GJB2 c.235delC variant associated with autosomal recessive nonsyndromic hearing loss and auditory neuropathy spectrum disorder

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Abstract

Autosomal recessive nonsyndromic hearing loss (ARNSHL) is a genetically heterogeneous neurosensory disorder, usually characterized by congenital or prelingual hearing loss, and not accompanied by other clinical features (Xia et al., 2015; Meena and Ayub, 2017). ARNSHL accounts for 45-52.5% of cases of inherited hearing loss, which occurs in approximately 1/1000-2000 newborns (Duman and Tekin, 2012; Duan et al., 2015; Xia et al., 2015). Individuals with ARNSHL usually present difficulty in language development and social interactions.

Since variants in the gap junction protein beta 2 gene (\(GJB2\)) were first identified as causative of ARNSHL in 1997 (Kelsell et al., 1997), to date (January, 2019), at least 72 pathogenic variants in other 72 genes have been causally associated with ARNSHL according to the Hereditary Hearing Loss Homepage (https://hereditaryhearingloss.org).

Keywords: Auditory neuropathy spectrum disorder, exome sequencing, hearing loss, \(GJB2\) gene, \(GJB2\) c.235delC variant.

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Peripheral blood samples were obtained from all participants, and genomic DNA was extracted using a saturated phenol-chloroform extraction method (Yuan et al., 2015). The present study was reviewed and approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University (Changsha, China), in accordance with the Declaration of Helsinki. Written informed consent forms were provided by all participants.

A series of auditory evaluations, including pure tone audiometry (PTA), tympanometry, acoustic reflex (AR) thresholds, auditory brainstem responses (ABR), transient evoked otoacoustic emission (TEOAE), and distortion product otoacoustic emission (DPOAE) were performed. Potential inner ear congenital malformations were evaluated with magnetic resonance imaging (MRI). Audiometric thresholds were evaluated at frequencies 250, 500, 1000, 2000, 4000, and 8000 Hz by PTA. Hearing acuity is considered ‘normal’ at a threshold within 25 decibels (dB), and the degree of hearing loss was classed as mild (26-40 dB), moderate (41-60 dB), severe (61-80 dB), or profound (> 81 dB) (Asghari et al., 2017). TEOAE and DPOAE were tested using GN otometrics-Madsen capella™. Fast-Screen mode and 80 dB hearing level were set for TEOAE examination. DP1, 65 dB hearing level for f1, and 55 dB hearing level for f2 were set for DPOAE.

Three micrograms of the patient’s genomic DNA was used for exome sequencing. It was first sonically sheared and then enriched, hybridized, and captured by the Agilent SureSelect Human All Exon V4 kit at BGI-Shenzhen (Shenzhen, China), according to the manufacturer’s protocol. The library with the targeted exome was sequenced using the Illumina HiSeq 2000 platform. The mean sequencing depth was 101.78, and 99.43% of the targeted exome was covered. Single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) were detected. Alleles with a frequency > 0.5% in the following databases were screened out based on the SNPs database (dbSNP version 138), 1000 genomes project (1000 genomes release phase 3), HapMap project (2010-08_phase II + III), Exome Sequencing Project 6500 (ESP6500) (Zheng et al., 2016), Exome Aggregation Consortium, and an in-house exome database of BGI. The functional effects of non-synonymous SNPs in the coding regions were predicted by Sorting Intolerant from Tolerant (SIFT, http://sift.jcvi.org/) and Polymorphism Phenotyping version 2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/).
Sanger sequencing was performed to identify whether candidate variants co-segregated with ARNSHL phenotype in the family, using an ABI3500 sequencer (Applied Biosystems, Foster City, CA, USA) (Zheng et al., 2016). Primer sequences for the pathogenic variant in the GJB2 gene were designed and synthesized as follows: forward, 5'-TCGCAATTATGATCCTCGGTG-3' and reverse, 5'-CTCCCCCGTATGAACCTCC-3'. The function effects of possible candidate variants were further predicted using MutationTaster (http://www.mutationtaster.org/).

The patient’s audiological evaluation revealed profound bilateral sensorineural hearing loss, a Type A tympanometric curve, and absent AR and ABR. TEOAE and DPOAE were absent in the patient’s left ear. TEOAE and low amplitude DPOAE at 4000 or 8000 Hz were elicited in the patient’s right ear. MRI showed no anomaly in the patient’s inner ears. The patient’s clinical phenotype was also consistent with ANSD, a disorder of the auditory pathway characterized by the absence of ABR and the presence of OAE (Manchaiah et al., 2011). PTA of his parents showed normal hearing level.

Exome sequencing generated 104,662 SNPs and 16,813 InDels. After screening out common and nonpathogenic variants, homozygosity for the c.235delC variant (rs80338943, a known pathogenic variant, NM_004004.5) in the GJB2 gene was found, and there were no other potentially causative variants for hearing loss.

Homozygosity for the c.235delC variant in the GJB2 gene was confirmed in the patient by Sanger sequencing (Figure 1B). His parents were found to be heterozygous for this variant (Figure 1C). The GJB2 c.235delC variant was not detected in the 200 healthy controls (Figure 1D), and it is predicted to be disease-causing by MutationTaster, resulting in a shift in the reading frame at codon 79 and a premature stop codon at codon 81, p.(L79Cfs*3).

Variants in the GJB2 gene are the primary cause of ARNSHL and responsible for 5-43% of nonsyndromic hearing loss in different ethnicities (Kenesson et al., 2002; Duan et al., 2015). Presently at least 400 pathogenic variants in the GJB2 gene are known on the basis of the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). Mutation spectrum and frequency in the GJB2 gene vary with ethnicity (Zheng et al., 2015).

In this study, by exome sequencing, a homozygous GJB2 c.235delC variant, known to be pathogenic (Dai et al., 2015), was found in an individual with hearing loss, inherited from first-cousin normal-hearing heterozygous parents. Variants in other causative genes for hearing loss were excluded. Exome sequencing is a powerful strategy for accurate diagnosis of ARNSHL or ANSD, a highly genetically heterogeneous disorder.

The GJB2 gene encodes connexin 26, a gap-junction protein, expressed in the human and rat cochlear cells (Kelsell et al., 1997). Connexin 26 consists of an N-terminal helix, four transmembrane helices (TM1-4), two extracellular loops (E1 and E2), a cytoplasmic loop (CL), and a C-terminus (Maeda et al., 2009). The protein is involved in recycling potassium ions (Kelsell et al., 1997), ATP release, intercellular signaling, hearing function regulation (Zhao et al., 2005), cochlear development, and active cochlear amplification (Chen et al., 2014). Connexin 26 knockout mice displayed congenital hearing loss and cochlear developmental disorders (Chen et al., 2014). Conditional knockout mice showed severe hearing loss and DPOAE reductions (Zhu et al., 2013).

The c.235delC variant in the GJB2 gene, predicted to produce a truncated protein, was reported in different populations, especially in East Asia (Dai et al., 2015; Taniguchi et al., 2015). The GJB2 c.235delC variant involving the TM2 domain is predicted to be a disease-causing alteration by MutationTaster. It generates a truncated protein p.(L79Cfs*3) missing important functional segments, including CL, TM3, E2, TM4, and C-terminal segments. The glutamine (p.Q80) in the TM2 segment of connexin 26 interacts with arginine (p.R32) in the TM1 segment, thus the variant may interfere with the interplay between the two TM domains, disturb the appropriate folding and/or oligomerization of connexins, and lead to defective gap junction channels (Maeda et al., 2009).

GJB2 variants have been reported in 7.5% of patients with ANSD (Carvalho et al., 2016). Our patient was diagnosed with ANSD due to the presence of right ear OAE, but the absence of ABR, and this is the first report of the GJB2 c.235delC variant in connection with ANSD. ANSD may result from an abnormality in the inner hair cells (IHC), in the synapse between IHC and auditory nerve, or in the auditory nerve itself (Starr et al., 1996). Connexin 26 is expressed in the cochlear basement membrane on which the hair cells lie (Kelsell et al., 1997). Connexin 26 expression contributes to IHC functional maturation (Johnson et al., 2017), thus GJB2-associated ANSD may be caused by immature IHCs.

In conclusion, our study shows a novel association of homozygosity for the c.235delC variant in the GJB2 gene with the phenotypes of ARNSHL and ANSD.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Author contributions

HX and HD conceived and designed the study. HX, XH, HBX, YAZ, LG, ZY, JL and HD conducted the experiments. HX, XH and HD analyzed the data. HX and HD wrote the manuscript. All authors read and approved the final version.

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