canals were enlarged compared to those of the father of proband II:3 (without c.975G > A). In addition, she had difficulties in maintaining the standing position and vertical instability in the Romberg test with open and closed eyes (Appendix B. Supplementary Fig. 2B).

4. Discussion

We revealed a novel hemizygous transition c.975G > A in the *POU3F4* gene in two deaf half-brothers from one Yakut family (Eastern Siberia, Russia) with identical inner ear abnormalities specific to the X-linked deafness-2 (DFNX2, MIM 304400). The c.975G > A transition leads to a stop codon (p.Trp325*) in the evolutionary conservative and functionally significant homeodomain of the POU3F4 (Brain 4) protein (Appendix B. Supplementary Fig. 3). Transition c.975G > A in the *POU3F4* gene had not previously been reported in the 1000 Genomes [13], the ESP6500 [14], and the ExAC projects [15]. In our study the c.975G > A (p.Trp325*) variant was not found in other examined *GJB2*-negative patients with hearing loss (deaf Yakut males, n = 68) and in the control samples (healthy Yakut females, n = 123).

The comprehensive clinical examination of both half-brothers revealed the association of novel truncating transition c.975G > A (p.Trp325*) in the *POU3F4* gene with inner ear malformations (“corkscrew” cochlea with an absence of modiolus) and mixed (sensorineural and conductive) progressive bilateral hearing loss. These clinical features correspond to early reported cases of the male patients with hemizygous pathogenic variants in the *POU3F4* gene associated with DFNX2 [16–30]. Moreover, the enlargement of semicircular canals and the postural disorders manifesting as a moderate vertical instability according to the Romberg test (Appendix B. Supplementary Fig. 2C) were detected by additional MRI and computed stabilometry in two affected siblings with c.975G > A (p.Trp325*) in the *POU3F4* gene. Thus, we believe that our findings expand currently available clinical information [16–30] about the male patients with X-linked deafness-2 (DFNX2, MIM 304400) caused by pathogenic variants in *POU3F4* gene.

In addition, thorough clinical examination of mother of the probands who was heterozygous for c.975G > A (p.Trp325*) revealed some clinical features of the X-linked deafness-2: inner ear malformations (acoustic canal abnormalities with a cylindrical shape on the left side and a conic shape on the right side), different types of hearing thresholds (with the better ear – up to 20 dB at low frequencies and up to 25 dB at high frequencies), the enlargements of semicircular canals, and the postural disorders manifesting as a moderate vertical instability according to the Romberg test. Similar clinical findings were observed in some cases in females heterozygous for pathogenic variants in the *POU3F4* gene [29–37]. Available literature data on the clinical findings in females from the DFNX2-affected families are summarized in Supplementary Table 2 (Appendix A). Variable hearing impairment were found in not less than 43% of the female carriers of the *POU3F4* pathogenic variants and radiological abnormalities were detected in 26% of them (Appendix A. Supplementary Table 2). Observed phenotypic variability in the female carriers is probably due to variation in the degree of skewing of X-inactivation and/or mosaicism for pathogenic *POU3F4* variants that requires further studies.

5. Conclusion

Novel hemizygous transition c.975G > A (p.Trp325*) in the *POU3F4* gene was revealed in two deaf half-brothers from one Yakut family (Eastern Siberia, Russia) with identical inner ear abnormalities specific to X-linked deafness-2 (DFNX2). The data from comprehensive clinical evaluation (computed tomography, audiological examination, magnetic resonance imaging and stabilometric examination) of four members of this family expand clinical information both for the DFNX2-affected males and for the female carriers of the pathogenic variants in the *POU3F4* gene.

Competing interests

All authors declare that they have no competing interests.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijporl.2017.11.001.

References

[1] M.L. Marazita, L.M. Ploughman, B. Rawlings, E. Remington, K.S. Arnos, W.E. Nance, Genetic epidemiological studies of early-onset deafness in the U.S. school-age population, Am. J. Med. Genet. 46 (1993) 486–491.

[2] A.L. Mehl, V. Thomson, The Colorado newborn hearing screening project, 1992-1999: on the threshold of effective population-based universal newborn hearing screening, Pediatrics 109 (2002) E7.

[3] C.C. Morton, W.E. Nance, Newborn hearing screening - silent revolution, N. Engl. J. Med. 354 (2006) 2151–2164, http://dx.doi.org/10.1056/NEJMra050700.

[4] Y.J. de Kok, S.M. van der Maarel, M. Bitner-Glindzicz, I. Huber, A.P. Monaco, S. Malcolm, M.E. Pembrey, H.H. Ropers, F.P. Cremers, Association between X-linked mixed deafness and transitions in the POU domain gene POU3F4, Science 267 (1995) 685–688.

[5] M. Bitner-Glindzicz, P. Turnpenny, P. Hoglund, H. Kaariainen, E.M. Sankila, S.M. van der Maarel, Y.J. de Kok, H.H. Ropers, F.P. Cremers, M. Pembrey, Further mutations in Brain 4 (POU3F4) clarify the phenotype in the X-linked deafness, DFN3, Hum. Mol. Genet. 4 (1995) 1467–1469.

[6] Y.J. de Kok, E.R. Vossenaar, C.W. Cremers, N. Dahl, J. Laporte, L.J. Hu, D. Lacombe, N. Fischel-Ghodsian, R.A. Friedman, L.S. Parnes, P. Thorpe, M. Bitner-Glindzicz, H.J. Pander, H. Heilbronner, J. Graveline, J.T. den Dunnen, H.G. Brunner, H.H. Ropers, F.P. Cremers, Identification of a hot spot for microdeletions in patients with X-linked deafness type 3 (DFN3) 900 kb proximal to the DFN3 gene POU3F4, Hum. Mol. Genet. 5 (1996) 1229–1235.

[7] R.A. Friedman, Y. Bykhovskaya, G. Tu, J.M. Talbot, D.F. Wilson, L.S. Parnes, N. Fischel-Ghodsian, Molecular analysis of the POU3F4 gene in patients with clinical and radiographic evidence of X-linked mixed deafness with perilymphatic gusher, Ann. Otol. Rhinol. Laryngol. 106 (1997) 320–325.

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

[9] P.J. Douville, S. Atanasoski, A. Tobler, A. Fontana, M.E. Schwab, The brain-specific POU-box gene Brn4 is a sex-linked transcription factor located on the human and mouse X chromosomes, Mamm. Genome 5 (1994) 180–182.

[10] P.D. Stenson, M. Mort, E.V. Ball, K. Shaw, A.D. Phillips, D.N. Cooper, The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine, Hum. Genet. 133 (2014) 1–9, http://dx.doi.org/10.1002/0471250953.bi0113s39.

[11] M. Landrum, J. Lee, G. Riley, W. Jang, W. Rubinstein, D. Church, D. Maglott, ClinVar. URL: http://www.ncbi.nlm.nih.gov/books/NBK174587/(Accessed July, 2017).

[12] N.A. Barashkov, V.G. Pshennikova, O.L. Posukh, F.M. Teryutin FM, A.V. Solovyev, L.A. Klarov, G.P. Romanov, N.N. Gotovtsev, A.A. Kozhevnikov, E.V. Kirillina, O.G. Sidorova, L.M. Vasilyeva, E.E. Fedotova, I.V. Morozov, A.A. Bondar, N.A. Solovyeva, S.K. Kononova, A.M. Rafailov, N.N. Sazonov, A.N. Alekseev, M.I. Tomsky, L.U. Dzhemileva, E.K. Khusnutdinova, S.A. Fedorova, Spectrum and frequency of the GJB2 gene pathogenic variants in a large cohort of patients with hearing impairment living in a subarctic region of Russia (the Sakha Republic), PLoS One 25 (2016) e0156300, http://dx.doi.org/10.1371/journal.pone.0156300.

[13] 1000 Genomes Project Consortium, A. Auton, L.D. Brooks, R.M. Durbin, E.P. Garrison, H.M. Kang, J.O. Korbel, J.L. Marchini, S. McCarthy, G.A. McVean, G.R. Abecasis, A global reference for human genetic variation, Nature 526 (2015) 68–74, http://dx.doi.org/10.1038/nature15393.

[14] Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA. URL: http://evs.gs.washington.edu/EVS/. (Accessed July, 2017).

[15] M. Lek, K.J. Karczewski, E.V. Minikel, K.E. Samocha, E. Banks, T. Fennell, A.H. O'Donnell-Luria, J.S. Ware, et al., Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans, Nature 536 (2016) 285–291, http://dx.doi.org/10.1038/nature19057.

[16] M.H. Song, H.K. Lee, J.Y. Choi, S. Kim, J. Bok, U.K. Kim, Clinical evaluation of DFN3 patients with deletions in the POU3F4 locus and detection of carrier female using MLPA, Clin. Genet. 78 (2010) 524–532, http://dx.doi.org/10.1111/j.1399-0004.2010.01426.x.

[17] C.W. Cremers, A.F. Snik, P.L. Huygen, F.B. Joosten, F.P. Cremers, X-linked mixed deafness syndrome with congenital fixation of the stapedial footplate and perilymphatic gusher (DFN3), Genet. Hear. Impair. Adv. Otorhinolaryngol. 61 (2002) 161–167.

[18] H.K. Lee, M.H. Song, M. Kang, J.T. Lee, K.A. Kong, S.J. Choi, K.Y. Lee, H. Venselaar, G. Vriend, W.S. Lee, H.J. Park, T.K. Kwon, J. Bok, U.K. Kim, Clinical and molecular characterizations of novel POU3F4 mutations reveal that DFN3 is due to null function of POU3F4 protein, Physiol. genomics 39 (2009) 195–201, http://dx.doi.org/10.1152/physiolgenomics.00100.2009.

[19] Q.J. Wang, Q.Z. Li, S.Q. Rao, Y.L. Zhao, H. Yuan, W.Y. Yang, D.Y. Han, Y. Shen, A novel mutation of POU3F4 causes congenital profound sensorineural hearing loss in a large Chinese family, Laryngoscope 116 (2006) 944–950.

[20] A.P. Vore, E.H. Chang, J.E. Hoppe, M.G. Butler, S. Forrester, M.C. Schneider, L.L. Smith, D.W. Burke, C.A. Campbell, R.J. Smith, Deletion of and novel missense mutation in POU3F4 in 2 families segregating X-linked nonsyndromic deafness, Arch. Otolaryngol. Head. Neck. Surg. 131 (2005) 1057–1063.

[21] N. Oh, S. Kupka, F. Mirghomizadeh, R. Arold, R. Zimmermann, N. Blin, H.P. Zenner, M. Pfister, Clinical and molecular genetic analysis of monozygotic twins displaying stapes gusher syndrome (DFN3), HNO 51 (2003) 629–633.

[22] C. Schild, E. Prera, N. Lüblinghoff, S. Arndt, A. Aschendorff, R. Birkenhäger, Novel mutation in the homeobox domain of transcription factor POU3F4 associated with profound sensorineural hearing loss, Otol. Neurotol. 32 (2011) 690–694, http://dx.doi.org/10.1097/MAO.0b013e318210b749.

[23] J. Li, J. Cheng, Y. Lu, Y. Lu, A. Chen, Y. Sun, D. Kang, X. Zhang, P. Dai, D. Han, H. Yuan, Identification of a novel mutation in POU3F4 for prenatal diagnosis in a Chinese family with X-linked nonsyndromic hearing loss, J. Genet. Genomics 37 (2010) 787–793, http://dx.doi.org/10.1016/S1673-8527(09)60096-5.

[24] S. Naranjo, K. Voesenek, E. de la Calle-Mustienes, A. Robert-Moreno, H. Kokotas, M. Grigoriadou, J. Economides, G. Van Camp, N. Hilgert, F. Moreno, B. Alsina, M.B. Petersen, H. Kremer, J.L. Gómez-Skarmeta, Multiple enhancers located in a 1-Mb region upstream of POU3F4 promote expression during inner ear development and may be required for hearing, Hum. Genet. 128 (2010) 411–419, http://dx.doi.org/10.1007/s00439-010-0864-x.

[25] T. Parzefall, S. Shivatzki, D.R. Lenz, B. Rathkolb, K. Ushakov, D. Karfunkel, Y. Shapira, M. Wolf, M. Mohr, E. Wolf, S. Sabrautzki, M.H. de Angelis, M. Frydman, Z. Brownstein, K.B. Avraham, Cytoplasmic mislocalization of POU3F4 due to novel mutations leads to deafness in humans and mice, Hum. Mutat. 34 (2013) 1102–1110, http://dx.doi.org/10.1002/humu.22339.

[26] W.X. Gong, R.Z. Gong, B. Zhao, HRCT and MRI findings in X-linked non-syndromic deafness patients with a POU3F4 mutation, Int. J. Pediatr. Otorhinolaryngol. 78 (2014) 1756–1762, http://dx.doi.org/10.1016/j.ijporl.2014.08.013.

[27] H. Moteki, A.E. Shearer, S. Izumi, Y. Kubota, H. Azaiez, K.T. Booth, C.M. Sloan, D.L. Kolbe, R.J. Smith, S. Usami, De novo mutation in X-linked hearing loss-associated POU3F4 in a sporadic case of congenital hearing loss, Ann. Otol. Rhinol. Laryngol. 124 (2015) 169–176, http://dx.doi.org/10.1177/0003489415575042.

[28] K.M. Stankovic, A.M. Hennessey, B. Herrmann, L.A. Mankarious, Cochlear implantation in children with congenital X-linked deafness due to novel mutations in POU3F4 gene, Ann. Otol. Rhin. Laryngol. 119 (2010) 815–822.

[29] A. Pollak, U. Lechowicz, A. Kędra, P. Stawiński, M. Rydzanicz, M. Furmanek, M. Brzozowska, M. Mrówka, H. Skarżyński, P.H. Skarżyński, M. Ołdak, R. Płoski, Novel and de novo mutations extend association of POU3F4 with distinct clinical and radiological phenotype of hearing loss, PLoS One 12 (2016) e0166618, http://dx.doi.org/10.1371/journal.pone.0166618.

[30] A. Kanno, H. Mutai, K. Namba, N. Morita, A. Nakano, N. Ogahara, T. Sugiuchi, K. Ogawa, T. Matsunaga, Frequency and specific characteristics of the incomplete partition type III anomaly in children, Laryngoscope 127 (2016) 1663–1669, http://dx.doi.org/10.1002/lary.26245.

[31] S. Marlin, M.P. Moizard, A. David, N. Chaissang, M. Raynaud, L. Jonard, D. Feldmann, N. Loundon, F. Denoyelle, A. Toutain, Phenotype and genotype in females with POU3F4 mutations, Clin. Genet. 76 (2009) 558–563, http://dx.doi.org/10.1111/j.1399-0004.2009.01215.x.

[32] C.W. Cremers, P.L. Huygen, Clinical features of female heterozygotes in the X-linked mixed deafness syndrome (with perilymphatic gusher during stapes surgery), Int. J. Pediatr. Otorhinolaryngol. 6 (1983) 179–185.

[33] P.D. Phelps, W. Reardon, M. Pembrey, S. Bellman, L. Luxom, X-linked deafness, stapes gushers and a distinctive defect of the inner ear, Neuroradiology 33 (1991) 326–330.

[34] C. Piussan, A. Hanauer, N. Dahl, M. Mathieu, C. Kolski, V. Biancalana, S. Heyberger, V. Strunski, X-linked progressive mixed deafness: a new microdeletion that involves a more proximal region in Xq21, Am. J. Hum. Genet. 56 (1995) 224–230.

[35] B. Arellano, R. Ramírez Camacho, J.R. García Berrocal, M. Villamar, I. del Castillo, F. Moreno, Sensorineural hearing loss and Mondini dysplasia caused by a deletion at locus DFN3, Arch. Otolaryngol. Head. Neck. Surg. 126 (2000) 1065–1069.

[36] J. Li, J. Cheng, Y. Lu, Y. Lu, A. Chen, Y. Sun, D. Kang, X. Zhang, P. Dai, D. Han, H. Yuan, Identification of a novel mutation in POU3F4 for prenatal diagnosis in a Chinese family with X-linked nonsyndromic hearing loss, J. Genet. Genomics 37 (2010) 787–793, http://dx.doi.org/10.1016/S1673-8527(09)60096-5.

[37] A.M. Waryah, Z.M. Ahmed, M.A. Bhinder, D.I. Choo, R.A. Sisk, M. Shahzad, S.N. Khan, T.B. Friedman, S. Riazuddin, S. Riazuddin, Molecular and clinical studies of X-linked deafness among Pakistani families, J. Hum. Genet. 56 (2011) 534–540, http://dx.doi.org/10.1038/jhg.2011.55.