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Short Communication

Major Contribution of *GREB1L* Alterations to Severe Inner Ear Malformation Largely in a Non-mendelian Fashion

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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.

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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.

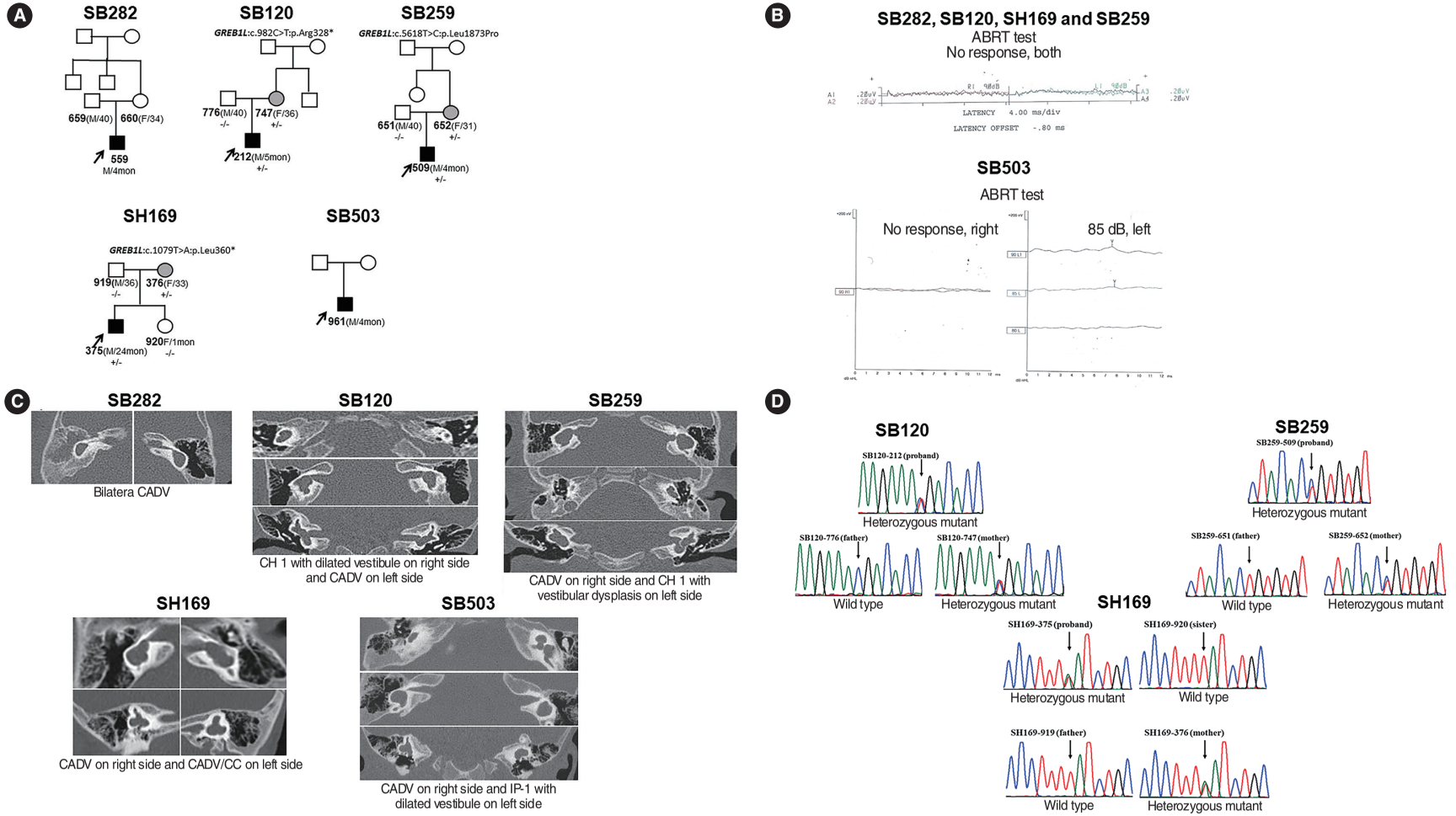

Fig. 1. Pedigrees, genotypes, and phenotypes of the five probands. (A) Black-filled symbols represent hearing-impaired individuals, clear symbols denote individuals with normal hearing, and gray-filled symbols indicate unaffected individuals who are heterozygous for the causative *GREB1L* variant in 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 *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, novel) 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 Ensemble 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.

Table 1. *GREB1L* gene variants detected in three probands with cochleovestibular anomalies

Subject ID (sex/age)	*GREB1L* [NM_001142966]: [NP_001136438]	Genomic position (GRCh37/hg19); dbSNP ID	Variant segregation	Zygosity	Family history of hearing loss	CADD phred (v1.4)	REVEL score	Global MAF/ KRGDB (n=1,722)	Severity/ phenotype	Other phenotypes	ACMG classification	Criteria applied	Reference
SB120-212 (male/ 5 mo)	c.982C>T: p.Arg328*	Chr18:19020262; rs1555648043	Unaffected carrier mother	het	No	36.00	NA	Absent	Profound bilateral hearing loss/cochlear hypoplasia type 1 (R): CADV (L)	-	Pathogenic	PVS1, PS1, PM2, PM5	[2]
SB259-509 (male/ 24 mo)	c.5618T>C: p.Leu1873Pro	Chr18:19102628; novel	Unaffected carrier mother	het	No	28.90	0.543	Absent	Profound bilateral hearing loss/bilateral CC	-	VUS	PM2	This study, novel
SH169-375 (male/ 24 mo)	c.1079T>A: p.Leu360*	Chr18:19021370; novel	Unaffected carrier mother	het	No	36.00	NA	Absent	Profound bilateral hearing loss/CADV (R): between CC and CADV (L)	-	Likely pathogenic	PVS1, PM2	This study, novel

CADD, Combined Annotation Dependent Depletion; REVEL, Rare Exome Variant Ensemble Learner; KRGDB, Korean Reference Genome database; ACMG, American College of Medical Genetics and Genomics; het, heterozygote; NA, not applicable; R, right; CADV, cochlear aplasia with dilated vestibule; L, left; CC, common cavity; VUS, variant of uncertain significance.
CADD: https://cadd.gs.washington.edu/ [10]; REVEL: https://sites.google.com/site/revelgenomics/ [11]; Global minor allele frequency database (gnomAD): https://gnomad.broadinstitute.org [12]; 1000Genomes: https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/; ESP6500: https://evs.gs.washington.edu/EVS/; KRGDB: http://152.99.75.168:9090/KRGDB/menuPages/intro.jsp [13].

CONFLICT OF INTEREST

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

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Conceptualization: CBY, BJK. Data curation: HJ, NY, JHH, SYL. Formal analysis: JHH, NY, GHS. Funding acquisition: CBY, BJK. Methodology: GHS. Project administration: JHH. Visualization: HJ. Writing–original draft: BJK. Writing–review & editing: SHO, CBY.

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