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

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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 1gene 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.[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.[2] To date, pathogenic variants in 3 genes have been identified including *EYA1*, *SIX1* and *SIX5*. Besides, several genes (e.g., *SHARPIN*) have been reported to be associated with the pathogenesis of such disease.[3–6] 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.[7] Furthermore, *EYA1* associated genome recombination and chromosome abnormality (chromosome 8) may be responsible for the onset of BOR/BO syndrome.[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.[10]

Up to now, very few studies have been focusing on the BO syndrome.[11,12] Meanwhile, little is known about the molecular mechanism of such disease.[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,

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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 bcl2fastq 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, III-2, III-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.lle584Val) heterozygous and *GJB2* c.109G>A (p.Val37lle) 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.lle584Val) and *GJB2* c.109G>A (p.Val37lle) 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	POU3F4	?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	DIAPH1	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	TMPRSS3
Deafness, autosomal dominant 3B	GJB6	Deafness, autosomal recessive 9	OTOF
Deafness, autosomal dominant 4A	MYH14	Deafness, autosomal recessive 10	TMPRSS3
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	OTOG
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	GRXCR1
Deafness, autosomal dominant 17	MYH9	?Deafness, autosomal recessive 26	GAB1
Deafness, autosomal dominant 20	ACTG1	Deafness, autosomal recessive 28	TRIOBP
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	ESRRB
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	DCDC2
Deafness, autosomal dominant 67	OSBPL2	Deafness, autosomal recessive 67	LHFPL5
?Deafness, autosomal dominant 68	HOMER2	Deafness, autosomal recessive 68	S1PR2
Deafness, autosomal dominant 69, unilateral or asymmetric	KITLG	Deafness, autosomal recessive 70	PNPT1
?Deafness, autosomal dominant 70	MCM2	Deafness, autosomal recessive 74	MSRB3
?Deafness, autosomal dominant 71	DMXL2	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*)

Table 1

(continued).

Disease	Gene	Disease	Gene
Deafness, autosomal recessive 84B	OTOGL	Waardenburg syndrome, type 1	PAX3
Deafness, autosomal recessive 86	TBC1D24	Waardenburg syndrome, type 2A	MITF
Deafness, autosomal recessive 88	ELMOD3	Waardenburg syndrome, type 2D	SNAI2
Deafness, autosomal recessive 89	KARS1	Waardenburg syndrome, type 2E, with or without neurologic involvement	SOX10
Deafness, autosomal recessive 91	SERPINB6	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 97	MET	Waardenburg syndrome, type 4C	SOX10
Deafness, autosomal recessive 98	TSPEAR	Waardenburg syndrome/ocular albinism, digenic	MITF
?Deafness, autosomal recessive 99	TMEM132E	Waardenburg syndrome/albinism, digenic	TYR
Deafness, autosomal recessive 100	PPIP5K2	Branchiootorenal syndrome 1, with or without cataracts	EYA1
?Deafness, autosomal recessive 101	GRXCR2	Branchiootorenal syndrome 2	SIX5
?Deafness, autosomal recessive 102	EPS8	Jervell and Lange-Nielsen syndrome	KCNQ1
?Deafness, autosomal recessive 103	CLIC5	Jervell and Lange-Nielsen syndrome 2	KCNE1
?Deafness, autosomal recessive 104	RIPOR2	Deafness, digenic, GJB2/GJB3	GJB3
Deafness, autosomal recessive 105	CDC14A	Deafness, digenic GJB2/GJB6	GJB6
Deafness autosomal recessive 106	EPS8L2	Deafness, congenital heart defects, and posterior embryotoxon	JAG1
Deafness, autosomal recessive 107	WBP2	Sinoatrial node dysfunction and deafness	CACNA1D
?Deafness, autosomal recessive 108	ROR1	Epiphyseal dysplasia, multiple, with myopia and deafness	COL2A1
?Deafness, autosomal recessive 109	ESRP1	Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract	ABHD12
?Deafness, autosomal recessive 110	COCH	{Deafness, mitochondrial, modifier of}	TRMU
Deafness, autosomal recessive 111	MPZL2	Hypoparathyroidism, sensorineural deafness, and renal dysplasia	GATA3
?Deafness, autosomal recessive 112	BDP1	Corneal endothelial dystrophy and perceptive deafness	SLC4A11
Deafness, autosomal recessive 113	CEACAM16	Nephropathy with pretibial epidermolysis bullosa and deafness	CD151
Deafness, autosomal recessive 114	GRAP	Leber congenital amaurosis with early-onset deafness	TUBB4B
?Deafness, autosomal recessive 115	SPNS2	Renal tubular acidosis with deafness	ATP6V1B1
Deafness, autosomal recessive	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	FGF3
Alport syndrome, autosomal recessive	COL4A4	?Microtia, hearing impairment, and cleft palate (AR)	HOXA2
Norrie disease	NDP	?Microtia with or without hearing impairment (AD)	HOXA2
Usher syndrome, type 1B	MYO7A	Cerebellar ataxia, deafness, and narcolepsy, autosomal dominant	DNMT1
Usher syndrome, type 1C	USH1C	Deafness and myopia	SLITRK6
Usher syndrome, type 1D	CDH23	Keratoderma, palmoplantar, with deafness	GJB2
Usher syndrome, type 1D/F	CDH23	Deafness, neurosensory, without vestibular involvement, autosomal dominant	ESPN
Usher syndrome, type 1D/F	PCDH15	Hystrix-like ichthyosis with deafness	GJB2
Usher syndrome, type 1F	PCDH15	Keratitis-ichthyosis-deafness syndrome	GJB2
Usher syndrome, type 1G	USH1G	?Peripheral neuropathy, myopathy, hoarseness, and hearing loss	MYH14
Usher syndrome, type 1J	CIB2	Dementia, familial Danish	ITM2B
?Usher syndrome, type 1M	ESPN	Multiple synostoses syndrome 1	NOG
Usher syndrome, type IV	ARSG	Ayme-Gripp syndrome	MAF
Usher syndrome, type 2A	USH2A	Woodhouse-Sakati syndrome	DCAF17

(*continued*)

Table 1

(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	LRP2
Usher syndrome, type IIC, 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	ATP6V1B2	COMMAD syndrome	MITF
Perrault syndrome 1	HSD17B4	Charcot-Marie-Tooth disease, X-linked recessive, 5	PRPS1
?Perrault syndrome 2	HARS2	Otospondylomegaepiphyseal 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	DIAPH3
Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome	POLD1	Wolfram-like syndrome, autosomal dominant	WFS1
Macrothrombocytopenia and progressive sensorineural deafness	MYH9	PCWH syndrome	SOX10
Brown-Vialetto-Van Laere syndrome 1	SLC52A3	Refsum disease	PHYH
Brown-Vialetto-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	Otospondylomegaepiphyseal 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 Axenfeld anomalies	FOXC1
Arts syndrome	PRPS1	Axenfeld-Rieger syndrome, type 3	FOXC1
Bart-Pumphrey syndrome	GJB2	Baraitser-Winter syndrome 1	ACTB
DOOR syndrome	TBC1D24	?Dystonia, juvenile-onset	ACTB
Epstein syndrome	MYH9	Heimler syndrome 2	PEX6
Fechtner syndrome	MYH9	LEOPARD syndrome 3	BRAF
Mental retardation, X-linked syndromic, Turner type	HUWE1	Warburg-Cinotti syndrome	DDR2
Vohwinkel syndrome	GJB2	Turnpenny-Fry syndrome	PCGF2
Wolfram syndrome	WFS1	?RHYNS syndrome	TMEM67
Bartter syndrome, type 4a	BSND	Lymphatic malformation 6	PIEZO1
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
Duane-radial ray syndrome	SALL4	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal recessive 4	DGUOK
Mohr-Tranebjaerg syndrome	TIMM8A	Bone marrow failure syndrome 1	SRP72
Muckle-Wells syndrome	NLRP3	Branchiooculofacial syndrome	TFAP2A
SESAME syndrome	KCNJ10	CATSHL syndrome	FGFR3
Thiamine-responsive megaloblastic anemia syndrome	SLC19A2	Chanarin-Dorfman syndrome	ABHD5

(*continued*)

Table 1

(continued).

Disease	Gene	Disease	Gene
Histiocytosis-lymphadenopathy plus syndrome	SLC29A3	CHARGE syndrome	CHD7
Pituitary hormone deficiency, combined, 3	LHX3	Escobar syndrome	CHRNG
Charcot-Marie-Tooth disease, type 1E	PMP22	Hypogonadotropic hypogonadism 2 with or without anosmia	FGFR1
Cowchock syndrome	AIFM1	Hypogonadotropic hypogonadism 3 with or without anosmia	PROKR2
Cardiospondylocarpofacial syndrome	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 without anosmia	CHD7	Kniest dysplasia	COL2A1
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 type 1	BCS1L
LADD syndrome	FGFR2	Craniometaphyseal dysplasia	ANKH
LADD syndrome	FGFR3	Craniometaphyseal dysplasia, autosomal recessive	GJA1
Marshall syndrome	COL11A1	Oculodentodigital dysplasia	GJA1
CINCA syndrome	NLRP3	Piebaldism	KIT
Muenke syndrome	FGFR3	Piebaldism	SNAI2
Achondroplasia	FGFR3	Neuropathy, hereditary sensory, type IE	DNMT1
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
Temtamy preaxial brachydactyly syndrome	CHSY1	Keipert syndrome	GPC4
Wolfram syndrome 2	CISD2	Meier-Gorlin syndrome 1	ORC1
?Otofaciocervical syndrome	EYA1	Hypophosphatemic rickets, AR	DMP1
Crouzon syndrome	FGFR2	Cockayne syndrome, type A	ERCC8
Mucopolysaccharidosis type VI (Maroteaux-Lamy)	ARSB	Marshall-Smith syndrome	NFIX
LADD syndrome	FGF10	Krabbe disease	GALC
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	NDUFS4
Fibrodysplasia ossificans progressiva	ACVR1	Ciliary dyskinesia, primary, 1, with or without situs inversus	DNAI1
Linear skin defects with multiple congenital anomalies 1	HCCS	Mitochondrial recessive ataxia syndrome (includes SANDO and SCAE)	POLG
Combined oxidative phosphorylation deficiency 13	PNPT1	Fanconi anemia, complementation group D2	FANCD2
Combined oxidative phosphorylation deficiency 24	NARS2	Fanconi anemia, complementation group C	FANCC
Simpson-Golabi-Behmel syndrome, type 1	GPC3	Fanconi anemia, complementation group A	FANCA
Desanto-Shinawi syndrome	WAC	Fanconi anemia, complementation group E	FANCE
Nephrotic syndrome, type 14	SGPL1	LEOPARD syndrome 1	PTPN11
Otopalatodigital syndrome, type I	FLNA	?Lichtenstein-Knorr syndrome	SLC9A1
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 (MNGIE type)	TYMP	Hajdu-Cheney syndrome	NOTCH2
Symmetric circumferential skin creases, congenital, 2	MAPRE2	Peroxisomal acyl-CoA oxidase deficiency	ACOX1
IFAP syndrome with or without BRESHECK syndrome	MBTPS2	Primrose syndrome	ZBTB20
Mental retardation and microcephaly with pontine and cerebellar hypoplasia	CASK	Charcot-Marie-Tooth disease, type 4D	NDRG1
Hypophosphatemic rickets, X-linked dominant	PHEX	Ichthyotic keratoderma, spasticity, hypomyelination, and dysmorphic facies	ELOVL1
Mullegama-Klein-Martinez syndrome	STAG2	Opitz GBBB syndrome, type II	SPECC1L

(*continued*)

Table 1

(continued).

Disease	Gene	Disease	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	SH3TC2	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 Sanctis-Cacchione syndrome	ERCC6
Retinitis pigmentosa 59	DHDDS	?Hydroxykynureninuria	KYNU
Osteolysis, familial expansile	TNFRSF11A	Sclerosteosis 1	SOST
{Paget disease of bone 2, early-onset}	TNFRSF11A	Van Buchem disease	SOST
Symphalangism, proximal, 1A	NOG	Keutel syndrome	MGP
Warsaw breakage syndrome	DDX11	Galactose epimerase deficiency	GALE
CAPOS syndrome	ATP1A3	Baraitser-Winter syndrome 2	ACTG1
SHORT syndrome	PIK3R1	Coenzyme Q10 deficiency, primary, 2	PDSS1
Rickets, vitamin D-resistant, type IIA	VDR	Lissencephaly 5	LAMB1
Osteopetrosis, autosomal recessive 1	TCIRG1	Zimmermann-Laband syndrome 2	ATP6V1B2
Congenital disorder of glycosylation, type In	RFT1	Thyroid hormone resistance, autosomal recessive	THRB
Coenzyme Q10 deficiency, primary, 6	COQ6	Frontotemporal dementia and/or amyotrophic lateral sclerosis 2	CHCHD10
Coenzyme Q10 deficiency, primary, 1	COQ2	Ectodermal dysplasia/short stature syndrome	GRHL2
?Combined oxidative phosphorylation deficiency 34	MRPS7	Arterial calcification, generalized, of infancy, 1	ENPP1
Amyloidosis, hereditary, transthyretin-related	TTR	Cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma syndrome	SNAP29
Klippel-Feil syndrome 1, autosomal dominant	GDF6	Spondyloepiphyseal 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)	TWNK
Epileptic encephalopathy, early infantile, 80	PIGB	Salt and pepper developmental regression syndrome	ST3GAL5
Cleidocranial dysplasia	RUNX2	Phosphoribosylpyrophosphate synthetase superactivity	PRPS1
Camurati-Engelmann disease	TGFB1	Chondrodysplasia punctata, X-linked recessive	ARSL
Myhre syndrome	SMAD4	Combined oxidative phosphorylation deficiency 11	RMND1
Stankiewicz-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
Opitz-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 cyrptorchidism	NUP188
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	PNKP
Osteopathia striata with cranial sclerosis	AMER1	Neurodevelopmental disorder with hearing loss	RERE
	PPP1R15B	Sensorineural hearing loss, association with	TMTC2

(*continued*)

Table 1

(continued).

Disease	Gene	Disease	Gene
Microcephaly, short stature, and impaired glucose metabolism 2			
Combined oxidative phosphorylation deficiency 30	TRMT10C	Short stature, microcephaly & hearing loss	RPS23
Mitochondrial complex I deficiency, nuclear type 31	TIMMDC1	Pendred syndrome, hearing loss	SCARB2
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	COQ7
Deafness	COL9A3	Non-syndromic hearing loss	IFNLR1
Deafness	MYO1C	Hearing loss, non-syndromic	RAI1
Sensorineural hearing loss, bilateral	MYO1F	Bilateral sensorineural hearing loss	SLITRK5
Sensorineural hearing loss	TCF21	Central hypothyroidism & hearing loss	TBL1X
Deafness	TMPRSS5	Global developmental delay & deafness	SYNGAP1
Sensorineural deafness, nonsyndromic	MYO1A	Parkinsonism, early-onset, with distal spinal amyotrophy, 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	PANX1
Keratoderma-Ichthyosis-Deafness syndrome	VPS33B	Hypotonia, motor delay, absent deep tendon 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	C1QBP	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-oesphageal 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	GLI3
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*)

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	ACO2	Acute neurological failure and deafness	MFN2
Progressive sensorineural hearing loss and migraine	ATP1A2	Deafness, profound, suppressor of	EEF1AKNMT
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	LRP5	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	FITM2	Deafness	LRP1
Deafness, hypotony, neurological regression, complex I deficiency, complex IV deficiency, and mtDNA depletion	RRM2B	Deafness	MIA
Enlarged vestibular aqueduct	FOXI1	Deafness	SOX2
Hearing loss, adult-onset	DNAH2	Hearing loss	SPANXC
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	GRM7
Hearing loss, adult-onset	NTN1	Hearing loss, adult-onset	ZAN
Hearing loss, adult-onset	SIK3	Hearing loss, adult-onset	LRIG3
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	SLC12A1		
Other related genes	PITX2		
Other related genes	GSTP1		
Other related genes	CDKN1C		

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 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	None	None
III-2	Female	31	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.

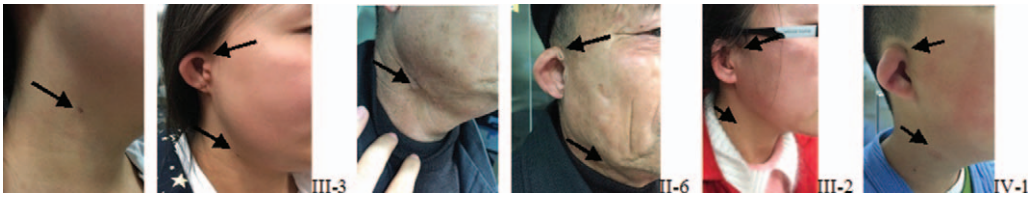

Figure 1. Clinical features of III-3,II-6, III-2, IV-1. The lower right arrow indicates right-sided branchial cleft fistula; the lower left arrow indicates bilateral pre-auricular pit.

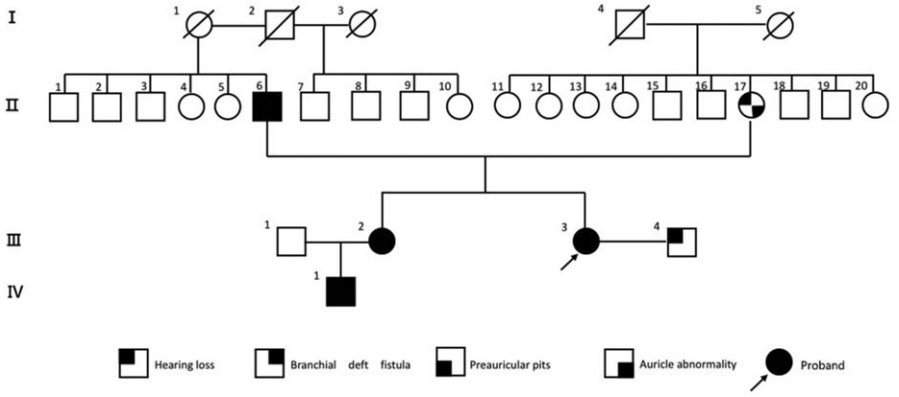

Figure 2. Pedigrees of family with BO syndrome.

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

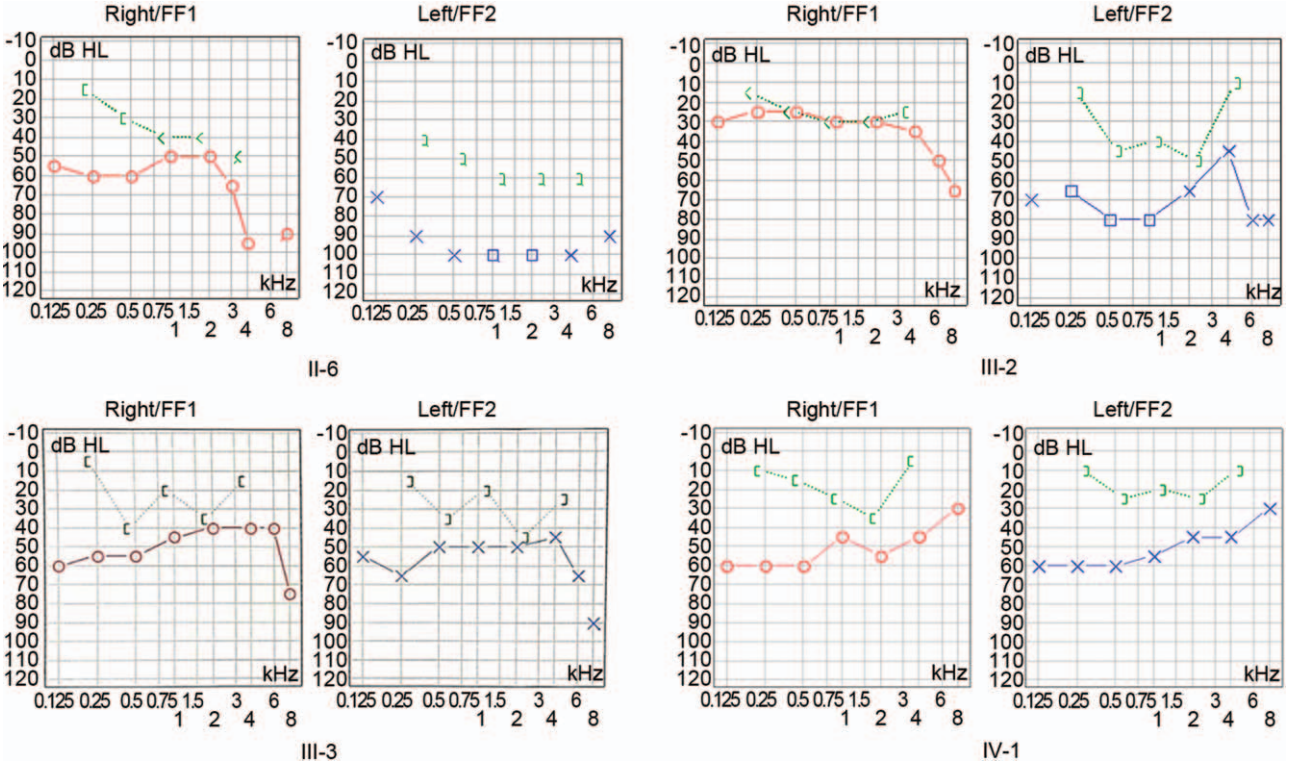

Figure 3. The pure tone audiometry of the affected individuals.

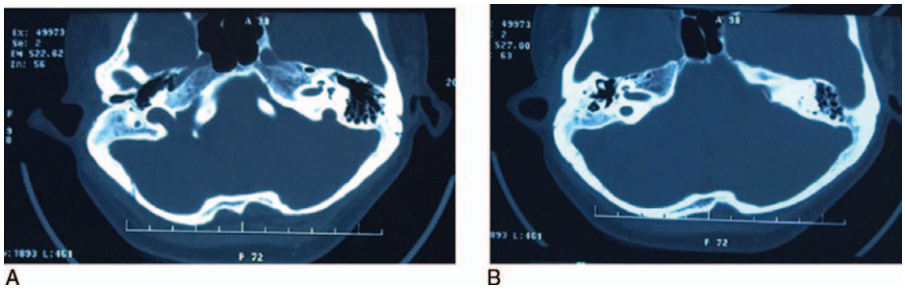

Figure 4. Axial CT images of the temporal bone from subject III-3 (A) Partial or complete loss in the auditory ossicle. (B) Dilatation in the bony auditory tube and defect in the internal ear and cochlea.

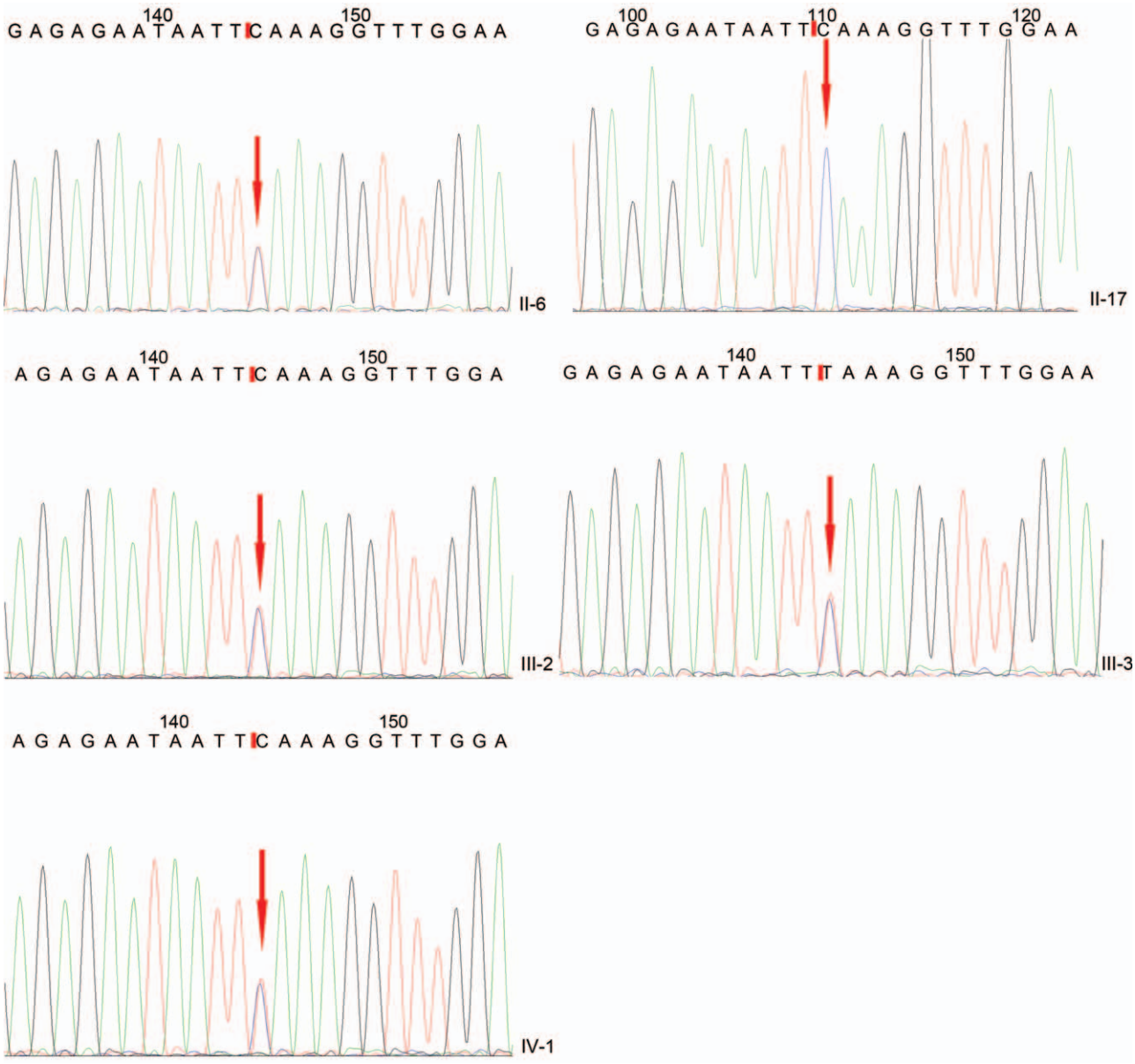

Figure 5. Sanger sequencing for exon 17 of EYA1 c.1627C>T gene mutation. III-3: proband. II-6 and II-17: parents of the proband; III-2: the elder sister of the proband; IV-1: the nephew of the proband.

Table 3

Candidate variants identified by Sanger sequencing.

Gene	Mutation site	III-3 Prophet	II-6	II-17	III-2	IV-1
EYA1	c.1627C>T (p.Gln543Ter)	Heterozygous	Heterozygous	No variation	Heterozygous	Heterozygous
HSD17B4	c.1750A>G (p.Ile584Val)	Heterozygous	Heterozygous	No variation	Heterozygous	No variation
GJB2	c.109G>A (p.Val37Ile)	Heterozygous	No variation	Heterozygous	No variation	No variation

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. EYA1: EYA transcriptional coactivator and phosphatase 1; GJB2 = Gap junction protein beta 2; HSD17B4 = Hydroxysteroid 17-beta dehydrogenase 4.
The affected members verified the results after the protestor detected the mutation site.

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) 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 by L Spahiu in BOS.[15] 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: otofaciocervical 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

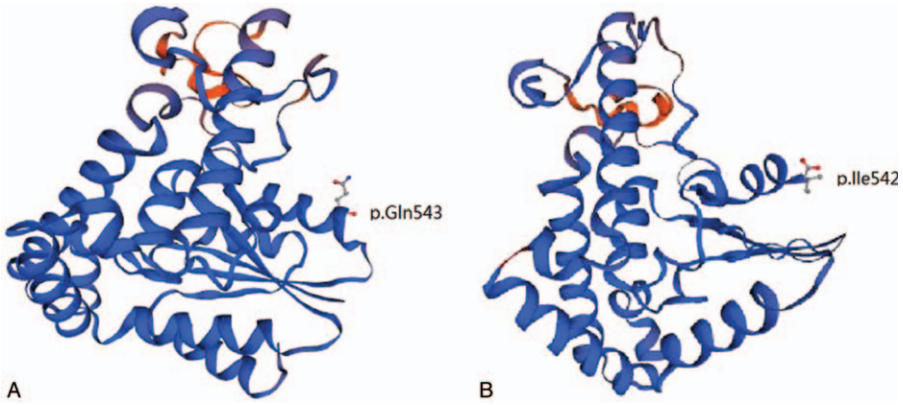

Figure 6. Model of protein sequence in the wild type (A) and mutation type (B). The p.Gln543 resulted in translation determination in advance. The mutation type was the p.Ile542 based on the EYA protein sequence prediction.

previous L Spahiu study, our case with *EYA1* c.1627C>T (p.Gln543Ter) 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.Gln543Ter) in different families.

In terms of *HSD17B4* c.1750A>G (p.lle584Val) and *GJB2* c.109G>A (p.Val37lle), they may induce changes of amino acid sequences.[18,19] 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.lle584Val) and *GJB2* c.109G>A (p.Val37lle) 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, there was a termination of p.lle542 in the mutated EYA1 protein. On this basis, we speculated that *EYA1* c.1627C>T (p.Gln543Ter) would affect the protein function encoded by the mutation. *EYA1* c.1627C>T (p.Gln543Ter) may be closely associated with the auricle anomaly, branchial cleft fistula and the hearing loss of this family.

There are some limitations in this study. We failed to obtain all the blood samples from the generation II, and only obtained the blood samples from II-6 and II-17. Besides, we did not conduct the functional test for the gene mutation related protein, as well as the molecular mechanism 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 first-generation sequence for the screening 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

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Software: Shuang Wu.
Validation: Erdengqieqieke Ye.
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Writing – review & editing: Jianbing Ding.

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