Molecular Genetic Etiology and Revisiting the Middle Ear Surgery Outcomes of Branchio-Oto-Renal Syndrome: Experience in a Tertiary Referral Center

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Objectives: To explore the phenotypes and genotypes of patients with branchio-oto-renal (BOR) and branchio-otic (BO) syndrome, and to analyze the middle ear surgery outcomes qualitatively and quantitatively, proposing a factor usefully prognostic of surgical outcomes.
Study design: Retrospective cohort study.
Setting: Tertiary referral center.
Patients: Eighteen patients with BOR/BO syndrome in 12 unrelated Korean families.
Intervention: Middle ear surgery, including either stapes surgery or ossicular reconstruction.
Main Outcome Measure: Clinical phenotypes, genotypes, and middle ear surgery outcomes
Results: Eight probands (66.7%) were confirmed genetically; the condition segregated as a dominant or de novo trait. Six *EYA1* heterozygous variants were identified by exome sequencing and multiplex ligation-dependent probe amplification. All variants were pathogenic or likely pathogenic based on the ACMG/AMP guidelines. Two novel *EYA1* frameshift variants (p.His373Phefs*4 and p.Gln543Asnfs*90) truncating a highly conserved C-terminal Eya domain were identified, expanding the genotypic spectrum of *EYA1* in BOR/BO syndrome. Remarkably, middle ear surgery was individualized to ensure optimal audiological outcomes and afforded significant audiological improvements, especially in BOR/BO patients without enlarged vestibular aqueducts (EVAs). A significant difference in air-bone gap closure after middle ear surgery was noted between the two groups even after adjusting for confounders: −20.5 dB in ears without EVAs (improvement) but 0.8 dB in ears with EVAs (no change or deterioration). Furthermore, the success rate was significantly associated with the absence of EVA.
Conclusions: The results of this study were against the notion that middle ear surgery is always contraindicated in patients with BOR/BO syndrome, and an EVA could be a negative prognostic indicator of middle ear surgery in BOR/BO patients. This may aid to determine the strategy of audiological rehabilitation in patients with BOR/BO syndrome.
Key Words: Branchio-otic syndrome—Branchio-oto-renal syndrome—Enlarged vestibular aqueduct—*EYA1*—Middle ear surgery.

Otol Neurotol **44:**e319–e327, 2023.

INTRODUCTION

Branchio-oto-renal (BOR) syndrome is characterized by branchial anomalies (branchial cleft or sinus, preauricular pits, or auricular deformity), hearing loss, and renal anomalies (1). The condition is rare and segregates in an autosomal-dominant manner (2). BOR syndrome is also termed branchio-otic (BO) syndrome if renal abnormalities are absent. BOR/BO syndrome is clinically and genetically heterogeneous (3). The causative mutated genes include *EYA1*, *SIX1*, and *SIX5*, but no genetic cause has been identified in approximately 50% of cases (2–4). The genotypic spectrum of BOR/BO syndrome includes variants in *EYA1* (40–75%), *SIX1* (2%), and *SIX5* (0–3.1%) (2,3). Chang et al. (5) developed diagnostic criteria based on the major findings (branchial anomalies, deafness, preauricular pits, and renal anomalies) and minor phenotypes (external,

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Acknowledgments: This research was supported and funded by SNUH Kun-hee Lee Child Cancer and Rare Disease Project, Republic of Korea (grant no. FP-2022-00001-004 by S-Y.L.).

Supplemental digital content is available in the text.

DOI: 10.1097/MAO.0000000000003880

middle, and inner ear anomalies, preauricular tags, and other features).

Although hearing impairment is very penetrant, the type, severity, and progression of hearing loss vary (6). Mixed hearing loss is the most common, followed by conductive or sensorineural hearing loss. Progressive hearing loss has been documented in some patients (7,8). As most patients evidenced mixed or conductive hearing loss, middle ear surgery would seem to be indicated. However, a systematic review found that ossicular reconstruction or stapes surgery is usually unsuccessful (9). Only a few patients enjoyed favorable outcomes after mastoidectomy with creation of a neo-oval window or placement of an incus homograft prosthesis. To the best of our knowledge, a comprehensive quantitative analysis of the effects of middle ear surgery has never been elucidated in the literature. Given this, prognostic factors of surgical outcomes would be invaluable.

Here, we explore the phenotypes and genotypes of a rather large BOR/BO cohort. Furthermore, we qualitatively and quantitatively analyze the middle ear surgery outcomes and propose a factor usefully prognostic of surgical outcomes.

MATERIALS AND METHODS

Participants

This study was approved by the Institutional Review Board of our institution (IRB nos. 2022-045-1298 and 0905-041-281); the need for informed patient consent was waived. We retrospectively reviewed the in-house database on syndromic hereditary deafness. Of 273 cases, those who met the BOR/BO criteria of Chang et al. (5) or those with pathogenic variants of known causative genes (*EYA1*, *SIX1*, and *SIX5*) were included. Ultimately, 18 patients from 12 unrelated Korean families were identified. We present the clinical phenotypes, demographics, imaging data, audiological profiles, and genotypes.

Audiological Evaluation

Type of hearing loss was divided into sensorineural, conductive, and mixed. When the air-bone gap (ABG) was >10 dB, if the bone conduction (BC) hearing threshold was ≤15 dB, hearing loss was conductive (otherwise it was mixed). If the ABG was ≤10 dB and the air conduction (AC) hearing threshold >15 dB, hearing loss was sensorineural (10). Hearing loss severity was classified as mild, moderate, moderately severe, severe, or profound (cutoffs: 25, 40, 55, 70, and 90 dB, respectively). Hearing loss progression was defined when the difference between the highest and the lowest mean hearing thresholds was >10 dB and the slope of the regression line >0.5 dB/year (11). Hearing loss severity and progression in subjects who underwent cochlear implantation or middle ear surgery were based on the hearing data before surgery. The mean hearing threshold was calculated as the average of the BC and AC hearing thresholds of 0.5, 1, 2, and 4 kHz.

Molecular Genetic Testing

Genomic DNA was extracted from peripheral blood using a standard procedure and subjected to target panel sequencing consisting of four genes associated with BOR/BO syndrome, including EYA1, SIX1, SIX5, and TFAP2A. The Agilent SureSelectXT Human all Exon 50-Mb kit was used to target the exon regions of the genomes, and these target regions were sequenced using the Illumina Hiseq sequencing system with 100-bp paired-end reads. The target panel sequencing metrics were 59.0× and 97.0% in mean depth of coverage and quality threshold (>10×), respectively. If these data were inconclusive, whole exome sequencing and multiplex ligation-dependent probe amplification (MLPA) were conducted to define the underlying molecular genetic etiology. Reads were aligned using the University of California Santa Cruz hg19 reference genome browser (https://genome.ucsc.edu/) running Lasergene ver. 14 software (DNASTAR, Madison, WI). As described previously (12–18), strict filtering was performed when retrieving genetic etiologies. Candidate variants were validated using Sanger sequencing, and segregation studies were performed using paternal DNA samples whenever possible. Specifically, the SALSA MLPA P461 DIS probemix kit (MRC-Holland, Amsterdam, the Netherlands) was used to detect copy number variants of *EYA1*. Amplification products were run on the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA), and the results were analyzed using Gene Marker 1.91 software (SoftGenetics, State College, PA). All variants identified were classified in accordance with the ACMG/AMP guidelines for hearing loss (19,20).

Temporal Bone Computed Tomography

The high-resolution computed tomography (CT) of the temporal bone yielded continuous cross-sectional images at 0.5- to 1.0-mm intervals (21). An enlarged vestibular aqueduct (EVA) satisfied the Valvassori criterion of midpoint ≥1.5 mm (22).

Middle Ear Surgery and Outcomes

Middle ear surgery was performed by two experienced surgeons (S.H.O and M.W.S.); either stapes surgery or ossicular reconstruction was scheduled. Surgery was individualized to ensure optimal audiological outcomes (Fig. 1). The customization of the piston wire prosthesis involves bending and shaping the wire component of the piston wire prosthesis to fit the unique anatomy of each patient, accounting for differences in angle, length, and orientation. The bone conduction and the air conduction hearing thresholds at different octave frequencies were evaluated via pure tone audiometry at least twice: before surgery (baseline) and at the last follow-up. We analyzed the preoperative BC and AC hearing thresholds and the postoperative AC threshold. The mean threshold was the average of the BC and AC thresholds at 0.5, 1, 2, and 4 kHz. ABGs were calculated by subtracting preoperative BC thresholds from the AC thresholds. Successful outcomes of middle ear surgery were defined as satisfying at least one of the following criteria: 1) postoperative ABG <20 dB or 2) hearing gain >15 dB (23).

Statistical Analyses

All analyses were performed using the R statistics package (ver. 3.6.1, R Foundation for Statistical Computing,

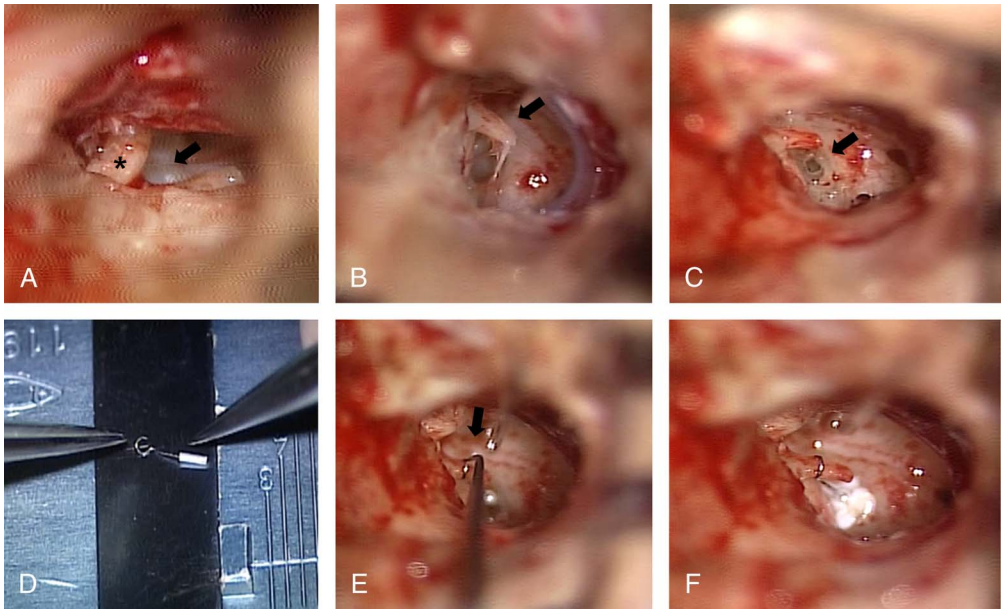

FIG. 1. Representative middle ear surgery in a patient with BOR syndrome. *A*, An abnormal malleus (*asterisk*) and a wide angle between the malleus and the incus (*black arrow*). *B*, A tilted, cone-shaped long process of the incus (*black arrow*) with no stapes. This reduces the distance between the incus and the footplate. *C*, Fenestration performed using a laser (*black arrow*). *D*, Trimming of the piston wire (which was bent, thus crooked) to completely grasp the cone-shaped long process. *E*, Pushing of the wire superiorly to a point of impingement (*black arrow*) after the wire was crimped. *F*, The piston wire supported with soft tissue.

Vienna, Austria). The Wilcoxon rank sum test was used to compare age at middle ear surgery, external ear anomalies, preoperative BC thresholds, preoperative AC thresholds, postoperative AC thresholds, postoperative ABG, and postoperative hearing gain according to the presence of EVA. The Fisher's exact test was used to compare categorical variables such as sex, genetic diagnosis, laterality, and type of middle ear surgery between the two groups. The Fisher's exact test was also used to qualitatively analyze the surgical outcomes, depending on the presence of EVA, in our cohort with the results of systematic review. All statistical tests were two-tailed, and $p < 0.05$ was considered significant.

RESULTS

Clinical Phenotypes

Eighteen patients (nine males and nine females) from 12 unrelated Korean families were identified. The clinical phenotypes, major and minor criteria, are summarized (see Table, Supplemental Digital Content 1, http://links.lww.com/MAO/B608, which shows clinical features according to the diagnostic criteria proposed by Chang et al. [5]). Fourteen patients (77.8%) had branchial anomalies, 16 (88.9%) hearing loss, and 14 (77.8%) preauricular pits. Of the 12 patients who underwent kidney imaging, 5 (41.7%) evidenced kidney hypoplasia or multicystic dysplastic kidneys. Seven patients (38.9%) exhibited external ear anomalies (microtia, cup ears, or external auditory canal hypoplasia). Temporal bone CT performed in 16 patients revealed middle ear anomalies, including bony fusion between malleolus handle and incus, abnormal obtuse angle articulation of malleoincudal joints, atretic oval window, aberrant position of stapedial crus, and small size middle ear cavity, in 12 of total 16 patients (75.0%), and inner ear anomalies, including incomplete cochlear turn, cochlear hypoplasia, EVA, dysplastic semicircular canal, and aplasia of the semicircular canal, in 14 patients (87.5%). Eight patients (50%) evidenced bilateral EVAs. The average size of vestibular aqueduct of these patients was 2.5 mm (range, 1.8–3.2 mm) (see Table, Supplemental Digital Content 2, http://links.lww.com/MAO/B609, which demonstrates the size of EVA). Sixteen patients (89%) exhibited BOR/BO syndrome (15 meeting three major criteria and 1 meeting two major and two minor criteria); two patients (11%) exhibited atypical BOR/BO syndrome (meeting one major and one minor criterion); the condition segregated with *EYA1* heterozygous variants (Table 1).

Audiological Characterization

Pure tone audiometry data (including BC information) were available for 16 patients (32 ears). Of these, 15 (46.9%), 10 (31.3%), and 7 (21.9%) ears evidenced conductive, mixed, and sensorineural hearing loss, respectively; a subset of the cohort (n = 9) underwent follow-up audiogram (>1 yr). The slope of the regression line was 0.7 ± 4.0 dB/year, and hearing loss progression was evident in five patients (55.6%; P5-1, P5-2, P9, P10-1, and P10-3).

Genotypes

The DNA of all probands was subject to comprehensive molecular genetic analyses. Of 13 probands, 8 were genetically confirmed; the condition segregated as a dominant or de novo trait. Five *EYA1* heterozygous variants were identified (Fig. 2) using target or exome sequencing. All affected amino acid residues were located in the highly conserved

TABLE 1. *Subject demographics*

					Initial HL				Major Criteria				Minor Criteria					
No	Sex	Age	Gene	Variant	EVA	Type (R/L)	Degree (R/L)	Progres-Sion of HL	Surgery	Final Hearing Rehab	Branchial Anomalies	HL	Preauricular Pits	Renal Anomalies	External Ear Anomalies	Middle Ear Anomalies	Inner Ear Anomalies	Preauricular Tags	Other Anomalies	Typical
P1	M	6.7	*EYA1*	c.1319G > A: p.Arg440Gln	–	Cond	Mod-sev	ND	B)MES	no	O	O	O	O	O	O	O	–	–	O
P2	F	5.5	*EYA1*	c.1319G > A: p.Arg440Gln	O	Mixed	Sev/prof	–	–	B)HA	O	O	O	O	–	O	O	–	–	O
P3	M	10.2	Not detected		O	Cond	Mild/mod	–	–	no	O	O	O	NA	–	–	O	–	–	O
P4	F	6	*EYA1*	EYA1 whole deletion	–	Mixed/sens	Mod/mild	ND	–	no	O	O	O	–	O	O	O	–	–	O
P5-1	F	5.6	Not detected		–	Sens	Mild	O	R)MES	B)HA	O	O	O	NA	–	O	O	–	–	O
P5-2	F	0.5	Not detected		NA	Cond	Mod/prof	O	–	B)HA	O	O	O	–	–	NA	NA	–	–	O
P6-1	M	7.3	*EYA1*	c.1081C > T: p.Arg361*	O	Cond	Mild	ND	L)MES	no	O	O	O	–	–	O	O	–	–	O
P6-2	F	0.7	*EYA1*	c.1081C > T: p.Arg361*	O	Mixed	Mod-sev	ND	R)MES	B)HA	O	O	O	–	O	O	O	–	–	O
P7-1	M	7.3	*EYA1*	c.1276G > A: p.Gly426Ser	NA	Sens	Normal	–	–	no	O	–	–	NA	–	NA	NA	–	–	–
P7-2	M	0.4	*EYA1*	c.1276G > A: p.Gly426Ser	–	Cond	Mod/mod-sev	–	–	L)HA	–	O	–	–	O	–	–	–	–	–
P8	M	5.5	*EYA1*	c.1081C > T: p.Arg361*	–	Cond	Mod-sev/mod	ND	B)MES	no	–	O	O	O	O	O	O	–	O	O
P9	M	4.1	Not detected		–	Cond	Mod-sev	O	R)MESL) BCD	L)BCD	O	O	O	–	O	O	–	–	O	O
P10-1	F	12.2	Not detected		O	Mixed	Mod/mod-sev	O	L)MES	B)HA	O	O	O	NA	–	O	O	–	–	O
P10-2	F	18.8	Not detected		O	NA	NA	ND	–	no	O	O	O	NA	–	–	O	–	–	O
P10-3	M	13.5	Not detected		O	Mixed/cond	Prof/mild	O	–	no	O	O	O	O	–	O	O	–	–	O
P10-4	F	46.5	Not detected		–	Sens/mixed	Normal/ Mod-sev	ND	–	no	O	O	O	NA	–	O	O	–	–	O
P11	M	31.5	*EYA1*	c.1623_1626dupp. Gln543Asnfs*90	–	Sens/mixed	Mild/	ND	L)MES	no	O	O	O	NA	–	O	O	–	–	O
P12	F	19.4	*EYA1*	c.1360G > T: p.Gly454Cys	O	NA	NA	ND	–	no	–	O	–	O	O	–	O	–	–	O

B indicates bilateral; BCD, bone conduction device implant; Cond, conductive; F, female; HA, hearing aids; HL, hearing loss; L, left; M, male; MES, middle ear surgery; Mod, moderate; Mod-sev, moderately severe; NA, not available; ND, not determined; Prof, profound; R, right; rehab, rehabilitation; Sens, sensorineural; Sev, severe.

C-terminal Eya domain. Three *EYA1* variants (c.1081C > T:p.Arg361*, c.1276G > A:p.Gly426Ser, and c.1319G > A:p.Arg440Gln) have been reported previously; two (c.1117_1118delCA: p.His373Phefs*4 and c.1623_1626dup: p.Gln543Asnfs*90) are novel. The two frameshift variants (p.His373Phefs*4 and p.Gln543Asnfs*90) that truncate the *Eya* domain were predicted to undergo nonsense-mediated mRNA decay, thus PVS1 of the ACMG/AMP rule. These variants were absent from the global allele frequency database (https://gnomad.broadinstitute.org/) and the Korean Reference Genome Database (http://152.99.75.168:9090/KRGDB/welcome.jsp), which applied PM2 of the ACMG/AMP rule. The affected amino acid residues (His373 and Gln543) are conserved among several species (http://genome.ucsc.edu/). In five probands (P3, P4, P5-1, P9, and P10-1) with inconclusive results from exome sequencing, a large deletion, including *EYA1*, was identified by MLPA in one proband (P4). Based on the ACMG/AMP guidelines (Table 2), our *EYA1* variants are "pathogenic" (p.Arg361*, p.His373Phefs*4, and p.Gln543Asnfs*90) and "likely pathogenic" (p.Gly426Ser and p.Arg440Gln).

Middle Ear Surgery Results

During follow-up (2.8 ± 3.3 yr), eight patients (10 ears) underwent middle ear surgery, of whom five used hearing aids before surgery (P1, P5-1, P6-2, P8, and P10-1); only three required hearing aids after surgery (P5-1, P6-2, and P10-1). One patient (P9) underwent a Bonebridge BC implant to treat congenital aural atresia and is currently using a BC hearing aid (Table 1). The detailed middle ear anomalies identified during surgery, including a stapes attached to the promontory, absence of the anterior crus or footplates, abnormalities of the incudostapedial joint, and a small stapes, are summarized in Table 3. Eight patients (10 ears) underwent middle ear surgery; eight and two ears underwent stapes surgery and ossicular reconstruction, respectively. During stapes surgery, the fixation and the geometric alignment of the ossicles were evaluated, resulting in the selection of incudostapedotomy for seven patients and malleostapedotomy for one patient. Specifically, in one subject (P9) with various ossicular anomalies identified during the surgery, laser stapedotomy using a "customized" piston wire prosthesis was performed. The piston wire was tailored to fit the specific anomalies by trimming and bending it to completely grasp the cone-shaped long process of the incus and positioning the fenestration site of the footplate. The remaining two patients underwent ossicular reconstruction; the columella was placed over the head of the stapes.

The pre- and postoperative audiograms are shown in Figure 3. The preoperative average BC, AC, and ABG were 17.0 ± 12.9, 59.0 ± 13.5, and 42.0 ± 9.8 dB, respectively; the postoperative BC, AC, and ABG were 19.1 ± 12.4, 45.4 ± 14.6, and 28.4 ± 9.6 dB, respectively (see Table, Supplemental Digital Content 3, http://links.lww.com/MAO/B610, which demonstrates hearing threshold before and after middle ear surgery).

Of the 10 ears that underwent middle ear surgery, 6 (60.0%) were successfully treated, while 4 were not. Importantly, all six ears with successful outcomes had normal vestibular aqueducts (Fig. 3A). Of the four ears with unsuccessful outcomes, three ears evidenced EVAs (Fig. 3B). A significant difference in ABG closure after surgery was noted between

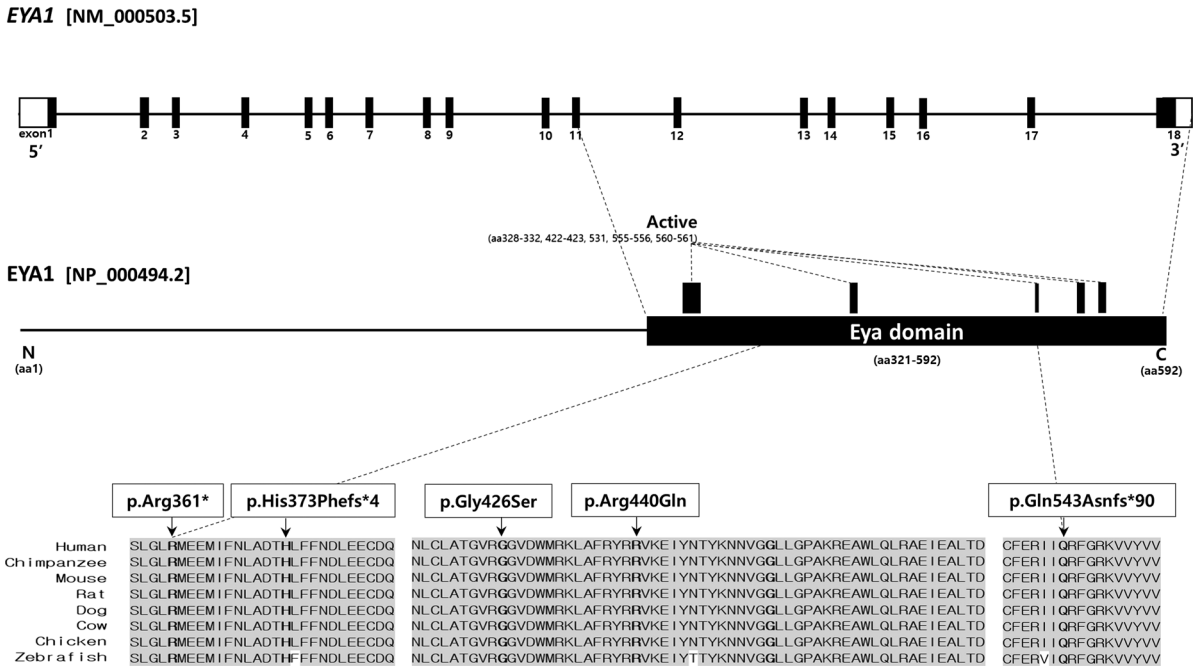

FIG. 2. Genotypes and domain maps of *EYA1* variants. The domain structure of EYA1 was constructed based on the universal protein resource (UniProt) database. The five variants are located in EYA domain. Conservation of the affected residues among species is shown for the five *EYA1* variants identified in this study.

TABLE 2. *EYA1* variants in the current study and in silico prediction analysis

Genomic Position: Change (GRCh37/hg19)	HGVS		Location (Exon/Domain)	Zygosity/ Inheritance	In Silico Predictions		Alternative Allele Frequency		ACMG/AMP 2018 Guideline		Clinvar
	Nucleotide Change	Amino Acid Change			CADD Phred	REVEL	KRGDB (1722 Individuals)	GMAF (gnomAD)	Criteria	Classification	Classification
Chr8:72128968C-T	c.1319G > A	p.Arg440Gln	Exon14/The phosphatase domain of Eya	Het/Autosomal dominant (de novo)	33.0	0.806	Absent	Absent	PS1, PS2_moderate PM2, PP3, PP4	Likely Pathogenic	Pathogenic (PMID35046468)
Chr8:72156897G-A	c.1081C > T	p.Arg361*	Exon12/The phosphatase domain of Eya	Het/Autosomal dominant	35.0	NA	Absent	Exome (0/250018) Gnome (Absent)	PVS1, PS1, PM2, PP4	Pathogenic	Pathogenic (PMID18177466)
Chr8:72129011C-T	c.1276G > A	p.Gly426Ser	Exon14/The phosphatase domain of Eya	Het/Autosomal dominant	25.7	0.801	0.002058	Exome (0.0001433) Gnome (0.00003185)	PS1, PP1, PP3, PP4	Likely Pathogenic	Conflicting (PMID30221713)
Chr8:72156860TG	c.1117_1118delCA	p.His373Phefs*4	Exon12/The phosphatase domain of Eya	Het/Autosomal dominant	NA	NA	Absent	Absent	PVS1, PM2, PP4	Pathogenic	No data
Chr8:72123462G-GAATT	c.1623_1626dup	p.Gln543Asnfs*90	Exon17/The phosphatase domain of Eya	Het/Autosomal dominant (de novo)	NA	NA	Absent	Absent	PVS1, PS2_moderate, PM2, PP4	Pathogenic	No data

Refseq transcript accession number NM_000503.5; Refseq protein accession number NP_000494.2. Sequence Variant Nomenclature (https://mutalyzer.nl/).
CADD indicates Combined Annotation Dependent Depletion (https://cadd.gs.washington.edu/); gnomAD: The Genome Aggregation Database (https://gnomad.broadinstitute.org/); Het, heterozygote; HGVS: Human Genome Variation Society (https://www.hgvs.org/); KRGDB: Korean Reference Genome Database (http://152.99.75.168:9090/KRGDB/welcome.jsp); MAF, minor allele frequency; NA, not available; REVEL: Rare Exome Variant Ensemble Learner (https://sites.google.com/site/revelgenomics/); VUS, variant uncertain significance.

the two groups: −20.5 ± 7.6 dB (range, 10.0–33.8 dB) in ears without EVAs (improvement) and 2.5 ± 5.4 dB (range, −8.8–1.3 dB) in ears with EVAs (no improvement or worsening) (p = 0.022, Wilcoxon rank sum test) (see Figure, Supplemental Digital Content 4, http://links.lww.com/MAO/B611, which demonstrates hearing threshold and hearing gain before and after surgery). The presence/absence of an EVA was not associated with any between-group difference in sex, genetic diagnosis, laterality, age at surgery, type of middle ear surgery (ossicular reconstruction or stapes surgery), or the preoperative BC or AC hearing threshold (Fisher's exact test and Wilcoxon rank sum test; see Table, Supplemental Digital Content 5, http://links.lww.com/MAO/B612, which shows the comparison according to the presence of EVA).

In a systematic review of the results of middle ear surgery in BOR/BO syndrome, three studies reported the presence or absence of EVA in 10 ears and the success of the surgery (23). There were two ears without EVA and eight ears with EVA, but none of them showed hearing improvement (see Table, Supplemental Digital Content 6, http://links.lww.com/MAO/B613, which shows previously reported middle ear surgery results in BOR/BO patients).

DISCUSSION

We herein expanded the genotypic spectrum of BOR/BO-causing *EYA1* variants and evaluated the middle ear surgery outcomes both qualitatively and quantitatively. Remarkably, customized middle ear surgery significantly improved the audiological outcomes of BOR/BO patients without EVAs. We are the first to suggest that an EVA could be negatively prognostic of middle ear surgery outcomes. This may aid to determine the strategy of audiological rehabilitation. The results of this study were against the notion that middle ear surgery is always contraindicated in patients with BOR/BO syndrome.

EYA1, a transcriptional cofactor that is evolutionarily conserved, forms a bipartite transcription factor, known as the EYA1-SIX1 complex, which plays a crucial role in the development of the otic vesicle and branchial arch-derived organs (1,24,25). In this study, we identified two novel frameshift variants (p.His373Phefs*4 and p.Gln543Asnfs*90) that truncate the Eya domain, thus expanding the EYA1 genotypic spectrum of BOR/BO syndrome. Variants of *EYA1* that cause BOR/BO syndrome are typically clustered in the Eya domain, and variants that affect functional domains such as the Eya domain are likely to have morphological and functional consequences, resulting in complex phenotypes, including hearing impairment with middle or inner ear anomalies (26,27). Middle ear malformations include ossicular anomalies, a narrow space, and a patulous Eustachian tube, whereas inner ear malformations include a hypoplastic apical cochlear turn, a funnel-shaped internal auditory canal, a hypoplastic or absent lateral semicircular canal, and EVAs (6). BOR/BO syndrome is associated with several types of hearing loss, of which the mixed type is the most common, followed by conductive hearing loss (8). Attempts have been made to improve the conductive component of

TABLE 3. *Middle ear anomalies and intraoperative findings in patients with BOR/BO syndrome*

		Ossicles							Operation	
				Stapes						
Patient	Side	Malleus	Incus	Suprastructure	Footplate	Fixation	Ossicular Chain	EVA	Name	Procedure
P1	Rt	–	–	Attached to promontory	–	No	–	No	Stapedotomy	1) PWP 4.25 mm was crippled around the LP of incus
	Lt	–	–	Slanted to promontory, anterior crus was absent	Absent	Yes	ISJ is connected with fibrous band	No	Stapedotomy	1) Vestibulotomy (vestibular endosteum was remained); 2) PWP 6 mm was inserted and hanged to the incus
P5-1	Rt	Fixation of head	–	Hypotropic with no capitulum (intact crura)	–	No (RWR is not clear)	Anomalous and obtuse angle of ISJ	No	Ossiculoplasty (Sc)	1) PORP 2.5 mm (titanium) inserted as Sc and cartilage interface
P6-1	Lt	Fixation	Tilted LP	–	–	Yes	–	Yes	Laser Stapedotomy	1) Anterior malleolar ligament detachment was done; 2) PWP (0.6 × 4.5 mm) was hanged to the incus (not fixed well)
P6-2	Rt	–	Mild hypomobile, but moves well with the malleus; thick and tilted LP	Tall	–	Yes	–	Yes	Laser stapedotomy	1) PWP (0.4 × 5.5 mm) was hanged to the LP of incus
P8	Rt	–	Anterior displaced LP	Absent	Membranous	No	Incudomalleolar dislocation	No	Laser stapedotomy	1) PWP (0.4 × 6.0 mm) was crimped over LP of incus (hard to fix due to anterior displaced LP)
	Lt	–	Abnormal	–	–	No	–	No	Ossiculoplasty (Sc)	1) Malleus head and incus were removed; 2) PORP 0.5 mm (titanium) inserted as Sc
P9	Rt	Abnormal handle (but mobile)	Cone-shaped appearance and tilted LP	Absent	–	Yes	Abnormal angle between malleus and Incus, Absence of ISJ	No	Laser stapedotomy	1) Fenestration was trimmed with 0.6 mm perforator; 2) PWP (0.4 × 3.5 mm, short length due to ossicle anomaly) was designed with more tilted wire and crooked end of the hook; 3) more crooked the end of hook bite the LP of the incus and fixed; 4) PWP was moved toward the body of the incus for more fixation due to cone-shaped LP
P10-1	Lt	–	Short, crooked LP	Small	–	Yes	–	Yes	Laser malleostapedotomy	1) Incus was removed due to short and crook of LP; 2) PWP was hanged over the neck of malleus
P11	Lt	Fixation of anterior malleolar head	Mobile, but anomalous long process	–	–	Yes	–	No	Laser stapedotomy	1) Anterior malleolar ligament was cut for mobilization of the malleus; 2) The length of PWP (4.75 mm) was cut due to short distance between incus and footplate of the stapes; 3) PWP (0.6 × 4.5 mm) was crimped over the incus

ISJ indicates incudostapedial joint; PWP, piston wire prosthesis; Sc, columella over stapes head.

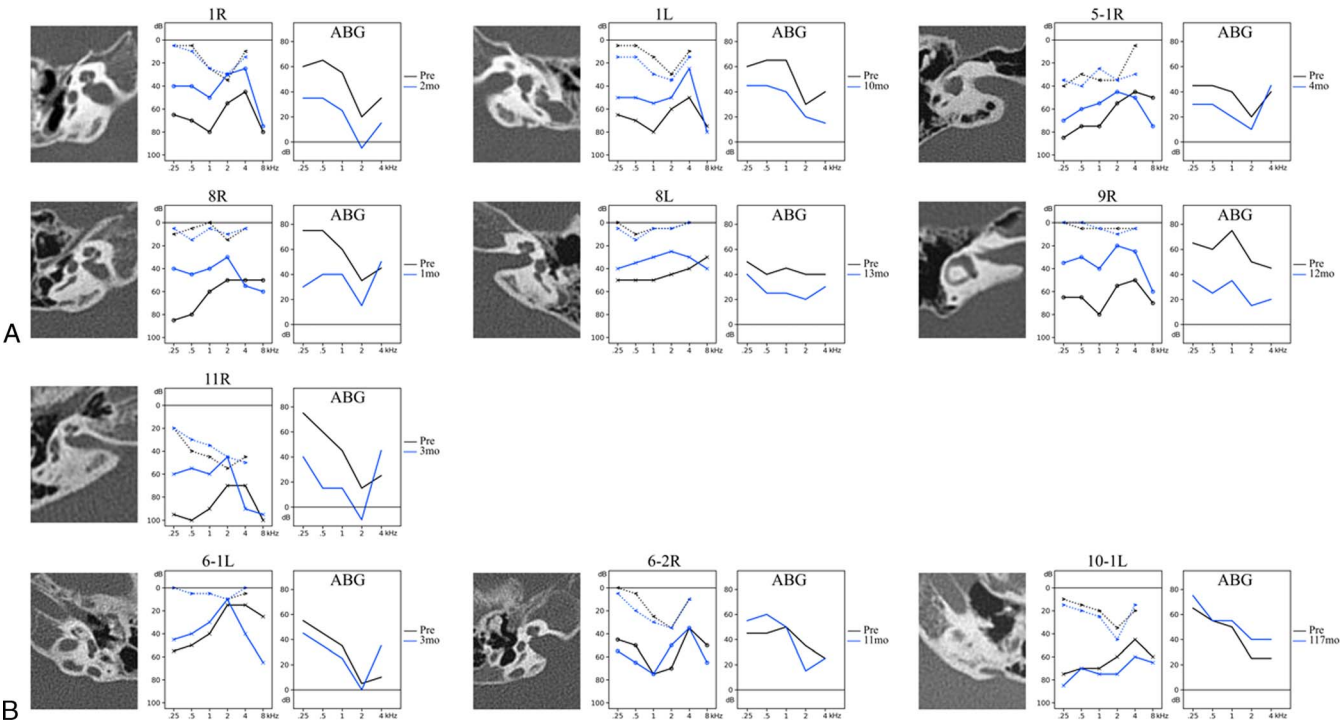

FIG. 3. Comparison of audiograms before and after surgery. *A*, Seven ears with normal vestibular aqueduct. *B*, Three ears with enlarged vestibular aqueduct. In each case, the left side shows the audiogram and the right side shows the air-bone gap by frequency.

loss by correcting middle ear anomalies. However, one systematic review using the same criteria for audiological improvement as present study showed that 11 studies reported poor outcomes in 25 of 28 ears (89.3%) (see Table, Supplemental Digital Content 6, http://links.lww.com/MAO/B613, which shows previously reported middle ear surgery results in BOR/BO patients). Surgery is complicated by the complexity of middle ear anomalies. For example, an abnormal stapes angle or a thickened long process may compromise prosthesis fixation or sound transmission after surgery (28). Inner ear anomalies may also contribute to poor outcomes after middle ear surgery. In particular, an EVA was closely associated with poor outcomes of exploratory tympanotomy, in line with our data and those of the cited review. No BOR/BO patient, especially those with EVAs, evidenced audiological improvement after middle ear surgery (28–30) (see Table, Supplemental Digital Content 6, http://links.lww.com/MAO/B613, which shows previously reported middle ear surgery results in BOR/BO patients). However, we found significant improvements after individualized middle ear surgery in BOR/BO patients without EVAs. Although the lack of comprehensive evaluation of EVA in BOR/BO patients in the previous studies could not elucidate the association between the presence or the absence of EVA and middle ear surgery outcomes, we revealed that ABG closure after middle ear surgery (i.e., hearing gain) was significantly affected by EVA status after adjustment for confounders. An EVA may be negatively prognostic of middle ear surgery outcomes. Mechanistically, an EVA may act as a pathological third window, precluding adequate energy transmission in the inner ear despite appropriate middle ear reconstruction. These insights may aid the audiological rehabilitation of patients with BOR/BO syndrome.

Our study had several limitations. First, the work was retrospective in nature; thus, uncontrolled variables may have introduced bias. Second, because our patient number was small, we lack the statistical power to draw any conclusion on the effectiveness of middle ear surgery. Third, the follow-up was rather short (although over 1 yr in 61.1%, 11 of 18). Middle ear surgery failure is usually attributable to prosthesis loss or slippage, and a longitudinal study is required. Lastly, some BOR/BO patients without EVA still had significant ABG after middle ear surgery, suggesting that the presence of additional unexpected anomalies or pathological third windows before or during exploration may affect incomplete ABG closure. However, we showed that middle ear surgery is possible to correct conductive components in non-EVA patients with BOR/BO syndrome. This is a good example for precision medicine in syndromic hereditary deafness.

In conclusion, we expand the genotypic spectrum of *EYA1* variants causing BOR/BO syndrome and describe the outcomes of middle ear surgery both qualitatively and quantitatively. In BOR/BO patients without EVAs, audiological outcomes improved significantly. This finding, and the fact that an EVA is surgically negative prognostic, may aid audiological rehabilitation. The results of this study were against the notion that middle ear surgery is always contraindicated in patients with BOR/BO syndrome.

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