Phenotypic Characterization of Hereditary Hearing Impairment Linked to DFNA25

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Objectives: To clinically characterize a family with non-syndromic sensorineural hearing loss linked to the DFNA25 gene and to assess whether mitochondrial mutations influence the penetrance of the phenotype.

Design: Longitudinal clinical and basic science molecular genetic study.

Setting: Academic medical center and molecular genetic research laboratory.

Participants: Members of a family with dominant high-frequency sensorineural hearing loss.

Interventions: Questionnaires, serial audiograms, and interviews correlated with molecular genetic data.

Main Outcome Measures: Symptoms, age at onset, serial audiometric data, and the presence or absence of 4 deafness-associated mitochondrial mutations.

Results: Affected individuals typically manifest a high-frequency, slowly progressive sensorineural hearing loss in the postlingual period. The mode of inheritance is autosomal dominant with age-dependent penetrance. Male affected members tended to report an earlier onset of hearing loss than female members. In those inheriting the DFNA25-associated haplotype from an affected mother, hearing loss invariably developed by the second decade of life, whereas those inheriting the DFNA25 haplotype from an affected father often maintained hearing levels comparable to those of age-matched control subjects, even into the seventh decade of life. None of 4 deafness-associated mitochondrial mutations screened (1555A>G, 7445A>G, Cins7472, and 7511T>C) were found to segregate in the family.

Conclusions: It is difficult to differentiate delayed-onset high-frequency sensorineural hearing loss inherited as a simple mendelian trait like DFNA25-associated hearing loss from that due to noise exposure or presbycusis, disorders that may also have a genetic component. An awareness of the clinical presentation of such hearing loss may help clinicians identify hearing loss attributable to genetic causes and improve care for these patients.

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To 1000 children are affected with significant hearing loss at birth or before they reach adulthood. Up until the 1970s, maternal rubella and other environmental factors such as ototoxic drugs caused a significant proportion of congenital hearing loss. With the introduction of universal immunization programs and improved perinatal health care, the proportion of congenital hearing impairment due to these factors has been greatly reduced. Today, at least 50% of congenital and early-onset deafness is genetic in origin. Genetic hearing loss can be further classified into (1) non-syndromic hearing loss, which is generally sensorineural and is not associated with any other clinical manifestations, and (2) syndromic loss, which can be sensorineural or conductive and is associated with a wide variety of other abnormalities not confined to the ear.

In the past 10 years, progress in the Human Genome Project and the availability of mouse models of deafness and cochlear-specific complementary DNA libraries have resulted in a huge expansion in our understanding of genetic deafness. Hearing loss is a component of more than 400 syndromes, and, so far, more than 30 genes associated with syndromic hearing loss have been identified. To date, more than 70 loci have been reported for non-syndromic deafness, including approximately 40 autosomal dominant, 30 autosomal recessive, and 8 X-linked, and approximately 30 genes have been cloned. Thus, many more genes associated with deafness await discovery.
In general, nonsyndromic hearing loss inherited as an autosomal recessive trait tends to be an early-onset or congenital profound sensorineural hearing loss, whereas autosomal dominant inheritance has a delayed onset and is progressive, ranging from mild to moderate or severe sensorineural loss.\(^5\) Mutations in GJB2, the gene that encodes the connexin 26 protein, are the most common cause of recessive deafness in the United States.\(^6,7\) and dominant low-frequency sensorineural hearing loss is most commonly caused by mutations in the Wolfram syndrome 1 gene.\(^8,9\) However, the genetic heterogeneity of nonsyndromic, high-frequency sensorineural hearing loss makes it difficult to identify the responsible gene or locus from the audiometric pattern alone.

In this report, we present the clinical findings in a large American family of Czech descent with autosomal dominant, nonsyndromic, high-frequency sensorineural hearing loss. Our group has previously mapped the DFNA25 gene segregating in this family by linkage analysis to chromosome 12q21-24.\(^10\) Herein we describe the age at onset, frequencies affected, penetrance, phenotype rate, and progression with age of the hearing loss in affected family members. In addition, because DFNA25-associated hearing loss appeared to be more severe and to occur earlier when inherited from the mother rather than the father, we investigated whether mitochondrial modifier genes might influence the phenotype.

**METHODS**

**DATA COLLECTION**

To date, 155 members of this family in 4 generations have been examined.\(^10\) This study was approved by the Institutional Review Board of the University of Michigan, Ann Arbor, and all participants gave informed consent. Family members completed questionnaires that assessed the age at onset of hearing loss, previous surgery or ear problems, noise exposure, and other risk factors for hearing loss. Clinical evaluation consisted of pure-tone audiometry, tympanometry, and otoacoustic emissions, when available. Previous audiograms were reviewed as available.

Audiometric data were obtained by means of field visits or standard testing in audiology offices in the subjects’ communities. During the field visits, pure-tone thresholds were obtained using a Beltone 120 audiometer with ER3-A earphones and EAR link foam eartips (Beltone Electronics Corp, Chicago, Ill). Tasco T250 earmuffs (Texas American Safety Company, Brownwood, Tex) were positioned during air conduction testing. When air conduction thresholds were poorer than 20 dB hearing level (HL), bone conduction thresholds were obtained at 2000 and 4000 Hz, and tympanometry was performed. Children as young as 27 months underwent evaluation using play audiometry. Ambient noise levels were measured throughout the test sessions with a sound-level meter with model 4153 microphone and model 1625 octave band filter (Bruel & Kjaer, Naerum, Denmark). Noise levels were below the proposed American National Standards Institute standards for noise levels, allowing measurement of 0-dB HL air conduction thresholds with insert earphones and earmuffs.\(^11\) We recorded air conduction thresholds for 500, 1000, 2000, 4000, and 6000 Hz. For some patients, threshold data for 125 and 8000 Hz were available.

The proband (III:14, Figure 1) underwent additional testing at the University of Michigan, including auditory brainstem response, transient evoked otoacoustic emissions, immittance testing, vestibular evaluation, computed tomography of the temporal bone, and karyotype analysis. The vestibular evaluation included electronystagmography, caloric testing, rotational chair testing, dynamic posturography, and the modified Clinical Test of Sensory Integration and Balance.

**ANALYSIS OF AUDIOMETRIC DATA**

Hearing thresholds obtained in subjects were compared with age- and sex-matched controls from an unselected population, including individuals with acquired hearing loss due to noise exposure or other causes.\(^12\) To differentiate hearing loss caused by DFNA25 from hearing loss caused by presbycusis, these standards were preferred to International Organization for Standardization 7029 standards, which were developed from otologically healthy populations.\(^13\) The reference ranges for frequencies from 500 to 6000 Hz were used and grouped by age in 5-year increments from 20 through 70 years of age. Children were divided into 3 groups based on age (6-9, 10-12, and 13-15 years) to facilitate comparison with published reference ranges.\(^14\) When the subject’s age fell between 2 age categories, eg, between 20 and 25 years, normative data for the higher age were used.

Individuals were classified according to the number of abnormal air conduction thresholds summed from both ears in the 500- to 6000-Hz frequency range. A hearing threshold was considered abnormal if it was greater than the 90th percentile of corresponding age-matched data\(^13\) for an adult, or greater than 1 SD above the mean for the corresponding age-matched data for children.\(^14\) For purposes of linkage analysis, individuals with at least 4 abnormal hearing thresholds were classified.

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**Figure 1.** Pedigree of the family with DFNA25 gene–associated hearing loss demonstrates selected family members. Solid symbols indicate affected family members; dots, haplotype carriers; clear symbols, spouses or unaffected individuals who lack the haplotype common to affected members; symbols with shaded lower half, phenocopies; squares, male members; and circles, female members.
as affected; 0 or 1 abnormal thresholds, within the reference range; and 2 or 3 abnormal thresholds, indeterminate.

The classification of the audiometric patterns of loss was adapted from those proposed by the European Work Group on Genetics of Hearing Impairment. High-frequency hearing loss was present when the average of the worst 2 thresholds at the higher frequencies was more than 15 dB greater than the average of the worst 2 thresholds at the lower frequencies. High-frequency losses that were 30 dB greater than lower-frequency thresholds were defined as steeply sloping, as opposed to gently sloping for high-frequency thresholds within 30 dB of the low-frequency thresholds. An audiometric profile was constructed from all available audiograms, including serial audiometric data as available, to determine whether there was any progression of the hearing loss with age.

AGE AT ONSET

Age at onset was determined by each person’s recollection of the onset of hearing loss, or if uncertain, by the earliest age at which the person wore hearing aids. If anamnestic data were not available for a patient who did not use hearing aids, the age at which a hearing loss was first documented was used.

GENETIC SCREENING FOR MITOCHONDRIAL MUTATIONS

To investigate whether a mitochondrial mutation might explain why the DFNA25-associated hearing loss appeared more severe when inherited from the mother, we screened family DNA samples for 4 mitochondrial mutations implicated in nonsyndromic hearing loss. Three of the mutations (7445A>G, Cins7472, and 7511T>C) are within the tRNAse (UCN) gene, whereas 1555A>G occurs within the 12SrRNA gene. Although Cins7472 has been reported only in families with sensorineural hearing loss, ataxia, and myoclonus, we included this mutation in our screen because the neurological manifestations may occur only rarely in some families with this mutation. Because siblings can be assumed to share the same mitochondrial DNA as their mothers, only selected DNA samples representative of the family were chosen for mitochondrial mutational screening. The DNA samples included those from individuals who inherited the DFNA25 haplotype by maternal or paternal transmission, unaffected individuals carrying the affected haplotype, and control individuals from the Centre d’Etude du Polymorphisme Humain pedigrees.

The DNA samples underwent screening for the mitochondrial mutations by means of restriction fragment length polymorphism assays. Assays were performed under conditions as previously described using HaeIII for 1555A>G, Xbal for 7445A>G, XcmI for Cins7472, and MbolI for 7511T>C. To avoid false-positive results due to incomplete digestion, when possible, restriction enzymes were chosen for which the mutation would create rather than abolish a novel cleavage site. Primers were chosen to amplify fragments that contained a second cleavage site as an internal control. The Cins7472 mutation did not create or abolish a restriction site for a rare-cutting enzyme, and there was no cleavage site in the wild-type sequence that could be used as an internal control. Therefore, the XcmI restriction site was created by a substitution of 1 of the base pairs of the sense primer before amplification of the fragment as previously described. In addition, a second mitochondrial DNA fragment was amplified containing an XcmI site as an external control and assayed in parallel.

The mitochondrial DNA fragment flanking each mutation site was amplified by polymerase chain reaction and incubated with the relevant restriction enzyme. The products of the restriction digest for each mutation were resolved by electrophoresis on 1.5% agarose gels and visualized with ethidium bromide.

RESULTS

Twenty-four family members had high-frequency sensorineural hearing loss that met the criteria for affected status and unambiguously shared the haplotype common to other affected family members (Figure 1). Fifteen members had hearing losses that were steeply sloping (III:2, III:4, III:11, III:14, III:15, III:17, III:20, IV:1, IV:2, IV:7, IV:9, IV:13, IV:17, V:1, and V:2), and 8 had losses that were gently sloping (II:9, III:1, III:9, III:12, IV:3, IV:12, IV:15, and V:3). Representative audiograms of 2 affected individuals are shown in Figure 2A. Individual IV:5 had an atypical flat pattern of sensorineural loss. All family members who had inherited at least 50% of the disease haplotype from their mothers had hearing loss that met criteria for affected status.

Three family members (III:5, III:8, and III:19) had significant sensorineural hearing loss but did not carry the haplotype common to the affected members (Figure 2B). Because their hearing loss is most likely due to causes other than a mutation in the DFNA25 gene, they are defined as phenocopies. All 3 phenocopies were male and had a significant history of occupational noise exposure. Although all 3 met criteria for affected status, their low-frequency thresholds tended to be better than those of affected family members of similar age. None of the 3 phenocopies had affected children.

Six individuals who did not meet criteria for affected status shared at least part of the haplotype common to the affected members. Two of these haplotype carriers shared less than 50% (III:6 and IV:14), whereas 3 (III:7, IV:4, IV:11, and IV:18) shared 60% or more of the common haplotype (data not shown). Individuals III:6 and III:7 were older than 60 years when undergoing testing (audiograms shown in Figure 2C), whereas the other carriers ranged in age from 28 to 45 years when undergoing testing. All 6 carriers had inherited the haplotype common to the affected members from their affected fathers, in contrast to the complete penetrance observed in family members who inherited the disease haplotype from their mothers.

AGE AT ONSET AND PROGRESSION OF HEARING LOSS

Determining the age at onset of hearing loss in this family was difficult because of the lack of audiologic data from early childhood and the length of time between suspicion and documentation of hearing loss. Despite the family history of dominant hearing loss, many family members had their first hearing test as part of this study, often because they lacked insurance coverage for hearing tests. Only 1 individual, V:2, had hearing loss suspected at birth, which was later confirmed by results of diagnostic auditory brainstem response at 3 months of age. Male members tended to report an earlier onset of hearing loss than female members, with more than half recalling hearing loss that led to academic difficulties in early childhood (Figure 3). In most cases, the
Hearing loss was diagnosed in the postlingual period. One individual recalled learning difficulties in school, but his hearing loss was not diagnosed until adulthood. No family members had undergone any ear surgery other than tympanostomy tubes.

In general, the hearing loss was slowly progressive over decades (Figure 4). Consistent with the data regarding age at onset, male members appeared to be more severely affected at younger ages (Figure 4A). The curve for male members older than 60 years reflects better thresholds than for those aged 40 to 59 years, largely owing to more steeply sloping losses in the oldest group. In the aggregate, the degree of hearing loss for female members appeared quite similar before 60 years of age (Figure 4B). The curve for female members older than 60 years is based on only 1 person, who is one of the more severely affected family members. Many members reported noise exposure from factory machinery, farming, and/or recreational activities.

**OTHER TESTING**

Consistent with the degree of hearing loss, no repeatable auditory brainstem response waveforms or transient evoked otoacoustic emissions were obtained from the proband. No ipsilateral or contralateral bilateral stapedial reflexes were obtained. No temporal bone abnormalities were evident on computed tomographic scan findings. Vestibular testing revealed no objective indications of peripheral or central vestibular system involvement. Results of the modified Clinical Test of Sensory Integration and Balance indicated normal ability to maintain upright stance under a
variety of changing sensory input conditions. Results of karyotype analysis were without abnormalities.

SCREENING FOR DEAFNESS-ASSOCIATED MITOCHONDRIAL MUTATIONS

For all family members and controls undergoing testing, results were negative for the 7445A>G, Cins7472, 7511T>C, and 1555A>G deafness-associated mitochondrial mutations.

Although newborn hearing screening programs seek to improve early detection of congenital hearing impairment, it is equally important to identify infants and children at risk for delayed-onset hereditary hearing loss. Autosomal dominant hearing loss typically has a variable age at onset and variable severity among family members, and penetrance is often incomplete and age dependent.3

In the family with DFNA25-associated hearing loss, only 1 of 24 affected members received a diagnosis of hearing loss at birth. More than half of the family members had noticed their loss or had it diagnosed by the study before 30 years of age, whereas the age at onset of the remainder ranged from 31 to 60 years. As family members become educated about hereditary hearing loss in their family, they can better advocate for their children to undergo diagnostic testing at birth and serial audiologic monitoring. Such longitudinal data will yield better estimates of the true age at onset and lead to a better understanding of the natural history of hearing loss in their family.

High-frequency hearing loss associated with aging likely results from genetic24 and environmental factors. Although the heterogeneity of high-frequency hearing loss makes it difficult to distinguish hearing loss attributable to DFNA25 from that caused by other genes, the phenotypic overlap with presbycusis suggests that DFNA25 may contribute to presbycusis in the general population. In this large family, it was possible to map the gene, despite the presence of 3 phenocopies, who usually cannot be identified a priori.

We found an apparent increase in severity of the phenotype when the deafness was inherited from an affected mother instead of an affected father, with all haplotype carriers inheriting the haplotype from an affected father. This variability in expression could be explained by modifier genes, genomic imprinting, or chance alone. Modifier genes are wild-type or disease alleles that alter the clinical severity of the disease caused by pathogenic mutations at another locus.25 One such modifier gene is DFNM1, which rescues the recessive deafness phenotype caused by DFNB26.26 Analysis of our data from a previous genome scan revealed no common haplotype at the DFNM1 locus that would explain why some who inherited the DFNA25 haplotype remained unaffected (A.S.T. and M.M.L., unpublished data, March 2002). None of the 4 deafness-associated mitochondrial mutations screened in this study were found in the family. However, other mitochondrial mutations cannot be ruled out, as the entire mitochondrial genome was not sequenced. The variability in expression of the DFNA25 phenotype could result from other deafness modifier genes as yet unknown.

Genomic imprinting has been demonstrated for certain chromosomal regions such as 15q11-q13, in which paternally and maternally inherited alleles cause different phenotypes, eg Prader-Willi syndrome and Angelman syndrome, even when the alleles are identical at the DNA sequence level.25 At present, no evidence exists to implicate chromosome 12q21-24 as a region of genomic imprinting, but it remains an intriguing hypothesis.

A recent report of bilateral severe profound sensorineural hearing loss and other syndromic features caused by a 12q22-24.1 deletion in a child suggested that haploinsufficiency for DFNA25 is responsible for the hearing loss.27 Although the deletion is contained within the DFNA25 candidate region, this child’s deafness may also result from the deletion of contiguous genes.

Although recent years have seen remarkable advances in the molecular genetics of hearing loss, trans-
lating this research into clinical practice has occurred more slowly. The key to recognizing genetic hearing loss is the family history. Many patients may be unaware of their family history or may attribute the hearing loss within the family to coincidence. Autosomal dominant hearing loss may affect any frequency and will not always manifest as a midfrequency “cookie-bite” sensorineural hearing loss. After identification of the DFNA25 gene, it will be possible to examine its role in presbycusis and/or susceptibility to noise in the general population.

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