A Dutch Family With Hearing Loss Linked to the DFNA20/26 Locus

Longitudinal Analysis of Hearing Impairment

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Objectives: To perform linkage analysis and to outline hearing loss characteristics in a family exhibiting a nonsyndromic, autosomal dominant type of progressive sensorineural hearing loss.

Design: Genetic analysis was performed using microsatellite markers. Audiometric data were collected and analyzed longitudinally. Sigmoidal dose-response curves enabled us to perform nonlinear regression analysis per frequency and on phoneme recognition scores. Speech recognition scores were compared with those of DFNA2, DFNA5, DFNA9, and presbyacusis subjects.

Subjects: Affected family members of a Dutch family (W99-060).

Results: We revealed linkage of hearing loss to the DFNA20/26 locus (maximum logarithm of odds score, 3.1 at $\theta=0.04$) and reduced the critical region from 12 to 9.5 centimorgans. Patients younger than 15 years already showed gently downsloping audiograms. At ages 15 to 20 and 25 to 40 years, hearing loss was profound at 8 kHz and 1 to 4 kHz, respectively. The 0.25- to 0.5-kHz thresholds showed more gradual progression by about 1.5 to 2 dB/y. From about age 40 years onward, hearing was residual. Hearing impairment took a more severe course than in a known DFNA20 family. Score recognition in DFNA20/26 subjects was better than in DFNA9 subjects at any pure-tone average (1-4 kHz) threshold. Compared with subjects having DFNA2 and DFNA5, speech recognition in those with DFNA20/26 scored better at threshold levels below 85 dB hearing level, but worse at levels above 90 dB. Compared with presbyacusis subjects, those with DFNA20/26 scored better in speech recognition at levels below 100 dB and worse at levels above 100 dB.

Conclusions: Autosomal dominant hearing loss is linked to the DFNA20/26 locus in this Dutch family. The critical region is reduced from 12 to 9.5 centimorgans. Phenotypically, patients are more severely affected than those of a known DFNA20 family.

Arch Otolaryngol Head Neck Surg. 2004;130:281-288

Received for publication Jan 27, 2004. Rejected for publication Jan 28, 2004. Accepted for publication Feb 17, 2004.

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In 1999, we began our investigations of hearing loss in a large Dutch family (W99-060) spanning 6 generations (Figure 1) with the approval of the institutional review board. Fourteen family members had a history of progressive hearing impairment that first manifested in adolescence. After having obtained written and informed consent, we obtained pure-tone and speech audiograms from 22 individuals using standard procedures and, in some cases, a portable audiometer. Previously obtained audiograms were retrieved for 13 individuals. Blood samples were collected from 11 presumably affected and 22 unaffected persons for linkage analysis. Special attention was paid to the presence of syndromic features possibly accompanying hearing loss.

**METHODS**

Genomic DNA was extracted from peripheral blood lymphocytes according to established procedures. Analysis of polymorphic markers involved amplification by polymerase chain reaction. Each reaction contained 100 ng of genomic DNA and 30 ng of each primer in 15 µL of Supertaq buffer (50mM potassium chloride, 1.5mM magnesium chloride, 10mM Tris-hydrochloride [pH 9.0], 0.1% Triton X-100, and 0.01% [wt/vol] gelatin) in the presence of phosphorus 32–dCTP with 0.06 U of Supertaq (HT Biotechnology Ltd, Cambridge, England). Amplification was achieved by 35 cycles of 1 minute at 94°C, 2 minutes at 55°C, and 3 minutes at 72°C with microsatellite markers. The radiolabeled polymerase chain reaction products were mixed with 15 µL of sample buffer (95% formamide, 20mM EDTA, and 0.05% bromphenol blue) and heated to 95°C for 3 minutes; 4 µL of this mixture was separated on a 6.6% denaturing polyacrylamide gel. Two-point logarithm of odds (lod) scores were calculated using the MLINK subroutine.
tine of the computer program LINKAGE (version 5.1) on the basis of autosomal dominant inheritance. For the calculation, the relative prevalence of the disease allele was assumed to be 0.0001; penetrance, 95%. A relative prevalence of 0.001 was assumed for phenocopies. The cutoff age for unaffected family members was 20 years.

AUDIOMETRIC ANALYSIS

Audiometric configuration and threshold asymmetry were evaluated according to the criteria and classification established by the European Work Group on Genetics of Hearing Impairment. Serial audiometry was available for 8 patients and suitable for longitudinal analysis for 5 of them (VI:4, IV:19, V:6, V:15, and V:21). Nonlinear longitudinal regression analysis (air-conduction threshold on age) was performed using a commercial program (Prism 3.02; GraphPad, San Diego, Calif). The bone conduction threshold was measured to exclude conductive hearing loss. One-way analysis of variance was used to detect significant differences between any groups or subgroups of patients. Pooling was only performed where it was permitted according to the results of such tests. All these data enabled us to construct age-related typical audiograms (ARTA).

Speech-recognition scores were measured using (phonetically balanced) standard consonant-vocal-consonant (CVC) syllables (Dutch CVC word lists). The phoneme score was analyzed in relation to age and pure-tone average (PTA) of the thresholds at 1, 2, and 4 kHz (PTA1-4kHz). Nonlinear regression analysis was performed using a sigmoid response curve with variable slope. Details can be found in a previous report.

Analysis of variance and the t test (with the Welch correction if the Bartlett test demonstrated significantly unequal variances) were used to compare the results with those previously obtained in (1) a group of patients with presbyacusis; (2) a group of DFNA9 subjects; and (3) a group of DFNA5 subjects.

VESTIBULOOCULAR EXAMINATION AND IMAGING TECHNIQUES

Seven family members (IV:7, IV:11, IV:22, V:5, V:6, V:15, and V:4) underwent vestibular and ocular motor tests. These included evaluation of vestibuloocular responses using electro-nystagmography with computer analysis and saccadic, smooth pursuit, and optokinetic nystagmus responses. Vestibular stimulation comprised rotatory and caloric tests. Details and normal values have been previously described. A computed tomographic scan of the petrosal bones of subject IV:22 was performed (Somatom Plus 4; Siemens, Forchheim, Germany).

RESULTS

The hearing loss trait in the family (Figure 1) exhibits an autosomal dominant pattern of inheritance. The case histories and physical examinations excluded syndromic involvement. Most of the patients dated their first symptoms of hearing loss to the first 2 decades of life. Given the normal speech and language development and the substantial progression of hearing loss, especially in the second decade, the hearing loss is expected to be mainly postlingual in origin.

LINKAGE ANALYSIS RESULTS

Because of the apparent similarity of the present type of hearing loss to that associated with DFNA5, this locus was tested first with polymorphic markers (eg, D7S673, D7S2444, and D7S2493). The locus was excluded by lod scores lower than −2 (data not shown). Subsequently, a genome scan was initiated, and after exclusion of about one third of the genome, linkage was detected with marker D17S928 (17q25) at a maximum 2-point lod score of 3.1 at θ=0.04. This marker flanks the DFNA20 interval on the telomeric side. Additional markers derived from this region were tested, and 2-point lod scores were calculated (Table).

The most likely haplotypes were constructed to determine the borders of the critical region (Figure 1). This revealed that individual IV:2 had only the allele for marker D17S668 as seen with the affected haplotype. A genotyping error was excluded by analyzing DNA from 2 independent samples. Since both parents had died, we were unable to determine whether allele 2 was derived from the affected mother. Therefore, we decided to determine the critical region primarily on the basis of the remaining part of the pedigree.

On the centromeric side, the critical region is flanked by D17S784 as can be deduced from a recombination event seen in the affected individual V:5. Individual V:10 (33 years old at examination) also displays a recombination event suggesting that marker D17S784 is the proximal flanking marker. However, for this individual, non-penetrance cannot be excluded. The given location of the marker D17S1830 relative to D17S784 is based on physi-
Regarding the allele of marker D17S668 in individual IV:2 being derived from the haplotype that carries the mutation, the critical region based on the given marker order would be delimited to the interval between D17S761 and D17S928. However, because of the extent of genetic heterogeneity and environmental causes of hearing loss, the results for individual IV:2 must be regarded with caution. Unfortunately, patient IV:1 refused to participate in this study. Assuming that 1 gene is involved in the hearing loss in the 4 families known to be linked to 17q25,3,10,23 our data reduce the critical region from 12 centimorgans (cM),19 between D17S1806 and D17S668, to 9.5 cM between D17S764 and D17S668. In the recently published high-resolution recombination map of Decode,13 this distance measures only 6.1 cM.

**Figure 2.** Representation of the critical regions of family W99-060 of the present study, a known DFNA20 family,13 and the USH1G locus.20 Order and distances in centimorgans of the underlined markers are according to the Marshfield genetic map (available at: http://research.marshfieldclinic.org/genetics) (left) and/or the Decode genetic map (right).10 Additional markers are located according to their positions in the Human Genome Working Draft (April 2003 freeze; available at: http://genome.ucsc.edu) and the Celera database.

**Figure 2**

The available audiograms of 10 affected cases are shown in Figure 3. Before they were 15 years old, the patients had already shown gently downsloping audiograms. By ages 15 to 20 and 25 to 40 years, hearing loss had become severe to profound at 8 kHz and 1 to 4 kHz, respectively. The thresholds at 0.25 to 0.5 kHz showed gradual progression at an average increase of about 1.5 to 2 dB/yr. There was residual hearing (ie, mainly at the lower frequencies) from about age 40 years onward. **Figure 4** shows the age-related typical audiograms of the present family and, for the sake of comparison, of a known DFNA20 family.13

The plots in Figure 5 combine the longitudinal analyses in the suitable cases with the (cross-sectional) data in the other cases. Cross-sectional data reported by Elfenbein et al13 are included for the purpose of comparison. The bottom panel shows the phoneme recognition score in relation to age. The threshold data showed much higher progression at the high frequencies than at the lower frequencies. The maximum rate of progression culminated within the age range of 10 to 35 years and varied in individual cases between about 3 and 8 dB/yr. Onset ages were in the range of 5 to 25 years, showing an apparent decrease at increasing frequencies in some cases. At age 15 to 35 years, 50% of the final degree of deterioration had developed, and by age 30 to 50 years, 90% of the final degree of deterioration had developed.

Appreciable deterioration of speech recognition (score <90%) began between ages 15 and 40 years and showed large intersubject variations (Figure 5, bottom panel). At age 20 to 45 years, recognition scores deteriorated maximally (range, 5%-20% per year). With few exceptions, speech recognition became problematic (maximum phoneme score <50%) from about age 25 to 45 years and onward. Between ages 30 and 60 years, speech recognition was almost completely lost except in 1 patient.

In relation to the corresponding threshold level (ie, PTA1-4 kHz), the phoneme recognition score was relatively good compared with that previously obtained at our clinic in patients with presbyacusis.14 DFNA2,8 DFNA5,9 and DFNA9.9 The slope at which the phoneme recognition score decayed with increasing PTA level appeared to be steeper than in the aforementioned different groups of patients (Figure 6).8,9,14 The recognition score in DFNA20/26 subjects was better than in DFNA9 subjects at any PTA. Compared with DFNA2 and
DFNA5 subjects, DFNA20/26 subjects scored better in speech recognition at PTAs lower than 85 dB HL (hearing level), but worse at PTAs higher than 90 dB. Compared with presbyacusis subjects, those with DFNA20/26 scored better in speech recognition at PTAs lower than 100 dB and worse at PTAs higher than 100 dB.

Figure 3. Serial audiograms of 10 family members (A–J) shown for the right (R) and left (L) ears separately (air-conduction threshold in decibels hearing level [dB HL]). Note that the panels are ordered (top left to bottom right) by age in years at the last visit. Some of the serial audiograms have been omitted for clarity.
DFNA20 interval. The locus for these 2 families was designated DFNA26. Flanking markers for the DFNA26 locus have not been reported so far. Extensive clinical comparison with these DFNA26 families is prohibited by the present lack of reported audiometric data.

The originally reported type of hearing impairment associated with DFNA20 showed progressive sensorineural hearing impairment with a relatively late onset (age 20 years) that predominantly affected the high frequencies. The pattern of hearing loss was suggested to resemble presbyacusis but having an onset that is 30 years earlier than normal. Recently, audiometric data were reported for 4 affected family members by Elfenbein et al., who described downsloping sensorineural hearing loss first evident at 6 kHz and later at 8 kHz. This pattern could be demonstrated in some patients in their early teenage years but was clearly evident only by age 24 to 29 years. By the end of the third and fifth decades, clear differences were found at some frequencies between affected and unaffected persons. With increasing age, hearing loss increased at all frequencies, ultimately manifesting as a “corner audiogram” configuration.

The hearing loss of the present Dutch DFNA20/26 family shows some similarities with that of the American family reported by Ellenbein et al., but the audiometric data also revealed apparent differences. We demonstrated that hearing loss was profound by age 15 to 20 and 25 to 40 years at 8 and 1 to 4 kHz, respectively. Loss at the lower frequencies (ie, 0.25-0.5 kHz) showed more gradual progression at an average increase of about 1.5 to 2 dB/year. Affected individuals have only residual hearing from an age of about 40 years onward. Thus, hearing impairment in the Dutch DFNA20/26 family has a more severe appearance than that in the American family (Figure 4). Higher threshold levels were attained at an earlier age at any given frequency. Obviously, comparing purely longitudinal data of both families would be more appropriate, but Figure 4 may give some indication of the difference in severity.

The DFNA20/26 patients in the present family showed better maximum speech recognition scores in relation to the level of pure-tone hearing impairment at levels below 80 to 90 dB HL than was found in patients with DFNA2, DFNA3, DFNA9, or presbyacusis. However, owing to a steeper slope of the trend line pertaining to DFNA20/26, these patients showed lower scores at levels above 90 dB than the DFNA2 and DFNA5 patients and scores similar to presbyacusis subjects at about 100 dB (Figure 6). Ellenbein et al. mentioned the proband’s poor speech recognition scores but did not provide details; they included data on acoustic emissions but no detailed data on speech recognition scores. We did not evaluate otoacoustic emissions.

A survey of the critical region for candidate genes for DFNA20/26 suggested that the P4HB gene, encoding the beta subunit of prolyl 4-hydroxylase, was the most promising. The P4HB protein catalyzes the formation of 4-hydroxyproline in collagens and thereby is important for the structure and function of collagen. In addition, the protein functions as protein disulfide isomerase and as a cellular thyroid hormone binding protein. However, a disease-causing mutation in this gene could not be demonstrated in the DFNA20 family or in the present family.

The difference in phenotype between the previously described American DFNA20 family, based on data of only 4 family members, and the present family does not exclude the involvement of the same causative gene. Different types of mutations might lead to different phenotypes. However, it can also be hypothesized that different genes are causing different traits linked to the DFNA20/26 interval. An example is the recent localization of a gene for Usher syndrome type 1G (USH1G) to 17q24-q25 overlapping with the DFNA20 interval as it was described by Morell et al.. Since the critical region for USH1G is flanked by D17S1830 on the telomeric side, this locus does not overlap with the critical region determined for the Dutch family (Figures 1 and 2). Therefore, we conclude that the distal part of chromosome 17q harbors at least 2 causative genes for hearing loss.

The mouse mutation jackson-shaker (js) associated with deafness and vestibular impairment is located in the region of mouse chromosome 11, homologous to human

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**VESTIBULOOCULAR EXAMINATION AND IMAGING RESULTS**

While caloric testing revealed no abnormalities, patient IV:7 exhibited vestibular hyporeflexia and asymmetrical responses to rotatory tests. Severe vestibular hyporeflexia and an enhanced cervicoocular reflex were noted in family member IV:22. Vestibular testing in 6 other participants (IV:11, IV:22, V:5, V:6, V:15, and VI:4) revealed no abnormalities. The middle and inner ear structures of family member IV:22 had a normal appearance on computed tomographic scans.

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**COMMENT**

The Dutch family in the present study shows postlingual, nonsyndromic, progressive, sensorineural hearing loss with probably no or very limited vestibular involvement. This is the fourth DFNA family found to have hearing loss linked to chromosome 17q25. The critical region, originally described by Morell et al., is located between markers D17S1806 and D17S668 and occupies an interval of about 12 cM (Figure 2). Yang and Smith described 2 unrelated American families with progressive autosomal dominant hearing loss with linkage to a region overlapping the DFNA20 interval. The locus for these 2 families was designated DFNA26. Flanking markers for the DFNA26 locus have not been reported so far. Extensive clinical comparison with these DFNA26 families is prohibited by the present lack of reported audiometric data.

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**Figure 4. Age-related typical audiograms of family W99-060, the present family, and of a known American DFNA20 family.** Italic indicates age in years. HL indicates hearing level.
Figure 5. Plots combining individual longitudinal data (with connection lines) and separate cross-sectional data from our subjects (open circles) for binaural mean air-conduction threshold and phoneme recognition scores against age. Black triangles and squares indicate cross-sectional and longitudinal data, respectively, from the American family described by Elfenbein et al.13 HL indicates hearing level.
chromosome 17q25. Given the vestibular impairment, it seems more likely that the mutated gene in the js mouse is the orthologue of the USH1G gene than that of the gene for nonsyndromic hearing loss in the present family. Already 3 genes have been found to be involved in both Usher syndrome and in nonsyndromic hearing loss. Therefore, the identification of the disease-causing mutations is needed to elucidate whether DFNA20 and DFNA26 are caused by mutations in the same gene. This will also show whether the USH1G gene is involved.

The present research was successful in mapping the causative gene for hearing loss in a Dutch family to the DFNA20/26 interval and in refining its critical region. The present report is the second to provide detailed tone and speech audiometric data for this locus. The publication of additional data available from other DFNA20/26 families is needed to improve phenotypic comparison. Clinical features of the 2 available families show some audiometric similarity. However, members of the Dutch family appear to be more severely affected at an earlier age. As yet, no gene or disease-causing mutations have been identified for DFNA20/26. It has been previously suggested that DFNA20 might represent a suitable model of presbyacusis. The present data, however, do not demonstrate any striking similarity between the phenotype of DFNA20/26 and presbyacusis.

Submitted for publication October 30, 2002; final revision received June 17, 2003; accepted August 12, 2003.

This study was financially supported by the Netherlands Organization for Health Research and Development (project No. 920-03-100), the Hague; and the Heinsius Houbolt Foundation and the Nijmegen Otorhinolaryngology Foundation, Nijmegen, the Netherlands.

We acknowledge the contributions of Wendy H. M. de Jong and Janneke J. C. van Lith-Verhoeven to the genetic analysis and of Marion H. F. B. Bohnen to the clinical part of this study.

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Figure 6. Plot showing relationship between phoneme recognition score (percentage of correct responses) and binaural mean pure-tone average of the thresholds at 1, 2, and 4 kHz (PTA1-4kHz). The PTA1-4kHz is shown schematically by lines drawn for DFNA2, DFNA5, and DFNA9 families and the Dutch DFNA20/26 family. Presbyacusis (presby) is represented by a dotted line. HL indicates hearing level.