A New Autosomal-Dominant Locus (DFNA12) Is Responsible for a Nonsyndromic, Midfrequency, Prelingual and Nonprogressive Sensorineural Hearing Loss

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Objective: This study aimed to report on the audiologic findings of a nonsyndromic autosomal-dominant hearing loss of which the gene (DFNA12) recently was found to map to chromosome 11q22–24. The study also aimed to propose and evaluate an algorithm based on the audiometric findings to discriminate between affected and unaffected family members before genetic linkage analysis.
Study Design: The study design was a retrospective analysis of the audiometric data of genetically affected and unaffected patients.
Setting: The study was conducted at a tertiary referral center.
Patients: A total of 17 genetically affected and 54 unaffected family members were studied.
Interventions: Pure-tone audiometry with air and bone conduction and construction and evaluation of an algorithm were performed.
Main Outcome Measures: The type and degree of hearing loss as compared to age and gender-dependent values according to the International Organization for Standardization 7029 standard were measured. For this comparison, the variable "hearing standard deviations" (HSD) is introduced and is defined as the number of standard deviations that a hearing threshold is lying above the age and gender-related median at the given frequency. A description of the algorithm and an evaluation in terms of α- and β-error also were measured.
Results: The hearing loss is nonsyndromic, sensorineural, moderate-to-moderately severe (pure-tone average, 51 dB at age 18 years), with an early onset (probably prelingual) and no progression. It affects all frequencies but mainly the midfrequencies (500, 1,000, and 2,000 Hz). The algorithm consists of an analysis of variance to determine the frequency that is most sensitive for the genetic trait under study and on the ranking of the family members according to their hearing loss (HSD) at this frequency. Individual persons are labeled as "affected" or "unaffected" according to this ranking. **Key Words:** Sensorineural hearing loss—Genetics—Midfrequency—Autosomal dominant—Audiology.
Am J Otol **19**:718–723, 1998.

In a recent article, we reported the results of a genetic analysis in a family with nonsyndromic autosomal-dominant hearing loss. Genetic linkage analysis was performed, and the gene was found to map to a 36-cM interval located on chromosome 11q22–24. The candidate region for the gene did not overlap with other known deafness loci on this chromosome, and the novel gene locus was named DFNA12 (1). Only one family has been linked to this locus by now.

The prevalence of congenital hearing loss is approximately 1–3 per 1,000 births (2,3), half of which is assumed to be of genetic origin. In addition, an unknown fraction of the postlingual types of hearing loss also is of genetic origin (4). In recent years, several loci for inherited hearing loss have been reported, a review of which can be found on the hereditary hearing loss homepage (Van Camp G, Smith RJH. http://dnalab-www.uia.ac.be/dnalab/hhh). Hearing losses can be classified into prelingual versus postlingual or stable versus progressive. Most often, those categorizations are performed by audiologists or otologists. Yet, no strict criteria exist, and the heterogeneity of the phenotypes makes it difficult to ensure whether the hearing loss is prelingual or not and whether the hearing loss is progressive or not. Several criteria have been suggested (5,6), but they are based merely on empiric interpretations, and they fail to provide a solid statistical rational. The current article reports on a statistical analysis of the audiologic data of the DFNA12-affected patients in an attempt to define the type of hearing loss on more solid grounds.

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In addition, when performing genetic linkage analysis, each family member has to be labeled "affected" or "unaffected" before the actual genetic linkage analysis. For all gene localization studies in hereditary deafness published up to now, this labeling has been based solely on the "expert" analysis of the audiometric findings. This expert analysis is subject to error, especially because in most hereditary hearing losses, the degree of hearing loss is not extreme but rather moderate. The current article proposes a mathematical algorithm to discriminate between affected and unaffected family members.

MATERIALS AND METHODS

Family collection

A pedigree of 10 generations and 238 members of a Belgian family with autosomal-dominant hearing loss was worked out, starting from a propositus who presented with familial sensorineural hearing loss. Audiograms and blood samples were obtained after informed consent. Pure-tone audiometry was performed, and air and bone conduction thresholds were established according to routine procedures. In case of hearing loss, anamnestic data were obtained and previous audiograms were collected if available.

Statistical analysis

The audiometric data were statistically analyzed. Five-parameter statistics and box-and-whisker plots were used as descriptive statistics (7). Pure-tone averages (PTA) were defined as the average of the thresholds at 500, 1,000, and 2,000 Hz. To label an audiogram in terms of normality, the thresholds were compared to the age- and gender-related distribution as defined by the International Organization for Standardization (ISO) 7029 standard (ISO 7029 [1984], "Acoustics—threshold of hearing by air conduction as a function of age and sex for otologically normal persons" [International Organization for Standardization, Geneva]). Thus, for each frequency, the threshold can be expressed as the number of standard deviations below or above the median value for the given age and gender (further called hearing standard deviations [HSD]). From this number of standard deviations, the corresponding percentile can be found in any table of a normal distribution. For example, the median hearing loss at 500 Hz for a normal male at 70 years of age is 8 dB according to the ISO 7029 standard with a positive standard deviation of 10 dB. A hearing loss of 25 dB can be expressed as 1.7 HSD, namely 1.7 standard deviations (17 dB) above the median, and this corresponds to the 96th percentile (or P96). Nonparametric statistics (Mann–Whitney U test) were used to compare the hearing thresholds of affected patients to those of unaffected patients.

Before linkage analysis can be done, the audiometric results of all the family members have to be labeled affected or unaffected. This has always been done visually by trained otolaryngologists. In an attempt to find out whether the expert interpretation of the audiometric results can be replaced by a calculated categorization, a tentative algorithm was evaluated. It is assumed that a genetic hearing loss is present with a preference for some frequencies. The algorithm is meant to detect the cases which are affected, based on the audiogram. The algorithm is a two-staged procedure: 1. Look for the audiometric frequency with the highest interindividual variability; 2. Find the cases with a big hearing loss at this frequency, expressed as asrSD's in such a way that only affected cases are withheld.

RESULTS

Genetic analysis

Of the 238 members of the pedigree, 163 belonged to the family and 75 were related by marriage. A pure-tone audiogram was obtained from 76 members, and the blood of 70 members was collected for genomic DNA analysis.

The diagnosis of sensorineural hearing loss was based on expert analysis of the audiograms. This expert analysis was based roughly on the following criteria:

1. Family members were considered to be affected if they had a bilateral sensorineural hearing loss exceeding the 95th percentile of an age- and gender-dependent control curve of the general population (ISO 7029 standard).
2. Family members were considered to be unaffected if their hearing thresholds were better than 20 dB hearing loss or better than the 50th age- and gender-related percentile.
3. In case of an abnormal audiogram that was atypical compared with that of other patients of this family, in case of doubt on the genetic cause of the hearing loss, or in case of a hearing loss between the 50th and 95th percentile, the patient was labeled "uncertain" regarding his or her affected status.

Of the 76 family members who were investigated audiometrically, 15 were scored as definitely affected and 45 as definitely unaffected. Only the blood of these members was further processed for linkage analysis. This analysis showed 17 patients to carry the DFNA12 gene. Table 1 lists the correlation between the audiometric analysis and the DNA status.

Anamnestic family data

The anamnestic data and, if available, the audiometric history of the affected family members are summarized in Table 2. Three patients (18%) were not aware of any hearing loss; three patients (18%) reported the onset of their hearing loss at ages ranging from 35–47 years. Four patients (24%) reported a hearing loss from primary school onward, and seven patients (41%) presumed their hearing loss to be prelingual. Of four patients, an audiogram before the age of 10 years was available. The hearing loss ranged from 50–70 dB, and no deterioration was

TABLE 1. *Correlation between audiometry and DNA status*

	DFNA 12 +	DFNA12 −	
Audio +	15		15
Audio +?	1	7	8
Audio −?	1	2	3
Audio −		45	45
Audio NA		3	3
Total	17	57	74

DFNA 12+, carrier of the DFNA 12 gene; DFNA12-, not a carrier of the DFNA 12 gene; NA, not available.

TABLE 2. *Patient history*

Actual age (years)	Reported age of onset (years)	Age of first audiogram (years)	First PTA (dB)	Evolution
40	0			
85	0			
36	0			
17	0	3.5	55	No deterioration
19	0	5	70	No deterioration
15	0	6	50	No deterioration
6	2.5	4	55	5 dB deterioration
47	10			
70	<12			
33	<12			
34	<12			
52	35			
53	40			
49	47			
44				
22				
23				

PTA, pure tone average.

TABLE 3. *Hearing loss of affected patients*

Frequency (Hz)	HL (HSD)
125	4.2
250	5.0
500	6.9
1,000	7.6
2,000	5.9
4,000	3.2
8,000	2.1

HL, hearing loss; HSD, hearing standard deviations.

measured during a follow-up time ranging from 2–14 years.

Statistical analysis

The audiometric results of the patients genetically diagnosed as affected are plotted in Figure 1, which shows a midfrequency sensorineural hearing loss of 57 dB as PTA. At all frequencies, the hearing loss of the genetically affected patients is significantly worse than that of the unaffected patients (Mann–Whitney U, p < 0.001). To eliminate the gender and age effect in the interindividual variation, the hearing loss of each individual was compared to the age- and gender-related median (refer to Materials and Methods section for details). Table 3 lists the hearing loss of the affected patients expressed as HSD. Here, also, the hearing loss is significantly worse than that of the unaffected patients (Mann–Whitney U, p < 0.001).

Because this study reports on a cross-sectional audiometric evaluation, it is not possible to give the exact time

course of the hearing for each individual. Yet, plotting the hearing threshold of each individual on an age–hearing loss plot gives a good approximation of the evolution with age. This is done for frequencies of 250, 1,000, and 4,000 Hz in Figure 2. The best linear fit can be calculated according to the ISO formula:

$$H_{md,Y} = \alpha \, (Y\text{-}18)^2 + H_{md,18}$$

where the median hearing threshold for a person of age Y ($H_{md, Y}$) is expressed as a function of age $(Y - 18)^2$ with $H_{md, 18}$ being the median hearing threshold at age 18 years and α being the slope of the linear function (expressed as deterioration in decibels/year^2). Table 4 resumes the values of the coefficient α for each frequency compared with the ISO values of α for males and females.

It can be readily seen that the values of coefficient α are lower in the affected patients than in the normal population, from which it can be inferred that the average hearing deterioration with age in the affected patients does not exceed the normal deterioration with age.

For the evaluation of the tentative algorithm, the interindividual variation for each frequency was determined in the entire study population (affected and unaffected patients). The results are listed in Table 5. Because the interindividual variation was maximal at 1,000 Hz, the hearing loss at this frequency was compared between the genetically affected and the unaffected group. This is shown in Figure 3.

DISCUSSION

A Belgian family with nonsyndromic autosomal-dominant hearing loss was investigated. The disease gene was named DFNA12 and was found to map to chromosome 11q22–24. This article focused on the analysis of the audiologic data.

Hearing evolution with time

It is impossible to draw definite conclusions on the evolution of the hearing loss based on a cross-sectional examination. Yet, we believe our analysis yields approximate values for both the "onset" hearing loss and the slope of the hearing deterioration with age. The onset hearing loss can be inferred from the value of $H_{md, 18}$, as calculated by the linear fit according to the ISO formula. This is the hearing loss at age 18 years. In the normal
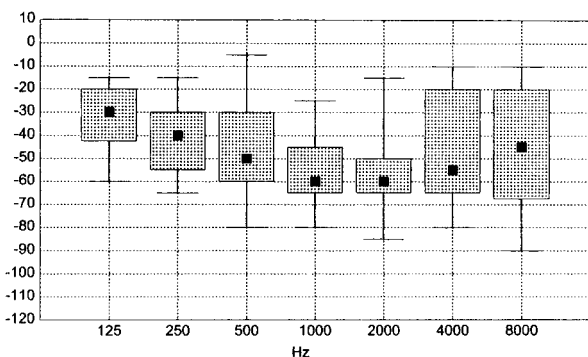

FIG. 1. Box-and-whisker plot of the audiometric data of 17 affected family members. Bars, minimum to maximum; large rectangles, 25–75%; small squares, median values. The hearing loss is highest over the midfrequencies (500–2,000 Hz).
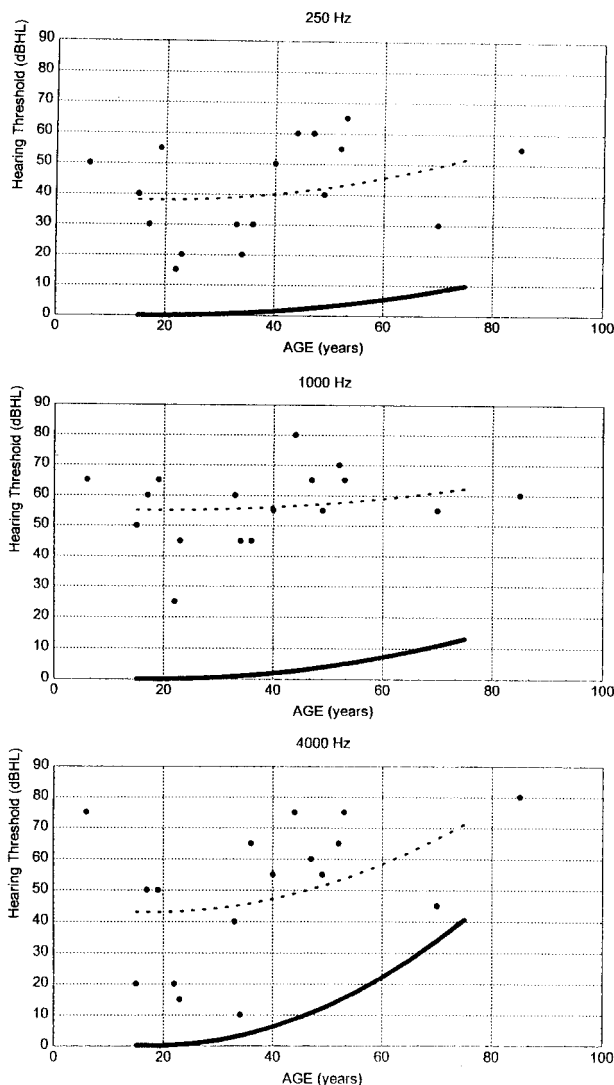
FIG. 2. Hearing thresholds (dBHL) for 250 Hz (top), 1,000 Hz (mid), and 4,000 Hz (bottom) plotted in function of age. The dots represent the thresholds of the affected patients. The dotted line is the best linear fit through the affected patients according to the International Organization for Standardization (ISO) 7029 formula (refer to text). The solid line is the best linear fit of the normal population according to the ISO 7029 standard. At all frequencies, the slope of the dotted line is similar to the slope of the solid line. Thus, the hearing deterioration of the affected patients does not exceed the normal hearing deterioration with age. The "onset" hearing loss is likely to be the same as the hearing at age 18 years and is 38 dB at 250 Hz, 55 dB at 1,000 Hz, and 43 dB at 4,000 Hz.

TABLE 4. *Slope α of hearing deterioration*

Frequency (Hz)	α			$H_{md,18}$ (dB HL) affected cases
	Normal males	Normal females	Affected cases	
125	0.0030	0.0030	0.0040	31
250	0.0030	0.0030	0.0040	38
500	0.0035	0.0035	0.0020	45
1,000	0.0040	0.0040	0.0020	55
2,000	0.0070	0.0060	0.0030	54
4,000	0.0160	0.0090	0.0090	43
8,000	0.0220	0.0150	0.0150	33

$H_{md,18}$, median hearing loss at age 18 years; HL, hearing level.

audiometric data at this age are available, they show hearing losses of >50 dB (age, 3.5–6 years) with no further deterioration (Table 2).

The hearing loss is sensorineural and most prominent in the midfrequencies, although all frequencies are affected. The slope of the linear fit is slightly less than the slope of the linear fit of the normal population (Table 4). This means that the hearing deterioration in the affected patients does not exceed the normal age-dependent hearing deterioration. Consequently, the current hearing loss may be labeled as nonprogressive. This is in line with the anamnestic data, because most affected persons mention no or only slight progression with age. In addition, when audiometric follow-up data are available, no deterioration is seen.

Audiometric definition of the affection status

Statistical analysis of audiometric data is not only important in the characterization of the type of hearing loss but also may contribute to a more reliable diagnosis of affected patients. For genetic linkage analysis to succeed, it is important that affected and unaffected patients are distinguished without error. Labeling an unaffected person as affected is an α-type of error, and labeling an affected person as unaffected is a β-type of error. In families with nonsyndromic hearing impairment, the only material that is available for a more objective evaluation is the audiogram. So far, the interpretation of the audiogram was a matter of expertise. For the current study, the ISO P95 values were plotted on top of each audiogram, and three otologists with experience in family investigation for genetic purposes were asked to agree on labeling each audiogram as positive (+), negative (−), positive with doubt (+?), or negative with doubt (−?). As listed in Table 1, the labels (+) and (−) correlated perfectly with the genetic diagnosis, whereas only 13% and 66% of the labels (+?) resp (−?) appeared to correspond with the final genetic diagnosis (Table 1). However, we still are uncertain regarding this expert audiometric diagnosis because one mistake may jeopardize gene localization studies. That is why an attempt was made to find a more objective way to interpret the audiogram by developing an algorithm. For this algorithm, the age- and gender-dependent variances are ruled out to not have them interfere with the genetically induced variance. For this purpose, the hearing
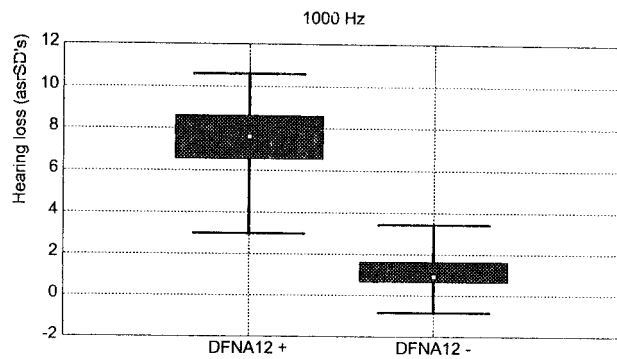
population, $H_{md,18}$ equals 0 dB at all frequencies. In the affected patients, $H_{md,18}$ equals between 31 and 55 dB, with an average of 51 dB as PTA (Table 4). To be comparable with the ISO 7029 data, the onset age was set at 18 years. Yet, when the calculations are performed with an age of onset being 0 year, the results are quite alike, with the same hearing loss at onset (PTA, 51 dB). In addition, anamnestic data confirm the early onset. A majority of patients (11 of 17, or 65%) mention the hearing loss to be first noticed before or at primary school, and where

FIG. 3. Box-and-whisker plot of the hearing loss at 1,000 Hz (expressed as HSD; refer to Materials and Methods section) in the affected (DFNA12+) and the unaffected (DFNA12−) family members. Bars, minimum to maximum; large rectangles, 25–75%; small squares, median values. No unaffected patients have a hearing loss of >4 HSD, whereas all but one (16 of 17) of the affected patients have a hearing loss exceeding 4 HSD at 1,000 Hz.

TABLE 5. *Analysis of variance*

Frequency (Hz)	Mean (HSD)	Variance (HSD^2)
125	2.5	2.5
250	2.7	4.7
500	2.8	8.0
1,000	2.9	10.1
2,000	2.5	7.7
4,000	1.9	3.7
8,000	1.2	1.9

HSD, hearing standard deviation.

of each individual is expressed in terms of age- and gender-corrected values, as defined by the ISO 7029 standard. Thus, the concept of HSD was introduced. A hearing loss of 1 HSD means that the hearing of a given person is 1 standard deviation worse than the age- and gender-matched median, or that the hearing of the given person is situated at the 84th percentile. The actual algorithm then is based on the idea that a family with inherited hearing loss is a mixed population with affected and unaffected persons, resulting in an increased statistical variance in the audiometric results when compared with those of a normal population. If the genetic hearing loss has a preference for certain frequencies (e.g., the high frequencies), then the increased variance will be most prominent at these frequencies and these frequencies are more sensitive for the genetic effect. Therefore, the first step of the algorithm is to analyze the variances at the different frequencies (hearing expressed as HSD). If a marked pattern is seen (e.g., the variance is larger at the high frequencies), then it is assumed that the genetically affected patients will differ most explicitly from the unaffected patients at these frequencies. The second step of the algorithm then is to define an upper and a lower cutoff level. Hearing losses above the upper cutoff level are labeled as affected, those below the lower cutoff level as unaffected, and those between the lower and the upper cutoff level as uncertain. Setting the upper cutoff level high will result in too few persons labeled as affected, but setting it low will result in too large an α-error. Similarly, setting the lower cutoff level low will result in too few persons labeled as unaffected, but setting it high will result in too large a β-error. It is up to the geneticist to decide how many persons with a positive or negative affection status he or she needs for the linkage analysis and which α- and β-error he or she is prepared to accept. An elegant method is to present the family members ranked according to their hearing loss at the most sensitive frequency, expressed as HSD. To define the affected persons, one may count down from the persons with the

worst hearing to those with the best hearing. The geneticist will decide how far one is allowed to count down and will need enough patients to be labeled as affected, but he or she will be limited in counting down by the increasing risk of labeling unaffected patients as affected. This risk is the α-error and can be assessed by the cutoff level that defines the affected patients. With a cutoff level at 4 HSD, the α-error will be <0.01%. The α-error is 0.14% at 3 HSD and 2.3% at 2 HSD (data derived from a standard normal distribution with one-side testing). In the current family, the variance was most prominent in the midfrequencies with a maximum at 1,000 Hz (Table 5). No genetically unaffected patients showed hearing losses exceeding 4 HSD at this frequency (Fig. 3). In contrast, 16 (94%) of 17 affected patients had hearing losses of >4 HSD. Thus, defining the cutoff threshold at 4 standard deviations above the age- and gender-correlated median would yield an excellent selection of affected patients without the risk of erroneously labeling patients as affected. Four HSD may seem high and therefore too strict, but one must take into account that one is evaluating the most sensitive frequency. Thus, the likelihood of finding such extensive hearing losses at this frequency is higher than one might expect to find when examining all frequencies or merely the PTA. It would be interesting to evaluate this cutoff level in other families with a genetic hearing loss.

Similarly, one may label the persons as unaffected by counting up, starting from those with the better hearing. The β-error cannot be calculated, however, because the genetically affected subpopulation is not yet known at this moment in the procedure. However, it is less critical, because the study population will consist of many unaffected persons, such that even a lower cutoff level will yield enough persons, whereas the β-error will remain low. For the current family, a hearing loss of <0 HSD or 1 HSD at 1,000 Hz could be used to label the members as unaffected.

CONCLUSIONS

The phenotypic expression of the DFNA12 gene is a nonsyndromic, sensorineural hearing loss, affecting all frequencies, but especially the midfrequencies. The hearing loss is moderate-to-moderately severe, with a most probable onset at a prelingual age and without progression. The median hearing loss is 51 dB (PTA) at onset. The inheritance is autosomal dominant and fully penetrant. An algo-

rithm is proposed for a more objective analysis of the audiogram. This may lead to a more reliable diagnosis of affected patients. The authors propose this algorithm to be evaluated further in other families with inherited hearing loss.

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