Autosomal Dominant Inherited Hearing Impairment Caused by a Missense Mutation in COL11A2 (DFNA13)

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**Objective:** To analyze the phenotype in a 5-generation DFNA13 family with a missense mutation in the COL11A2 gene that causes autosomal dominant, presumably prelingual, nonsyndromic sensorineural hearing impairment.

**Design:** Family study.

**Setting:** University hospital department.

**Patients:** Twenty mutation carriers from a large American kindred.

**Methods:** Cross-sectional analysis using pure-tone threshold measurements at 0.25, 0.5, 1, 2, 4, and 8 kHz. The audiometric configuration was evaluated according to an existing consensus protocol. The significance of features relating to audiometric configuration was tested using 1-way analysis of variance. Progression was evaluated with linear regression analyses of threshold-on-age.

**Results:** Most individuals showed midfrequency (U-shaped) characteristics. The mean threshold in generations IV and V was 44 dB at 1, 2, and 4 kHz (midfrequencies); it was 29 dB at the other frequencies (0.25, 0.5, and 8 kHz). There was no significant progression beyond presbyacusis.

**Conclusion:** The trait in this family can be characterized as autosomal dominant, nonprogressive, presumably prelingual, midfrequency sensorineural hearing impairment.


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**GENETIC** linkage techniques have facilitated the identification of genes essential for normal auditory function. The initial step in this process is the localization of these genes using classic linkage techniques; since 1992, 38 loci for autosomal dominant nonsyndromic sensorineural hearing loss have been mapped, and 11 of the relevant genes have been cloned. The different gene loci for the nonsyndromic forms of hearing impairment have been called DFNA (Deafness) and are numbered in chronological order of discovery. Autosomal dominant loci are referred to as DFNA, autosomal recessive as DFNB, and X-linked as DFNX. An update of these genetic data can be obtained by consulting the Hereditary Hearing Loss Homepage.

The identification of these genes, in turn, has prompted studies to determine whether phenotypic-genotypic correlations exist. It is well known that different mutations in the same gene can produce a broad spectrum of phenotypes. For example, mutations in the myosin VIIA gene (MYO7A) cause Usher syndrome type 1B, DFNB2, and DFNA11, and mutations in the Pendred syndrome gene (PDS) cause Pendred syndrome and DFNB4. Mutations in the COL11A2 gene also cause syndromic and nonsyndromic hearing loss.

Two DFNA13 kindreds, one American and the other Dutch, recently were shown to have missense mutations in the COL11A2 gene. The COL11A2 gene encodes the α(XI) chain of type XI collagen. Type XI collagen is a minor fibrillar component of cartilage collagen. Mice with a targeted disruption of the col11a2 gene have hearing loss and, by electron microscopy, loss of organization of the collagen fibrils in the tectorial membrane.

A mutation in the COL11A2 gene was also identified as the cause of hearing impairment in persons with Stickler syndrome type 2 (STL2). This autosomal dominant syndrome is characterized by hearing impairment, midface hypoplasia, and arthropathy, but in contrast to the clas-
PATIENTS AND METHODS

An American family spanning 5 generations and comprising 67 members alive (Figure 1)\textsuperscript{10} showed autosomal dominant sensorineural hearing impairment, presumably affecting 38 persons (13 by history). Forty persons underwent general and otorhinolaryngological examinations, including audiometry. Individual V-13 was excluded from the study because the reliability of the hearing test was questioned. Genetic linkage analysis was performed and the hearing loss segregated with markers on chromosome 6p21-p22; this locus was designated DFNA13.\textsuperscript{3} Recently, a missense mutation in the COL11A2 gene was reported in this family.\textsuperscript{10} Two phenocopies (individuals III-8 and III-17) were identified. They had hearing loss confirmed by audiograms, but lacked the gene mutation; their audiograms could not be distinguished from those in the mutation carriers.

The present analysis of the hearing phenotype included only those persons carrying the COL11A2 gene missense mutation from whom at least one audiogram had been obtained (n=20). Corrections were made for presbyacusis sense mutation from whom at least one audiogram had been obtained (n=20). Corrections were made for presbyacusis by subtracting the ISO 7029 median norm (50th percentile [P50]) threshold for presbyacusis\textsuperscript{17} from each person's threshold, according to that person's age and sex. For persons older than 70 years, the P50 values for age 70 years (the maximum age for which normative data are available) were used. Audiograms (air conduction threshold, in decibels, hearing level) were recorded in a sound-shielded room following common clinical standards.

Audiometric configuration was classified according to the criteria formulated by the European Work Group on Genetics of Hearing Impairment.\textsuperscript{18} Midfrequency hearing impairment (a U-shaped audiogram) is defined as follows: a 15-dB difference or greater between the poorest thresholds in the midfrequencies (>0.5 to 2 kHz) and those at higher and lower frequencies. The low-frequency ascending pattern is defined as a difference of 15 dB or greater between the thresholds at the poorer low frequencies and the higher frequencies. A flat audiogram is defined as a difference of less than 15 dB from 0.25 to 8 kHz. High-frequency hearing impairment is subdivided into gently and steeply sloping configurations. The former type is defined as a 15- to 29-dB difference between the mean of 0.5 and 1 kHz and the mean of 4 and 8 kHz, while the latter implies a 30-dB difference or greater between the described frequencies.

One-way analysis of variance and regression analyses were performed on cross-sectional threshold-on-age data (last-visit audiogram) using a commercial program (Prism, PC version 2.01; GraphPad, San Diego, Calif). To evaluate whether significant progression (slope >0) occurred, linear regression analysis was performed using only the raw threshold-on-age data in generations IV and V (age range, 11-48 years); the affected persons in generation III (age range, 67-75 years) were substantially older. By excluding generation III, the potentially confounding effects of presbyacusis were avoided. All threshold-on-age data were used in an attempt to fit parabolic curves, similar to the fits on which the ISO 7029 norms\textsuperscript{17} are based: $Y = \text{offset} + [a(X-18)]^2$, where $Y$ is the binaural mean air conduction threshold (measured in decibels), $X$ is age (in years), and $a$ is acceleration of hearing deterioration (measured as decibels times years negatively squared). Chauvenet criterion was used to detect any outlying values, ie, data points pertaining to excessively large regression residues.\textsuperscript{17} We also determined whether each ISO 7029 value for $a$\textsuperscript{17} was within the calculated 95% confidence interval for $a$ (distribution).

To establish whether phenotypic-genotypic correlations exist, it is imperative to describe phenotypes, based on their genotypes, as thoroughly as possible. We present herein a detailed analysis of the clinical data of the American DFNA13 family, and relate these phenotype findings to the other COL11A2-linked disorders.

All 20 persons examined and known to carry the COL11A2 gene mutation showed sensorineural hearing impairment (Figure 2A-T). The individuals are given in ascending order of age at the last visit. Classification of audiometric configuration was based on the original data (ie, without presbyacusis correction). Of the 40 ears, 17 showed a U-shaped audiogram (Figure 2A, B, F, G, J, and M [right and left ears] and D, H, I, N, and O [left ear only]).
Other audiometric types included a flat configuration in 6 ears (Figure 2L, R, and T [right and left ears]), gently downsloping in 3 ears (Figure 2C, H, and K [right ear]), and steeply downsloping in 4 ears (Figure 2Q and S [right and left ears]). The latter persons had a history of significant noise exposure. Low-frequency ascending curves were found in 3 ears (Figure 2E [right and left ears] and N [right ear only]), and in 7 ears (Figure 2C and K [left ear only], D, I, and O [right ear only], and P [right and left ears]) the audiometric configuration could not be classified. The ears in Figure 2O (right ear only) and P (right and left ears) showed a threshold curve that gently sloped from the low frequencies to a dip at 4 kHz but ascended at 8 kHz. In the last 3 individuals, there was no history of noise exposure. In 4 ears (Figure 2C and K [left ear] and D and I [right ear]), although the configuration was gently downsloping, the degree of slope did not meet the classification criterion. In some persons (ie, the oldest patients), presbyacusis correction changed the audiometric configuration. In Figure 2Q-S, the audiogram could no longer be classified. In Figure 2R, the audiometric curve was similar to those seen in Figure 2O (right ear) and P (right and left ears), but with a dip at 2 kHz. After correction for presbyacusis, Figure 2T fits the description of a low-frequency ascending curve.

While the audiometric configuration was variable, the predominant type was identified as U-shaped (42.5% [17 of 40 ears]), even including data corrected for presbyacusis. The "mean audiogram" also showed a U-shaped configuration (Figure 3); the thresholds at 0.25 to 4 kHz dif-
The DFNA13 locus originally was mapped to chromosome 6p using a portion of this American family. Expansion of the pedigree permitted locus refinement and eventually the demonstration of a missense mutation in the \textit{COL11A2} gene that segregated with the hearing loss phenotype. The mutation, a C-to-T transition in exon 42, results in an arginine-to-cysteine substitution. The hearing loss may be caused by altered type II collagen spacing in the tectorial membrane, as suggested by histopathological and electron microscopic findings in mice with a \textit{col11a2} gene mutation.

The phenotypic characteristics of the family have been described only briefly, and the results of this study complement that description in detail. The sensorineural hearing impairment segregating in this family is autosomal dominant, presumably prelingual, and nonprogressive, and it affects the midfrequency range. Our presumption that it is prelingual is based on its lack of progression and our finding of a mean threshold in the younger generations that differs significantly from 0.
This pattern of hearing loss is similar to the hearing loss in the only other DFNA13 family (a Dutch kindred) that is known to segregate for a COL11A2 gene mutation. Affected persons in the Dutch family carry a G-to-A transition in exon 31 of the COL11A2 gene that results in a glycine-to-glutamate amino acid substitution. Their sensorineural hearing loss is presumably prelingual and clearly nonprogressive. Like their affected American counterparts, affected Dutch persons present with midfrequency loss. Age-corrected thresholds, however, are about 10 dB better at 0.25 to 4 kHz and about 25 dB worse at 8 kHz. This additional high-frequency hearing impairment in the Dutch family persists after correction for presbyacusis.

Syndromic COL11A2-associated hearing loss is somewhat different. As reported by Admiraal et al. in their study of a Dutch family with STL2 carrying a G-to-A transition that causes in-frame skipping of a 54-base pair exon encoding 18 amino acid residues within the triple helical and C-propeptide domains of the α(1)I(X) collagen molecule, the mean sensorineural threshold was 40 dB (n = 14). There was no substantial progression, and the audiometric configuration, as classified by criteria used in this study, showed substantial variability. Most cases were downsloping, and in addition to sensorineural hearing impairment, almost half of affected persons showed a conductive loss, perhaps attributable to the associated features of STL2. A fairly similar type of hearing loss was reported in a second family with STL2 in whom an in-frame deletion removes 3 repeats of 2 unspecified amino acids and glycine in the midportion of the α(1)I major triple helical domain.

Progression in hearing impairment in the American family is similar to the ISO 7029 standard curves for presbyacusis at 1 to 2 kHz (Figure 4C-D). At lower frequencies (0.25-0.5 kHz), there is more progression than predicted by the ISO 7029 (PS0) norm. At higher frequencies (4-8 kHz), the parabolic curve for the combined group of male and female patients is fairly similar to the standard presbyacusis norm for women, although there is a predominance of men in our series. These findings suggest that presbyacusis in the American DFNA13 family is less severe than normal at the higher frequencies. The possibility that presbyacusis is milder in this family because of other, as yet unidentified, genetic factors cannot be excluded. It is intriguing, however, that fairly similar observations have been reported in 2 other families with midfrequency hearing impairment at the DFNA8 or DFNA12 locus. These families, one Austrian and the other Belgian, show less progression of hearing loss with age than the normal population. Their deafness is presumably caused by mutations in the α(1)(X) chain of type XI collagen. J Pediatr. 1998;132:368-371.

Like the COL11A2 gene, the α-tectorin gene produces an important component of the tectorial membrane. Perhaps, by changing the mechanical properties of the tectorial membrane, it may be possible to modify the “wear and tear” effects of age on auditory function.

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