

## ORIGINAL RESEARCH

# A novel mutation of X-linked recessive deafness gene *POU3F4* in a boy with congenital deafness

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**Abstract**

**Purpose:** To report an interstitial deletion of Xq21.1 in chromosome X in a boy with congenital deafness.

**Methods:** The proband underwent a thorough physical examination and a detailed audiological and temporal bone computed tomography (CT) scan. Cochlear implantation was performed on the proband, and follow-up was conducted. High throughput sequencing and copy number analysis was made of peripheral blood samples from the proband, family members, and control subjects.

**Results:** Sensorineural hearing loss was present in the boy and temporal bone CT scan showed a bilateral incomplete partition type III anomaly (IP-III). Q21.1 (79.40–83.32 Mb) of chromosome X in the proband had a copy number deletion with a fragment size of about 3.92 Mb. Categories of auditory performance scores and SIR scores of the cochlea in this child improved after surgery.

**Conclusion:** Through the analysis of *POU3F4*, a novel mutation site with potentially pathogenic significance was found.

Level of Evidence: 5.

**KEYWORDS**

cochlear malformation, deafness, *POU3F4* gene, temporal bone CT

## 1 | INTRODUCTION

Congenital deafness is estimated to occur in 0.1%–0.3% of neonates.<sup>1</sup> Non-syndromic hearing loss caused by X-linked mutations accounts for 1%–2% of hereditary hearing loss.<sup>1–3</sup> Currently, four genes on the X chromosome are known to cause non-syndromic hearing loss, namely *COL4A6*,<sup>4</sup> *PRPS1*,<sup>5</sup> *POU3F4*,<sup>6</sup> and *SMPX*,<sup>7</sup> with *POU3F4* causing 40% of X-linked non-syndromic deafness cases.<sup>8</sup>

The human *POU3F4* gene is located on chromosome Xq21.1 and encodes POU3F4, a member of the POU domain family, that is

crucial to the development of the inner ear.<sup>6</sup> Incomplete partition type III (IP-III) cochlear malformation is found in patients with *POU3F4* mutations.<sup>9</sup>

Here, we report a novel 3.92 Mb deletion at Xq21.1 region in the affected individual by using copy number variation sequencing (CNV-seq) detection after deafness gene panel test. Because panel detection suggests a larger range of missing areas, CNV detection was selected. Patients with *POU3F4* gene mutations have uncertain outcomes after cochlear implantation in the literature. But cochlear implantation resulted in significant hearing improvement in our patient.

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## 2 | METHODS

### 2.1 | Ethics

This study was approved by the ethics committee of our hospital, and all subjects or their guardians provided written informed consent to the study.

### 2.2 | Subjects

The subject was a 19-month-old Han Chinese boy who had poor response to sound after birth and subsequently received cochlear implantation at the Department of Otolaryngology of our hospital. A detailed medical history was collected from the proband's parents and the proband. All family members of the proband suspected of having a hearing problem underwent detailed physical examination, including an examination by an otolaryngologist. Neural response telemetry (NRT) and impedance detection were performed post cochlear implantation in the proband, and impedance between two points was detected by NRT.

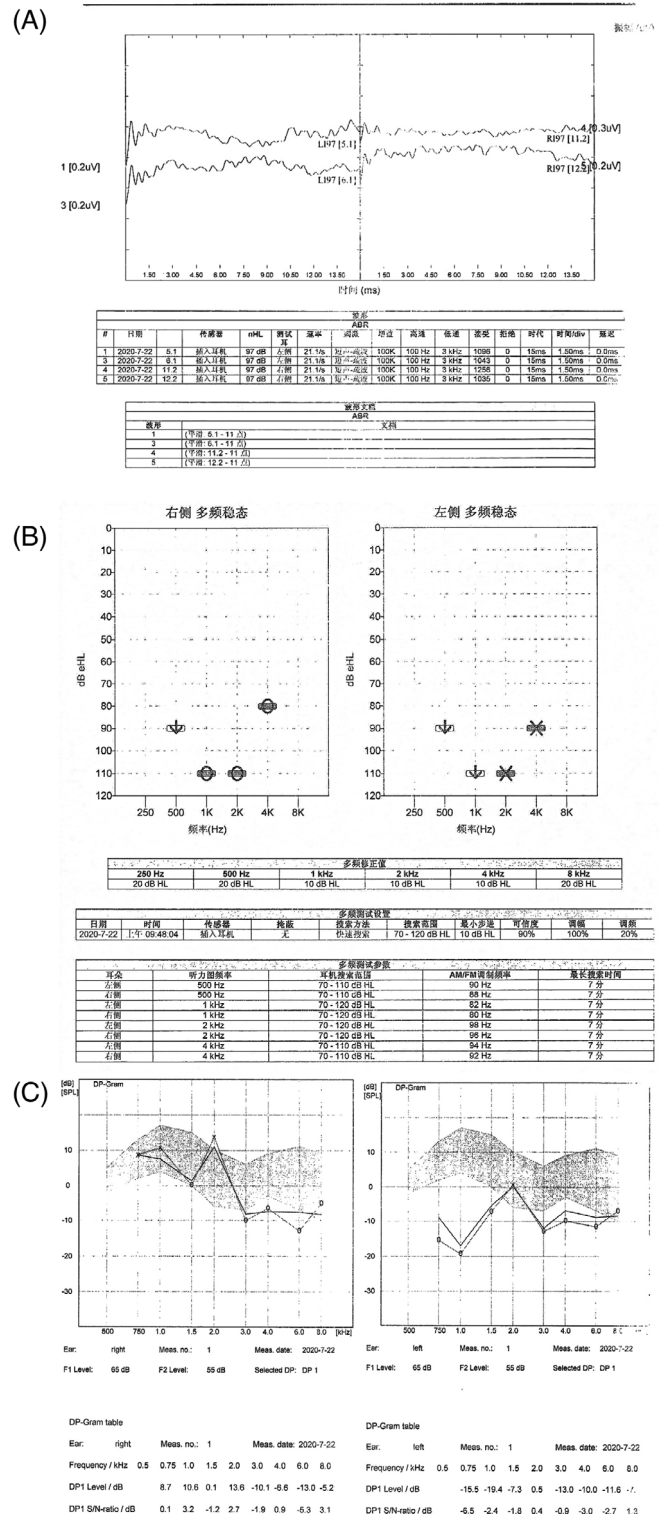
High-throughput sequencing Peripheral venous blood samples (5 ml, thrice) were obtained from the proband, his parents, and other family members suspected of hearing loss. In addition, venous blood samples from 50 normal controls were obtained from the molecular genetic database of our hospital. Genomic DNA was extracted using the Genomic DNA Extraction Kit according to the manufacturer's instructions (Qiagen., Valencia, CA). DNA concentration and purity were determined by spectrophotometry (Nanno-1000). High-throughput DNA sequencing was done at Boao Laboratory. The average sequencing coverage was about 0.198 using the JINGXIN BioelectronSeq 4000 System sequencing platform. The genetic information of the proband's mother was verified by first-generation Sanger sequencing.

### 2.3 | Copy number analysis

CNV-Seq was done for copy number analysis of the mother and the child. The CNVs were compared with genomic CNV polymorphism databases including Database of Genomic Variants (DGV, <http://dgv.tcag.ca/dgv/app/home>), Decipher (<https://www.deciphergenomics.org/browser>), and International Standards for Cytogenomic Arrays (ISCA). Diagnosis was made according to the American Society of Medical Genetics and Genomics ACMG Sequence Variation Classification Standards and Guidelines 2015.

## 3 | RESULTS

The medical history of the proband, and family history investigation revealed that only the patient was deaf, and both his parents had normal hearing. The resistance maps of the bilateral eardrum chambers of the proband were A-type with an acoustic response



**FIGURE 1** (A) ABR: Bilateral lead >90 dB; (B) ASSR shows bilateral very severe sensorineural deafness; and (C) DPOAE was not elicited bilaterally

value of 0.49 ml in the left ear and 0.40 ml in the right ear and acoustic reflection thresholds of 0.5, 1, 2, and 4 kHz not evoked. Auditory brainstem response in both ears for air conduction failed to elicit a response at 97 dBnHL. Response to the two aural



vestibular semicircular canals were irregular in shape. The bone wall of the right posterior semicircular canal and the horizontal semicircular canal were partially defective. The left posterior semicircular canal and jugular bulbar osseous septum were absent. Bilateral vestibular aqueducts were enlarged in the middle portion, but not in the outer orifice.

The hearing threshold values improved after cochlear implantation. Similarly, the Central Auditory Processing and Speech Intelligibility Rating scores both improved from 0 before cochlear implantation to 3 at 3 months and then to 5 at 6 months post cochlear implantation.

High-through sequencing and CNV analysis revealed that Q21.1 (79.40 Mb–83.32 Mb) of the patient's chromosome X had a copy number deletion with a fragment size of about 3.92 Mb, which was identified as a newly developed mutation through pedigree analysis, as shown in Figure 3. The deletion of the Xq21.1 region has not been reported in the DGV database, the general population's genomic polymorphism database. The Decipher and ISCA databases revealed two potentially pathogenic cases smaller than the deletion of the region found in the study.

## 4 | DISCUSSION

Human *POU3F4* gene is located on chromosome Xq21.1, and encodes POU3F4, which is a member of the POU domain family. About 50% of non-comprehensive X-linked hearing loss cases are caused by single nucleic acid mutation, gene deletion in the coding region of *POU3F4* or deletion of upstream regulatory elements.<sup>10</sup> Typical radiographic features can be distinguished from other X-linked hearing loss, such as cochlear incomplete partition type III, spherical enlargement of the internal auditory canal, abnormal communication between the cochlear basement gyrus and the internal auditory canal and thickening of the stapes plate.<sup>11</sup> In animal models, *POU3F4* mutation can block mesenchymal differentiation, leading to a variety of ear development malformations, which may explain why *POU3F4* mutation leads to hearing loss and imaging manifestations in humans.<sup>12,13</sup> So far, dozens of pathogenic mutations of the *POU3F4* gene have been identified, which are mainly missense mutation, insertion mutation and deletion mutation. Most of the pathogenic mutations occur in the POU specific domain and the POU homologous domain, and only a few occur in the upstream region of the POU domain. The phenotypes of these mutations vary mainly from mixed deafness to very severe sensorineural deafness. CNV is also an important cause of hereditary deafness, accounting for one-fifth of the molecular causes of all non-syndromic deafness.<sup>14-17</sup> However, in this study, there was copy number deletion of a fragment size of 3.92 Mb at q21.1(79.40-83.32 Mb) of X chromosome of the submitted samples. Through family analysis and literature search, the mutation appears to be novel and is the first reported such mutation in the world. The mutation results in the loss of all *POU3F4* gene sequences, with the loss of functional *POU3F4*, disrupting the normal development of structures in the middle and inner ear, and leading to hearing impairment. The report of this mutation further enriches the spectrum of human *POU3F4* gene mutations.

There have been many reports of syndromic hearing impairment caused by the deletion of large fragment in the XQ21 region of the

*POU3F4* gene and its adjacent genes. The specific phenotype depends on the size of CNVs and the content of pathogenic genes, mainly including *DFNX2*, attention deficit, mental retardation, and choroidal hemorrhage.<sup>18-22</sup> Although the deletion range in our case also covered other genes, the patient did not present other specific phenotypes, and there was no clear evidence of pathogenicity in the other genes at present, so syndromic hearing impairment was ruled out in our patient. However, it cannot be ruled out that the proteins encoded by other genes may interact with the mutated *POU3F4* gene and produce functional changes associated with the observed phenotypes, which need to be further identified by genome-wide analysis.

In the patient studied, sensorineural hearing loss was present, and temporal bone CT showed a bilateral incomplete cochlear partition deformity (IP-III). Nadol et al.<sup>23</sup> reported a significant correlation between the number of residual spiral ganglia cells and sensorineural hearing loss. IP-III malformed patients with the absence of a cochlear axis and an uncertain number of spiral ganglion cells have been considered as contraindications for cochlear implant (CI) surgery, and different studies have evaluated its postoperative effects. Kang et al.<sup>24</sup> found that the Meaningful Auditory Integration Scale and categories of auditory performance (CAP) scores of four patients with IP-III malformation after CI surgery were not significantly different from those of patients with normal cochlear development. They believed that appropriate auditory rehabilitation of such patients after CI surgery could help them approach hearing in those with normal cochlear development. Kim et al.<sup>25</sup> reported a case of IP-III malformation with bilateral CI surgery performed at 12 and 15 months after birth. After 12 months of postoperative follow-up, the average threshold of binaural hearing assistance was less than 40 dBHL, suggesting that auditory and speech rehabilitation of both ears could enable patients to achieve normal language development. We performed cochlear implant surgery, and the patient's hearing was improved after the surgery. The hearing thresholds, CAP scores, and SIR scores of the cochlea in this child with an IP-III malformation all improved after surgery compared with those before surgery. The CAP and SIR scores were zero points before surgery, three points 3 months after surgery, and five points 6 months after surgery, indicating good operative outcomes. However, its long-term effect remains to be further observed.

This study has some limitations. First, traditional detection methods have certain limitations for the precise location of CNVs breakpoints, such as rich complex repeating elements, high GC rate, pseudogenes, sequencing read length, and inability to accurately map back to the correct genome location in the area where the breakpoints are located. Second, as IP-M malformation is an X-linked recessive genetic disease, it has been reported that different mutation sites have different CI effects after surgery, and some mutation sites may lead to poor surgical results in patients. In this study, CAP, SIR and other hearing scores of patients with IP-III malformation after implantation of cochlear implant were much improved compared with those before implantation, and the surgical effect of this case was good. However, due to the lack of sufficient sample numbers about this mutation site, it is still necessary to further observe and study the long-term prognosis of patients with IP-III deformity after CI surgery. The *POU3F4* gene sits in the region on the X chromosome. This region is not covered by many important functional gene and is rich in height.

Conserved non-coding regions, which may lead to POU3F4 gene or correspondingly CNVs occurred more frequently in regions. To elucidate the exact mechanism of CNVs in POU3F4 gene, further analysis of repeat sequences around CNVs breakpoints is needed.

## 5 | CONCLUSION

To our knowledge, we reported for the first time a novel mutation site of POU3F4 and studied the long-term prognosis of the patient with IP-III deformity after CI surgery. Through the analysis of POU3F4, a novel mutation site with potentially pathogenic significance was found, suggesting a genetic etiology of deafness for the proband. This finding further enriches the mutation spectrum of POU3F4.

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## CONFLICT OF INTEREST

All the authors of this article declare that they have no conflict of interests.

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