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Etiologic Diagnosis of Nonsyndromic Genetic Hearing Loss in Adult vs Pediatric Populations

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Abstract

Objectives—Determine the diagnostic yield of a shared genetic testing algorithm in adult and pediatric populations with sensorineural hearing loss (SNHL) and recommend effective testing strategies to evaluate for genetic causes of deafness in patients presenting with idiopathic sensorineural hearing loss.

Study Design—Hospital-based cohort study.

Setting—University of Miami outpatient otology clinics between 2001 and 2010.

Subjects—Two hundred twenty-one adult and 163 pediatric patients with nonsyndromic sensorineural hearing loss.

Methods—Peripheral blood samples were screened for mutations in *GJB2* and *GJB6* and mitochondrial DNA mutations 1555A>G, 7444G>A, and 3243A>G. Audiometric data and family history were also collected.

Results—*GJB2*/*GJB6*-related deafness was diagnosed in 23 of 163 pediatric patients (14%) compared with only 3 of 221 adults (1%). All adults had a family history of hearing loss, and 2 patients noted deafness onset at birth. Nineteen *GJB2* mutations were identified with 35delG the most common mutation. The 35delG homozygous state was the most common pathogenic genotype (54%). Mitochondrial DNA (mtDNA) mutations were found in 6 adult probands (3%). No mtDNA mutations were found in pediatric patients.

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Author Contributions

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Conclusion—Testing for common *GJB2*/*GJB6* mutations in pediatric patients has considerable value in establishing an etiologic diagnosis for SNHL. Similar testing in adults is of very low yield except perhaps in cases of early-onset SNHL or strong family history. Mitochondrial DNA testing should be considered in adults with idiopathic SNHL.

Keywords

connexin 26; connexin 30; DFNB1; *GJB2*; *GJB6*; mitochondrial DNA; nonsyndromic sensorineural hearing loss; molecular genetic testing; hereditary hearing loss

Hearing loss is the most common sensory disorder, affecting all age groups, ethnicities, and genders. Newborn hearing screening has also established that hearing loss is the most common birth defect in industrialized countries and the most prevalent sensorineural disorder. One of every 500 newborns has bilateral permanent sensorineural hearing loss (SNHL) ≥40 dB HL.[1] A recent study noted that the prevalence of hearing loss has increased among adolescents to 19.5% among those aged 12 to 19 years.[2] The prevalence then greatly increases over age 70 years.

Although those with congenital vs adult-onset hearing loss are usually considered distinct populations during clinical evaluation, our knowledge of genetic contributions to hearing loss continues to rapidly expand. With more than 150 loci for deafness genes and over 70 of them being identified (http://hereditaryhearingloss.org/), we also know that hereditary hearing loss is a very heterogeneous condition. This makes strict genotype-phenotype correlations difficult to define. Genetic testing and genetic counseling play an important role in caring for patients with SNHL, as early intervention programs can significantly improve development and quality of life.[3]

The application of genetic testing for hearing loss has primarily been directed toward evaluation of congenital hearing loss since it is estimated that about 50% of all cases are genetic in origin. In this population, testing for common genetic mutations has considerable value in establishing an etiology for hearing loss. For instance, testing for *GJB2* mutations has a high diagnostic yield given that it accounts for up to 50% of autosomal recessive hearing loss and thus 20% of all congenital hearing loss.[4,5] However, it has not been determined if similar testing at the DFNB1 locus may have a role in establishing an etiologic diagnosis in adults with idiopathic hearing loss. MtDNA mutations have also been identified in up 3% of patients with sensorineural hearing loss, primarily adults.[6] A1555G, G7444A, and A3243G are some of the most commonly found mtDNA mutations associated with SNHL. Given the phenotypic variability in many forms of genetic deafness, we sought to determine if testing for several common mutations might be of value when applied to both adult and pediatric populations presenting with idiopathic nonsyndromic SNHL. After application of a shared genetic testing algorithm, the diagnostic yield of specific mutation testing would be determined for both adult and pediatric populations with SNHL. On the basis of our findings, we will recommend effective testing strategies to evaluate for genetic causes of deafness in patients presenting with idiopathic SNHL.

Methods

All subjects participating in the study were from the University of Miami Ear Institute. The cohorts consisted of 221 adult and 163 pediatric patients with nonsyndromic SNHL, recruited from the outpatient service between 2001 and 2010. The study was open to all patients with mild or greater SNHL, and inclusion was not based on family history or audiogram configuration. This study was approved by the University of Miami Institutional Review Board prior to data collection. Written informed consent was obtained from all participants and from parents of patients younger than 18 years. The age of the patients varied between 3 months and 80 years. Pediatric patients were defined as those younger than 18 years. Each patient underwent a full medical history and physical focusing on his or her otologic complaints. A questionnaire was completed by the patient or his or her family at the time of enrollment with specific questions pertaining to his or her hearing loss, family history, and hearing habilitation. Patients were questioned regarding family members with hearing loss to generate a 3-generation pedigree. Questions regarding hearing included severity, age of onset, and hearing habilitation.

Age-specific pure-tone audiometry was obtained when possible, using equipment in accordance with International Standards Organization (8253-1-3) standards. Pure-tone average (PTA) in decibels of hearing level (dB) was then calculated for each ear by averaging thresholds at 3 frequencies (0.5, 1, and 2 kHz). Severity of hearing loss was then categorized as mild (21–40 dB), moderate (41–70 dB), severe (71–95 dB), and profound (>95 dB) based on binaural PTA.

Genomic DNA was extracted from peripheral blood samples. All patients were screened for allelic variants in the coding exon (exon 2) of *GJB2* using polymerase chain reaction (PCR) amplification. For patients with heterozygous or no mutations in *GJB2*, further analysis of the noncoding exon (exon 1) was performed using direct sequencing. All patients were also screened for the 342-kb deletion of *GJB6* using PCR amplification. DFNB1 was diagnosed if patients had either biallelic pathologic mutations of *GJB2*, biallelic deletions of *GJB6* as described above, or 1 *GJB2* pathologic mutation with the 342-kb deletion of *GJB6*. Patients with 1 identified DFNB1-associated mutation were DFNB1 carriers. Mitochondrial DNA mutations 1555A>G, 7444G>A, and 3243A>G were screened using PCR amplification.

Fisher’s exact test was used to compare outcomes of testing between adult and pediatric patients. The level of significance used was $P = .05$.

Results

DFNB1 was diagnosed in 23 of 163 (14%) pediatric patients with hearing loss compared with only 3 of 221 adults (1%; $P = .0001$). Among pediatric patients, the average age at the time of DFNB1 diagnosis was 9 years. Sixteen of 23 pediatric patients had reported onset of hearing loss before age 1 year, whereas the remainder had onset before age 5 years. Nineteen *GJB2* mutations were identified with 35delG accounting for 45 of 102 (44%) mutant alleles. The 35delG homozygous state was the most common pathogenic genotype

(54%). The genotypes of the 26 patients with DFNB1-related hearing loss are outlined in Table 1.

The ages of adults diagnosed with DFNB1 were 18, 34, and 49 years. Table 2 outlines the clinical characteristics of these patients. Two of 3 noted onset at birth, and each noted a family history of hearing loss. One patient noted adult-onset unilateral hearing loss, which was moderate in severity. Of the 26 patients with DFNB1, 18 had severe or profound hearing loss.

Among pediatric patients, 21 of 163 (13%) were found to harbor at least 1 *GJB2* pathogenic allele and are thus DFNB1 carriers. Only 8 of 221 (4%; $P = .008$) of adults were found to be heterozygote carriers. Forty-four of 163 (27%) of pediatric patients were either DFNB1 homozygotes or carriers compared with 11 of 211 (5%) adult patients ($P = .001$).

Mitochondrial DNA (mtDNA) mutations were found in 6 of 221 adult probands (3%). No mtDNA mutations were found in 163 pediatric patients ($P = .041$). Average age at diagnosis of mtDNA-related hearing loss was 55 years. Two patients were found to carry the homoplasmic mtDNA A1555G mutation, 2 had the mtDNA G7444A, and 2 harbored the common MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) mutation A3243G in the mtDNA.

The clinical characteristics of those patients with mtDNA mutations were variable. One patient with the A1555G mutation had no history of aminoglycoside exposure but had progressive hearing loss starting around age 40 years and subsequently underwent cochlear implantation at age 80 years. The other patient with A1555G presented at age 53 years with progressive moderate to severe SNHL but reported aminoglycoside exposure as a child, and hearing loss starting around age 3 years. The 2 patients with G7444A mutations presented with adult-onset moderate SNHL and have been treated with coenzyme Q10 without progression of hearing loss. The first patient noted to have the A3243G (MELAS) mutation presented at age 29 years with several years of fluctuating hearing loss. He also reported a recent stroke and new-onset diabetes and was diagnosed with MELAS syndrome around that time. His audiogram showed mild to moderate SNHL, and subsequent testing confirmed the A3243G mutation. No additional clinical data were available on the second patient with A3243G.

Discussion

Molecular genetic testing continues to play an increasing role in determining the etiology of sensorineural hearing loss. It can be a valuable supplement to history and physical, audiologic testing, and radiographic imaging. However, our results show that testing for some of the most common mutations is highest yield when applied in a more targeted fashion as compared with generalized screening of patients with hearing loss. Our finding that 14% of pediatric patients with hearing loss were DFNB1 positive is similar to previous reports.[7] Even though DFNB1 patients may show phenotypic variability, our data show that DFNB1 testing will have a very low yield in establishing a diagnosis of genetic hearing loss

in adult populations. Two-thirds of the DFNB1-positive adult patients in our study presumably had congenital hearing loss and could have been diagnosed at an earlier age.

The most common DFNB1 mutation in our cohort, 35delG, has a carrier frequency of 1.3% to 4% depending on the population studied.[8] However, our study showed a much higher rate of DFNB1 carriers (13%) among pediatric patients with hearing loss. This suggests an unrecognized mutation may be contributing to hearing loss in pediatric DFNB1 carriers. Our previous work has shown that 85% of individuals with 2 identifiable DFNB1 mutations (ie, homozygotes or compound heterozygotes) have severe to profound hearing loss compared with only 38% in DFNB1 carriers. The mean binaural pure-tone average level among DFNB1 patients was 90 dB HL compared with 62 dB HL among DFNB1 carriers.[9]

Finding that 3% of adults with hearing loss harbored mitochondrial DNA mutations shows that this is a significant cause of nonsyndromic SNHL in our study population. Two patients had the mtDNA A1555G mutation, which is associated with aminoglycoside-induced hearing loss.[10] The prevalence of mtDNA-related SNHL in our adult cohort is greater than the typically reported rate of less than 1%.[11] Early identification of patients with mtDNA-related SNHL will affect genetic counseling regarding maternal inheritance, allow avoidance of known risk factors, and promote pharmacological strategies to prevent or ameliorate the progression of the hearing loss.[12] Although A1555G and A7445G mtDNA mutations have been associated with non-syndromic SNHL in children,[11] they were not identified in any of our pediatric patients.

The flow diagram in Table 3 summarizes our current paradigm for evaluating patients with SNHL with the following recommendations for genetic screening of nonsyndromic hearing loss:

1. In neonates with congenital hearing loss and no obvious family history: *Cx26* mutation screening by gene sequencing and cytomegalovirus IgM titers
2. The patient has a family history and other first-degree hearing-impaired relative(s): *Cx26* mutation screening and gene-specific mutation screening if the pedigree shows autosomal dominant inheritance
3. The pedigree suggests mitochondrial DNA inheritance (maternal inheritance): testing for the A1555G mutation (associated with aminoglycoside ototoxicity) and the A7445G mutation, after excluding *Cx26* mutations
4. If nonsyndromic deafness is suspected and both parents are deaf, *Cx26*-related deafness is strongly suspected: because *Cx26* deafness is the most common in the United States, the vast majority of marriages between deaf individuals who produce deaf offspring are between individuals with *Cx26*-related deafness
5. In patients with progressive SNHL, imaging studies are recommended to identify inner ear malformations. If an inner ear malformation is found (incomplete partition, dilated vestibular aqueduct): screening for *SLC26A4* mutations for Pendred syndrome/DFNB4.

Our study supports the considerable value of genetic testing in establishing an etiology for childhood hearing loss. Given its high yield, DFNB1 mutation screening should be considered an early step in the diagnostic evaluation of pediatric hearing loss. However, its role in adults seems considerably more limited and is yet to be defined. This study suggests adults with childhood-onset hearing loss or a strong family history may benefit from DFNB1 testing, but this is based on small numbers of patients. Mitochondrial DNA testing may be considered in adults with progressive idiopathic SNHL, particularly if there is a history of maternal inheritance or hearing loss after exposure to aminoglycosides.

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Table 1

DFNB1 Genotypes

Allele Pairs	Number
35delG/35delG	14
35delG/Cx30del	3
W77R/Q124X	2
167delT/167delT	1
R143W/IVS1+1G>A	1
P58R/H100Y	1
35delG/W77R	1
W44X/V371	1
35delT/644delT	1
G4D/V371	1

Table 2

Adults with DFNB1

Parameter	Patient 1	Patient 2	Patient 3
Sex	F	F	F
Age, y	18	34	49
Estimated onset of HL	Birth	Birth	3 y prior
Mutation	35delG homozygous	35delG/GJB6 heterozygote	G4D/V371
Severity of HL	Severe to profound	Profound	Moderate to severe, progressive
Audiogram configuration	Gently sloping	Gently sloping	Unilateral, sloping
Pattern of inheritance	Autosomal recessive	Autosomal recessive	Autosomal dominant

Abbreviations: F, female; HL, hearing loss.

Table 3

Diagnostic Algorithm for Unknown Cause of Sensorineural Hearing Loss

History, Physical, and Audiological Evaluation	
Clinical Test Results	**Genetic Testing**
Bilateral SNHL by appropriate hearing tests	*GJB2, GJB6*, mitochondrial DNA
Other common deafness genes	
Bilateral enlarged vestibular aqueduct by CT scan	*SLC26A4*
Auditory neuropathy by OAE and ABR	*OTOF*
Mild to moderate SNHL at low frequencies, dominant	*WFS1*
Moderate to severe SNHL mid-frequency, recessive	*TECTA*
Progressive mild to severe HL with vestibular impairment, late onset	*COCH*
Characteristic Features of Other Less Common Nonsyndromic Deafness Genes	
Autosomal dominant	
Low frequency	*DIAPH1*
Auditory and peripheral neuropathy, erythrokeratodermia variabilis (no deafness)	*GJB3, KCNQ4*
Prelingual	*GJB2, GJB6*
Asymptomatic vestibular dysfunction	*MYO7A*
Middle frequency	*COL11A2*
Cochleosaccular degeneration	*MYH9*
Autosomal recessive	
Prelingual or postlingual onset, reduced or absent vestibular function, retinitis pigmentosa	*MYO7A*
Prelingual or postlingual onset	*TMPRSS3*
Prelingual or postlingual onset	*STRC*
X-linked inheritance	
Characteristic CT (dilated internal auditory canal with abnormal connection between subarachnoid space and cochlear endolymph)	*POU3F4*

Abbreviations: ABR, auditory brainstem response; CT, computed tomography; HL, hearing loss; OAE, otoacoustic emission; SNHL, sensorineural hearing loss.