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A gene for autosomal dominant late-onset progressive non-syndromic hearing loss, *DFNA10*, maps to chromosome 6

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Late-onset non-syndromic hearing impairment is the most common type of neurological dysfunction in the elderly. It can be either acquired or inherited, although the relative impact of heredity on this type of loss is not known. To date, nine different genes have been localized, but none has been cloned. Using an extended American family in which a gene for autosomal dominant late-onset non-syndromic hearing impairment is segregating, we have identified a new locus, *DFNA10*, on chromosome 6.

INTRODUCTION

Hearing impairment is commonly classified by audiometric criteria as conductive, sensorineural or mixed, and quantitatively graded as mild (26–40 dB), moderate (41–55 dB), moderately-severe (56–70 dB), severe (71–90 dB) or profound (>90 dB) (1). In addition, by medical history, it is described as congenital or late-onset, and inherited or acquired. Inherited losses are subclassified as syndromic or non-syndromic to reflect the presence or absence of co-inherited physical abnormalities (2).

In the United States, one in 1000 neonates has congenital severe-to-profound hearing impairment of sufficient magnitude to preclude normal speech development (3); in half of these affected babies, the loss is inherited. The prevalence of hearing impairment increases with age, and by puberty, the number of affected persons doubles (4). With advancing years, median hearing thresholds insidiously decline to such an extent that 50% of octogenarians have a hearing loss greater than 25 dB (5). This age-related decrease in auditory acuity makes late-onset hearing impairment the most common type of neurological dysfunction of the elderly.

Both environmental and hereditary factors impact on age-related hearing impairment, however the relative contribution of each is not known. Most types of inherited late-onset non-syndromic hearing impairment appear to be autosomal dominant. To date, nine different genes have been localized although none has been cloned (Table 1). Using an extended American family in which a gene for autosomal dominant late-onset progressive non-syndromic hearing impairment is segregating, we have identified a new locus, *DFNA10*, on chromosome 6q.

Table 1. Location and screening markers for known autosomal dominant non-syndromic genes

Locus name	Location	Screening markers
DFNA1 (6)	5q31	D5S640, D5S410, D5S412
DFNA2 (7)	1p32	D1S432, MYCL1, D1S193
DFNA3 (8)	13q12	D13S143, D13S175, D13S292
DFNA4 (9)	19q13	D19S208, D19S224, ApoC2
DFNA5 (10)	7p15	D7S629, D7S673, D7S529
DFNA6 (11)	4p15.3	D4S1614, D4S412, D4S432
DFNA7 (Tranebjærg, L., pers. comm.)	1q21-q22	D1S194, D1S196, D1S210
DFNA8 (Kirschhofer, K., pers. comm.)	15q15-q21	THBS, D15S132, D15S123
DFNA9 (12)	14q12-q13	D14S252, D14S121, D14S49
DFNA10	6q22-q23	D6S267, D6S407, D6S472

Information available online at http//:alt-www.uia.ac.be/u/dnalab/hhh/html. Nomenclature used in this table has been approved by the HUGO Nomenclature Committee, Phyllis McAlpine, PhD, FCCMG, chair.

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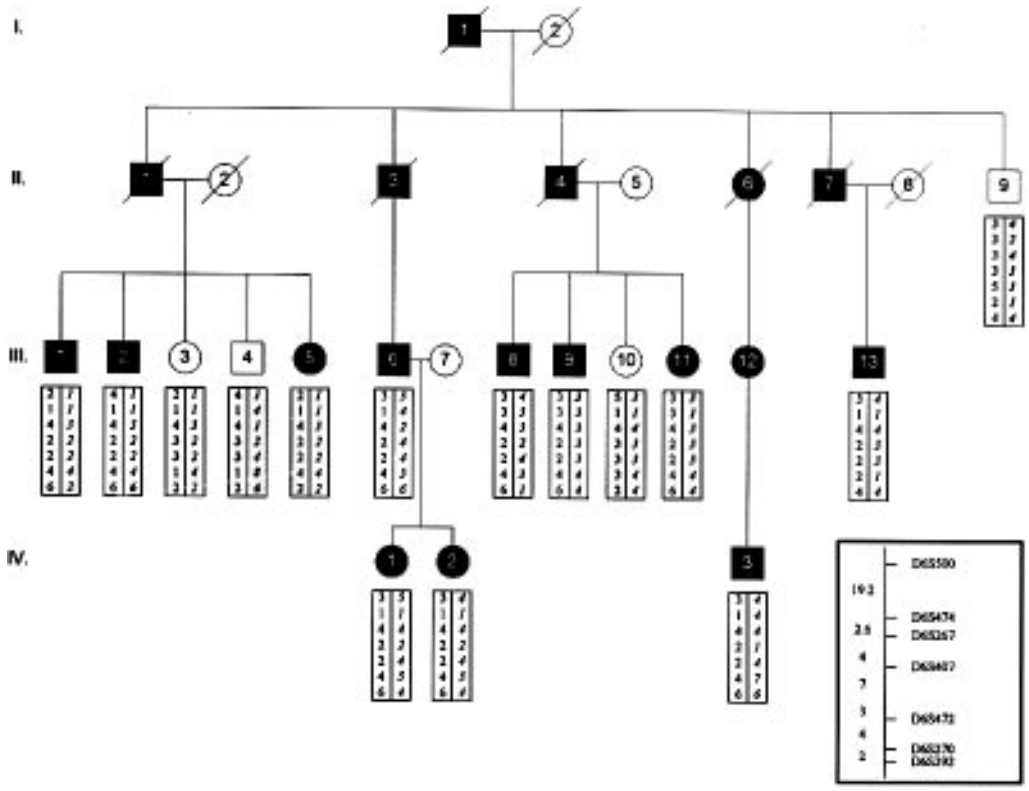

Figure 1. Extended pedigree localizing the *DFNA10* gene to the D6S474–D6S270 interval on chromosome 6. Two-point lod scores of 4.44 and 4.00 were obtained at θ = 0 with markers D6S472 and D6S407 (○, female; □, male; ●, effected female; ■, affected male).

RESULTS

Exclusion analysis

Linkage analysis was performed on a large multi-generational family from the United States (Fig. 1). Affected persons exhibited an inexorably progressive sensorineural hearing loss beginning in the second-to-fifth decades and leading ultimately to severe-to-profound hearing impairment requiring the use of amplification (Fig. 2). After excluding known loci for non-syndromic hearing loss using markers listed on the Hereditary Hearing Loss HOMEPAGE (http//:alt-www.uia.ac.be/u/dnalab/hhh/html), a genome-wide search was initiated. Two hundred and twenty-four highly polymorphic microsatellite markers spaced at approximately 15–20 cM intervals across the genome were typed, and areas of exclusion were calculated by two-point linkage analysis using MLINK (version 5.10) of the Linkage Package (13).

DFNA10 maps to chromosome 6q22.3-q23.2

Two-point lod scores of 4.44 and 4.00 were obtained at θ = 0 with markers D6S472 and D6S407 (Table 2). A multipoint map of the region containing *DFNA10* was constructed ordering the markers as shown in Figure 1. This order was supported with odds of 1000:1 with the exception of markers D6S472 and D6SS292 (only 700:1 better than inverting the pair). Multipoint linkage analysis of *DFNA10* within a five marker linkage map yielded a maximum lod score of 5.27 with marker D6S407. The 1-lod support interval places *DFNA10* within a 13.3 cM region on 6q (Fig. 3).

Table 2. Two-point lod scores for markers linked to *DFNA10*

Locus symbol	Locus name	Het.	Alleles	θ	Z_{max}
D6S474	GATA31	64%	5	8.4	1.10
D6S267	114xd12	75%	7	2.5	1.30
D6S407	198wg11	93%	12	0	4.00
D6S472	059yd9	56%	7	0	4.44
D6S270	127xb2	85%	9	8.6	2.22
D6S292	203za9	82%	10	12.2	1.60

Because lod scores are sensitive to estimates of allele frequency, two-point analysis was calculated using several different allele distributions for D6S472 and D6S407. As the frequency of the linked allele increased, the lod score with D6S472 quickly dropped below 3.0, but with D6S407, it remained above 3.0 for frequencies as high as 0.34. D6S407 has 12 alleles, the most common of which occurs at a frequency of 0.20. Repeating multipoint analysis using affecteds only also gave significant results, with a lod score between 3.46 and 3.66 over the D6S267–D6S472 interval.

DISCUSSION

The localization of *DFNA10*, defined by cross-overs, spans ~15 cM between markers D6S474 and D6S270. No obvious candidate genes have been mapped to this interval. Although regional assignments do include the laminin M chain gene (LAMM) (14), a nucleotide pyrophosphatase gene (NPPase)

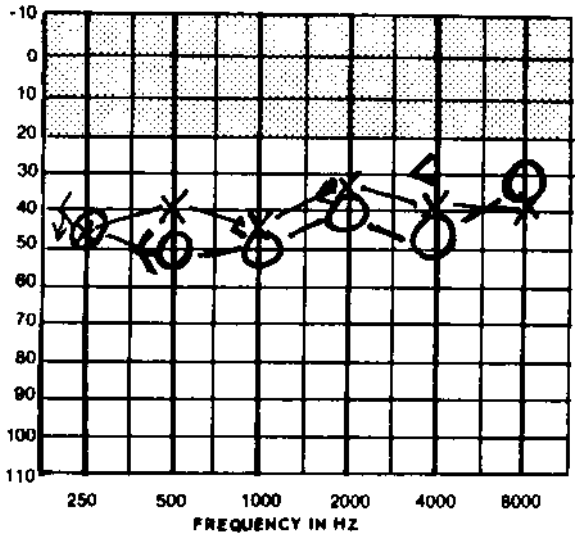

Figure 2. Audiogram of a 42-year-old male with progressive sensorineural hearing loss (*DFNA10*) (abscissa, frequency in Hertz; ordinate, hearing threshold level in dB; measurements of hearing sensitivity scaled so that 20 dB on the ordinate equals one octave on the abscissa; shaded area, normal hearing; ○, air conduction, right ear; ←, bone conduction, right ear; X, air conduction, left ear).

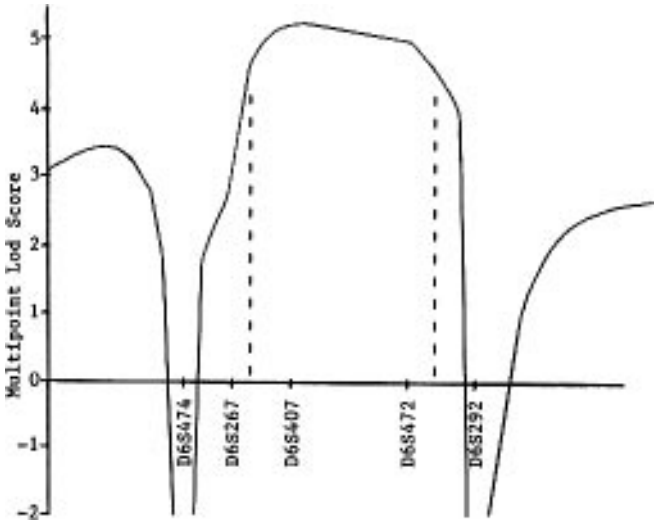

Figure 3. *DFNA10* placed in a multipoint map of the region constructed by ordering the markers as shown in Figure 1. D6S270 is not included as it was not possible to order this marker with respect to D6S472 and D6S292. The order of all markers is supported by odds of at least 1000:1 with the exception of D6S472 and D6S292 (700:1) (dashed line = 1-lod confidence interval).

(15), phospholamban (16) and connexin43 (GJA1) (17), none of these genes is known to be expressed in the inner ear or to belong to gene families important in hearing (Bussoli, T., and Fleming, J., pers. comm.).

Possible animal models of *DFNA10* based on synteny assignments and phenotype are the fused (*Fu*) and *Snell's waltzer* (*sv*) mouse mutants. The *Fu* phenotype is expressed in both homozygotes and heterozygotes and includes deafness, in addition to occasional tail shortening or kinking and a type of choreic behavior similar to, though more varied and chaotic than that of Japanese waltzing mice (18). The *sv* phenotype includes deafness, circling, hyperactivity and head tossing but expression is limited to homozygotes (19). The *sv* gene has been cloned and encodes an unconventional myosin, *Myo6* (20). Although the homologous gene has not yet been localized in humans, the position of *Myo6* on the mouse genome suggests that the human counterpart may map to a region more centromeric than the localization of *DFNA10* (20).

MATERIALS AND METHODS

Family data

The family in this study was ascertained through the University of Iowa Department of Otolaryngology-Head & Neck Surgery. A family history was obtained by questionnaire and personal interviews, and audiograms were reviewed on most persons. Blood samples were collected from cooperative family members.

Because age-of-onset of hearing loss was variable among affected persons, family members under 50 years of age who did not wear hearing aids were excluded from the linkage analysis. The portion of the extended family pedigree used to map *DFNA10* to chromosome 6 was limited to normal hearing individuals over 50 years of age and affected persons with hearing aids ($n = 9$) or documented hearing loss ($n = 2$). Amplification has been recommended for the latter two persons, both of whom have moderate-to-severe sensorineural hearing impairment.

Genotyping

DNA was prepared from peripheral blood lymphocytes (21) and amplified using polymorphic microsatellite primer pairs listed in the Genome Database (GDB). Amplification was performed by the polymerase chain reaction with 30 ng template DNA, 1 µl of each primer (10 µM), 1 µl each of 10 mM dATP, dTTP and dGTP, 1 µl [^{32}P]-dCTP together with 1 µl unlabeled 0.1 mM dCTP, polymerase buffer as supplied by the vendor, and 1 U *Taq* DNA polymerase (Amersham) under the following conditions: denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, for a total of 25 cycles followed by a post-PCR extension step at 72°C for 10 min. Reaction products were resolved on a 6% polyacrylamide gel, followed by drying and autoradiography.

Linkage analysis

Pairwise and multipoint linkage analyses were performed using the MLINK and LODSCORE modules of version 5.10 of the Linkage Program package (13). All analyses were performed with recombination ratios of $\theta_m/\theta_f = 1$. For construction of the multipoint linkage, genotypes (version 3) were obtained from the CHLC ftp site (ftp.chlc.org). The BUILD module of CRI-MAP version 2.4 was used to construct a multipoint map using different pairs as anchors for the BUILD runs (22). Markers were added in decreasing order of informativeness. The same multipoint map of five markers was obtained from each of the runs. The overall support for the map was evaluated using the FLIPS option of CRI-MAP to invert pairs of adjacent loci. This five marker map was used for the multipoint linkage analysis of *DFNA10* using the VITESSE version 1.0 program (23). Recombination distances between loci are based on reported data (24,25). The frequency

of the *DFNA10* gene was set at 0.001, and the disease was coded as fully penetrant and autosomal dominant.

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