Progression of Low-Frequency Sensorineural Hearing Loss (DFNA6/14-WFS1)

Ronald J. E. Pennings, MD; Steven J. H. Bom, MD; Kim Cryns, MSc; Kris Flothmann; Patrick L. M. Huygen, PhD; Hannie Kremer, PhD; Guy Van Camp, PhD; Cor W. R. J. Cremers, PhD

**Objective:** To assess the audiometric profile and speech recognition characteristics in affected members of 2 families with DFNA6/14 harboring heterozygous mutations in the \textit{WFS1} gene that cause an autosomal dominant nonsyndromic sensorineural hearing impairment trait.

**Design:** Family study.

**Setting:** Tertiary referral center.

**Patients:** Thirteen patients from 2 recently identified Dutch families with DFNA6/14 (Dutch III and IV).

**Methods:** Cross-sectional and longitudinal analyses of pure-tone thresholds at octave frequencies of 0.25 to 8 kHz were performed, and speech phoneme recognition scores were assessed. Progression was evaluated by linear regression analysis with and without correction for presbycusis.

**Results:** All individuals showed low-frequency hearing impairment. The 2-kHz frequency was more affected in the Dutch III family than in the Dutch IV family. Progressive hearing loss beyond presbycusis was found in the Dutch IV family and in 3 individuals in the Dutch III family. Annual threshold deterioration was between 0.6 and 1 dB per year at all frequencies. The speech recognition scores in the Dutch III family showed significantly more deterioration at increasing levels of hearing impairment compared with those in the Dutch IV family.

**Conclusion:** Both families showed an autosomal dominant, progressive, low-frequency sensorineural hearing impairment caused by heterozygous \textit{WFS1} mutations.


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Thirty-four years ago, the Vanderbilt University Hereditary Deafness Study Group\(^1\) described a large family with low-frequency sensorineural hearing impairment showing an autosomal dominant pattern of inheritance. Several years later, Konigsmark et al\(^2\) described 3 more families harboring a dominant low-frequency hearing impairment trait. Audiograms in all families displayed upward-sloping patterns. Today, characterization of nonsyndromic forms of hereditary hearing impairment is based more on genetic characteristics than on clinical findings. The loci for nonsyndromic autosomal dominant forms of hearing impairment are designated DFNA (DFN for deafness and A for autosomal dominant) and are numbered in chronological order of discovery. Forty loci are known to account for the autosomal recessive Wolfram syndrome.\(^3,6\) To our knowledge, 3 families with heterozygous mutations in the \textit{WFS1} gene have been described: USA 1 (L829P mutation\(^4\)), Dutch I (T699M\(^4\)), and Dutch II 8 (A716T 4). All families showed mild progression of hearing impairment; however, only in the Dutch II family did this persist beyond correction for presbycusis.\(^9\) Brodwolf et al\(^10\) recently described a German family with DFNA6/14 in whom linkage analysis showed a harboring of low- to mid-frequency hearing impairment. Young et al\(^11\) described a Newfoundland kindred harboring the same \textit{WFS1} mutation (A716T\(^+\)) as was detected in the Dutch II family.

This report describes the hearing impairment in 2 additional families with...
DFNA6/14, Dutch III and Dutch IV, that harbor 2 mutations in the WFS1 gene, G674E and G674V, respectively.

**METHODS**

In the Dutch III and Dutch IV families (Figure 1), the WFS1 gene was analyzed for mutations after audiograms of members of both families demonstrated low-frequency hearing impairment. Four affected individuals from the Dutch III family had a G674E mutation, and 9 from the Dutch IV family had a G674V mutation. From the pedigree, it was concluded that the deceased individual III:2 in the Dutch IV family also harbored the G674V mutation.

In this study, we assess the audiometric profile and speech recognition performance in affected family members with DFNA6/14 of different ages. Medical histories were taken, focusing on acquired and syndromic conditions. Otoscopy was performed and previous audiologic data were retrieved, including data on deceased individual III:2 in the Dutch IV family. Pure-tone thresholds (binaural means of air and bone conduction) at octave frequencies of 0.25 to 8 kHz and speech recognition scores (monaural means of maximum phoneme recognition scores) were obtained in a sound-treated room according to the International Organization for Standardization (ISO). Age-related typical audiograms were derived from the raw data at a sufficiently high number of different sound frequencies (P<.05 in the appropriate binomial distribution). Threshold data were also evaluated for progression after correction for age and sex for median norms (30th percentile) of presbycusis, according to the ISO 7029 norms.

Age-related typical audiograms were derived from the results of the cross-sectional regression analysis of the raw data. Individual longitudinal regression analysis, also including correction for presbycusis, was performed for individuals III:1, IV:1, and IV:2 in the Dutch III family. For cross-sectional regression analysis of speech audiometric data, maximum phoneme recognition scores (percent-correct) were derived from individual performance-intensity plots. Regression analysis was performed for performance-age plots (scores related to age) and performance-impairment plots (scores on means of pure-tone thresholds at 0.5, 1, and 2 kHz). Speech recognition scores were fitted by a linear regression line. The x-coordinate relating to a 90% score (X90) represented the onset age (in years) for X and the onset level for pure-tone audiogram thresholds (measured in decibels hearing level) at 0.5 to 2 kHz. The 95% confidence interval (CI) for X90 was obtained by nonlinear regression analysis using an alternative equation for the linear regression line, Y=slope(X-X90)+90, where Y is the binaural mean air conduction threshold measured in decibels hearing level. t Tests (including Welch correction if the Bartlett test detected unequal variances) were used to test differences in X90 between the families. Slope represented the deterioration rate relative to age and deterioration gradient for pure-tone audiogram threshold.

Analysis of covariance was used to compare slopes and intercepts between regression lines pertaining to different frequencies within a given family or pertaining to the families at a given frequency. Some slopes and intercepts were pooled where possible.

Vestibulo-ocular responses were evaluated in individuals IV:2 and IV:5 in the Dutch IV family using electronystagmography with computer analysis. Saccadic, smooth pursuit, optokinetic, and vestibular nystagmus responses were evaluated. Vestibular stimulation comprised rotatory and caloric tests. Details and normal values have been previously described.

**RESULTS**

Four individuals in the Dutch III family and 9 in the Dutch IV family showed low-frequency hearing impairment. The Dutch IV kindred also harbored a Duchenne-type muscular dystrophy trait; according to family history, 3 affected boys (Figure 1) without hearing impairment died at a young age.

Cross-sectional analysis of threshold-on-age data was performed in both families. The results are shown in Figure 2. The scattering of threshold data points was not substantially different between the 2 families. Both families showed significant progression. When individual frequencies were compared, analysis of raw data
showed no significant differences in progression between the families. In the Dutch IV kindred, no significant difference in progression among the frequencies was found using raw data. The pooled annual threshold deterioration was 1.0 dB. However, after correction for presbycusis, the Dutch IV family showed significant progression, which was not the case for individuals in the Dutch III family.

Age-related typical audiograms for the 2 families displayed ascending configurations from low-frequency thresholds (fairly flat at 0.25-1 kHz) of about 40 to 70 dB in the Dutch III kindred and 40 to 90 dB in the Dutch IV kindred (Figure 3). A flat threshold configuration was found at 2 kHz in the Dutch III family. In younger individuals, the thresholds at 4 to 8 kHz were close to normal, especially in the Dutch IV family.

In individuals III:1, IV:1, and IV:2 in the Dutch III family, longitudinal regression analysis of pure-tone audiograms was performed. Significant progression was detected in all of them (Figure 4), persisting beyond correction for presbycusis. Age-related typical audiograms derived for these analyses (plots not shown) were similar to those obtained for the cross-sectional analysis (Figure 3).

Figure 5 demonstrates results of the analyses of speech recognition scores for the Dutch III and Dutch IV families. The regression lines in the performance-age plots show a slow decline in score with increasing age. The mean onset age for individuals in the Dutch III family was 25 years (95% CI, 16-34 years); the mean deterioration rate was 0.8% per year (95% CI, 0.5%-1.1% per year). The values for the Dutch IV family were similar, with a mean onset age of 28 years (95% CI, 18-38 years) and a mean deterioration rate of 0.5% per year (95% CI, 0.1%-0.9% per year). A significant performance difference between the families was found only in the level of impairment (Figure 5B). In the Dutch III family, the mean deterioration gradient was almost 2% per decibel (95% CI, 1.4%-2.6% per decibel). In the Dutch IV family, it was 0.45% per decibel (95% CI, 0.3%-0.6% per decibel). There was no significant difference in mean onset level between the families (Dutch III, 58 dB hearing level [95% CI, 55-61 dB] and Dutch IV, 51 dB hearing level [95% CI, 42-60 dB]).

None of the patients reported substantial vestibular symptoms, and the 2 individuals examined showed normal ocular motor and vestibular responses.

**COMMENT**

The Dutch III (longitudinal analyses) and Dutch IV (cross-sectional analyses) families showed similar progression that persisted after correction for presbycusis. On evaluation of speech recognition scores, the performance-impairment plots were significantly different between the 2 kindreds, while the performance-age plots were similar.

Although nonsyndromic autosomal dominant hearing impairment is a heterogeneous condition, the subgroup of loci predominantly affecting the lower frequencies is homogeneous to some extent. DFNA1 was the first locus identified with a nonsyndromic autosomal domi-
nant hearing impairment trait. It is located on chromosome 5q31 and is characterized as a progressive low-frequency type of hearing impairment. Lynch et al\(^19\) identified this mutation in the \textit{DIAPH1} gene in a large Costa Rican family. To our knowledge, no other families showing linkage to the DFNA1 locus have been described.

Lesperance et al\(^20\) identified a second locus (DFNA6) for dominant low-frequency hearing impairment on chromosome 4p16.3 in the American family in whom the corresponding phenotype had been outlined by the Vanderbilt University Hereditary Deafness Study Group.\(^1\) Predominant involvement of the frequencies from 0.25 to 1 kHz was found. Recently, the raw data published in that report were reanalyzed in a cross-sectional analysis and no significant progression beyond presbycusis was found.\(^8\)

In the Dutch I family, Van Camp et al\(^21\) discovered a third locus associated with low-frequency sensorineural hearing impairment on chromosome 4p16.3, close to the DFNA6 locus but without an apparent overlap. Kunst et al\(^7\) described the audiometric presentation in this Dutch I family and demonstrated progression of hearing impairment, but not beyond that attributable to presbycusis.

The Dutch II family was linked to a larger chromosomal region comprising DFNA6 and DFNA14. Progression was mild but significant, and ranged from 0.5 dB per year at 0.25 kHz to 1.3 dB per year at 8 kHz. Significant progression persisted after correction for presbycusis.

**Figure 3.** Age-related typical audiograms for 5 families with DFNA6/14 (A, present families; B, previously described families\(^{1,7,8}\), with corresponding \textit{WFS1} mutations\(^4,12\) dB HL indicates decibels hearing level.

**Figure 4.** Longitudinal analyses of raw threshold (in decibels hearing level [dB HL]) data for frequencies ranging from 0.25 to 8 kHz in individuals III:1, IV:1, and IV:2 in the Dutch III family. Bold lines indicate significant progression.
sis. Recently, Brodewolf et al described an additional family linked to DFNA6/14 showing a nonprogressive low-frequency hearing impairment. Young et al have reported another low-frequency hearing impairment trait, designated DFNA38, in a Newfoundland family harboring the same mutation (A716T) in the WFS1 gene as was found in the Dutch II family.

Age-related typical audiograms for the clinically described families are depicted in Figure 3; they demonstrate 2 types, with (G674V and A716T) and without (T699M and L829P) progression beyond presbycusis at low frequencies (0.25-1 kHz). The G674E mutation in the Dutch III family seems to have caused a progression that is intermediate between these 2 extremes. Cross-sectional analysis of this family did not indicate progression after presbycusis correction, but this may have been because of a lack of sufficient number of observations. However, longitudinal analysis in 3 individuals in this family, involving more observations, demonstrated progression beyond presbycusis.

Speech recognition scores have also been evaluated for some individuals in the Dutch II family. The scores for younger individuals (<32 years) were within the 90% to 100% range, which is in line with the mean onset ages of 25 and 28 years found for the Dutch III and IV families, respectively. The mean onset levels for these 2 families ranged from 50 to 60 dB hearing levels. There was no substantial difference in pure-tone audiogram findings between the Dutch III and IV families, although the 2-kHz threshold seemed to be more affected in the Dutch III family (ie, in line with a flat threshold at 0.25-2 kHz). Speech performance scores relative to age were not substantially different. However, a significant difference in speech performance relative to the level of hearing impairment was detected. This difference may have been related to the worse pure-tone threshold found at 2 kHz in the Dutch III family.

The point mutations in these 2 families cause a missense mutation of the same amino acid, G674. This glycine is substituted by glutamic acid in the Dutch III family and by valine in the Dutch IV family. The phenotype relating to the A716T mutation was similar to that in these families.

Recently, it was demonstrated in 7 families that heterozygous mutations in the WFS1 gene are responsible for traits linked to DFNA6/14. In the original family demonstrating DFNA6, a key recombinant that excluded the DFNA14 candidate region had actually been based on a phenocopy. This led to an incorrect localization of DFNA6, while in fact DFNA6 and DFNA14 represent a single locus.

The WFS1 gene encodes the protein wolframin and is homozgyously mutated in Wolfram, or DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) syndrome. The minimum features required for the diagnosis are type 1 diabetes mellitus and optic atrophy. However, diabetes insipidus (described in 54%-58% of cases) and “deafness” (described in 51%-62%) are also common features of this syndrome. This autosomal recessive syndrome seems to be associated with a high-frequency hearing impairment, rather than the low-frequency impairment found in the present families.

This rare syndrome has a prevalence of 1 in 770000 in the United Kingdom. Wolframin, encoded by WFS1, is a transmembrane protein. It has been localized to the endoplasmic reticulum and probably plays a role in membrane trafficking, protein processing, and regulation of endoplasmic reticulum calcium homeostasis. However, its exact location and role in the cochlea remain obscure. Electrophysiologic, magnetic resonance imaging, and neuropathological studies of this syndrome have shown general progressive degeneration of the central and peripheral nervous systems, including the vestibulocochlear nerve. Ohata et al described an increased risk of hearing impairment and diabetes mellitus in heterozygous carriers. Unfortunately, no frequencies were specified and hearing impairment was defined as an overall threshold greater than 20 dB hearing level. Young et al described an individual in the Newfoundland family who was a homozygous carrier and who had diabetes mellitus at a young age and other clinical features reminiscent of Wolfram syndrome. However, this individual was not affected by optic atrophy. Therefore, it seems possible that carriers of the Wolfram syndrome show low-frequency hearing impairment that is similar to that found in DFNA6/14.

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Corresponding author: Ronald J. E. Pennings, MD, Department of Otorhinolaryngology, University Medical Centre St Radboud, PO Box 9101, 6500 HB Nijmegen, the Netherlands (e-mail: r.pennings@kno.azn.nl).

Figure 5. Cross-sectional analyses shown in performance-age plot of binaural means of percentage correct phoneme recognition scores relative to age (in years) (A) and the same score shown in performance-impairment plot relative to (binaural means of) pure-tone average (PTA) at 0.5 to 2 kHz (measured in decibels hearing level) (B). Linear regression lines are shown with Roman numerals indicating the families (III, solid circles; IV, open circles). Dotted lines and numbers relate to 90% correct scores.


