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 NEW ERA in hearing research has been taking place over the past few years with the advent of structural and functional genomics. The molecular and biological basis for hereditary hearing loss is being elucidated at a fast pace, with the hope that these discoveries will herald new treatments for deafness. More than half of cases of deafness are due to genetic causes and occur in association with other symptoms in the form of syndromic hearing loss or as an isolated finding, nonsyndromic hearing loss. To date, approximately 60 loci for nonsyndromic hearing loss have been mapped throughout the human genome, with each locus representing a family or families with dominant, recessive, or X-linked deafness.[1] The first reports of genes involved in nonsyndromic hearing loss appeared only in 1993 with the identification of a mitochondrial mutation in the 12S ribosomal RNA gene.[2] Since then, mutations leading to hearing loss have been identified in another mitochondrial gene, tRNA-ser(UCN); transcription factors POU3F4 and POU4F3 (*DFN3*, *DFNA15*); unconventional myosins myosin VIIA and myosin XV (*DFNB2*, *DFNB3*, *DFNA11*); connexins 26 and 31 (*DFNA3*, *DFNB1*, *DFNA2*); the formin-family member diaphanous (*DFNA1*); the extracellular matrix protein α-tectorin (*DFNA8*, *DFNA12*); the ion transporter pendrin (*DFNB4*); the K+ ion channel KCNQ4 (*DFNA2*); and in other genes for which no function has yet been assigned (*DFNA5*, *DFNA9*).[1,3] Most compelling is the fact that connexin 26 (*GJB2*) mutations contribute to about half of recessive prelingual deafness cases in some regions.[4]

Genes responsible for nonsyndromic hearing loss were identified first in the

SUBJECTS, MATERIALS, AND METHODS

FAMILY COLLECTION

A pedigree of family H was constructed by interviewing several key individuals. In an effort to the isolate the gene responsible for hearing loss, linkage analysis was performed on 12 affected and 11 unaffected individuals older than 40 years, as reported previously.[8] In this study, clinical analysis is reported on 11 family members identified as having hearing loss and 5 unaffected individuals, all older than 40 years. Approximately 10 mL of blood was collected by a physician. Genomic DNA was prepared from half of the blood by a "salting out" procedure for extracting DNA without the use of organic solvents.[9] In addition, DNA samples of 6 individuals from family H younger than 30 years were obtained and the samples were screened for the *POU4F3* deletion. Carriers of the deletion volunteered to undergo audiological testing. The project was approved by the Israel Ministry of Health Helsinki Committee.

MUTATION ANALYSIS

POU4F3 primers 629F (5′-CGGCTCTGGCTAATCTCAAG-3′) and 939R (5′-AAGGTCCAGTTTCTCAGCGA-3′) surrounding the deletion in affected family H members were used to amplify genomic DNA. The products were labeled by incorporation of [α^{32}P]–deoxycytidine triphosphate during polymerase chain reaction. Each sample was loaded onto a 6% polyacrylamide denaturing gel and electrophoresed at 70 W for 4.5 hours to resolve the 310-bp (wild-type) and 302-bp (family H mutant allele) fragments. Gels were dried and exposed to x-ray film.

AUDIOMETRY

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.[10] 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.

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mouse, demonstrating the value of the mouse for identifying and studying human deafness genes.[5,6] The structure and function of the mouse and human inner ear is remarkably similar, and thus the mouse has proved to be an invaluable model for studying human hearing loss. The mouse was instrumental in the identification of the *DFNA15* gene; targeted mutagenesis of the *Pou4f3* gene led to deafness and circling.[7] Once the human homolog of *Pou4f3* was found to map to 5q31, the region to which *DFNA15* was localized by linkage analysis, mutations in this small gene was found in hearing-impaired members of family H.[8]

The genetic heterogeneity of inherited deafness has made it difficult to make correlations between audiological and clinical symptoms and mutations in particular loci. To establish a correlation of phenotype with genotype for inherited hearing loss, we studied the audiological parameters of family H members harboring the *POU4F3* 8–base pair (bp) deletion and describe them in detail in this report.

RESULTS

MEDICAL HISTORY

The hearing impairment that occurred in members of family H older than 40 years with the *POU4F3* mutation was progressive bilateral sensorineural hearing loss.[8] The hearing loss is inherited in a dominant fashion and has been segregating in 5 generations, presumably from a founder born in 1843.[8] The affected individuals claimed that their hearing loss began in their 20s, although there are no audiograms to confirm this. There were no complaints of vestibular dysfunction; only individual 524 complained of tinnitus.

AUDIOLOGIC EVALUATION

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
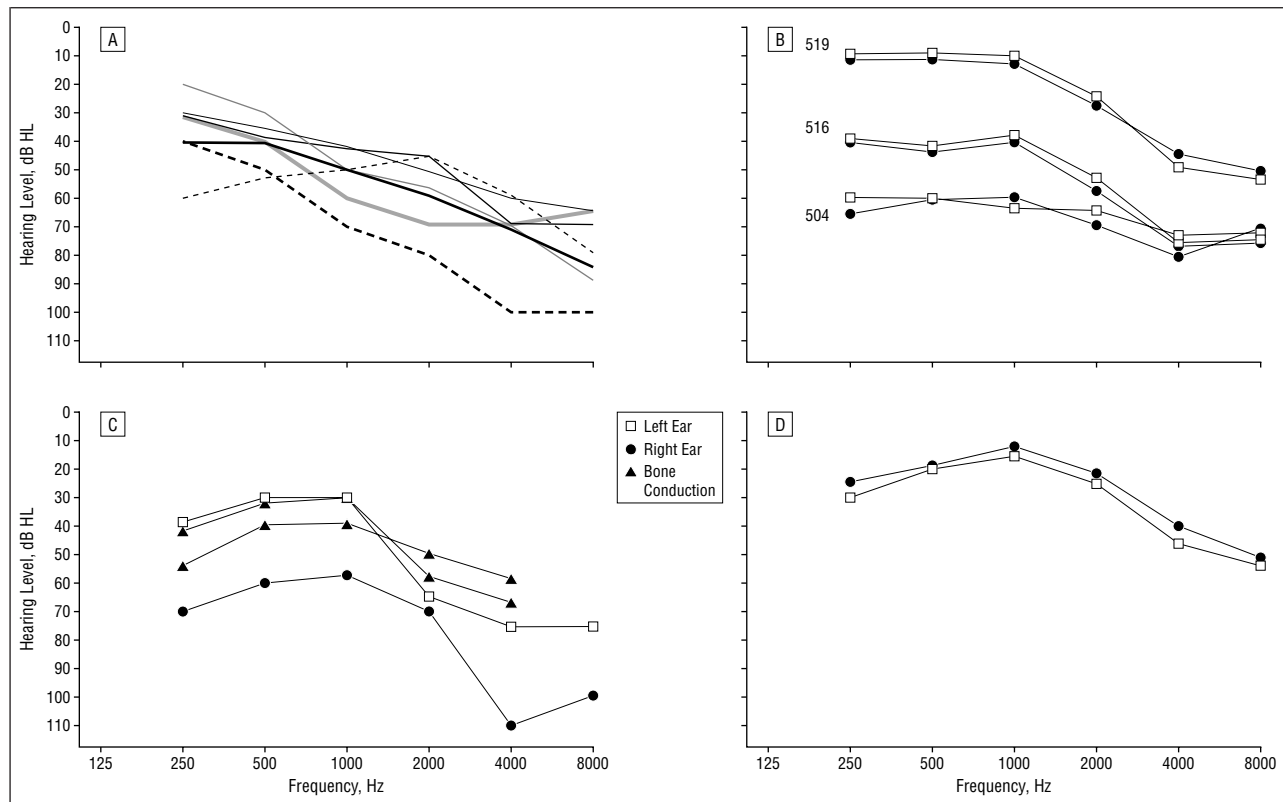
Figure 1. *Pure-tone audiograms of affected family H members. A, Audiograms of 7 individuals (500, 506, 510, 515, 518, 524, and 527) with sensorineural hearing loss (HL), all with the* POU4F3 *mutation. Only the better ear is shown for each person. B, Most recent audiograms for 3 individuals for whom more extensive testing was done, all harboring the* POU4F3 *deletion. C, Audiogram for patient 505, the individual with hearing loss, but without a* POU4F3 *mutation. D, Audiogram of individual younger than 30 years with the* POU4F3 *deletion.*

Table 1. Analysis of Pure-Tone Audiometry in Individuals With the *POU4F3* Mutation

Individual No.	Age, y	Hearing Level (dB) by Frequency					
		250 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz	8000 Hz
500	50	50	50	40	40	60	80
504	54	50	50	50	65	60	80
506	54	20	30	40	55	70	90
510	58	40	50	65	80	95	95
515	42	30	40	60	70	70	60
516	51	40	40	40	60	70	80
518	50	20	20	25	45	60	60
519	41	10	10	10	20	40	50
524	44	40	40	45	40	70	80
527	50	25	15	50	50	70	90
Median (mean)	50 (49)	35 (33)	40 (35)	43 (43)	53 (53)	70 (67)	80 (77)
SD	5.52	13.59	14.99	16.03	17.36	13.75	14.92

Table 2. Analysis of Discrimination and Speech Reception Threshold (SRT) Values

Patient No.	Discrimination, %	SRT, dB
500	100	45
504	80	50
506	92	40
510	80	50
515	86	45
516	92	40
518	84	35
519	96	10
524	Not done	Not done
527	96	40
Median (mean)	92 (90)	40 (39)
SD	7.33	12.10

41 years, this patient was retested and found to have further deterioration of his hearing, extending to 8000 Hz (Figure 1, B). Maximum speech discrimination of 100% was now reached at 55 dB. In distortion-product otoacoustic emissions, responses were detected below 2500 Hz in the right ear and 2000 Hz in the left ear. All tests demonstrated lack of response above the 2000- to 4000-Hz range, in accordance with the audiometry results.

Individual 516 tested positive for the 884del8 mutation in *POU4F3* (Figure 2). Pure-tone testing of indi-

vidual 516 at the age of 51 years 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,[8] 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 (I-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 audiologic test results between siblings at the ages tested,

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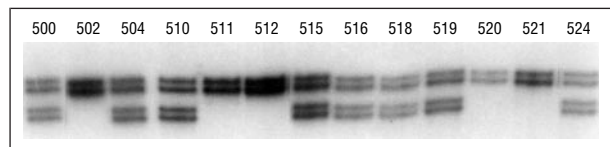
Figure 2. *Identification of* POU4F3 *deletion in family H. Primers surrounding the deletion were used to amplify genomic DNA. The top pair of bands represents the wild-type 310–base pair allele of* POU4F3*; the lower set of bands represents the mutant 302–base pair allele.*

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 midfrequency 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.[8] Progressive bilateral sensorineural hearing loss was the common theme in all family H members older than 40 years with the *POU4F3* mutation.[8] 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[8] and in the inner and outer hair cells of the mouse.[7] 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

Individual No.	Age, y	Hearing Level (dB) by Frequency						
		250 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz	6000 Hz	8000 Hz
1	30	0	5	10	5	0	20	0
2	25	15	15	20	10	15	50	30
3	28	20	10	10	10	25	Not done	50
Median (mean)	28 (28)	15 (12)	10 (10)	10 (13)	10 (8)	15 (13)	35 (35)	30 (27)
SD	2.52	10.41	5.00	5.77	2.89	12.58	21.21	25.17

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 audiovestibular 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 hearing loss, for example, using adenoviral and adeno-associated virus as gene transfer vectors in the cochlea.[13-15] Progressive hearing loss is particularly amenable to intervention since there is often a large window of opportunity before the hearing loss begins.

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