Diagnostic pitfalls for GJB2-related hearing loss: A novel deletion detected by Array-CGH analysis in a Japanese patient with congenital profound hearing loss

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Key Clinical Message
Here, we report a novel deletion (copy number variation: CNV) in the GJB2 gene observed in a Japanese hearing loss patient. The deleted segment started in the middle of the GJB2 gene, but the GJB6 gene remained intact. This partial deletion in the GJB2 gene highlights the need for further improvements in GJB2 screening.

KEYWORDS
CNV, GJB2 gene, novel deletion, profound hearing loss

1 | INTRODUCTION

GJB2 gene mutations are known to be the most common cause of hereditary hearing loss worldwide. Therefore, genetic testing for GJB2 mutations is one of the most important screening processes for the molecular diagnosis of deafness. However, some caution is due in the diagnosis of hearing loss based on GJB2 screening as several types of large deletions have been reported. Here, we report a novel deletion (copy number variation: CNV) in the GJB2 gene observed in a Japanese patient presenting with profound hearing loss. This deletion was observed in trans to a GJB2-mutated allele carrying the GJB2:NM_004004.5:c.427C>T:p.R143W mutation by PCR fragment analysis. It should be noted that this deletion was identified as homozygosity of c.427C>T:p.R143W by Sanger sequencing. Array-CGH analysis showed the deleted segment started in the middle of the GJB2 coding region and extended for at least eight thousand base pairs, although the GJB6 gene remained intact. The distal breakpoint downstream of the GJB6 gene differed from the breakpoints of the known DFNB1 locus deletions. This partial deletion in the GJB2 gene highlights the need for further improvements in GJB2 screening.
Inherited sensorineural hearing loss (HL) is an extremely heterogeneous group of sensory disorders in humans. The overall incidence is estimated to be one in approximately 1000 newborns. The GJB2 gene (MIM# 121011), which encodes the gap junction protein connexin 26 (Cx26), is the most common genetic etiology associated with congenital HL worldwide, with the mutation spectrums known to vary among different ethnic groups. The GJB2 gene is a small gene composed of two exons, one of which possesses a 678-bp coding sequence. As screening of the GJB2 gene is considered a standard, first-step approach in the diagnosis of genetic hereditary HL, it is not surprising that more than 300 mutations in the GJB2 gene sequence have been described (The Human Gene Mutation Database). In general terms, GJB2-related congenital HL develops through a biallelic status. Among patients with a single GJB2 heterozygous status, some caution is due in the diagnosis of hearing loss based on GJB2 screening as a large deletion located in the 13q12 region encompassing the GJB2 and GJB6 genes (the so-called DFNB1 locus) is sometimes seen in trans with GJB2-coding region variants. Therefore, the potential for such deletions to be present should be kept in mind. To date, six large deletions contributing to HL have been identified in the DFNB1 region.

Here, we report for the first time a novel large deletion in the GJB2 gene in one Japanese family with nonsyndromic HL. The present work highlighted the diagnostic pitfalls of GJB2-related hearing loss and could expand the pathogenic spectrum and strengthen our understanding of the complicated mechanisms by which GJB2 gives rise to HL.

2 | CLINICAL REPORT

2.1 | Ethics statement

This study was approved by the Ethical Committee for Clinical Research of Shinshu University and each participating institution as described previously. We obtained written informed consent from all the participants (or their parents) involved in this study.

One two-generation Japanese family with sporadic nonsyndromic sensorineural HL was introduced to the Department of Otolaryngology at the Toranomon Medical Hospital. The patient of the family was a 3-year-old girl who was referred for newborn auditory screening in both ears: Her wave V at 90 dB nHL did not show a bilateral response during auditory brainstem response testing at 5 weeks after birth. Her auditory steady-state response (ASSR) examination showed thresholds of 95-115 dB for the right ear and 115 dB for the left, indicating profound hearing loss. Conditioned orientation response (COR) testing showed reactions in the range of 90-100 dB, while her tympanometric results were normal. After confirmation of hearing loss, the patient started wearing hearing aids in both ears, but showed only limited audiological improvement and difficulty with language acquisition. Pure-tone audiometry at four frequencies (0.5, 1, 2, and 4 kHz) showed a bilateral configuration characteristic of profound hearing loss (>95 dB HL) (Figure 1A). At 3 years and 6 months of age, the patient underwent cochlear implantation in her left ear. She has since showed steady progress in language development as her ability to recognize words has increased dramatically. Her average hearing threshold (0.5, 1, and 4 kHz) is 30 dB when wearing the cochlear implant (Figure 1A). Her development was normal, and no other phenotypic abnormalities, apart from left fistula auris congenita, were noted. Her parents have no hearing impairment. Screening for congenital cytomegalovirus infection using her preserved umbilical cord specimen was negative for cytomegalovirus DNA. Temporal bone computed tomography (CT) scans also revealed no abnormalities.

2.2 | DNA analysis

Independent of PCR-based methods, the proband’s Invader assay results showed a GJB2:c.427C>T:p.R143W heterozygous pattern at both 2 and 4 hours (data not shown). Next, we considered that a second GJB2 mutation could be present in the other allele as the GJB2 gene can cause prelingual HL due to biallelic mutations. Sanger sequencing for the family members revealed that, quite unexpectedly, the affected girl had the GJB2:c.427C>T:p.R143W homozygote (Figure 1B), and the mother of the patient, but not the father, carried a p.R143W heterozygous mutation. Sanger sequencing also revealed that the GJB2:c.427C>T:p.R143W variant did not segregate with the phenotype and that it was inconsistent with the results of the Invader assay. Genotypes of two non-pathogenic variations (V27I and E114G, which are located upstream of GJB2:c.427C>T:p.R143W) also showed similar segregation in this family (data not shown). As the results of both the Invader assay and Sanger sequencing are highly reliable, as part of our molecular interpretation of these discrepancies, we hypothesized that a paternally transmitted deletion in the GJB2 gene might exist and that only one of the two alleles might be read in the direct sequence. To verify our hypothesis, we performed DNA assay with uniquely designed PCR primers sets including the R143R and R143W positions (allele-specific primers). In her mother, both of the allele-specific primers for the wild type and R143W mutation were amplified and PCR products of the expected size were confirmed as one band. For her father, no fragments were detected for R143W-specific PCR amplification, indicating an apparently wild-type sequence. For the proband, PCR products were obtained with the R143W-specific primers in both the upstream and downstream regions. On the other hand, no
FIGURE 1  Pedigree and genomic DNA analysis. A, The audiograms of both ears in the proband of the family revealed profound hearing loss. Black triangles: the hearing threshold level via cochlear implant. B, Direct sequencing electropherograms of GJB2 showing the p.R143W homozygote in the patient. C, Agarose gel electrophoresis of the PCR products in this family. In the patient and her mother, p.R143W-specific amplified bands are observed. PCR products for p.R143R_r allele-specific and upstream designed primers were absent (red arrowhead) in the patient only. For the wild-type sample, all the corresponding amplified PCR fragments were observed except for PCR amplification by p.R143W-specific primers.
FIGURE 2 Location of deletions at the DFNB1 locus on chromosome 13q12.11. A. Results of Array-CGH for the GJB2 gene region in the patient. Hybridization results are shown for the affected patient in whom an abnormality was identified by Array-CGH. For each panel, the x-axis marks the distance, in megabases, along the chromosome from the q telomere. The y-axis marks the hybridization ratio plotted on a log2 scale. The patient shows a hemizygous deletion involving in the GJB2 gene region based on analysis using CytoGenomics software. The area shown in red is depleted by one copy loss. The upper panel includes the region up to GJB6, and the lower panel is an expanded image showing the approximate eight thousand base pair deletion. B, Schematic map of seven deletions. All genomic configurations are derived from NC_000013.11 (Reference: GRCh38.p12 Primary Assembly).
of the transcriptional promoter sequence is thought to abolish gene transcription. The absence of this region is shown in Figure 2A. A deletion of approximately eight thousand base pairs (8 kb) from the GJB2 gene region was identified by Array-CGH, but this deletion did not extend to the GJB6 gene. This novel deletion starts from the middle of the GJB2 coding region sequence and spreads upstream of GJB2, differing from the breakpoint of the other known large DFNB1 locus deletions (Figure 2B). The deletion region deduced from the Array-CGH probe position was Chr13: 20763410-20771801 in GRCh37.5. Finally, we concluded that the HL in this patient was caused by a compound heterozygous mutation of GJB2:c.427C>T:p.R143W with a long deletion and was passed down by autosomal recessive inheritance.

3 | DISCUSSION

In this study, we identified a novel deletion in the GJB2 gene (hereafter, the 8 kb deletion), in cis with a p.R143W heterozygous status, in a Japanese patient with profound HL. Our DNA fragment analysis by PCR amplification indicated the existence of a broad deletion that appears to possess a breakpoint starting between p.E114 and p.R143 in the GJB2 coding region and extending upstream. Array-CGH was next implemented, revealing an overview of the new deletion (Figure 2B). We calculated the deletion to be approximately 8 kb in length, but that it did not affect the GJB6 gene. In this work, we detected for the first time the presence of a single large deletion in the GJB2 gene sequence. The long deletion identified in the GJB2 gene in this study was not identified in the DECIPHER databases (https://decipher.sanger.ac.uk) or our in-house controls (data not shown). The identified GJB2 deletion region contains the translational start codon, 5' UTR, and a transcriptional promoter region of the GJB2 gene. The absence of the transcriptional promoter sequence is thought to abolish GJB2 gene transcription. Previous functional studies of c.-22-2A>C (a splice-site mutation) and g.-77C>T (a mutation of the basal promoter region) did not yield any detectable Cx26 protein or mRNA, suggesting that no GJB2 transcripts would be produced from this deletion allele. The proband's father, harboring a heterozygous 8 kb deletion, is not conscious of any hearing loss, implying that the novel long deletion is a pathogenic variant in autosomal recessive inheritance HL. The p.R143W variant shows an extraordinarily high prevalence among congenital HL patients in Ghana, West Africa. Not limited to Africa, it is also commonly found in congenital HL patients among various populations. Bi-allelic-mutated individuals with GJB2 truncating mutations display profound HL. Considering the 8 kb deletion to be equivalent to a truncating mutation, there appears to be no contradiction in the fact that this patient shows a profound hearing impairment phenotype. According to several studies on the carrier frequency of GJB2 mutations, the p.R143W allele frequency is 0.42% (13/3062 alleles) – 0.67% (18/2686 alleles) in congenital HL patients among different ethnic groups, and a frequency of up to 0.1% (1/1018 alleles) was observed in the general Japanese population. The p.R143W mutation is mostly found in a compound heterozygous form, except for in Ghana. As p.R143W is a pathologic variation of relatively rare frequency (<1%), apart from inbred or familial HL accumulation, it is unlikely to occur in a homozygous p.R143W genotype. In genetic counseling of recessive families or simplexes in which a GJB2 homozygous mutation is detected, it is generally explained as parental heterozygous inheritance or uniparental disomy of chromosome 13, but the latter is extremely uncommon. Thus, when possible, segregation studies are needed to confirm whether both parents are carriers or not. Sanger sequencing, by overlooking a large deletion in the DFNB1 locus, may lead to a judgment of apparent GJB2 homozygosity. Even though the probability is very small, to avoid missing such large deletions in the DFNB1 locus, other DNA tests capable of detecting large deletions should be used in combination for screening the GJB2 gene. Recently targeted next-generation sequencing (NGS) has been applied to the high throughput and fast identification of pathological causes. In addition, the NGS strategy can be expected to provide an effective method for the identification of multi-exon deletions or duplications of target genes. Therefore, smooth progression to the analysis of gene dosage for confirmation may bring about precise diagnosis and appropriate genetic counseling for GJB2-related HL without the need for Sanger sequencing. Although the additional analysis may be required for further refinement of this novel deletion, the results of our study have furthered our knowledge of GJB2 and its pathogenicity.

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REFERENCES

1. Morton CC, Nance WE. Newborn hearing screening-a silent revolution. N Engl J Med. 2006;354:2151-2164.
2. Smith RJ, Bale JF Jr, White KR. Sensorineural hearing loss in children. Lancet. 2005;365:879-890.
3. Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ. Prevalent connexin 26 gene (GJB2) mutations in Japanese. Genet J Med. 2000;37:41-43.
4. Hamelmann C, Amedofu GK, Albrecht K, et al. Pattern of connexin 26 (GJB2) mutations causing sensorineural hearing impairment in Ghana. Hum Mutat. 2001;18:84-85.
5. Snoeckx RL, Huygen PL, Feldmann D, et al. GJB2 mutations and degree of hearing loss: a multicenter study. Am J Hum Genet. 2005;77:945-957.
6. Del Castillo I, Moreno F. Prevalence and evolutionary origins of the del(GJB6-D13S1830) mutation in the DFNB1 locus in hearing-impaired subjects: a multicenter study. Am J Hum Genet. 2003;73:1452-1458.
7. del Castillo FJ, Rodríguez-Ballesteros M, Alvarez A, et al. A novel deletion involving the connexin-30 gene, del(GJB6-d13s1854), found in trans with mutations in the GJB2 gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. J Med Genet. 2005;42:588-594.
8. Feldmann D, Le Maréchal C, Jonard L, et al. A new large deletion in the DFNB1 locus causes nonsyndromic hearing loss. Eur J Hum Genet. 2009;17:195-200.
9. Wilch E, Azaiez H, Fisher RA, et al. A novel DFNB1 deletion allele supports the existence of a distant cis-regulatory region that controls GJB2 and GJB6 expression. Clin Genet. 2010;78:267-274.
10. Tayoun AN, Mason-Suárez H, Frisella AL, et al. Targeted droplet-digital PCR as a tool for novel deletion discovery at the DFNB1 locus. Hum Mutat. 2016;37:119-126.
11. Bliznetz EA, Lalayants MR, Markova TG, et al. Update of the GJB2/DFNB1 mutation spectrum in Russia: a founder Ingush mutation del(GJB2-D13S175) is the most frequent among other large deletions. J Hum Genet. 2017;62:789-795.
12. Moteki H, Azaiez H, Sloan-Heggen CM, et al. Detection and confirmation of deafness-causing copy number variations in the STRC gene by massively parallel sequencing and comparative genomic hybridization. Ann Otol Rhinol Laryngol. 2016;125:918-923.
13. Matos TD, Caria H, Simões-Teixeira H, et al. A novel hearing-loss-related mutation occurring in the DFNB2 basal promoter. J Med Genet. 2007;44:721-725.
14. Gandía M, Del Castillo FJ, Rodríguez-Álvarez FJ, et al. A novel splice-site mutation in the GJB2 gene causing mild postlingual hearing impairment. PLoS ONE. 2013;8:e73566.
15. Tsukada K, Nishio S, Usami S. A large cohort study of GJB2 mutations in Japanese hearing loss patients. Deafness gene study consortium. Clin Genet. 2010;78:464-470.
16. Taniguchi M, Matsuo H, Shimizu S, et al. Carrier frequency of the GJB2 mutations that cause hereditary hearing loss in the Japanese population. J Hum Genet. 2015;60:613-617.
17. Álvarez A, del Castillo I, Pera A, et al. Uniparental disomy of chromosome 13q causing homozygosity for the 35delG mutation in the gene encoding connexin26 (GJB2) results in prelingual hearing impairment in two unrelated Spanish patients. J Med Genet. 2003;40:636-639.
18. Yan D, Ouyang XM, Angeli SI, Du LL, Liu XZ. Paternal uniparental disomy of chromosome 13 causing homozygous 35delG mutation of the GJB2 gene and hearing loss. Am J Med Genet A. 2007;143:385-386.
19. Sakuma N, Moteki H, Takahashi M, et al. An effective screening strategy for deafness in combination with a next-generation sequencing platform: a consecutive analysis. J Hum Genet. 2016;61:253-261.
20. Pasmant E, Parfait B, Luscan A, et al. Neurofibromatosis type 1 molecular diagnosis: what can NGS do for you when you have a large gene with loss of function mutations? Eur J Hum Genet. 2015;23:596-601.

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