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Cochleosaccular Dysplasia Associated With a Connexin 26 Mutation in Keratitis–Ichthyosis–Deafness Syndrome

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Abstract

Objective—The objective of this study was to characterize the temporal bone phenotype associated with a mutation of *GJB2* (encoding connexin 26).

Study Design—The authors conducted correlative clinical, molecular genetic, and postmortem histopathologic analysis.

Methods—The study subject was a male infant with keratitis–ichthyosis–deafness (KID) syndrome. We performed a nucleotide sequence analysis of *GJB2* and a histopathologic analysis of the temporal bones.

Results—The subject was heterozygous for G45E, a previously reported KID syndrome mutation of *GJB2*. The primary inner ear abnormality was dysplasia of the cochlear and saccular neuroepithelium.

Conclusions—*GJB2* mutations can cause deafness in KID syndrome, and possibly in other *GJB2* mutant phenotypes, by disrupting cochlear differentiation.

Keywords

Scheibe dysplasia; cochleosaccular dysplasia; connexin 26; *GJB2*; hearing; KID syndrome

INTRODUCTION

There are hundreds of genes in which mutations cause sensorineural hearing loss (SNHL) either alone or in combination with other abnormalities as part of a syndrome.¹ Some genes have

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both dominant and recessive mutant alleles, and some are associated with both syndromic and nonsyndromic forms of SNHL. One of these genes is *GJB2*, encoding connexin 26, a polypeptide component of gap junctions in the inner ear, skin, and other tissues. Gap junctions are intercellular channels, which allow passive movement of water and small solutes between adjacent cells. Mutations that decrease or eliminate connexin 26 function act as recessive mutant alleles that cause nonsyndromic SNHL. In contrast, dominant mutant alleles of *GJB2* are missense substitutions that act through dominant negative mechanisms to cause SNHL alone or in combination with cutaneous epithelial abnormalities.² One of the dominant *GJB2* mutant phenotypes, keratitis–ichthyosis–deafness (KID) syndrome,² also affects corneal epithelium and vision to cause a dual sensory deficit that is difficult to rehabilitate.³

A transgenic mouse model for dominant SNHL caused by the R75W mutation of *GJB2* led to the conclusion that SNHL may result from disruption of homeostatic potassium ion movements between cells in the mature organ of Corti.⁴ It is possible that other *GJB2* mutations affect differentiation of the cochlear duct epithelium, which is consistent with the pathophysiology of connexin mutations in other tissues.² The relevance of a conditional knockout mouse model to this question is unknown as a result of incomplete cochlear deletion of *GJB2* expression.⁵ Temporal bone findings in a human subject with nonsyndromic SNHL are similarly inconclusive because the reported *GJB2* genotype might have been artifactual or nonpathogenic.^{6,7}

Although there have been a few temporal bone histopathologic studies of patients with clinical features of KID syndrome, no genotype information was available to directly link the observed changes to *GJB2*.^{8–10} The pathogenesis of *GJB2*-related SNHL thus remains uncertain but is important because recessive *GJB2* mutations are the most common cause of childhood deafness in many populations.¹ The efficacy of potential molecular therapies may depend on if and when *GJB2* mutations disrupt cochlear development or later homeostatic processes. To differentiate among these possibilities, we examined the temporal bones of a deaf infant with KID syndrome,¹¹ and we performed a molecular genetic analysis to demonstrate the association of his clinical and histopathologic phenotypes with a *GJB2* mutation.

MATERIALS AND METHODS

Subjects

This study was approved by Institutional Review Boards at the Massachusetts Eye and Ear Infirmary and the National Institute of Arthritis and Musculoskeletal and Skin Diseases. The subject's mother gave written informed consent. The clinical phenotype was previously reported.¹¹

Genetic Analysis

Genomic DNA was extracted from peripheral blood, fixed skin biopsy specimen, and fixed brain tissue.⁷ We polymerase chain reaction-amplified and sequenced the coding exons and flanking intronic sequences of *GJA1*, *GJB1*, *GJB2*, *GJB3*, *GJB4*, *GJB5*, and *GJB6* with primers shown in Table I. We used an allele-specific assay to detect a reported genomic deletion of *GJB6*.¹²

Histopathologic Analysis

Temporal bone specimens were removed at 19.5 hours postmortem and prepared for light microscopy by fixation in 10% formalin, decalcification in ethylene-diamine-tetra-acetate, embedding in celloidin, serial sectioning at a thickness of 20 μ m, and staining of every tenth section with hematoxylin & eosin.¹³

RESULTS

Clinical Phenotype

The subject was a previously described white infant male with KID syndrome.¹¹ There was no family history of this phenotype. An auditory brainstem response (ABR) study at 4 days of age revealed no response to 45-, 65-, or 95-dBHL stimuli in either ear. His mother reported that he had frequent bilateral otorrhea with a distinctive odor. A subsequent attempt to repeat the ABR was unsuccessful as a result of inadequate sedation. At 6 months of age, he died of overwhelming sepsis.¹¹

GJB2 Genotype

Nucleotide sequence analysis of *GJB2* revealed a heterozygous point mutation (134G>A) predicted to substitute glutamate for a conserved glycine at amino acid position 45 (G45E; Fig. 1). This mutation was confirmed in DNA samples independently prepared from three independently obtained tissue sources in three different laboratories. Sequence analyses of both DNA strands and subcloned polymerase chain reaction amplification products clearly confirmed the presence of this mutation. We did not identify any mutations of other connexin genes implicated in hearing or cutaneous disorders (see “Methods”). We did not detect G45E in a maternal DNA sample, and a paternal DNA sample was not available. We sequenced the 15 kb of genomic DNA, including and adjacent to *GJB2* exon 2, but did not detect any heterozygous polymorphisms that could be used in a haplotype analysis to infer the parental origin of the allele with G45E.

G45E is a nonconservative substitution in the N-terminal portion of the first extracellular loop of connexin 26, in which most reported KID syndrome mutations are located.² G45E has been identified as a *de novo* mutation in another fatal case of KID syndrome in an Austrian (white) infant but not in normal controls,¹⁴ supporting its pathogenic role in our study subject.

Temporal Bone Phenotype

The specimens were well preserved with similar findings in both ears. We observed mild, bilateral chronic otitis media and mastoiditis (not shown). The osseous cochleae were normal and consisted of two and a half turns. Examination of the cochlear ducts revealed dysplasia of the neuro-sensory organ of Corti, which consisted of undifferentiated cells (Fig. 2A). There was hypercellularity of the stria vascularis, Reissner's membrane was partially collapsed, the tectorial membrane was deformed and embedded within surrounding cells, and neither the inner sulcus nor the spiral prominence was formed (Fig. 2A). The saccular neuroepithelium was dysplastic and missing hair cells, there was deformation and encapsulation of the otolithic membrane, and the membranous wall was collapsed onto the neuroepithelium (Fig. 2C). In contrast, the utricle and all three semicircular canals were normally developed (Fig. 3). This combination of findings comprises the classic developmental anomaly known as cochleosaccular, or Scheibe, dysplasia.¹³

We observed subnormal numbers of spiral ganglion neurons in the basal cochlear turns with normal numbers in the other turns. Peripheral dendrites extending from the spiral ganglion neurons to the organ of Corti were present in all turns.

DISCUSSION

Our study demonstrates the classic findings of cochleosaccular dysplasia in an infant with KID syndrome and the G45E mutation of *GJB2*. Our results are consistent with temporal bone histopathologic changes in three other infants with similar clinical phenotypes and unknown genotypes.⁸⁻¹⁰ The fulminant clinical course associated with G45E may reflect additional

effects on barrier function, immune function, or both.¹¹ Although our results cannot distinguish if the subject's father carried G45E or if it arose *de novo* on one of the parental alleles, the unaffected paternal phenotype and the observation that most KID syndrome mutations arise *de novo* support the latter hypothesis.²

G45E has been detected in Japanese individuals with nonsyndromic recessive deafness,¹⁵ but not in Japanese patients with KID syndrome. There are several possible explanations for this; first, differences in genetic background might account for the incomplete penetrance of cutaneous phenotypic abnormalities caused by some dominant *GJB2* mutations.¹⁴ Second, digenic inheritance of G45E with a mutation in another gene could cause KID syndrome. However, neither our case nor that reported by Janecke et al.¹⁴ had detectable mutations in other connexin genes. Finally, G45E in *cis* configuration with an inactivating mutation of the same *GJB2* allele would likely be a recessive allele associated with nonsyndromic deafness. In a large series of Japanese nonsyndromic recessive deafness subjects, 27 of 27 subjects with G45E also had the nonsense mutation Y136X.¹⁵ The authors stated that G45E was in *cis* configuration with Y136X in at least some samples, but data were not shown to indicate if the remaining samples had G45E in *cis* configuration with other mutations.¹⁵ Similarly, in Japanese individuals, G45E could also be in *cis* configuration with undetected mutations in noncoding regions of *GJB2* such as one of the large genomic deletions upstream of *GJB2*.¹² Despite the difference between Japanese and other study populations, these observations all support the etiologic association of G45E with deafness.

Our findings demonstrate that a *GJB2* mutation can cause deafness through disruption of cochlear epithelial differentiation. Although this might result from trans-dominant negative effects of G45E on other connexins, there are no published studies that clearly indicate this could not be a direct effect of loss of *GJB2* function. Because the normal human cochlea is fully differentiated at birth, gene replacement or suppression strategies for preventing or reversing *GJB2* deafness may be unsuccessful if they are administered postnatally, when deafness is typically diagnosed. However, the intact cochlear dendrites and neurons we observed would be potentially available for direct stimulation with a cochlear implant.

CONCLUSION

We have characterized the temporal bone phenotype associated with the G45E mutation of *GJB2* in a male infant with KID syndrome. His primary inner ear abnormality was dysplasia of the cochlear and saccular neuroepithelium. *GJB2* mutations can cause deafness in KID syndrome, and possibly in other *GJB2* mutant phenotypes, by disrupting cochlear differentiation.

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Fig. 1.

GJB2 genotype. Wild-type *GJB2* sequence and the heterozygous G-to-A mutation at nucleotide position 134 in the keratitis–ichthyosis–deafness syndrome subject. Predicted amino acid sequences are shown below the corresponding nucleotide sequence. 134G<A is predicted to substitute glutamate (E) for glycine (G) at amino acid position 45 of connexin 26.

Fig. 2.

Temporal bone phenotype of the keratitis-ichthyosis-deafness syndrome subject. (A) Dysplasia of the organ of Corti. (C) Dysplasia of the saccule. (B and D) Equivalent sections prepared at 14 hours postmortem from a normal 7-month-old female infant for comparison to A and C, respectively. All panels are shown at the same scale as A.

Fig. 3. Utricular phenotype. Low-power micrograph showing a normally developed and innervated utricle from the keratitis–ichthyosis–deafness syndrome subject.

Table I

Polymerase Chain Reaction Amplification and Sequencing Primers.

Gene	Exon	Forward Primer Sequence	Reverse Primer Sequence
<i>GJA1</i>	2	5'-GTAATTTGCAATCTGTGATCCTTG	5'-TTCACCTTACCATGCTCTTCAATA
	2	5'-GCACTTGAAGCAGATTGAGATAAA	5'-TTTGTCTCTGCACTGTAATTAGCC
	2	5'-AATATGCTTATTTCAATGGCTGCT	5'-TGTCTTTGTGTTTCTAAGCCTCA
<i>GJB1</i>	2	5'-CTACTGGCTCTTGAAGAGTTGA	5'-GCCGTAGCATTTCCTCTCTATGT
	2	5'-AGCGTTTGCTATGACCAATTCT	5'-AGCTAGCATGAAGACGGTGAAG
	2	5'-TTTATCTGCTCTACCCTGGCTAT	5'-AGTAATCCCCAGCAGGCAGAG
<i>GJB2</i>	2	5'-TGAGGTTGTGTAAGAGTTGGTGTT	5'-TTGATGAACTTCCTCTTCTCTCA
	2	5'-CTACGATCACTACTTCCCCATCTC	5'-AATCTTTGTGTTGGGAAATGCT
<i>GJB3</i>	1	5'-ATTCATTACATACGATGGTTTTTC	5'-CAGGTAGAGGAAGAGGAACTCAAT
	1	5'-TCATCTTCAAGCTCATCATTGAGT	5'-AAAAATATCTGCCAAAACGAAAGT
<i>GJB4</i>	1	5'-GCAGGTAGCACCCAGGTATAGA	5'-GTAGAGGCGGTGGAAGATATAGAG
	1	5'-GTACTTGCTGAGCCTCATCTTCA	5'-CCTCCTCACCACCCTGCTAC
<i>GJB5</i>	2	5'-GCTCAGAGCAAGTCTGTGATAAAT	5'-ATATTTGGGGTAGAATGAGTGGA
	2	5'-ATATGTCTGCAGCCTAGTGTTCAA	5'-TTGCACCTATGAGAGATGCTAGAG
<i>GJB6</i>	3	5'-AAGGCCTCTTCCACTAATAAACCT	5'-TTCAAAGATGATTCGGAAAAAGAT
	3	5'-TTAAAAAGCAGAAGGTTTCGGATAG	5'-AAACTCTTCAGGCTACAGAAGGAA