Genetic Findings of Sanger and Nanopore Single-Molecule Sequencing in Patients with X-Linked Hearing Loss and Incomplete Partition Type III

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Research

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Abstract

Background

POU3F4 is the causative gene for X-linked deafness-2 (DFNX2), characterized by incomplete partition type III (IP-III) malformation of the inner ear. The aim of this study was to investigate the clinical characteristics and molecular findings by Sanger or Nanopore single-molecule sequencing in IP-III patients.

Methods

Diagnosis of IP-III was mainly based on clinical characteristics including radiological and audiological findings. Sanger sequencing of *POU3F4* were carried out for these IP-III patients. For those patients with negative results for *POU3F4* Sanger sequencing, Nanopore long-read single-molecule sequencing was used to identify the possible pathogenic variants. Hearing intervention outcomes of hearing aids fitting and cochlear implantation were also analyzed. Grouped by different locations of *POU3F4* variants, aided PTA was further compared between patients in whom the variants located in the exon region or in the upstream region.

Results

In total, 18 male patients from 14 unrelated families were diagnosed with IP-III. 10 variants were identified in *POU3F4* by Sanger sequencing and 9 of these were novel (p.Val321Gly, p.Gln181*, p.Cys233*, p.Val215Gly, p.Arg282Gln, p.Trp57*, p.Gln316*, c.903_912 delins TGCCA and p.Arg205del). Four different deletions (DELs) that varied from 80 to 486 kb were identified 876-1503 kb upstream of *POU3F4* by Nanopore long-read single-molecule sequencing. Of them, de novo genetic mutations occurred in 21.4% (3/14) of patients with *POU3F4* mutations. Of these 18 patients, 7 had bilateral hearing aids (HAs) and 10 patients received unilateral cochlear implantation (CI). The mean aided pure tone average (PTA) for HAs and CI users were 41.1 ± 5.18 and 40.3 ± 7.59 dB HL respectively. The mean PTAs for whom the variants located in the exon and upstream regions were 39.6 ± 6.31 vs 43.0 ± 7.10 dB HL, which presented no significant difference ($p=0.342$).

Conclusions

Among IP-III patients, 28.6% (4/14) had no definite mutation in exon region of *POU3F4*, however, possible pathogenic deletions were identified in upstream region of this gen. De novo genetic mutations occurred in 21.4% (3/14) of patients with *POU3F4* mutation. Hearing intervention outcomes of IP-III patients presented no difference regardless of the variants locations on exon or upstream regions.

Introduction

Hearing loss is the most common sensory deficit with a prevalence of 1–2 in 1000 newborns [1], and genetic factors account for over 50% of cases [2]. With respect to genetic hearing loss, deafness is transmitted by an X-linked inheritance pattern in 2–3% of cases [2, 3]. To date, according to the Hereditary Hearing Loss website (<http://hereditaryhearingloss.org>), six loci (DFNX1–6) and five causative genes have been identified in X-linked hearing loss: *PRPS1* (OMIM 311850) for DFNX1, *POU3F4* (OMIM 300039) for DFNX2, *SMPX* (OMIM 300226) for DFNX4, *AIFM1* (OMIM 300169) for DFNX5, and *COL4L6* (OMIM 303631) for DFNX6.

The most common gene for DFNX is *POU3F4*, encoding a transcription factor that belongs to the POU-domain family (NM_000307.4) [4, 5]. *POU3F4* comprises a POU-specific domain and a POU-homeodomain, both of which influence DNA binding and specificity [6]. *POU3F4* is expressed in the developing neural tube, the supraoptic and paraventricular nuclei of the hypothalamus [5, 7], and the inner ear (mainly in the spiral ligament, Reissner's membrane and bony spiral lamina) [8–12]. DFNX2 was originally reported in 1971 as an X-linked pattern characterized in males by profound mixed deafness, vestibular abnormalities, and congenital fixation of the stapes with perilymphatic gusher in a Caucasian kindred [13]. In 2002, this type of cochlear abnormality was classified as incomplete partition type III (IP-III) [14]. Previous studies have identified over 90 mutations of *POU3F4* related to DFNX2 including missense/nonsense, insertions, deletions, or duplications (The Human Gene Mutation Database, <http://www.hgmd.cf.ac.uk>). Missense mutations in functional domains might change their structures, abolishing their original roles in cells or inducing endoplasmic reticulum stress [4, 15–17]. Frameshift truncations and extension mutations are also pathogenic as they cause functional deterioration by reducing protein stability and mislocations within the cell [18].

Phenotypically, DFNX2 predominantly manifests in male patients as severe-to-profound mixed hearing loss with cochlear anomalies characteristic of IP-III, dilated basal turn and absent modiolus [13, 14, 19]. Cochlear implantation (CI) is one of the main methods of hearing intervention in this population although it is associated with increased risk of cerebrospinal fluid (CSF) gusher and aberrant

electrode positioning in the internal auditory canal (IAC) [20]. Furthermore, CI outcomes were reported to be variable [21–23], probably because the studies were based on limited case series with low patient numbers.

In this study, we report the clinical characteristics and molecular findings by Sanger or Nanopore single-molecule sequencing in IP-III patients resulting from *POU3F4* anomalies as well as their hearing intervention outcomes.

Materials And Methods

Patients

Between January 2017 and October 2019, 18 male patients from 14 unrelated Chinese families diagnosed with IP-III malformations were recruited in the Department of Otolaryngology-Head and Neck Surgery, Shanghai Ninth People's Hospital Affiliated to Shanghai Jiaotong University School of Medicine. All 18 patients underwent a complete medical history inquiry and gave written, informed consent to participate in this study.

Clinical characteristics

Auditory evaluations included otoscopic examination, tympanogram, pure tone average (PTA) or auditory brainstem responses (ABR). The degree of hearing impairment was calculated as the average of the hearing levels at 0.5, 1.0, 2.0 and 4.0 kHz for the better ear. The severity of hearing loss was defined as mild (26–40 dB HL), moderate (41–60 dB HL), severe (61–80 dB HL), or profound (> 80 dB HL).

Temporal bone high-resolution computed tomography (HRCT) was performed using a 64-section CT scanner (Somatom Definition Flash, Siemens Medical Solutions, Germany). Bone algorithm reconstruction was used for image acquisition. The main parameters for CT scanning were as follows: 120 kV, 150 mAs, and 0.6 mm section thickness. Cochlea, IAC, vestibule, semicircular canals, vestibular aqueduct, nerve canals in the fundus of the IAC, stapes and cochlear nerves (CN) were rigorously investigated by HRCT.

Hearing interventions and outcome evaluations

Appropriate hearing interventions and follow-up evaluations were conducted for patients according to their age, level of hearing loss and individual factors. Aided PTA of hearing aid (HA) and CI was recorded as hearing outcomes. In cases of CI, these data were compared with 20 implantees in our center who had normal cochlear structures. The t-test was used to compare the aided PTAs using IBM SPSS Statistics, Version 19.0 (IBM Corp., Armonk, NY, USA). A *p*-value less than 0.05 was considered indicative of statistical significance and all of the tests were two-tailed.

Genetic analysis

Genomic DNA was extracted from blood samples using the Blood DNA kit (TIANGEN BIOTECH, Beijing, China). All probands, and other family members where possible, were screened for variants of *POU3F4* coding sequences using PCR amplification and bidirectional Sanger sequencing. Possible pathogenic effects of the missense mutations were evaluated using computational tools including Mutation Taster, PROVEAN and SIFT. The allele frequencies of the variants detected were also investigated in 200 Chinese patients with normal hearing. The 3D structures of proteins were modeled using the Swiss-Model website (<http://swissmodel.expasy.org>) and visualized using RasMol (v. 2.7.5) software.

For those with negative results of *POU3F4* Sanger sequencing, Nanopore long-read single-molecule sequencing was used to identify structural variants (SVs). The major types of SV included deletion (DEL), insertion (INS), duplication (DUP), inversion (INV), and translocation (TRA). Further annotation of SVs was performed using ANNOVAR(<https://github.com/WGLab/doc-ANNOVAR>) combining public databases such as 1000 genome phase3, DGV gold standard CNV, dbVar nstd37, and Decipher.

Results

Clinical data and hearing phenotypes

In total, 18 male patients with IP-III malformations were recruited for this study (Fig. 1). The patients were from 14 unrelated Chinese families including thirteen Han and one Zang family (Family 08). The mean age at identification of hearing loss was 1.1 years (range: 0.3–3.6). All patients had bilateral, prelingual, sensorineural or mixed deafness. The results of click-ABR of 14 pediatric patients ranged from 50 to > 97 dB nHL. With respect to the tympanograms, 67% (12/18) of them presented a type “A” tympanogram, 17% (3/18) had type “C”, and 11% (2/18) presented type “B”. Among them, 4 had other problems in addition to hearing loss (Table 1). To note, patient 4 –

1 was diagnosed with mild autism and had undergone bilateral tympanotomy tube insertion when he was 7 months old to treat "secretory otitis media (SOM)".

Table 1
Clinical and molecular genetic characteristics of patients with IP-III malformation

Family	Patient	Age at detection (y)	Age at visit (y)	Hearing loss level ^a	Tympanogram	Other problems	Hearing intervention (side and age at which CI ^b or HA ^c was fitted)	<i>POU3F4</i> variants
01	1-1	0.5	4.2	L 85; R 60 dB nHL	A	/	HA (L + R, 1.7y)	c.962T > G (p.Val321Gly)
02	2-1	0.4	2.4	L 80; R 70 dB nHL	A	/	CI (L, 4.0y) + HA (R, 0.7y)	c.541C > T (p.Gln181*)
03	3-1	0.4	2.3	L 65; R 65 dB nHL	A		CI (R, 2.7y) + HA (L, 0.5y)	c.699C > A (p.Cys233*)
04	4-1	0.5	2.1	L > 97; R 80 dB nHL	C	Mild autism	CI (L, 4.5y) + HA (R, 2.5y)	g.81548899_82006629delins ACCAATTGGTAGTACAAT
05	5-1	0.6	3.8	L 60; R 65 dB nHL	A	/	HA (L + R, 3.8y)	c.644T > G (p.Val215Gly)
	5-2	3.4	23.5	L 65; R 67.5 dB HL	A	/	HA (L + R, 14.0y)	c.644T > G (p.Val215Gly)
06	6-1	0.5	0.5	L > 97; R > 97 dB nHL	A	/	CI (R, 1.0y)	g.81806051_82292259del
	6-2	3.6	67.2	L 106; R 107.5 dB HL	A	/	None	g.81806051_82292259del
07	7-1	0.5	2.0	L > 97; R > 97 dB nHL		/	CI (R, 2.5y)	c.648dupG (p.Leu217ValfsTer9)
08	8-1	3.5	14.8	L 115; R 116 dB HL	C	/	CI (R, 15.0y)	c.845G > A (p.Arg282Gln)
	8-2	3.0	12.0	L 107.5; R 105 dB HL	C	Cataract	CI (R, 12.0y)	c.845G > A (p.Arg282Gln)
09	9-1	0.6	0.9	L 70; R > 97 dB nHL	A	Developmental retardation	CI (R, 1.2y) + HA (L, 0.9y)	c.171G > A (p.Trp57*) ^d
10	10-1	0.4	2.0	L > 97; R > 97 dB nHL	A	/	CI (R, 3.0y)	g.81839469_82004841del
	10-2	0.4	2.0	L > 97; R > 97 dB nHL	A	/	CI (R, 2.4y)	g.81839469_82004841del

^a dB nHL: click-ABR results, dB HL: PTA results.

^b CI: cochlear implantation. ^c HA: hearing aid.

^d De novo mutation.

Family	Patient	Age at detection (y)	Age at visit (y)	Hearing loss level ^a	Tympanogram	Other problems	Hearing intervention (side and age at which CI ^b or HA ^c was fitted)	<i>POU3F4</i> variants
11	11 - 1	0.3	0.7	L 65; R 60 dB nHL	A	/	HA (L + R, 0.7y)	c.946C > T (p.Gln316*)
12	12 - 1	0.4	0.7	L 50; R 55 dB nHL	B	/	HA (L + R, 1.0y)	c.903_912 delins TGCCA (p.Lys302AlafsTer25)
13	13 - 1	0.3	4.5	L 75; R 60 dB nHL	B	/	HA (L + R, 4.5y)	c.614_616delGAA (p.Arg205del) ^d
14	14 - 1	0.5	0.6	L 65; R 60 dB nHL	A	Atrial septal defect	HA (L + R, 0.6y)	g.81807331_81887213del ^d
^a dB nHL: click-ABR results, dB HL: PTA results.								
^b CI: cochlear implantation. ^c HA: hearing aid.								
^d De novo mutation.								

Among the mothers of all probands, subject 5 - 3 was 47 years of age and reported a history of left myringoplasty about two decades earlier. She presented mixed hearing loss in the left ear (PTA = 48.75 dB HL) and sensorineural hearing loss in the right ear (PTA = 40 dB HL). Meanwhile, HRCT scans showed unaffected inner ears.

Radiological findings

The HRCT images of all 18 patients showed typical IP-III anomalies: bilateral and symmetrical malformation, a relatively normal shape of outer coating with absence of cochlear modiolus and bony spiral lamina. There was a direct intercommunication between cochlea and IAC with a "cloudy like" characteristic (Fig. 2a I-III). There was a high jugular bulb in 83.3% (15/18) of patients (Fig. 2a IV). Stapes abnormalities were present in 11.1% (2/18) of patients and included a thickened stapes footplate and absence of fissula ante fenestram (Fig. 2a V). A vestibular aqueduct was occasionally visible, with a normal shape on axial CT images (Fig. 2a VI). The semicircular canals and vestibular aqueduct shapes were also normal. Postoperative HRCT was performed in six CI users. As examples, straight electrode arrays were successfully implanted on the right side in patients 7 - 1 and 10 - 2 (Fig. 2b I-II).

Mutations in *POU3F4* gene by Sanger sequencing

From Sanger sequencing, 12 patients from 10 families had hemizygous variants in *POU3F4* (Table 1) including 4 nonsense mutations, 3 missense variants, 2 frame-shift mutations and one indel mutation. Apart from c.648dupG identified in Family-07, the other 9 variants were novel. Besides, two variants p.Trp57* and p.Arg205del were de novo mutations for the patients from Family-09 and - 13 cause their mothers had no variants of *POU3F4*. All these mutations were not present in 200 normal hearing Chinese controls (150 Han and 50 Zang).

Four nonsense mutations, p.Gln181*, p.Cys233*, p.Trp57* and p.Gln316* were identified in Family-02, -03, -09 and - 11, respectively (Fig. 3a). Mutations p.Trp57* and p.Gln181* resulted in the loss of the entire POU-specific (POUs) domain and the POU homeodomain (POU_{HD}). In particular, proband 9 - 1 had a de novo p.Trp57* hemizygous mutation. p.Cys233* was located in the POU_s domain and p.Gln316* was present in the POU_{HD}. Both caused premature termination in *POU3F4*. These residues are highly conserved in species including primates, mammals and other vertebrates (Fig.S1ab).

Three missense variants p.Val321Gly, p.Val215Gly and p.Arg282Gln were identified in Family-01, -05 and - 08, respectively (Fig. 3a). These residues are also highly conserved in different species (Fig.S1ab). These three variants were predicted to be disease causing and deleterious by the three prediction tools used in this study (Table.S1).

Two frame-shift mutations c.648dupG and c.903_912 delins TGCCA were identified in Family-07 and -12, respectively. They caused amino acid mutations of p.Leu217ValfsTer9 and p.Lys302AlafsTer25, respectively (Fig. 3a), and resulted in premature termination of *POU3F4*.

The indel mutation c.614_616delGAA (p.Arg205del), identified in Family-13, was also a de novo mutation in this study. This residue is also highly conserved in different species (Fig.S1a). The predicted structures of wild-type and mutant proteins were observed and analyzed using RasMol (Fig.S1c).

Deletions identified in upstream of *POU3F4* by Nanopore long-read single-molecule sequencing

Sanger sequencing of *POU3F4* was negative in 4 families (Family-04, -06, -10 and -14), so Nanopore long-read single-molecule sequencing was conducted. A total of 40–50 Gb of reads with a mean length of 11.6–22 kb was obtained in each sample with an average coverage of 95.3%, and an average depth of 13–16× for the whole genome. Four different types of DELs were identified (Fig. 3b). The breakpoint junctions of the DELs were verified by Sanger sequencing and the sequences of primers were listed in Table S2.

In proband 4 – 1, we detected a 458 kb DEL and an 18 bp INS located at ChrX (g.81548899_82006629delinsACCAATTGGTAGTACAAT), approximately 1502 kb upstream of *POU3F4*. A total of 7 reads supported the DEL. The breakpoint junction and insertion were successfully sequenced in the proband and his mother (Fig. 3c I).

In proband 6 – 1, we identified a 486 kb DEL (g.81806051_82292259del), 1217 kb upstream of *POU3F4*. A total of 7 reads supported the DEL. The junction was successfully sequenced in the proband, his mother and his grandfather (Fig. 3c II).

In patient 10 – 1, a 165 kb DEL (g.81839469_82004841del) was detected, about 1503 kb upstream of the *POU3F4* gene. A total of 8 reads supported the DEL. The junction sequence was successfully amplified for the proband, his twin brother and their mother (Fig. 3c III).

In proband 14 – 1, we detected a 80 kb DEL (g.81807331_81887213del), 876 kb upstream of the *POU3F4* gene, and this mutation was successfully sequenced (Fig. 3c IV). His mother's genotype was normal. The proband had a de novo DEL located upstream of *POU3F4*.

Effect of hearing intervention and its correlation with genotypes

Appropriate hearing intervention was carried out in 94.4% (17/18) of patients in this study. Ten patients received unilateral CI with or without HA on the contralateral side. Seven patients wore bilateral HAs. Only one patient did not receive hearing intervention: Patient 6 – 2, 67 years of age, had congenital hearing loss, never had a HA and used sign language in daily life.

For 10 CI users, mean aided PTA was 40.3 ± 7.59 dB HL (range: 25.0–51.3 dB HL) 12 months after activation of the implant. Compared with the control group (age-matched CI recipients with normal cochlea, N = 20, PTA = 34.0 ± 5.74 dB HL), the result was not statistically different ($p = 0.20$). The genotypes of these IP-III patients consisted of 3 nonsense mutations (p.Gln181*, p.Cys233*, p.Trp57*), one frame-shift mutation (c.648dupG), one missense variant (p.Arg282Gln) and 3 upstream DELs (165–486 kb).

For 7 patients with bilateral HAs, mean aided PTA of the better ear was 41.1 ± 5.18 dB HL (range: 35.0–50.0 dB HL). The genotypes of them were p.Val215Gly (two siblings), p.Val321Gly, p.Arg205del, c.903_912 delins TGCCA, p.Gln316* and g.81807331_81887213del (80 kb DEL).

Based on the location of variants, 17 patients with HAs or CI were further divided into two groups: variants located in the exon region of *POU3F4* (ER group, N = 12) or the upstream region (UR group, N = 5). Mean aided PTA of the ER group was 39.6 ± 6.31 dB HL, and that of the UR group was 43.0 ± 7.10 dB HL. The difference was not significant ($p = 0.342$).

Discussion

In this study, we analyzed the clinical characteristics, molecular variants and hearing intervention outcomes in 18 patients with IP-III from 14 unrelated families. To the best of our knowledge, the largest cohort with IP-III in previous studies was 11 cases [24]. Ten different variants were identified in the exon region of *POU3F4*, of which nine were novel. Four novel DELs in the upstream region of *POU3F4* were identified by Nanopore long-read single-molecule sequencing. For the first time, this study also measured the hearing outcomes of IP-III patients grouped by different locations of variants in *POU3F4*.

For the clinical diagnosis of IP-III, it is difficult to predict IP-III malformation merely from the family history and audiological outcomes. In this study, 8 of 14 (57.1%) IP-III families contained sporadic cases and the level of hearing loss varied greatly. HRCT of temporal bone

appears to be more reliable for predicting *POU3F4* variants and is essential for IP-III diagnosis. HRCT images showed typical characteristic “cloudy like” anomalies: a relatively normal shape of outer coating, and direct intercommunication between the cochlea and IAC. Incomplete partitions occurred in 41% of inner ear malformations and IP-III constituted 2% of malformations in this region [4]. To note, HRCT examination is strongly recommended before invasive hearing intervention in children with mixed hearing loss. In this study, one patient was misdiagnosed with SOM as he did not undergo a CT scan before bilateral tympanotomy tube insertion.

71.4% (10/14) of unrelated IP-III patients had hemizygous variants in the exon region of *POU3F4* based on Sanger sequencing in this study. 28.6% (4/14) patients had 80–486 kb DELs upstream of *POU3F4* using Nanopore long-read single-molecule sequencing. In previous studies, DELs identified upstream of *POU3F4* varied from 2.6 kb to 1.75 Mb [25, 26]. Entire deletions, insertions and translocations of *POU3F4* were also recognized to be pathogenic in some cases [25, 27–29]. The DELs upstream of *POU3F4* were also located downstream of *SH3BGRL*. *SH3BGRL* was reported to play a role in gastric cancer, acute myeloid leukemia and breast cancer [30–32]. However, whether DELs located between *POU3F4* and *SH3BGRL* genes influence the function of *SH3BGRL* remains unknown.

Besides, 21.4% (3/14) de novo mutations of *POU3F4* were found including two mutations located in the exon region and one located in the upstream region. In a previous study, de novo genetic mutations occurred in 20% of patients with *POU3F4* mutations [27]. These results indicate that the high rate of de novo mutations is one reason for the relatively high incidence of sporadic cases of *POU3F4* variants. To note, two probands with de novo mutations located in the exon region (p.Trp57* and p.Arg205del) presented relatively asymmetric hearing loss (interaural threshold gap ≥ 15 dB nHL).

Hearing intervention outcomes were reported to vary greatly among IP-III patients [16, 25]. Stankovic et al reported limited auditory perception and language acquisition after CI in IP-III patients and commented that HA may sometimes be a better alternative than CI [23]. In our study, aided PTA values in HA users and CI recipients were 41.1 ± 5.18 dB HL and 40.3 ± 7.59 dB HL, respectively. These results showed that CI was also an optimal intervention method for IP-III patients. This study firstly focused on the correlation of genotype and hearing outcomes based on the variants locations. As a result, the aided PTA presented no difference regardless of the variants locations on exon or upstream regions ($p = 0.342$).

This study has three potential limitations. The first is the short follow-up period of 12 months for hearing outcomes. Long-term follow-up is still needed. The second limitation is the uncertainty over the pathogenicity of variants, especially DELs identified in the upstream region of *POU3F4*. Further functional studies are required at the animal level to obtain clear evidence. The final limitation of this study is the lack of sensitive indicators to predict outcomes of hearing intervention in IP-III patients. The benefits of CI vary greatly among individual patients [23, 24, 33]. It has also been demonstrated that the number and capability of spiral ganglion neurons (SGNs) are important factors for CI outcomes [34–36]. Animal studies have shown that radial bundle fasciculation and hair cell innervation of SGNs were impaired after *Pou3f4* was deleted from the otic mesenchyme [34]. A recent animal study verified that, with the absence of the mesenchyme-specific transcription factor *Pou3f4*, a portion of SGNs underwent apoptosis after birth resulting in an approximately 25% decrease in the overall SGN population [37]. Whether there is a neurophysiological or other indicator which might provide clinical prediction of the function of SGNs and CI outcomes is still unknown.

In conclusion, 28.6% (4/14) had no mutation in exon region of *POU3F4*, however, possible pathogenic deletions were identified in upstream region of this gene. De novo genetic mutations occurred in 21.4% (3/14) of patients with *POU3F4* mutation. Hearing intervention outcomes of IP-III patients presented no difference regardless of the variants locations on exon or upstream regions.

Abbreviations

IP-III: incomplete partition type III; DELs: deletions; PTA: pure tone average; CI: cochlear implantation; CSF: cerebrospinal fluid; IAC: internal auditory canal; HRCT: high-resolution computed tomography; CN: cochlear nerves; ABR: auditory brainstem responses; IT-MAIS: infant-toddler meaningful auditory integration scale; CAP: categories of auditory performance; MUSS: meaningful use of speech scale; SIR: speech intelligibility rating; SV: structural variants; INS: insertion; DUP: duplication; INV: inversion; TRA: translocation; SOM: secretory otitis media; NHS: newborn hearing screening; CISS: constructive interference in steady-state; HA: hearing aid; ER: exon region; UR: upstream region

Declarations

Ethics approval and consent to participate

All participants gave written, informed consent to participate in this study. The study was approved by the Ethics Committee of the Ninth People's Hospital, Shanghai Jiaotong University School of Medicine (No. SH9H-2019-T245-1).

Consent for publication

A consent for publication has been obtained.

Availability of data and materials

Please contact author for data requests.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HW, ZWH and YL designed the research; YC analyzed the data and wrote the manuscript; YC and QJJ performed genotyping and analyzed the data; YC, YWW, MDJ, JJQ, ZLW, JY and HBS participated in phenotyping and clinical data collection; HW, ZYW, ZHZ, HJ and LXH performed surgery. All authors read and approved the final manuscript.

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Figures

Figure 1

Pedigrees of the 14 unrelated families. Among them, 18 patients diagnosed with IP-III were enrolled in this study and they were numbered from 1-1 to 14-1.

a: HRCT images from some patients showing IP-III malformations

b: HRCT of CI patients showing position of electrodes

Figure 2

HRCT images of temporal bone and MRI of inner ear in some IP-III patients (a) HRCT images from some patients showing IP-III malformations. I-III: “Cloudy like” characteristics of the cochlea: absence of cochlear modiolus and bony spiral lamina, and direct intercommunication between cochlea and IAC; IV: High jugular bulb; VIII-IX: Thickened stapes footplate; V: Visible vestibular aqueduct. (b) HRCT of CI patients showing position of electrodes. I: Coronal scan of patient 7-1; II: Axial scan of patient 10-2.

Figure 3

Identification of *POU3F4* variants by Sanger sequencing and Nanopore long-read single-molecule sequencing (a) Schematic illustration of the exon region of *POU3F4* with mutations labeled. Four nonsense mutations, three missense variants, two frame-shift mutations and one indel mutation were identified. (b) Schematic illustration of the upstream region of *POU3F4* with DELs indicated. These DELs were located between *SH3BGRL* and *POU3F4* genes on the X chromosome. Four DELs varied from 80 to 486 kb. (c) The breakpoint junction sequence of families with DELs upstream of *POU3F4*. The red arrows indicate the breakpoints. An INS of 18 bp was also identified in Family-04.

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