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.


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) showed autosomal dominant sensorineural hearing impairment, presumably affecting 38 persons (15 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. Recently, a missense mutation in the COL11A2 gene was reported in this family. 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 includes only those persons carrying the COL11A2 gene mis-sense mutation from whom at least one audiogram had been obtained (n=20). Corrections were made for presbyacusis, but lacked the gene mutation; their audiograms could not be distinguished from those in the mutation carriers.

The present analysis of the hearing phenotype includes 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 by subtracting the ISO 7029 median norm (50th percentile [P50]) threshold for presbyacusis 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. Midfrequency hearing impairment (a U-shaped audiogram) is defined as: 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 are based: Y = 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. We also determined whether each ISO 7029 value for a^2 was within the calculated 95% confidence interval for a (t distribution).

Figure 1. Pedigree of the American DFNA13 family. Roman numerals indicate different generations; squares, men; circles, women; solid symbols, hearing impairment; +, gene carriers; and a, audiogram available. All individuals in generations I and II (except II-2) and person III-13 are deceased.

RESULTS

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 audimetric 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-

fered significantly from the one at 0 kHz. The mean thresholds at 0.25, 0.5, and 8 kHz appeared fairly similar (mean, about 29 dB) and so did the mean thresholds at the frequencies from 1 to 4 kHz (mean, about 44 dB). Thus, the difference between these grouped frequencies (15 dB) complied with the definition of a U-shaped configuration. These (raw) data (Figure 3) cover only generations IV and V to avoid any major influence of presbyacusis.

Analysis of variance disclosed significant intragenerational and thus age-related differences in threshold (raw data) for a given frequency (data not shown). Linear regression analysis was performed on the raw threshold-on-age data in the combined generations IV and V. Only the 0.5-kHz frequency showed mild, but significant, progression (0.3 dB/y), which vanished following presbyacusis correction (data not shown).

The age-corrected threshold shows ascending characteristics in generation III (Figure 2Q-T), which would have been even stronger if we had been able to use appropriate P50 values for the persons older than 70 years (Figure 2R-T). We attempted to circumvent this limitation by fitting ISO 7029 presbyacusis parabolas to the threshold data, as described in the “Patients and Methods” section (Figure 4A-F). The fitted offset values were included in the parabolic fit according to the usual median (P50) presbyacusis norms. The curves thus obtained were used to compare the apparent age-related progression with the expected progression in presbyacusis.

The threshold data (Figure 4) behave similar to presbyacusis in the midfrequency range (1-2 kHz). At 0.25 kHz, the ISO 7029 acceleration coefficient \( a \) (0.0030 dB·y\(^{-2}\)) is below the lower limit of the 95% confidence interval of the fitted value (0.0093 dB·y\(^{-2}\); 95% confidence limits, 0.0056-0.0130 dB·y\(^{-2}\)). The same applies to the values at 0.5 kHz (ISO 7029 value, 0.0035 dB·y\(^{-2}\); fitted value, 0.0075 dB·y\(^{-2}\); 95% confidence limits, 0.0046-0.0100 dB·y\(^{-2}\)). At an age relating to generation III, the difference is maximal (Figure 4A-B). The thresholds at 4 and 8 kHz show accelerations that are fairly close to those in normal women (Figure 4E-F, generation III), although there were more men (n=14) than women (n=6) in our series.

### COMMENT

The DFNA13 locus originally was mapped to chromosome 6p using a portion of this American family.\(^9\) Expansion of the pedigree permitted locus refinement and eventually the demonstration of a missense mutation in the **COL11A2** gene that segregated with the hearing loss phenotype.\(^10\) 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 **col11a2** gene mutation.\(^10\)

The phenotypic characteristics of the family have been described only briefly,\(^10\) 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.

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**Figure 3.** The “mean audiogram”±1 SD based on the raw thresholds of the analyzed cases in generations IV and V.

**Figure 4.** Analysis of progression in threshold (circles indicate the raw binaural mean threshold for air conduction) with advancing age. A, 0.25 kHz; B, 0.5 kHz; C, 1 kHz; D, 2 kHz; E, 4 kHz; F, 8 kHz. The continuous curve is the fitted parabola for the present patients (men and women), and the dashed (women) and dotted (men) curves are the parabolas for ISO 7029 norms given the same offset as the patients. The asterisk in A and B represents outlying values (one case excluded from the analyses).
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 α2(XI) 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 α2(XI) 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 (P50) 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.

Accepted for publication August 11, 2000.

This study was supported in part by grant RO1-DC03544 from the National Institutes of Health, Bethesda, Md (Dr Smith); by the Heinsius Houbolt Foundation, Wassenaar, the Netherlands (Dr Cremers); and by the Nijmegen KNO-Research Foundation, Nijmegen, the Netherlands (Dr Cremers).

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