Clinical Characterization of Genetic Hearing Loss Caused by a Mutation in the POU4F3 Transcription Factor

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Objectives: To describe the detailed auditory phenotype of DFNA15, genetic hearing loss associated with a mutation in the POU4F3 transcription factor, and to define genotype-phenotype correlations, namely, how specific mutations lead to particular clinical consequences.

Design: An analysis of clinical features of hearing-impaired members of an Israeli family, family H, with autosomal dominant–inherited hearing loss.

Setting: Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; Department of Audiology, Rabin Medical Center, Petah Tiqwa, Israel; and audiological centers.

Participants: Clinical features of 11 affected and 5 unaffected individuals older than 40 years from family H were studied. Mutation analysis was performed in 6 presymptomatic individuals younger than 30 years; clinical features were analyzed in 4 of these family H members.

Interventions: Hearing was measured by pure-tone audiometry and speech audiometry on all participating relatives of family H. Immittance testing (tympanometry and acoustic reflexes), auditory brainstem response, and otoacoustic emissions were done in a selected patient population.

Results: The patients presented with progressive high-tone sensorineural hearing impairment, which became apparent between ages 18 and 30 years. The hearing impairment became more severe with time, eventually causing significant hearing loss across the spectrum at all frequencies.

Conclusions: Our results indicate that POU4F3 mutation-associated deafness cannot be identified through clinical evaluation, but only through molecular analysis. Intrafamilial variability suggests that other genetic or environmental factors may modify the age at onset and rate of progression.


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A complete medical history of each affected individual was obtained to ensure that the hearing loss was not a result of infection, head trauma, acoustic trauma, or use of ototoxic drugs. Hearing was measured by pure-tone audiometry and speech audiometry on 20 members of family H (500, 502, 504, 505, 506, 510, 511, 512, 515, 516, 518, 519, 520, 521, 524, 527 and 4 presymptomatic individuals). The thresholds were compared with the age- and sex-related median of normal hearing standards set by the International Organization for Standardization (ISO) 7029 standard (ISO 7029 [1984]) (data not shown). Four individuals, 504, 505, and 516 and 519, were tested further with immittance testing (tympanometry and acoustic reflexes), and auditory brainstem response (ABR). Individual 519 also underwent click-evoked otoacoustic emission and distortion-product otoacoustic emission. Six individuals younger than 30 years were tested for the POU4F3 mutation, and in 4 cases, pure-tone audiometry and speech audiometry was also performed.

For otoacoustic emission tests, an ILO88 Otodynamic Analyzer (Version 3.0; Otodynamics, London, England) was used to elicit and record click-evoked otoacoustic emissions. Following sealing of the probe into the ear canal, 2080 rectangular clicks (80-microsecond duration) were presented unilaterally and subsequently averaged. The accepted responses were averaged in 2 subaverages of 1040 sweeps; poststimulus intervals started at 3 milliseconds after the stimulus and ended 20 milliseconds later. The correlation coefficient between the 2 subaverages was calculated and displayed. The otoacoustic emission response was derived following the nonlinear paradigm of the ILO system.

The criterion for hearing impairment in family H is a hearing threshold below the 95th percentile of the standard reference curves (ISO 7029 standard). There was little consistency in the shape of the audiograms between affected individuals with the POU4F3 mutation, ranging from a flat to a sloping curve (Figure 1, A and B). The hearing thresholds for the better ear at different frequencies are given in Table 1. The mean±SD score on speech discrimination tests was 90%±7.33% (Table 2).

Individual 519 tested positive for the 884del8 mutation in POU4F3 (Figure 2). He was first tested at age 39 years and pure-tone testing revealed bilateral sensorineural hearing loss at 4000 Hz (40- to 50-dB threshold). The speech reception threshold was at 10 dB and reached a maximum discrimination score of 100% at 45 dB. At age
At the age of 51 years, individual 516 demonstrated bilateral moderate sensorineural hearing loss in the low frequencies, with a threshold of 40 dB at 1000 Hz and a more severe hearing loss in the higher frequencies with a maximal loss of 80 dB at 8000 Hz (Figure 1, B). The speech reception thresholds were 40 dB in the right ear, with a maximal discrimination of 92% at 75 dB; and 45 dB in the left ear, with a maximal discrimination of 88% at 80 dB. Type A tympanometry was established bilaterally. Acoustic reflexes were absent in both ears. The ABR was
normal with respect to latency and shape at 100 dB (absolute wave V latencies, interpeak latencies, and interaural differences).

Individual 504 tested positive for the 884del8 mutation in POU4F3 (Figure 2). Pure-tone testing of individual 504, taken at the age of 54 years, demonstrated bilateral moderate to severe sensorineural hearing loss, with a flat audiogram (Figure 1, B). The speech reception threshold was 60 dB with a maximal discrimination of 100% at 90 dB. Type A tympanometry was established in both ears. Acoustic reflexes were absent in both ears. The ABR at 105 dB sound pressure level was normal with respect to latency and shape.

Individual 505 does not carry the 884del8 POU4F3 mutation, although he has a moderate sensorineural hearing loss in the left ear, and moderate to severe mixed hearing loss in the right ear (Figure 1, C). Testing was performed at age 53 years. High-tone loss began at 2000 Hz. The speech reception thresholds were 60 dB in the right ear, with a discrimination of 96% at 95 dB; and 40 dB in the left ear, with a discrimination of 100% at 75 dB. Type A tympanometry was established in both ears. Acoustic reflexes were absent in both ears. These results suggest there is otosclerosis in the right ear. No ABR response was present in the right ear. The ABR of the left ear showed prolonged absolute latencies of the fifth peak (1-V interval is 4.55 milliseconds at 100 dB).

**PRESYMPTOMATIC DIAGNOSIS**

Mutation analysis for POU4F3 was performed on 6 family H members between the ages of 23 and 30 years with one affected parent, only a portion of whom complained of hearing loss. Five of 6 of these individuals were positive for the POU4F3 mutation. To determine whether audiological testing was consistent with the mutation at these ages, 4 also underwent audiometry (3 with the mutation and 1 without) (Table 3).

There was essentially no difference in the audiological test results between siblings at the ages tested, whether they harbored the mutation or not. Audiograms for 2 individuals with the mutation revealed mild hearing loss, with a slight decrease at high frequency. They reported being aware of their hearing loss. Both showed a similar curve pattern, namely, higher thresholds at both low and high frequencies and lower thresholds in the mid-frequency range (Figure 1, D). One other individual in this family also showed this type of audiogram (individual 500).

Deafness has been segregating in family H for 5 generations, presumably from a founder born in 1843. Progressive bilateral sensorineural hearing loss was the common theme in all family H members older than 40 years with the POU4F3 mutation. The hearing loss is inherited in an autosomal dominant fashion. Penetrance was complete in individuals older than 40 years.

POU4F3 is a member of the family of POU transcription factors involved in development, and in particular, for proper differentiation of inner and outer hair cells. In family H, a truncated protein presumably impairs high-affinity binding of this transcription factor via a dominant-negative mechanism. Based on the fact that hearing loss is progressive in family H, POU4F3 may also be involved in maintenance of hair cells. During development and childhood, one functioning allele may produce a sufficient amount of POU4F3 required to regulate other genes, but with increasing age, a decline in POU4F3, in conjunction with environmental factors, modifier genes, and dysfunctional cellular repair processes, may lead to premature hearing loss.

POU4F3 is specifically expressed in the human cochlea and in the inner and outer hair cells of the mouse. Otoacoustic emission tests in individual 519 suggests that the outer hair cells are malfunctioning, which is consistent with this individual testing positive for the 884del8 mutation. Normal ABR suggests a functional central auditory pathway in hearing-impaired individuals with POU4F3 mutations. Therefore, our results demonstrate that there is a correlation between the cellular and clinical phenotypes.

Individual 519 was 38 years old when first examined. Bilateral sensorineural hearing loss at 4000 Hz (40- to 50-dB threshold) with a normal discrimination score was found. This pattern is typical of noise-induced hearing loss. Yet, a careful medical history excluded any significant noise exposure. At age 41 years, progression of the hearing impairment to 40 to 70 dB at 3000 to 8000

**Table 3. Analysis of Pure-Tone Audiometry in Individuals Younger Than 40 Years With a POU4F3 Mutation**

<table>
<thead>
<tr>
<th>Individual No.</th>
<th>Age, y</th>
<th>250 Hz</th>
<th>500 Hz</th>
<th>1000 Hz</th>
<th>2000 Hz</th>
<th>4000 Hz</th>
<th>6000 Hz</th>
<th>8000 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>10</td>
<td>15</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>Not done</td>
<td>50</td>
</tr>
<tr>
<td>Median (mean)</td>
<td>28 (28)</td>
<td>15 (12)</td>
<td>10 (10)</td>
<td>10 (13)</td>
<td>10 (8)</td>
<td>15 (13)</td>
<td>35 (35)</td>
<td>30 (27)</td>
</tr>
<tr>
<td>SD</td>
<td>2.52</td>
<td>10.41</td>
<td>5.00</td>
<td>5.77</td>
<td>2.89</td>
<td>12.58</td>
<td>21.21</td>
<td>25.17</td>
</tr>
</tbody>
</table>
Hz was observed. Otoacoustic emission tests performed on this individual showed that he did have emissions in the middle frequencies, but no emissions were measured in the higher frequencies.

Careful audiological evaluation was essential to distinguish the hearing loss in individual 505 from that of other members of family H. Had individual 505 been misdiagnosed, DFNA15 could not have been identified. Initially, this individual was presumed to be a phenocopy, that is, an individual with a similar clinical presentation (phenotype), but due to a different cause, either genetic or environmental. However, upon closer inspection of his audiological characteristics, the phenotype was shown to be different from the remainder of the family, and thus audiologically, not a phenocopy (Figure 1, C).

The following audiological parameters were consistent between family H affected members with the POU4F3 mutation: the hearing impairment is sensorineural, progressive, and bilateral. Audiological characteristics, such as configuration of audiogram (low, middle, high frequency), shape of slope, severity, and age at onset, are variable. In general, audiometric patterns ranged from middle to high frequency, with a sloping profile and moderate to severe hearing loss. Ideally, one would like to predict which gene might be mutated in a group of nonrelated individuals based on clinical parameters, and then perform mutation analysis for 1 or 2 candidate genes. The variability between audiological characteristics among individuals with the POU4F3 884del8 mutation, even within one nuclear family, makes it difficult to make a direct correlation between genotype and phenotype.

Genetic counseling for hereditary hearing loss has changed substantially in the past 2 years with the discovery that 30% to 50% of prelingual genetic deafness is associated with connexin 26 mutations.11,12 Thus far, no single gene has been found to be responsible for a significant portion of postlingual hearing loss, which may be distributed more evenly among many genes. The lack of genotype-phenotype correlations will make screening difficult, since screening for many genes, especially large ones, may be impractical clinically. In this family, however, conclusive presymptomatic diagnosis can be made due to the ease with which mutation screening can be performed. Postlingually deaf individuals typically request presymptomatic diagnosis for their children so that they can prepare for the impending hearing loss.

Our understanding of the molecular basis of deafness and the biological function of the proteins involved in auditory transduction is progressing at a fast pace. As more mutations contributing to hearing loss are identified, and thorough audiological analyses are performed, comprehensive genotype-phenotype correlations can be made for inherited nonsyndromic hearing loss. This will enable a new generation of treatments of audivestibular disorders to be developed based on advances in genomics, including sensory hair cell regeneration and gene therapy. In vivo gene therapy is currently being developed for inherited nonsyndromic hearing loss. This will enable a new generation of treatments of audivestibular disorders to be developed based on advances in genomics, including sensory hair cell regeneration and gene therapy. In vivo gene therapy is currently being developed for inherited nonsyndromic hearing loss. This will

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