Audiometric Characteristics of a Dutch Family Linked to DFNA15 With a Novel Mutation (p.L289F) in POU4F3

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Objective: To report on the audiometric characteristics of a large Dutch family linked to DFNA15 with a novel mutation (p.L289F) in POU4F3 (OMIM 602460).

Design: Clinical investigation.

Setting: Tertiary referral center.

Patients: Family members from a large 5-generation pedigree with sensorineural hearing impairment segregating as an autosomal dominant trait.

Main Outcome Measures: Cross-sectional and longitudinal analyses of pure-tone audiometric data, and cross-sectional analyses of speech audiometry data.

Results: Overall, a flat to gently downsloping audiometric configuration was observed with a progression rate of approximately 0.8 dB/y across most frequencies. Speech recognition scores remained fairly good in relation to age and hearing level compared with a group of patients with presbycusis. Interindividual variability was observed in terms of subjective onset age and audiometric configuration. Two mutation carriers, who reported vestibular symptoms, underwent vestibular examination and showed hypofunction of the vestibular labyrinth.

Conclusions: The audiometric phenotype of the Dutch family linked to DFNA15 with a novel mutation in POU4F3 is comparable to that observed in the original Israeli family linked to DFNA15. Relatively good speech recognition scores suggest outer hair cell involvement. DFNA15 may represent a cochleovestibular disorder.


At present, 54 loci for autosomal dominant nonsyndromic hearing impairment (DFNA1-54) have been described. The causative genes have been identified in 21 of these cases. A thorough phenotype description of each novel locus and mutation is necessary to identify the clinical significance of all the new findings and, for counseling purposes, to gain insight into the natural course of the specific genetic disease.

The autosomal dominant hearing impairment disorder DFNA15 is characterized by bilateral progressive, adult-onset sensorineural hearing impairment without concomitant vestibular features. It was described for an Israeli family, designated family H. Hearing impairment in that family appeared to be moderate to severe, and the audiometric configuration varied between flat and downsloping. Age at onset was between 18 and 30 years. The DFNA15 locus resides on human chromosome 5q31, and a mutation in the POU4F3 gene was shown to be responsible for the disorder. In affected members of family H, an 8–base pair deletion in POU4F3 (884del8) was identified, resulting in a frame shift that causes premature termination of the POU4F3 protein. The annotation at protein level is I295fsX4.

POU4F3 is a member of the POU family of transcription factors and is strongly expressed in mouse cochlear and vestibular hair cells. It is essential for the differentiation and survival of hair cells. Targeted deletion of Pou4f3 results in profound deafness and impaired balance due to complete loss of auditory and vestibular hair cells in mice homozygous for the deletion.

Herein we report on the clinical characteristics of a large Dutch family linked to DFNA15 with a novel mutation in POU4F3. The identification of this novel mutation, p.L289F, and its effect on protein function will be reported separately. Audiometric data were analyzed and compared with those obtained for family H.
**METHODS**

**PATIENTS**

After written informed consent had been obtained from all participating family members, a family investigation was performed and a pedigree of Dutch family W05-549 was constructed (Figure 1). The study was approved by the local medical ethics committee. A medical history was taken from all participants, paying special attention to hearing impairment and vestibular symptoms. Furthermore, concomitant disease, the use of medication, and any other possible cause of acquired deafness were ruled out. After micro-otoscopic examination, pure-tone audiometry was performed. Clinically affected individuals also underwent speech audiometry. Vestibular function was tested in affected family members who reported vestibular symptoms. Blood samples were collected from 32 affected family members, 19 presumably unaffected family members, and 10 spouses for the purpose of linkage analysis. Available previous medical records and audiograms were traced to enable individual longitudinal analysis.

Analyses of audiometric data, as described in the following section, were performed on data pertaining to mutation carriers of the present family.

**AUDIOMETRY AND DATA ANALYSIS**

The audiometric examination consisted of conventional pure-tone audiometry in a sound-treated room. The individual 95th percentile threshold values of presbycusis in relation to the patient’s sex and age were derived for each frequency using the ISO 7029 method. Individuals were considered affected if the best-hearing ear showed thresholds mainly or exclusively beyond the 95th percentile threshold values for presbycusis.

Cross-sectional, binaurally averaged threshold data (air-conduction level in decibels of hearing level) were plotted against age for each frequency and analyzed by means of linear regression analysis (threshold on age). The regression coefficient (slope) was called the annual threshold deterioration (ATD) and expressed in decibels per year. Progression was significant if the 95% confidence interval of the ATD did not include zero. If significant progression was present, age-related typical audiograms (ARTA) were constructed following a method described previously. Individual longitudinal regression analysis of binaural mean threshold values on age was performed only in clinically affected persons with 3 or more consecutive measurements and an overall follow-up period of at least 3 years. Individual progression was considered significant when significant progression was observed in at least 3 of 6 frequencies (P < .05 in binomial distribution). The level of significance used in all tests was P = .05.

Speech audiometry was performed using phonetically balanced, standard Dutch consonant-vowel-consonant word lists. The maximum phoneme recognition score (mean value of percentage correct for both ears) was obtained from monaural performance-intensity curves and was analyzed in relation to age and pure-tone average (mean value for both ears) at frequencies of 1, 2, and 4 kHz (PTA1,2,4 kHz). Nonlinear regression analysis was performed using a dose-response curve with a variable slope to fit individual longitudinal scores for the p.L289F mutation carriers. The age at onset and the onset level (ie, X90) were defined at a recognition score of 90% in cross-sectional performance vs age or performance vs impairment plots, respectively. Between the scores of 90% and 75%, a straight line was also fitted to simplify the results and to obtain an estimate of local average slope that was called the deterioration rate in the performance vs age plot and called the deterioration gradient in the performance vs impairment plot. A previously described group of patients with presbycusis was used as a reference group.

**VESTIBULO-OCULAR EXAMINATION AND DATA ANALYSIS**

Two mutation carriers (IV-48 and IV-62) underwent vestibular and ocular motor tests. The tests included evaluation of the vestibulo-ocular reflex (velocity-step test), using electronystagmography with computer analysis and saccadic, smooth pursuit, and optokinetic nystagmus responses. Vestibular stimulation consisted of rotatory tests. Details and normal values have been described previously.

Figure 1. Simplified pedigree of the Dutch family W05-549 with nonsyndromic autosomal dominant hearing loss caused by a mutation (p.L289F) in POU4F3. PC indicates phenocopy.
Figure 2. Individual audiograms of p.L289F mutation carriers from the Dutch family with hearing loss linked to DFNA15, ordered by age (from top left to bottom right) at last visit. Shown are air conduction (AC) threshold levels for the right (open circles) and for left (crosses) ears. Above each audiogram, the pedigree number and age in years are given. The line with solid circles represents the 95th percentile AC threshold levels of presbycusis (P95) for a given age and sex according to ISO 7029.9

*Data for these individuals were excluded from cross-sectional analysis.
RESULTS

Forty persons were clinically affected by hearing loss according to patient history, 34 of whom were alive (Figure 1). Blood samples were obtained from 60 individuals for genetic analysis. Audiometry was performed and/or audiograms could be retrieved from elsewhere in 60 individuals. These data showed that 32 individuals were affected. An autosomal dominant pattern of inheritance was apparent. The case histories and physical examination results excluded syndromic involvement. Most clinically affected individuals reported bilateral, slowly progressive hearing loss with a mean subjective onset age of 35 years (range, childhood to 60 years). Two individuals (IV:48 and IV:62) reported varying vestibular symptoms, including instability (especially in the dark), vertigo, and a tendency to fall. Vestibular function test results for individual IV:62 showed symmetrical hyporeflexia with time constants of 7 and 10 seconds for both nystagmus directions. Individual IV:48 showed asymmetrical velocity-step test responses with time constants of 11 seconds (marginally low) and 28 seconds (too long) for both nystagmus directions.

Genetic analysis revealed the presence of a total of 30 POU4F3/p.L289F mutation carriers, all of whom were clinically affected. Two clinically affected individuals (IV:36 and IV:71) did not harbor the mutation in POU4F3 and may thus represent phenocopies (Figure 1).

Figure 3. Single-snapshot (open circles) thresholds are shown for each frequency separately. The line in each panel represents the result of the cross-sectional linear regression analysis for each frequency. The average threshold deterioration (ATD) values in decibels per year are derived from cross-sectional linear regression analysis. HL indicates hearing level.

Audiometry

Pure-Tone Thresholds Related to Age

The last-visit audiograms pertaining to all mutation carriers are shown in Figure 2. Overall, the threshold levels deteriorated with advancing age. However, some interindividual variability in audiometric configuration and the degree of hearing impairment was observed. A flat to gently downsloping audiometric configuration applied to most audiograms, but mid-frequency (V:39, 16 years of age) and steeply downsloping (IV:48, 44 years of age; IV:57, 45 years; and III:62, 67 years) audiograms occurred as well (Figure 2). Individuals IV:48 and IV:57 showed more steepness at the high frequencies at 44 and 45 years of age, respectively, than that predicted by presbycusis, whereas steepness and thresholds at these frequencies conformed with presbycusis in individual III:62 at age 67 years. Flat configurations were shown for individuals V:12 (age, 18 years), V:6 (25 years), IV:19 (47 years), IV:10 (55 years), and III:18 (74 years). Audiogram data pertaining to individuals IV:50 (age, 45 years) and III:33 (73 years) were excluded from cross-sectional analyses because of outlier behavior; that is, each had a threshold beyond the 95% tolerance limits of the linear regression line in cross-sectional analysis (Figure 2).

The cross-sectional threshold-on-age data were plotted against age for each separate frequency in Figure 3.
Linear regression analysis was performed on these data and showed significant progression with advancing age for each frequency. Values of ATD ranged from 0.7 to 1.4 dB/y (Figure 3).

Individual longitudinal data were available for 12 mutation carriers. Linear regression analysis was performed on individual threshold-on-age data and showed significant progression only for individuals IV:50, III:33, and IV:62 at ages 30 to 45, 30 to 73, and 32 to 75 years, respectively (data not shown). The data pertaining to the other 9 mutation carriers who did not show significant progression covered relatively short time intervals and, for some mutation carriers, the available serial audiograms were obtained at relatively advanced ages.

Based on the results of cross-sectional linear regression analysis, ARTA were constructed (Figure 4A). These showed a flat to gently downsloping configuration with a fairly similar ATD of 0.7 to 0.9 dB/y at frequencies of 0.25 to 2 kHz. The ARTA from the original Israeli family H3,14 were added for comparison (Figure 4B). At ages younger than 50 years, threshold levels from the present Dutch family were worse than those for family H; at 50 years of age, threshold levels were similar; and at 60 and 70 years of age, family H showed worse threshold levels, mainly in the mid and high frequencies. Threshold levels deteriorated faster in family H than they did in the Dutch family, primarily in the mid and high frequencies.

Speech Recognition Scores

Figure 5 shows the single-snapshot measurements of the phoneme scores in the p.L289F mutation carriers (open circles) of binaural mean phoneme recognition scores against age (A) and against binaural mean pure-tone average at 1, 2, and 4 kHz (PTA1,2,4 kHz) (B). The solid line is a sigmoidal curve fitted to these measurements as previously described.11 The dashed curve represents data fitted to patients with presbycusis only.12 The horizontal dotted line at 90% correct represents onset age (56 years [A]) or onset level (59 dB HL [B]) after which speech recognition deteriorates. HL indicates hearing level.
deteriorated by 0.6% per year on average. The 90% recognition score was found at a PTA1,2,4 kHz level of 39 dB and deteriorated by 0.8% per decibel between the scores of 90% and 75%.

The p.L289F mutation carriers showed better speech recognition scores than did patients with presbycusis at matching ages (Figure 5A). The hearing impairment scores for patients with the p.L289F mutation appeared to be better at similar levels of impairment than those for the patients with presbycusis (Figure 5B).

This report presents a detailed audiometric analysis of a Dutch family linked to DFNA15 with a novel mutation (p.L289F) in the POU4F3 gene. This is the second family linked to DFNA15 that has been identified worldwide. The emerging clinical picture is consistent with the report on the original Israeli family H.3,3

Based on the available clinical data, an early adult to midlife onset of hearing impairment applies to the present family. Most often, a high-frequency type of hearing impairment was observed with a downsloping audiometric configuration. However, mid-frequency as well as flat audiometric configurations also occurred (Figure 2).

Linear regression analysis of cross-sectional audiometric data showed significant progression for each frequency separately. The ARTA (Figure 4) showed a flat to gently downsloping audiometric configuration with a progression rate of approximately 0.8 dB/y across the frequencies of 0.25 to 2 kHz. At 4 to 8 kHz, the progression rate was slightly higher at 1.0 to 1.4 dB/y.

Although all affected family members reported a slowly progressive type of hearing impairment, analysis detected significant progression in 3 individuals with a sufficiently high number of suitable longitudinal data. This is probably because sequential audiograms were not available for each person or, when available, covered only a short time interval or were obtained at relatively old ages, when progression may have already stopped.

A slowly progressive type of hearing impairment with a flat audiometric configuration may go unnoticed for a long time. In some cases this results in a discrepancy between the subjective onset age and the onset age estimated by backward extrapolation of hearing thresholds. This discrepancy has been shown for other autosomal dominant types of hearing impairment with a flat to gently downsloping configuration, such as DFNA15,15 DFNA11,16 DFNA21,17 and DFNA31,18,19 (previously linked to DFNA13). Thus, the characteristics of hearing impairment in the present family may explain why the subjective onset age varies so greatly between the different mutation carriers.

Speech recognition scores remained fairly good with advancing age and with progression of hearing impairment as measured by pure-tone audiometry. DFNA2,11 DFNA5,20 and DFNA1116,21 are other autosomal dominant hearing impairment disorders in which speech recognition scores remain relatively good. For these disorders the underlying defect is localized mainly in outer hair cells,20,22-27 Relative sparing of inner hair cell function may account for the preservation of speech recognition. This suggests outer hair cell involvement in DFNA15 and would confirm findings in Pou4f3-null mice.6,7 Speech recognition scores for DFNA2, DFNA5, and DFNA11 are comparable to those obtained for the present Dutch family as well as for the Israeli family H.3,11,16,20

In mice, Pou4f3 (also known as Brn3.1 or Brn-3c) is expressed in both inner and outer hair cells of the cochlea and in hair cells in the vestibular labyrinth.6,7 Targeted deletion of the gene results in deafness and vestibular dysfunction.6,7 In the Israeli family H, no vestibular involvement was reported.2,3 However, no vestibular testing was performed in Israeli mutation carriers, so vestibular involvement cannot be entirely excluded. In the present Dutch family, 2 mutation carriers reported vestibular symptoms and underwent vestibular function tests. The test results showed a hypofunction of the vestibular labyrinth in one of them and problematic results in the other. It may be interesting to subject an additional subset of mutation carriers of the present Dutch family to vestibular function tests to assess whether DFNA15 represents a cochleovestibular disorder similar to DFNA9,28-30 and DFNA1110 and as demonstrated in mice with a targeted deletion of Pou4f3.6,7

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