Severe inner ear malformation (IEM), including common cavity (CC) or cochlear aplasia with dilated vestibule (CADV), is challenging in terms of auditory rehabilitation and genetic counseling [1]. Little is known regarding its genetic etiologies, although occasional reports have suggested involvement of GREB1L (growth regulation by estrogen in breast cancer 1-like) gene [2,3]. Alterations of GREB1L, a neural crest regulatory molecule, have been reported to cause kidney anomalies [4]. Interestingly, most pedigrees showed maternal transmission, leading to hypotheses of genomic imprinting or effects on male fertility [4]. Four variants of GREB1L have been reported as candidate variants for profound sensorineural hearing loss under various inheritance modes (DFNA80 [MIM: #619274]: de novo, autosomal dominant with or without reduced penetrance [3]. However, the genetic etiology and mode of inheritance of severe IEM remain largely unknown. Through this study, we suggest that GREB1L alterations are the major etiology of CC/CADV, and they manifest the phenotype largely in a non-Mendelian fashion. Our results point towards the novel concept that severe IEM could develop due to autosomal genetic alterations, but frequently in a non-Mendelian fashion.

Five unrelated nonsyndromic hearing loss families (SNUBH-CADV/CC cohort) with severe IEM on both sides and CADV/CC on at least one side were recruited from 2012 to 2019 at Seoul National University Bundang Hospital. Five probands of the five pedigrees all showed profound deafness requiring bilateral cochlear implants (CIs) for appropriate auditory rehabilitation. Their audiologic and radiologic data were rigorously reviewed. During the same period, 421 CI recipients, including 220 pediatric subjects, were also ascertained to have severe to profound hearing loss at the same hospital. The 215 pediatric CI recipients other than the five patients recruited here had conditions including enlarged vestibular aqueduct (EVA, n=36), incomplete partition type 1 (IP-1, n=3), and IP-3 (n=3).

Exome sequencing (ES) was performed for 150 of the 220 pediatric CI recipients. ES data were not available for 70 patients. In detail, some patients with EVA (n=36) and IP-3 (n=3) were directly sequenced for SLC26A4 and POU3F4, while a subset of subjects with nonsyndromic hearing loss and deafness (n=11) carrying GJB2 variants were genetically diagnosed after a screening panel was performed, and some participants at the beginning of the study (n=20) underwent panel sequencing instead of ES. Family-based trio ES was performed in four of the five CADV/CC families (SB120, SB259, SB282, and SH169). ES followed by bioinformatics analysis narrowed down the candidate variants [5,6]. The pathogenic variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guideline and the recently specified ACMG/Association for Molecular Pathology (AMP) hearing loss rules [7,8]. Pedigrees, audiograms, and abnormal radiologic findings from the five CADV/CC families are displayed in Fig. 1.
Fig. 1. Pedigrees, genotypes, and phenotypes of the five probands. (A) Black-filled symbols represent hearing-impaired individuals, clear symbols denote individuals with normal hearing, and gray-filled symbols indicate unaffected individuals who are heterozygous for the causative \( \text{GREB1L} \) variant in the pedigree (non-Mendelian inheritance). Black arrows represent the probands. (B) Auditory brain stem response threshold (ABRT) testing showed no response on both sides in all individuals except SB503, with 85 dB on the left side. (C) Temporal bone computed tomography revealed bilateral inner ear malformations. (D) A Sanger sequencing chromatogram confirmed the presence of each potential causative variant of \( \text{GREB1L} \) in the SB120, SB259, and SH169 pedigrees. CADV, cochlear aplasia with dilated vestibule; CH 1, cochlear hypoplasia type 1; CC, common cavity; IP-1, incomplete partition type 1.
All steps in this study were approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB-B-1007-105-402). Written informed consent was obtained from all individuals or their guardians (for minors).

The SNUBH-CADV/CC cohort constituted 1.19% (5/421) of all CI recipients and 2.27% (5/220) of pediatric recipients. We identified three heterozygous variants of GREB1L, including one novel missense variant (c.5618T>C) and two nonsense variants (one novel) (c.982C>T and c.1079T>A) from three CADV/CC families (SB259, SB120, and SH169), while the genetic etiology was not determined in two CADV/CC families (SB282 and SB503) (Fig. 1, Table 1). Public databases including Global minor allele frequency and Korean Reference Genome Database and in silico studies including Rare Exome Variant Ensembl Learner and Combined Annotation Dependent Depletion further demonstrated the pathogenic potential of three heterozygous variants of the GREB1L gene, which were classified as pathogenic (c.982C>T), likely pathogenic (c.1079T>A) and VUS (c.5618T>C), respectively, according to the ACMG/AMP guidelines (Table 1). Each candidate variant was confirmed to be present through Sanger sequencing.

No convincing GREB1L variants were detected in any of the other 145 pediatric CI recipients with available ES data, giving rise to a statistically significant predilection of GREB1L variants exclusively in the CADV/CC cohort (Fisher’s exact test, P<0.001). Noticeably, all three families showed discordant segregation between GREB1L variants and CADV/CC among family members. Specifically, all probands carrying GREB1L variants were boys who always inherited the GREB1L variant from their normal-hearing mothers (Fig. 1).

Our study showed a statistically significant, and most likely causal, relationship between GREB1L variants and CADV/CC, since GREB1L variants were exclusively detected in CADV/CC subjects. A significant genetic load of GREB1L variants in CADV/CC was also suggested by the detection rate of 60% in the SNUBH-CADV/CC cohort.

Of particular note, the segregation of GREB1L variants in all three families did not conform to conventional Mendelian inheritance. Our observations are consistent with the previous detection of a GREB1L variant descended from a normal-hearing mother [3] and the proposed pathogenesis of kidney agenesis caused by GREB1L variants [4]. Intrafamilial variability might exist, or this finding could be potentially related to the fact that GREB1L is an androgen-regulated gene [9]. However, the carrier mothers in the cohort showed no overt hearing or renal phenotypes. Thus, genomic imprinting of GREB1L with preferential expression of the maternal mutant allele (silencing of the paternal allele) could be a possibility for further research. GREB1L alterations should be suspected as a major genetic contributor to severe IEMs, potentially through a non-Mendelian inheritance pattern.
CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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