A mutation of EYA1 gene in a Chinese Han family with Branchio-Oto syndrome

Rui Han, PhD\textsuperscript{a,b,c}, Yan Xia, MS\textsuperscript{a}, Zhijuan Liu, BS\textsuperscript{a}, Shuang Wu, MS\textsuperscript{a}, Erdengqieqieke Ye, MS\textsuperscript{a}, Ling Duan, BS\textsuperscript{a}, Jianbing Ding, PhD\textsuperscript{b,c,1}, Xiaolin La, MD\textsuperscript{a}

Abstract
Branchio-Oto (BO) syndrome is one of the common syndromic forms of hearing loss. In this study, we aimed to characterize the clinical and genetic features of BO syndrome in a Chinese deaf family.

The proposita in this study was a 29-years-old Chinese female with hearing loss, microtia, anterior concave auricle, and right branchial fistula. The family members agreed to undergo clinical examination. We collected blood samples from 7 family members, including 4 affected by the syndrome. Genomic DNA was extracted and subjected to Sanger sequencing. In addition, bioinformatics software SWISS MODEL was used to predict the protein encoded by EYA transcriptional coactivator and phosphatase 1 (EYA1) gene.

Intra-familial consistency can be observed in the clinical phenotypes of BO syndrome in this family. EYA1 c.1627C>T (p. Gln543Ter) mutation was identified as the pathogenic cause in this family.

This study reports a mutation associated with BO syndrome in a Chinese Han family. We highlight the utility of genetic testing in the diagnosis of BO syndrome. Thus, we believe that this report would provide a basis for the diagnosis of similar diseases in the future.

Abbreviations: BO = Branchio-Oto, BOR = Branchio-oto-renal syndrome, EYA1 = EYA transcriptional coactivator and phosphatase 1, GJB2 = Gap junction protein beta 2, HSD17B4 = Hydroxysteroid 17-beta dehydrogenase 4, MAF = minimum allele frequency, PTA = Pure tone audiometry.

Keywords: Branchio-Oto-syndrome, EYA transcriptional coactivator and phosphatase 1 gene mutation, target sequence capture sequencing

1. Introduction
Branchio-oto-renal syndrome (BOR, OMIM 113650), an autosomal dominant disorder, is featured by hearing loss, renal malformations and branchial arch anomalies.\cite{1} In the patients without aberrant renal anomalies, such condition is also defined as Branchio-Oto (BO) syndrome (OMIM 602588). The incidence of BOR/BO syndrome is estimated to be 1/40,000, and is responsible for 2% of deafness in children.\cite{2} To date, pathogenic variants in 3 genes have been identified including EYA1, SIX1 and SIX3. Besides, several genes (e.g., SHARPIN) have been reported to be associated with the pathogenesis of such disease.\cite{3,4,5,6} Among these genes, EYA1 localized on 8q13.3 is considered as the most common type of pathogenic gene with about 40% of the BOR/BO patients carrying mutation.\cite{7} Furthermore, EYA1 associated genome recombination and chromosome abnormality (chromosome 8) may be responsible for the onset of BOR/BO syndrome.\cite{8,9}

EYA1 may be affected by the gene dosage effects that may lead to differential phenotype in the BOR patients in a family. The amount of encoding protein decided the development of the branchial arch, ear and kidneys. Upon the amount of encoding protein surpassed a certain threshold, the gene activity would display.\cite{10}

Up to now, very few studies have been focusing on the BO syndrome.\cite{11,12} Meanwhile, little is known about the molecular mechanism of such disease.\cite{13,14} In this study, we identified a c.1627C>T (p.Gln543Ter) mutation in EYA1 as the pathogenic cause in a Chinese Han family with BO syndrome.

2. Materials and methods
2.1. Subjects
We collected the pedigree information of 7 members of a family over 3 generations at the First Affiliated Hospital of Xinjiang Medical University, China. The family members II-6, III-2, III-3,
and IV-1 received detailed family history inquiry and physical examination. Focal points of the clinical evaluation were cervical branchial cleft fistula, preauricular pit and auricle shape. For audiological tests, both air and bone-conducted pure tone audiometry (PTA) was performed. Each patient signed the informed consent. The study protocols were approved by the Ethical Committee of Xinjiang Medical University.

2.2. Karyotyping analysis and gene determination

3 mL of peripheral blood of patients II-6, II-17, III-1, III-2, III-3, III-4 and IV-1 were extracted. The 7 samples were cultured with fetal bovine serum (FBS) at 37°C at a constant temperature for 72 hours. G band method was utilized for the preparation of chromosomal sections. The karyotyping was obtained based on 10 chromosomal karyotypes from 30 metacinesis.

2.3. Methods

Upon sample collection, genomic DNA was extracted from peripheral blood samples (3 mL) of II-6, II-17, III-1, III-2, III-3, III-4, and IV-1 using FlexiGene DNA kits (Qiagen, product number 51206), target sequence capture technique was utilized for the screening of gene mutation in Kangxu Medical Institution (Peking, China). For DNA library preparation of the targeted next-generation sequencing, genomic DNA was fragmented to an average size of 180 bp. DNA repair, adapter ligation and PCR enrichment were performed as recommended by the Illumina protocols. The amplified DNA was captured using the DNA chips (Agilent, product number 5067–1522) and DNA 1000 Reagent (Agilent, product number 5067–1504). The DNA probes were designed to tile along the exon and partial intron regions of the 461 deafness genes (Table 1). Then NEXTSEQ500 (Illumina) was used for sequencing according to the concentration and depth requirement of DNA sample in the captured library and the manufacturer’s protocols.

2.4. Data analysis, variant annotation, and result verification

The files were transformed in bcl format, which was created by the sequencing platform, to fastq format using bcftools software. Then we assessed the sequencing quality using a series of bioinformatics software, including BWA, Samtools, Picard, and Genome Analysis Toolkit. Assessing factors includes the total count of reads, the percentage of reads that match human genome sequence, the percentage of reads that locate inside the target sequence of the target gene, average sequencing depth, and coverage uniformity (the percentages of sequencing depth greater than 1X, 5X, 10X, 15X, 20X, 25X, and 30X). Next, add annotation to the genetic variants using PolyPhen-2,2,2 software (http://genetics.bwh.harvard.edu/pph2/), ANNOVAR software (originally designed by Dr. Kai Wang), HGMD database, dbSNP database, and 1000 Genome database. In the last, verify the selected variants using Sanger method.

2.5. Bioinformatic analysis

The sequencing results were referred to the dbSNP database in the NCBI, Hapmap database, the 1000 genome database, and the SNP database of normal control provided by the Kangxu Medical Institution. Potential pathogenic variants were filtered using the minimum allele frequency threshold of 0.001 or less for dominant inheritance. Subsequently, the mutation was compared with the 183 mutations of EYA1 gene in the HGMD database (http://www.hgmd.org/) and that reported in the recent 5 years. The mutation-induced amino acid changes were judged using the SIFT, PolyPhen 2, Swiss-Model (http://swissmodel.expasy.org/) and Mutation Taster software (http://www.mutationtaster.org/), respectively. Co-segregation of the disease phenotype and the candidate variants were confirmed by Sanger sequencing of the family members. Moreover, their influences on the protein structure and function were evaluated, combined with the prediction of mutation pathogenicity. Swiss-Model software was utilized for the online homologous modeling of the EYA1 protein. The EYA1 protein encoded by the genes with mutation was predicted subsequently based on the mutation types.

3. Results

3.1. Clinical characterization

In this study, we included the pedigree information of 7 members of the families in 3 generations. The II-6, II-17, III-2, III-3 and III-4 were congenital deafness patients by consulting their case reports. They did not receive the neonatal screening. The neonates of IV-1 did not pass the neonatal screening, and was finally diagnosed with congenital deafness in our department. II-6 showed severe hearing loss, cervical branchial cleft fistula, preauricular pit and cup ear (right ear). II-17 showed severe hearing loss, mental deficiency, and right auricle malformation and absence of external acoustic meatus. III-2 and IV-1 showed severe hearing loss, cervical branchial cleft fistula, preauricular pit and cup ear (right ear). III-3 showed progressive hearing loss, cervical branchial cleft fistula, preauricular pit and cup ear (right ear, Table 2 and Fig. 1). Among the 4 lineal relations, 4 (II-6, II-3, II-3, IV-1) were diagnosed with BO syndrome. The pedigree analysis was in line with the features of autosomal dominant inheritance (Fig. 2). Ultrasonograms showed no anomalies in their (II-6, III-2, III-3, IV-1) kidney or urogenital tract. Their urine routine and blood test for renal function were normal.

Pure tone audiometry (PTA) indicated a significant gap between air- and bone-conducted hearing thresholds, suggesting mixed hearing loss with both sensorineural and conductive impairment (Fig. 3). High resolution CT axial view for temporal bone indicated partial or complete defect of auditory ossicle, together with osseous eustachian tube dilatation and cochlea malformation (Fig. 4).

3.2. Genetic observations and sanger sequencing

Based on the target sequence capture technique, screening of genetic deafness gene was performed by using the peripheral blood collected from the proband. Three suspicious variations were identified including EYA1 c.1627C>T (p.Gln543Ter) heterozygous, HSD17B4 c.1750A>G (p.Ile584Val) heterozygous and GJB2 c.109G>A (p.Val37Ile) heterozygous. No studies had reported the EYA1 c.1627C>T (p.Gln543Ter). Such gene was in line with the autosomal dominant inheritance. The HSD17B4 c.1750A>G (p.Ile584Val) and GJB2 c.109G>A (p.Val37Ile) were acknowledged pathogenic mutations, which were consistent with the autosomal recessive inheritance. Co-segregation of the disease phenotype and the candidate variants were confirmed by Sanger sequencing of the family members (Fig. 5). Sanger sequencing validation results were shown in Table 3.
### Table 1
Details of hereditary deafness gene panel.

| Disease | Gene       | Disease                      | Gene       |
|---------|------------|------------------------------|------------|
| Deafness, X-linked 1 | PRPS1 | Deafness, autosomal dominant 73 | PTPRQ |
| Deafness, X-linked 2 | POLUIF4 | Deafness, autosomal dominant 74 | PDE1C |
| Deafness, X-linked 4 | SMPX | Deafness, autosomal dominant with peripheral neuropathy | GJB3 |
| Deafness, X-linked 5 | AIFM1 | Deafness, autosomal recessive 1A | GJB2 |
| Deafness, X-linked 6 | COL4A6 | Deafness, autosomal recessive 1B | GJB6 |
| ??Deafness, X-linked 7 | GPRASP2 | Deafness, autosomal recessive 2 | MYO7A |
| ??Deafness, Y-linked 2 | TBL1Y | Deafness, autosomal recessive 3 | MYO15A |
| Deafness, autosomal dominant 1 | DIAF1 | Deafness, autosomal recessive 4, with enlarged vestibular aqueduct | SLC26A4 |
| Deafness, autosomal dominant 2A | KCNQ4 | Deafness, autosomal recessive 6 | TMIE |
| Deafness, autosomal dominant 2B | GJB3 | Deafness, autosomal recessive 7 | TMC1 |
| Deafness, autosomal dominant 3A | GJB2 | Deafness, autosomal recessive 8 | TMPLSS3 |
| Deafness, autosomal dominant 3B | GJB6 | Deafness, autosomal recessive 9 | OTOF |
| Deafness, autosomal dominant 4A | MYH14 | Deafness, autosomal recessive 10 | TMPLSS3 |
| Deafness, autosomal dominant 4B | CEACAM16 | (Deafness, autosomal recessive 12, modifier of) | ATP2B2 |
| Deafness, autosomal dominant 5 | GSDME | Deafness, autosomal recessive 12 | CDH23 |
| Deafness, autosomal dominant 6 | WFS1 | Deafness, autosomal recessive 15 | GIPC3 |
| Deafness, autosomal dominant 8 | TECTA | Deafness, autosomal recessive 16 | STRC |
| Deafness, autosomal dominant 9 | COCH | Deafness, autosomal recessive 18A | USH1C |
| Deafness, autosomal dominant 10 | EYA4 | Deafness, autosomal recessive 18B | OTOF |
| Deafness, autosomal dominant 11 | MYO7A | Deafness, autosomal recessive 21 | TECTA |
| Deafness, autosomal dominant 12 | TECTA | Deafness, autosomal recessive 22 | OTOA |
| Deafness, autosomal dominant 13 | COL11A2 | Deafness, autosomal recessive 23 | PCDH15 |
| Deafness, autosomal dominant 14 | WFS1 | Deafness, autosomal recessive 24 | RDX |
| Deafness, autosomal dominant 15 | POU4F3 | Deafness, autosomal recessive 25 | GPCR1 |
| Deafness, autosomal dominant 17 | MYH9 | ?Deafness, autosomal recessive 26 | GAB1 |
| Deafness, autosomal dominant 20 | ACTG1 | Deafness, autosomal recessive 28 | TR0BP |
| Deafness, autosomal dominant 22 | MYO6 | Deafness, autosomal recessive 29 | CLDN14 |
| Deafness, autosomal dominant 22, with hypertrophic cardiomyopathy | MYO6 | Deafness, autosomal recessive 30 | MYO3A |
| Deafness, autosomal dominant 23 | SIX1 | Deafness, autosomal recessive 31 | WHRN |
| Deafness, autosomal dominant 25 | SLC17A8 | Deafness, autosomal recessive 32, with or without immotile sperm | CDC14A |
| Deafness, autosomal dominant 26 | ACTG1 | Deafness, autosomal recessive 35 | ESR9B |
| Deafness, autosomal dominant 28 | GRHL2 | Deafness, autosomal recessive 36 | ESPN |
| Deafness, autosomal dominant 34, with or without inflammation | NLRP3 | Deafness, autosomal recessive 37 | MYO6 |
| Deafness, autosomal dominant 36 | TMC1 | Deafness, autosomal recessive 39 | HGF |
| ??Deafness, autosomal dominant 37 | COL11A1 | Deafness, autosomal recessive 42 | ILDR1 |
| Deafness, autosomal dominant 38 | WFS1 | ?Deafness, autosomal recessive 44 | ADCY1 |
| Deafness, autosomal dominant 39, with dentinogenesis | DSPP | Deafness, autosomal recessive 48 | CIB2 |
| Deafness, autosomal dominant 40 | CRYM | Deafness, autosomal recessive 49 | MARVELD2 |
| Deafness, autosomal dominant 41 | P2RX2 | Deafness, autosomal recessive 53 | COL11A2 |
| ?Deafness, autosomal dominant 44 | CCDC50 | Deafness, autosomal recessive 57 | PDZD7 |
| Deafness, autosomal dominant 56 | TNC | Deafness, autosomal recessive 59 | PJVK |
| Deafness, autosomal dominant 64 | DIABLO | Deafness, autosomal recessive 61 | SLC26A5 |
| Deafness, autosomal dominant 65 | TBC1D24 | Deafness, autosomal recessive 63 | LRTOMT |
| ?Deafness, autosomal dominant 66 | CD164 | ?Deafness, autosomal recessive 66 | CDDC2 |
| Deafness, autosomal dominant 67 | OSBP2 | Deafness, autosomal recessive 67 | UHPL5 |
| ?Deafness, autosomal dominant 68 | HOMER2 | Deafness, autosomal recessive 68 | SIPR2 |
| Deafness, autosomal dominant 69, unilateral or asymmetric | KITLS | Deafness, autosomal recessive 70 | PNP1 |
| ?Deafness, autosomal dominant 70 | MCM2 | Deafness, autosomal recessive 74 | MSRB3 |
| ?Deafness, autosomal dominant 71 | DMX2 | Deafness, autosomal recessive 76 | SYNE4 |
| ?Deafness, autosomal dominant 72 | SLC44A4 | Deafness, autosomal recessive 77 | LOXHD1 |
| Deafness, autosomal recessive 79 | TPRN | Usher syndrome type 3B | HARS1 |
| Deafness, autosomal recessive 81 | CLPP | (Retinal disease in Usher syndrome type IIA, modifier of) | PDZD7 |
| Deafness, autosomal recessive 84A | PTPRQ | Pendred syndrome | SLC26A4 |

(continued)
| Disease | Gene   | Disease Gene | Disease | Gene |
|---------|--------|--------------|---------|------|
| Deafness, autosomal recessive 84B | OTOGL | Waardenburg syndrome, type 1 | PAX3 | |
| Deafness, autosomal recessive 86 | TB1C024 | Waardenburg syndrome, type 2A | MITF | |
| Deafness, autosomal recessive 88 | ELMOD3 | Waardenburg syndrome, type 2D | SNA12 | |
| Deafness, autosomal recessive 89 | KARS1 | Waardenburg syndrome, type 2E, with or without neurologic involvement | SOX10 | |
| Deafness, autosomal recessive 91 | SERPNB6 | Waardenburg syndrome, type 3 | PAX3 | |
| Deafness, autosomal recessive 93 | CABP2 | Waardenburg syndrome, type 4A | EDNRB | |
| Deafness, autosomal recessive 94 | NARS2 | Waardenburg syndrome, type 4B | EDN3 | |
| Deafness, autosomal recessive 95 | MET | Waardenburg syndrome, type 4C | SOX10 | |
| Deafness, autosomal recessive 97 | TSPEAR | Waardenburg syndrome/ocular albinism, digenic | MITF | |
| Deafness, autosomal recessive 98 | TMEM132E | Waardenburg syndrome/ocular albinism, digenic | TYR | |
| Deafness, autosomal recessive 99 | PPP2SK2 | Branchiootorenal syndrome 1, with or without cataracts | EYA1 | |
| Deafness, autosomal recessive 100 | GRXCR2 | Branchiootorenal syndrome 2 | SIX5 | |
| Deafness, autosomal recessive 101 | EPS8 | Jervell and Lange-Nielsen syndrome | KCNQ1 | |
| Deafness, autosomal recessive 102 | CLIC5 | Jervell and Lange-Nielsen syndrome 2 | KONE1 | |
| Deafness, autosomal recessive 103 | RIPOR2 | Deafness, digenic, GJB2/GJB3 | GJB3 | |
| Deafness, autosomal recessive 104 | CDC14A | Deafness, digenic GJB2/GJB6 | GJB6 | |
| Deafness, autosomal recessive 105 | EPS5L2 | Deafness, congenital heart defects, and posterior embryotoxon | JAG1 | |
| Deafness, autosomal recessive 106 | WBP2 | Sinoatrial node dysfunction and deafness | CACNA1D | |
| Deafness, autosomal recessive 107 | ROR1 | Epithelial dysplasia, multiple, with myopia and deafness | COL2A1 | |
| Deafness, autosomal recessive 108 | ESPP1 | Polynephropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract | ABHD12 | |
| Deafness, autosomal recessive 109 | COCH | (Deafness, mitochondrial, modifier of) | TRMU | |
| Deafness, autosomal recessive 110 | MPZL2 | Hypoparathyroidism, sensorineural deafness, and renal dysplasia | GATA3 | |
| Deafness, autosomal recessive 111 | BDP1 | Corneal endothelial dystrophy and perceptive deafness | SLC4A11 | |
| Deafness, autosomal recessive 112 | CEACAM16 | Nephropathy with pretibial epidermolysis bullosa and deafness | CD151 | |
| Deafness, autosomal recessive 113 | GRAP | Leber congenital amaurosis with early-onset deafness | TUBB4B | |
| Deafness, autosomal recessive 114 | SPNS2 | Renal tubular acidosis with deafness | ATP6V1B1 | |
| Deafness, autosomal recessive 115 | GJB3 | Retinitis pigmentosa, X-linked, and sinorespiratory infections, with or without deafness | RPGR | |
| Alport syndrome, X-link | COL4A5 | ?Split-hand/foot malformation 1 with sensorineural hearing loss | DLX5 | |
| Alport syndrome, autosomal dominant | COL4A3 | Sensorineural deafness with mild renal dysfunction | BSND | |
| Alport syndrome, autosomal recessive | COL4A3 | Deafness, congenital with inner ear agenesis, microtia, and microdontia | FGFR3 | |
| Alport syndrome, autosomal recessive | COL4A4 | ?Microtia, hearing impairment, and cleft palate (AR) | HOX2A | |
| Alport syndrome, autosomal recessive | COL4A4 | Nephropathy with or without hearing impairment (AD) | HOX2A | |
| Norrie disease | NDP | Cerebellar ataxia, deafness, and narcolepsy, autosomal dominant | DNMT1 | |
| Usher syndrome, type 1B | MYO7A | Deafness and myopia | SLITRK6 | |
| Usher syndrome, type 1C | USH1C | Keratoderma, palmoplantar, with deafness | GJB2 | |
| Usher syndrome, type 1D | CDH23 | Deafness, neurosensory, without vestibular involvement, autosomal dominant | ESPN | |
| Usher syndrome, type 1D/F | CDH23 | Hystrix-like ichthyosis with deafness | GJB2 | |
| Usher syndrome, type 1E | PCDH15 | Keratitis-ichthyosis-deafness syndrome | GJB2 | |
| Usher syndrome, type 1F | PCDH15 | ?Peripheral neuropathy, myopathy, hoarseness, and hearing loss | MYH14 | |
| Usher syndrome, type 1G | USH1G | Dementia, familial Danish | ITM2B | |
| Usher syndrome, type 1H | CIB2 | Multiple synostoses syndrome 1 | NOD | |
| Usher syndrome, type 1I | ESPN | Ayme-Gripp syndrome | MAF | |
| Usher syndrome, type 1J | ARSG | Woodhouse-Sakati syndrome | DCAF17 | |

(continued)
| Disease                                                                 | Gene      | Disease                                                                 | Gene      |
|------------------------------------------------------------------------|-----------|-------------------------------------------------------------------------|-----------|
| Usher syndrome, type 2C                                                | ADGRV1    | Johanson-Blizzard syndrome                                              | UBR1      |
| Usher syndrome, type 2C, GPR98/PDZD7 digenic                          | ADGRV1    | Donnai-Barrow syndrome                                                 | LRPA2     |
| Usher syndrome, type 1C, GPR98/PDZD7 digenic                          | PDZD7     | Lysyl hydroxylase 3 deficiency                                          | PLOD3     |
| Usher syndrome, type 2D                                                | WHRN      | Bartter syndrome, type 4b, digenic                                      | CLCNKA    |
| Usher syndrome, type 3A                                                | CLRN1     | Bartter syndrome, type 4b, digenic                                      | CLCNKB    |
| ?Cataracts, growth hormone deficiency, sensory neuropathy, sensorineural hearing loss, and skeletal dysplasia | IARS2     | MEDNIK syndrome                                                         | AP1S1     |
| Tietz albinism-deafness syndrome                                        | MITF      | Duane retraction syndrome 3                                            | MAFB      |
| Deafness, congenital, with onychodystrophy, autosomal dominant         | ATP6B1B2  | COMMAD syndrome                                                         | MITF      |
| Perrault syndrome 1                                                    | HSD17B4   | Charcot-Marie-Tooth disease, X-linked recessive, 5                      | PRPS1     |
| ?Perrault syndrome 2                                                    | HARS2     | Otospondyloepiphysial dysplasia, autosomal recessive                     | COL11A2   |
| Perrault syndrome 3                                                    | CLPP      | 3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome | SERAC1    |
| Perrault syndrome 4                                                    | LARS2     | Leukoencephalopathy, cystic, without megalencephaly                     | RNASET2   |
| Perrault syndrome 5                                                    | TWNK      | Mandibulofacial dysostosis, Guion-Almeida type                          | EFTUD2    |
| Perrault syndrome 6                                                    | ERAL1     | Auditory neuropathy, autosomal dominant 1                               | DAPHS1    |
| Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome | POLD1  | Wolfram-like syndrome, autosomal dominant                               | WF5       |
| Macrophthrombocytopenia and progressive sensorineural deafness         | MYH9      | PCWH syndrome                                                           | SXO10     |
| Brown-Valetto-Van Laere syndrome 1                                      | SLC52A3   | Rieger disease                                                          | PBYH      |
| Brown-Valetto-Van Laere syndrome 2                                      | SLC52A2   | Treacher Collins syndrome 1                                            | TCOF1     |
| Craniofacial-deafness-hand syndrome                                     | PAX3      | Treacher Collins syndrome 2                                            | POLR1D    |
| Paragangliomas 1, with or without deafness                             | SDHD      | Treacher Collins syndrome 3                                            | POLR1C    |
| Deafness, dystonia, and cerebral hypomyelination                        | BCAP31    | Weissenbacher-Zweymuller syndrome                                       | COL11A2   |
| ?Myopathy, congenital, with neuropathy and deafness                    | SPTBN4    | Otospondyloepiphysial dysplasia, autosomal dominant                      | COL11A2   |
| Growth retardation with deafness and mental retardation due to IGF1 deficiency | IGF1     | 3MC syndrome 1                                                          | MASP1     |
| Congenital anomalies of kidney and urinary tract syndrome with or without hearing loss, abnormal ears, or developmental delay | PBX1     | Apert syndrome                                                          | FGFR2     |
| ABCD syndrome                                                           | EDNRB     | Rieger or Auenfeld anomalies                                            | FOXC1     |
| Arts syndrome                                                           | PRPS1     | Arlzenfeld-Rieger syndrome, type 3                                      | FOXC1     |
| Bart-Pumphrey syndrome                                                  | GB2       | Baraitser-Winter syndrome 1                                             | ACTB      |
| DOOR syndrome                                                           | TBC1D24   | 3-methylglutaconic aciduria with deafness                              | ACTB      |
| Epstein syndrome                                                       | MYH9      | Heimer syndrome 2                                                       | PEH6      |
| Fechtner syndrome                                                       | MYH9      | LEOPARD syndrome 3                                                      | BRAF      |
| Mental retardation, X-linked syndromic, Turner type                     | HUWE1     | Warburg-Cinotti syndrome                                                | DDR2      |
| Vohwinkel syndrome                                                      | GB2       | Tumpenny-Fry syndrome                                                   | PGCF2     |
| Wolffram syndrome                                                       | WFS1      | ?RiHNS syndrome                                                         | TMEM67    |
| Bartter syndrome, type 4a                                               | BSNF      | Lymphatic malformation                                                  | PIEZ01    |
| Bjornstad syndrome                                                     | BCS1L     | Congenital disorder of glycosylation, type Ig                          | ALG12     |
| Chudley-McCullough syndrome                                            | GPSM2     | Mitochondrial DNA depletion syndrome 5 (encephalomyopathic with or without methylmalonic aciduria) | SUCLA2 |
| Duanne-radial ray syndrome                                              | SALL4     | Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal recessive 4 | DGUOK     |
| Mohr-Tranebjerg syndrome                                               | TIMM8A    | Bone marrow failure syndrome 1                                          | SRP72     |
| Muckle-Wells syndrome                                                   | NLRP3     | Branchioculofacial syndrome                                             | TFAP2A    |
| SESAME syndrome                                                         | KNU10     | CATSHL syndrome                                                         | FGRP3     |
| Thiamine-responsive megaloblastic anemia syndrome                       | SLC19A2   | Chianarin-Dorfman syndrome                                              | ABHD5     |

(continued)
| Disease                                      | Gene    | Disease                                      | Gene    |
|----------------------------------------------|---------|----------------------------------------------|---------|
| Histiocytosis-lymphadenopathy plus syndrome  | SLC29A3 | CHARGE syndrome                              | CHD7    |
| Pituitary hormone deficiency, combined, 3    | LHX3    | Escobar syndrome                             | CHN5    |
| Charcot-Marie-Tooth disease, type 1E         | PMP22   | Hypogonadotropic hypogonadism 2 with or      | FGR1    |
|                                              |         | without anosmia                              |         |
| Cowcock syndrome                             | AIRM    | Hypogonadotropic hypogonadism 3 with or      | PRKRI    |
|                                              |         | without anosmia                              |         |
| Cardiospondylocarposacral disease            | MAP3K7  | Frontonasal dysplasia 1                      | ALX3    |
| Optic atrophy plus syndrome                  | OPA1    | Mannosidosis, beta                           | MANBA   |
| Townes-Brocks syndrome 1                     | SALL1   | D-bifunctional protein deficiency            | HSD17B4 |
| Hypogonadotropic hypogonadism 5 with or      | CHD7    | Kniest dysplasia                             | COL2A1  |
| without anosmia                              |         |                                              |         |
| Dyskeratosis congenita, autosomal dominant 3 | TINF2   | Spondyloperipheral dysplasia                 | COL2A1  |
| ?CHARGE syndrome                             | SEMA3E  | Canavan disease                              | ASPA    |
| Coffin-Lowry syndrome                        | RPS6KA3 | Biotinidase deficiency                       | BTD     |
| IVIC syndrome                                | SALL4   | Mitochondrial complex III deficiency, nuclear| BCS1L    |
|                                              |         | type 1                                       |         |
| LADD syndrome                                | FGFR2   | Craniometaphyseal dysplasia                  | ANKH    |
| LADD syndrome                                | FGFR3   | Craniometaphyseal dysplasia, autosomal recessive | GA1    |
| Marshall syndrome                            | COL11A1 | Oculodentodigital dysplasia                  | GA1     |
| CNCA syndrome                                | NLRP3   | Prebaldism                                   | KIT     |
| Muenke syndrome                              | FGR3    | Prebaldism                                   | SNA2    |
| Achondroplasia                               | FGR3    | Neuropathy, hereditary sensory, type IE       | DNM1    |
| Stickler syndrome, type I                    | COL2A1  | Neurofibromatosis, type 2                    | NF2     |
| Stickler syndrome, type II                   | COL11A1 | Facial paresis, hereditary congenital, 3      | HOXB1   |
| Stickler syndrome, type III                  | COL11A2 | Xeroderma pigmentosum, group A               | XPA     |
| Stickler syndrome, type IV                   | COL9A1  | Xeroderma pigmentosum, group B               | ERCC3   |
| ?Stickler syndrome, type V                   | COL9A2  | Xeroderma pigmentosum, group D               | ERCC2   |
| Tantemy preaxial brachydactyly syndrome      | CHSY1   | Keipert syndrome                             | GPC4    |
| Wolfzaw syndrome                             | CSD2    | Meier-Gorlin syndrome 1                      | ORC1    |
| ?Otofaciocervical syndrome                   | EYA1    | Hypophosphatemic rickets, AR                 | DMP1    |
| Crouzon syndrome                             | FGR2    | Cockayne syndrome, type A                    | ERSR8C  |
| Mucopolysaccharidosis type VI (Maroteaux-Lamy)| AFSB    | Marshall-Smith syndrome                      | NFK     |
| LADD syndrome                                | FGFR1   | Krabbe disease                               | GAC     |
| Alstrom syndrome                             | ALMS1   | Peroxisome biogenesis disorder 9B            | PEX7    |
| Branchiootic syndrome 1                      | EYA1    | Rhizomelic chondrodysplasia punctata, type 1  | PEX7    |
| Branchiootic syndrome 3                      | SIX1    | Mitochondrial complex I deficiency, nuclear type 1 | NDUF54 | |
| Fibrodysplasia ossificans progressiva        | AORR1   | Ciliary dyskinesia, primary, 1, with or without situs inversus | DNA1 |
| Linear skin defects with multiple congenital anomalies 1 | HCCS  | Mitochondrial recessive ataxia syndrome (includes SANDO and SCAE) | POLG |
| Combined oxidative phosphorylation deficiency 13| PNPT1 | Fancioni anemia, complementation group D2  | FANCO2  |
| Combined oxidative phosphorylation deficiency 24| NARS2 | Fancioni anemia, complementation group C  | FANCC  |
| Simpson-Golabi-Behmel syndrome, type 1        | GPC3    | Fancioni anemia, complementation group A      | FANCA  |
| Desanto-Shinawi syndrome                      | WAC     | Fancioni anemia, complementation group E      | FANC3  |
| Nephritic syndrome, type 14                   | SGPL1   | LEOPARD syndrome 1                           | PTN11   |
| Otopalatodigital syndrome, type I            | FLNA    | ?Lichtenstein-Knorr syndrome                 | SLO9A1  |
| Otopalatodigital syndrome, type II           | FLNA    | Emberger syndrome                            | GATA2   |
| Frontometaphyseal dysplasia 1                | FLNA    | Mannosidosis, alpha-, types I and II         | MAN2B1  |
| Burn-McKeown syndrome                        | TXNL4A  | Congenital disorder of glycosylation, type Ip | ALG11   |
| Mitochondrial DNA depletion syndrome 1 (MNIE type) | TYMP | Hajdu-Cheney syndrome                        | NOTCH2  |
| Symmetric circumferential skin creases,     | MAPRE2  | Peroxisomal acyl-CoA oxidase deficiency      | ACX1    |
| con genital, 2                               |         |                                              |         |
| IFAP syndrome with or without BRESHECK       | MBT8S2  | Primrose syndrome                            | ZBTB20  |
| syndrome                                    |         |                                              |         |
| Mental retardation and microophaly with pontine and cerebellar hypoplasia | CASK | Charcot-Marie-Tooth disease, type 4D | NDRG1 |
| Hypophosphatemic rickets, X-linked dominant   | PHEX    | Ichthyotic keratoderma, spasticity,           | ELOV1   |
|                                              |         | hypomyelination, and dysmorphic facies       |         |
| Mullegama Klein-Martinez syndrome            | STAG2   | Optiz GBBB syndrome, type II                 | SPEC1L  |

(continued)
| Disease 1 | Gene   | Disease 2 | Gene   |
|-----------|--------|-----------|--------|
| Fraser syndrome 1 | FRAS1 | 3-methylglutaconic aciduria, type VIII | HTRA2 |
| Spondylocarpotarsal synostosis syndrome | FLNB | Galloway-Mowat syndrome 5 | TPRKB |
| HSD10 mitochondrial disease | HSD17B10 | Bone marrow failure syndrome 4 | MYSM1 |
| CHIME syndrome | PIGL | Osteogenesis imperfecta, type I | COL1A1 |
| Charcot-Marie-Tooth disease, type 2J | MPZ | Osteogenesis imperfecta, type IV | COL1A2 |
| Charcot-Marie-Tooth disease, type 4C | ShH3TC2 | Osteogenesis imperfecta, type IV | COL1A1 |
| Charcot-Marie-Tooth neuropathy, X-linked dominant, 1 | GJB1 | Osteogenesis imperfecta, type XII | SP7 |
| Charcot-Marie-Tooth disease, axonal, type 2N | AARS1 | De Santis-Cacchione syndrome | ERC06 |
| Retinitis pigmentosa 59 | DHDDS | ?Hydroxykynureninuria | KNYU |
| Osteopetrosis, autosomal recessive 1 | TNFRSF11A | Sclerosteosis 1 | SOST |
| Sympathalangism, proximal, 1A | NOG | Keutel syndrome | MGP |
| Warsaw breakage syndrome | DDX11 | Galactose epimerase deficiency | GALE |
| CAPOS syndrome | ATP1A3 | Baraitser-Winter syndrome 2 | ACTG1 |
| SHORT syndrome | PK3R1 | Coenzyme Q10 deficiency, primary, 2 | PDSS1 |
| Rickets, vitamin D-resistant, type IIA | VDR | Lissencephaly 5 | LAMB1 |
| Congenital disorder of glycosylation, type In | TOR6G1 | Zimmermann-Laband syndrome 2 | ATPI6V1B2 |
| Coenzyme Q10 deficiency, primary, 6 | COQ6 | Thyroid hormone resistance, autosomal recessive | THRB |
| Coenzyme Q10 deficiency, primary, 1 | COQ2 | Frontotemporal dementia and/or amyotrophic lateral sclerosis 2 | CHCHD10 |
| ?Combined oxidative phosphorylation deficiency | ? | Ectodermal dysplasia/short stature syndrome | GRHL2 |
| Amyloidosis, hereditary, transthyretin-related | TTR | Arterial calcification, generalized, of infancy, 1 | ENP1 |
| Klippel-Feil syndrome 1, autosomal dominant | GDF6 | Spondyloepiphysyeal dysplasia with congenital joint dislocations | CHST3 |
| Saethre-Chotzen syndrome with or without eyelid anomalies | TWIST1 | Charcot-Marie-Tooth disease, dominant intermediate E | INF2 |
| Multiple synostoses syndrome 4 | GDF6 | Neuropathy, hereditary sensory and autonomic, type IA | SPTLC1 |
| Acrofacial dysostosis 1, Nager type | SF3B4 | Multiple congenital anomalies-hypotonia-seizures syndrome 2 | PIGA |
| Combined oxidative phosphorylation deficiency 37 | MICOS13 | Mitochondrial DNA depletion syndrome 7 (hepatocerebral type) | TWINK |
| Epileptic encephalopathy, early infantile, 8D | PIGB | Salt and pepper developmental regression syndrome | ST3GAL5 |
| Cleidocranial dysplasia | RUNX2 | Phosphoribosylpyrophosphate synthetase superactivity | PRPS1 |
| Camurati-Engelmann disease | TGF8 | Chondrodysplasia punctata, X-linked recessive | APRL |
| Myfry syndrome | SMAD4 | Combined oxidative phosphorylation deficiency 11 | RMND1 |
| Starkiewicz-isidor syndrome | PSMD12 | Mental retardation-hypotonic facies syndrome, X-linked | ATRX |
| ?Abruzzo-Erickson syndrome | TBX22 | Metaphyseal chondrodysplasia, Murk Jansen type | PTH1R |
| Paget disease of bone 3 | SQSTM1 | Growth retardation, developmental delay, facial dysmorphism | FTO |
| Ohdo syndrome, X-linked | MED12 | Diabetes mellitus, neonatal, with congenital hypothyroidism | GLIS3 |
| Optiz-Kaveggia syndrome | MED12 | Infantile-onset multisystem neurologic, endocrine, and pancreatic disease | PTRH2 |
| Feingold syndrome 1 | MYCN | Charcot-Marie-Tooth disease, dominant intermediate G | NEFL |
| Peroxisome biogenesis disorder 3B | PEX12 | Familial cold autoinflammatory syndrome 2 | NLRP12 |
| Peroxisome biogenesis disorder 1A (Zellweger) | PEX1 | Motor delay, C1 dysplasia, hearing loss, tracheomalacia and cryptorchidism | NLRP1B8 |
| Paget disease of bone 5, juvenile-onset | TNFRSF11B | Failure to thrive, hearing loss, hepatomegaly & pericardial effusion | PMM2 |
| Brittle cornea syndrome 2 | PRDM5 | Microcephaly, early-onset seizures, developmental delay & hearing loss | PKN2 |
| Osteopatia striata with cranial sclerosis | AMER1 | Neurodevelopmental disorder with hearing loss | RERE |
|                | PPP1R15B | Sensorineural hearing loss, association with       | TMT2C |

(continued)
| Disease                          | Gene            | Disease                          | Gene            |
|---------------------------------|-----------------|----------------------------------|-----------------|
| Microcephaly, short stature, and impaired glucose metabolism 2 | TRMT10C         | Short stature, microcephaly & hearing loss | RPS23          |
| Combined oxidative phosphorylation deficiency 30 | TIMMDC1         | Pendred syndrome, hearing loss | SCARBP2        |
| Mitochondrial complex I deficiency, nuclear type 31 |                  |                                  |                 |
| Diarrhea 3, secretory sodium, congenital, syndromic | SPINT2          | Sensorineural hearing loss, nonsyndromic | OTOR           |
| Epileptic encephalopathy, early infantile, 73 | RNF13           | High frequency hearing loss, progressive | NIN            |
| Multiple sulfatase deficiency | SUMF1           | Spasticity and sensorineural hearing loss | C007           |
| Deafness | COL9A3          | Non-syndromic hearing loss | IFNL1R1         |
| Deafness | MYO1C           | Hearing loss, non-syndromic | RA1            |
| Sensorineural hearing loss, bilateral | MYO1F           | Bilateral sensorineural hearing loss | SLITRK5        |
| Sensorineural hearing loss | TGF21           | Central hypothyroidism & hearing loss | TBL1X          |
| Sensorineural deafness, nonsyndromic | MYO1A           | Parkinsonism, early-onset, with distal spinal atrophy, cataracts and sensory-neural deafness | PARK7          |
| Sensorineural hearing loss | TJP2            | Leigh-like syndrome, developmental delay, encephalopathy, sensorineural hearing loss with sepsis like features | ACY1           |
| Hearing loss, non-syndromic, autosomal dominant | TJP2            | Intellectual disability, sensorineural hearing loss, skeletal defects and primary ovarian failure | PANK1          |
| Keratoderma-Ichthyosis-Deafness syndrome | VPS33B          | Hypotonia, motor delay, absent deep tendons reflexes & sensorineural hearing loss | TRPV4          |
| Agenesis of corpus callosum, retinopathy & deafness | CDK10           | Intellectual disability, macrocephaly, hyperlaxity of finger joints and hearing loss | CHD4           |
| Intellectual disability, deafness, Duane anomaly, obesity and diabetes type 2 | DIPK2B          | Sensorineural hearing loss, developmental delay, hypoglycaemia & combined OXPHOS deficiency | MRPS2          |
| left ventricular hypertrophy, diabetes, sensorineural hearing loss, neurogenic abnormalities and exercise intolerance | C10BP           | Foveal dysplasia, pulmonary abnormalities, and sensorineural hearing loss | MPDZ           |
| Cone-rod degeneration with sensorineural hearing loss | CEP78           | Global developmental delay, epilepsy, hypotonia, hearing loss, hyperopia, and strabismus | DMBX1          |
| Congenital cataracts, hearing loss and low serum copper and ceruloplasmin | SLC33A1         | Sensorineural hearing loss, developmental delay with inguinal/umbilical hernia | PLS3           |
| Microcephaly, intellectual disability, seizures & hearing loss | SPATA5          | Language disorder, hearing loss, gastro-esophageal reflux disease, failure to thrive, and short stature | SOS1           |
| Mitochondrial DNA depletion syndrome with hearing loss | TK2             | Intellectual disability, cerebellar ataxia & atrophy, hearing loss, progressively coarsening facial features & macrocephaly | SNX14          |
| Cerebellar ataxia, myoclonic epilepsy, cataract, deafness and hyperlactatemia | DNA2            | Congenital disorder of glycosylation 2m with hypertrophic cardiomyopathy, hearing loss and short stature | SLC35A2        |
| Inner ear malformations & deafness | GREB1L          | Brain abnormalities, transposition of the great arteries, ventricular septal defect, renal anomaly and hearing loss | HERC2          |
| Ataxic neuropathy, cachexia and deafness | KIF5A           | Preaxial polydactyly IV, developmental delay, sensorineural hearing loss, skeletal & genitourinary anomalies | GLB3           |
| Keratitis-ichthyosis-deafness syndrome, modifier of | KRT17           | Retinitis pigmentosa, hearing loss, premature ageing, short stature, mild intellectual disability and distinctive gestalt | EXOSC2         |
| Deafness, non-syndromic, autosomal recessive | SLC22A4         | Developmental delay, spasticity, arthrogryposis, microcephaly, short stature, ventricular septal defect, and hearing loss | PHGDH          |
| Deafness, developmental regression, and leukoencephalopathy | PEX5            | Muscle-eye-brain disease, Walker-Warburg syndrome, Epileptic Encephalopathy-West syndrome, and sensorineural hearing loss | B3GALNT2       |
| Spastic paraplegia, sensorineural-deafness, blindness and seizures | SELENOI         | Diabetes, hypothyroidism, hypogonadism, short stature, ID, obesity, deafness, high myopia, microcephaly and alopecia | MANF           |

(continued)
3.3. Prediction of mutation pathogenicity using mutation
taster software

Mutation Taster software predicted that the mutation of
EYA1 c.1627C>T (p.Gln543Ter) may be pathogenic mutations.
Swiss-Model was used for the prediction of protein structure in
the wild type and mutation type [EYA1 c.1627C>T (p.
Gln543Ter)] According to the protein sequence model of the
wild type and mutation type, termination was observed which
then significantly alternated the protein structure accordingly
(Fig. 6).

### Table 1
(continued)

| Disease | Gene     | Disease | Gene |
|---------|----------|---------|------|
| Cohen syndrome, cutis verticis gyrata & sensorineural deafness | VPS13B | Congenital anomalies of the kidney and urinary tract, intellectual disability, deafness, and growth retardation | ZBTB24 |
| Truncal ataxia, hypotonia, developmental delay & hearing loss | AC02 | Acute neurological failure and deafness | MFN2 |
| Progressive sensorineural hearing loss and migraine | ATP1A2 | Deafness, profound, suppressor of | EEF1AK1NMT |
| Elliptocytosis, midface hypoplasia, impaired growth and hearing loss | AMMECR1 | Macular degeneration, age related, exercise fatigue, atrial fibrillation and deafness | CKMT2 |
| Inflammatory vitreoretinopathy, hearing loss & developmental delay, early-onset | CAPN5 | Deafness | APOD |
| Seizures, hearing loss & dysmorphic features | CDK20 | Deafness | REST |
| Primary microcephaly & sensorineural hearing loss | CDK5RAP2 | Deafness | CCS |
| Mild cone-rod dystrophy and sensorineural hearing loss | CEP250 | Deafness | CEMIP |
| Seizures, hearing loss & dysmorphic features | HIVEP1 | Deafness | CLU |
| Hearing loss, non-syndromic, autosomal recessive | LRPS | Deafness | FBXO2 |
| Sensorineural hearing loss and short stature | MAP2K2 | Deafness | GJB4 |
| Developmental delay, poor growth and sensorineural hearing loss | MARS2 | Deafness | GJC3 |
| Hearing loss, age-related, association with | SOD2 | Deafness | LHFPL6 |
| Deafness-dystonia syndrome | RTM2 | Deafness | LRP1 |
| Deafness, hypotony, neurological regression, complex I deficiency, complex IV deficiency, and mtDNA depletion | RPM2B | Deafness | MIA |
| Enlarged vestibular aqueduct | FOX11 | Deafness | SOX2 |
| Hearing loss, adult-onset | DNA42 | Hearing loss | SPANX |
| Hearing loss, adult-onset | DUOX2 | Hearing loss | MYH7B |
| Hearing loss, adult-onset | LAMA2 | Hearing loss | OBSCN |
| Hearing loss, adult-onset | LRIG1 | Hearing loss | PRKCB |
| Hearing loss, adult-onset | NEDD4 | Hearing loss, adult-onset | GPM7 |
| Hearing loss, adult-onset | NTN1 | Hearing loss, adult-onset | ZAN |
| Hearing loss, adult-onset | SIK3 | Hearing loss, adult-onset | LRG3 |
| Hearing loss, age-related | SLC7A8 | Hearing loss, adult-onset | NEFH |
| Hearing loss, age-related | TiAM1 | Hearing loss, adult-onset | ACAN |
| Other related genes | CATSPER2 | Other related genes | Other related genes |
| Other related genes | SLC12A4 | Other related genes | Other related genes |
| Other related genes | PIT2 | Other related genes | Other related genes |
| Other related genes | GSTP1 | Other related genes | Other related genes |
| Other related genes | CDKN1C | Other related genes | Other related genes |

### Table 2
Patient characteristics for the partial family members.

| Family | Gender | Age | Karyotype | Congenital deafness | Left auricle, external ear / pre-auricular fistula | Right auricle, external ear / pre-auricular fistula | Branchial cleft fistula | Renal malformation |
|--------|--------|-----|-----------|---------------------|-----------------------------------------------|-----------------------------------------------|-----------------------|-------------------|
| II-6   | Male   | 65  | 46,XY     | Yes, self-reported  | Normal/available                               | Malformation/ available                      | Left and right sides | None              |
| II-17  | Female | 61  | 46,XX     | Yes, self-reported  | Normal/ not available                          | Malformation/ not available                  | None                  | None              |
| III-1  | Male   | 33  | 46,XY     | No                  | Normal/ not available                          | Normal/ not available                        | Normal/ not available | None              |
| III-2  | Female | 51  | 46,XX     | Yes, self-reported  | Normal/ not available                          | Malformation/ available                      | Left and right sides | None              |
| III-3  | Female | 29  | 46,XX     | Yes, self-reported  | Normal/ not available                          | Malformation/ available                      | Left and right sides | None              |
| III-4  | Male   | 30  | 46,XY     | Yes, self-reported  | Normal/ not available                          | Normal/ not available                        | None                  | None              |
| IV-1   | Male   | 10  | 46,XY     | Yes, medical screening | Normal/ not available                           | Malformation/ available                      | Right side           | None              |

III-3: proband; III-4: The husband of the proband; II-6 and II-17: parents of the proband; III-1 and III-2: the brother-in-law and sister of the proband; IV-1: the nephew of the proband.
Clinical phenotypes of 7 members of the Branchio-Oto (BO) syndrome family were observed.
4. Discussion

The diagnosis of BO is affected by the high genetic heterogeneity, and its diagnosis is still a challenge in clinical settings. Currently, 3 BOR related pathogenic genes (i.e., EYA1, SIX1, and SIX5) have been identified. About 40% of such cases present EYA1 mutation.[3,13] Branchio-oto-renal syndrome (BOR, OMIM 113650), an autosomal dominant disorder, is featured by hearing loss, renal malformations and branchial arch anomalies.[1] In the

![Image](https://via.placeholder.com/150)

Figure 1. Clinical features of III-3, II-6, III-2, IV-1. The lower right arrow indicates right-sided branchial cleft fistula; the lower left arrow indicates bilateral pre-auricular pit.

![Image](https://via.placeholder.com/150)

Figure 2. Pedigrees of family with BO syndrome.

![Image](https://via.placeholder.com/150)

Figure 3. The pure tone audiometry of the affected individuals.
Figure 4. Axial CT images of the temporal bone from subject III-3 (A) Partial or complete loss in the auditory ossicle. (B) Dilatation in the bony auditory tube and defect in the internal ear and cochlea.

Figure 5. Sanger sequencing for exon 17 of EYA1 c.1627C>T gene mutation. III-3: proband. II-6 and II-17: parents of the proband; III-2: the elder sister of the proband; IV-1: the nephew of the proband.
patients without aberrant renal anomalies, such condition is also defined as Branchio-Oto (BO) syndrome (OMIM 602588).

In 2004, based on a large sample Branchio-oto-renal syndrome (BOR) study, Chang et al summarized the major clinical manifestations as hearing loss (98.5%), pre-auricular fistula (83.6%), malformation of branchial cleft (68.5%) and renal malformation (38.2%).[3] In the patients without aberrant renal anomalies, such condition is also defined as Branchio-Oto (BO) syndrome (OMIM 602588). In this study, 4 cases were diagnosed with BO syndrome based on these criteria, and the 4 cases showed high consistency in the clinical manifestations including deafness, pre-auricular fistula, malformation of branchial cleft, deformity of external and internal ear.

Unlike the previous studies,[1–3,14] there were no obvious heterogeneity among the individuals. CT scan for the temporal bone of the proband showed that there were agenesia in the auricular bone and cochlea, which was consistent with the hearing loss revealed by pure tone audiometry (PTA). There were no aberrant changes in the renal ultrasonography and renal function evaluation. Then the cases were obtained from a typical family with BO syndrome.

In this study, we also investigated the molecular mechanism of the disease. Heterozygous mutations to 3 genes were identified including EYA1 c.1627C>T, HSD17B4 c.1750A>G and GJB2 c.109G>A. In cases with EYA1 c.1627C>T (p.Gln543Ter) HSD17B4 may lead to generation of non-functioning protein. After searching the HGMD Pro and PubMed databases, there were no reporting on the pathogenicity of such variation. But the c.1627C>T (p.Gln543Ter) of EYA1 has been reported in L Spahiu in BOS.[13]

To date, 183 mutations of EYA1 have been reported to be associated with the BO/BOR syndrome (http://www.hgmd.cf.ac.uk/). EYA1 c.1627C>T (p.Gln543Ter) was not a polymorphism and the frequency was extremely low after referring to the 1000 Genomes and dbSNP databases. EYA1 gene showed a type of autosomal dominant inheritance, and the clinical manifestations of the individuals in the family showed high consistency. After Sanger sequencing, the cases with BO syndrome carrying the heterozygous mutation of EYA1 gene. Such variation led to generation of termination codon, which resulted in termination of translation. EYA1 was a key gene for mammalian organogenesis and mutations, which resulted in multiple organ malformation. Similar to other EYA family members, EYA1 possessed a highly conservative 271-amino acid Cterminal EYA domain and a divergent N-terminal transactivation domain for protein–protein interactions.[16] The human EYA1 is the homologous gene of drosophila eye absent gene (EYA), which is initially expressed at week 4 to 6 of human embryo development. The EYA1 is highly expressed in the human embryo kidney. The proteins encoded by EYA1 are crucial for the development of branchial arch, ear, and kidney.[17] Since the role of EYA1 in development is crucial, it is related to 4 diseases as a pathogenic gene: ototaciocervical syndrome, anterior segment anomaly, BO syndrome, and BOR syndrome.[10] According to the protein prediction software, mutation in such site may lead to functional loss of protein. The evidence of ACMG was based on PVS1, PM2, and PP3. Meanwhile, the clinical genotypes were completely consistent with the BO syndrome. Therefore, we speculated that EYA1 c.1627C>T (p.Gln543Ter) was the most suspicious pathogenic factor for BO syndrome in this family. The sequencing results for this family may provide some clinical suggestions for the surgeons. It is not recommended to evaluate the familiar pathogenicity based on the analysis report given by the third party. It is necessary to focus on the published literatures to promote the diagnosis of the family genetic disease. Unlike the
previous L Spahiu study, our case with EYA1 c.1627C>T (p. Glu543Ter) did not show renal anomalies. In future, further studies are required to investigate the potential mechanisms associated with the different clinical features induced by EYA1 c.1627C>T (p.Glu543Ter) in different families.

In terms of HSD17B4 c.1750A>G (p.Ile584Val) and GJB2 c.109G>A (p.Val37Ile), they may induce changes of amino acid sequences. Since HSD17B4 and GJB2 gene were in a type of autosomal recessive inheritance, further tests were required to accomplish the gene test for the HSD17B4 and GJB2 in the family members, in order to verify the pathogenicity of HSD17B4 c.1750A>G (p.Ile584Val) and GJB2 c.109G>A (p.Val37Ile) in the family.

To date, Swiss-Model is the most commonly used software for the prediction of the 3-dimensional structure of protein. The Swiss-Model was used for the single modeling of EYA1 protein. Based on the EYA1 sequence, the Swiss-Model would select the protein structure with wide coverage and the high similarity for the modeling. Then the 3-dimensional structure model was established for the wild type and mutation types of EYA1 protein (Fig. 6). Based on the mutation prediction of 3-dimensional protein structure with wide coverage and the high similarity for the protein. Furthermore, the sample size for the rare disease is usually small, which cannot accomplish the verification for the BO syndrome cases. The targeted next-generation sequencing was performed to the III-3 in this study due to financial reasons. The other 4 with clinical symptoms received the whole exon sequencing of mutation sites targeted the III-3. We could not find the genes associated with the hearing loss of II-17 as the whole exon sequencing was not performed to the II-17 with low intelligence, auricle anomaly, and hearing loss.

In summary, we reported a rare family of BO syndrome in a Chinese Han family, in which 4 showed highly consistent symptoms. We highlight that combining molecular tests with the analysis of clinical phenotypes would contribute to the timely diagnosis and treatment of BO syndrome. The targeted next-generation sequencing technique which used in this article is an effective method for the diagnosis of rare disease and genetic single gene disorders. Our study contributed to the summarization of EYA1 gene mutation in the HGMD databases.

**Author contributions**

Data curation: Xiaolin La.
Formal analysis: Ling Duan.
Methodology: Yan Xia, Zhijuan Liu.

**Software** Shuang Wu.
**Validation** Erdengqieqie Ye.
**Writing – original draft** Rui Han.
**Writing – review & editing** Jianbing Ding.

**References**

[1] Melnick M, Bixler D, Nance WE, et al. Familial branchio-oto-renal dysplasia: a new addition to the branchial arch syndromes. Clin Genet 1976;9:25–34.
[2] Fraser FC, Sproule JR, Halal F. Frequency of the branchio-oto-renal (BOR) syndrome in children with profound hearing loss. Am J Med Genet 1980;7:341–9.
[3] Abdelhak S, Kalatzis V, Heilig R, et al. A human homologue of the Droso phila eyes absent gene underlies branchio-oto-renal (BOR) syndrome and identifies a novel gene family. Nat Genet 1997;15:157–64.
[4] Rui RG, Xu PX, Silvius D, et al. SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1-SIX1-DNA complexes. Proc Natl Acad Sci USA 2004;101:8090–5.
[5] Hoskins BC, Cramer CH, Silvius D, et al. Transcription factor SIX5 is mutated in patients with branchio-oto-renal syndrome. Am J Hum Genet 2007;80:800–4.
[6] Brophy PD, Alasti F, Darbro BW, et al. Genome-wide copy number variation analysis of a branchio-oto-renal syndrome cohort identifies a recombination hotspot and implicates new candidate genes. Hum Genet 2013;132:1339–50.
[7] Chang EH, Menezes M, Meyer NC, et al. Branchio-oto-renal syndrome: the mutation spectrum in EYA1 and its phenotypic consequences. Hum Mutat 2004;23:582–9.
[8] Schmidt T, Bierhals T, Kortum F, et al. Branchio-otic syndrome caused by a genomic rearrangement: clinical findings and molecular cytogenetic studies in a patient with a pericentric inversion of chromosome 8. Cytogenet Genome Res 2014;142:1–6.
[9] Vervoort VS, Smith RJ, O’Brien J, et al. Genomic rearrangements of EYA1 account for a large fraction of families with BOR syndrome. Eur J Hum Genet 2002;10:757–66.
[10] Wang YG, Sun PS, Qi YL, et al. A novel mutation in EYA1 in a Chinese family with Branchio-oto-renal syndrome. BMC Med Genet 2018;19:139.
[11] Pan X, Ding X, Song XM. Branchiootic syndrome: a case report in a family. Zhong Hua Kou Qiang Yi Xue Za Zhi 2010;45:775.
[12] Sun SP, Lu W, Zhu LY. Branchio-otic syndrome misdiagnosed into preauricular fistula. Zhonghua Er Bi Hou Tou Jing Wai Ke Za Zhi 2014;49:330–2.
[13] Orten DJ, Fischer SM, Sorensen JL, et al. Branchio-oto-renal syndrome (BOR): novel mutations in the EYA1 gene, and a review of the mutational genetics of BOR. Hum Mutat 2008;29:537–44.
[14] Zou MZ, Zhu HM, Wei QJ, et al. Clinical and genetic characteristics of a Chinese family with branchio-otic syndrome. Zhong Hua Kou Qiang Yi Xue Za Zhi 2013;29:1306–9.
[15] Spahiu L, Merovci B, Ismaili Jaha V, et al. Case Report: a novel mutation of the EYA1 gene in a patient with Branchio-oto-renal syndrome. Balk J Med Genet 2016;19:91–4.
[16] Buller C, Xu X, Marquis V, et al. Molecular effects of Eya1 domain mutations causing organ defects in BOR syndrome. Hum Mol Genet 2001;10:2775–81.
[17] Kumar S, Deffanabacherk K, Marres HA, et al. Genome wide search and genetic localization of a second gene associated with autosomal dominant branchio-oto-renal syndrome: clinical and genetic implications. Am J Hum Genet 2000;66:1715–20.
[18] Lee Y, Kim C, Park Y, et al. Next generation sequencing identifies abnormal Y chromosome and candidate causal variants in premature ovarian failure patients. Genomics 2016;108:209–15.
[19] Abe S, Usami S, Shinkawa H, et al. Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet 2000;37:41–3.