Am. J. Hum. Genet. 61:924–927, 1997

A Novel Locus for Autosomal Dominant Nonsyndromic Hearing Loss, DFNA13, Maps to Chromosome 6p

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Summary

Nonsyndromic hearing loss (NSHL) is the most common type of hearing impairment in the elderly. Environmental and hereditary factors play an etiologic role, although the relative contribution of each is unknown. To date, 39 NSHL genes have been localized. Twelve produce autosomal dominant hearing loss, most frequently postlingual in onset and progressive in nature. We have ascertained a large, multigenerational family in which a gene for autosomal dominant NSHL is segregating. Affected individuals experience progressive hearing loss beginning in the 2d–4th decades, eventually making the use of amplification mandatory. A novel locus, DFNA13, was identified on chromosome 6p; the disease gene maps to a 4-cM interval flanked by D6S1663 and D6S1691, with a maximum two-point LOD score of 6.409 at D6S299.

Introduction

Inherited hearing impairment can be classified as syndromic or nonsyndromic, reflecting the presence or absence of inherited physical abnormalities, respectively. To date, 12 dominant, 17 recessive, 8 X-linked, and 2 mitochondrial loci for nonsyndromic hearing loss have been identified (Van Camp et al. 1997). Numbered sequentially on the basis of time of discovery, dominant loci carry the prefix DFNA, recessive loci the prefix DFNB, and X-linked loci the prefix DFN. Mitochondrial mutations are designated by the site of the mutation (e.g., A1555G, T7445C).

With the exception of single families linked to DFNA3 (Chaib et al. 1994), DFNA8 (Kirschhofer et al. 1996), and DFNA12 (Verhoeven et al. 1997), the typical phenotype of persons with autosomal dominant nonsyndromic hearing loss (ADNSHL) is one of late-onset, progressive sensorineural hearing impairment (Van Camp et al. 1997). Although advancing age is invariably associated with a decline in auditory acuity so that ~50% of octogenarians have a hearing loss >25 dB (Morton 1991), the relative contribution of ADNSHL to this prevalence figure is unknown.

In this article, we describe a large multigenerational nonconsanguineous American kindred of northern European extraction in which persons with hearing impairment experience progressive, postlingual hearing impairment beginning in the mid-frequencies and slowly progressing to all frequencies. All affected persons require bilateral amplification for effective communication.

Patients, Material, and Methods

Family Data

The family in this study was ascertained through the University of Iowa Department of Otolaryngology—Head and Neck Surgery. Family history was obtained by questionnaire and telephone interviews. Medical records and audiograms were reviewed, and blood samples were collected from all consenting individuals (27) (fig. 1).

Genotyping

Genomic DNA was extracted from whole blood by following a standard protocol (Grimberg et al. 1989), quantitated by spectrophotometric readings at optical density$_{260}$, and diluted to 30 ng/μl for amplification by PCR. After testing the family for linkage to the known ADNSHL and ARNSHL (autosomal recessive) loci (Van Camp et al. 1997), short tandem repeat polymorphism (STRP) screening set version 6, developed from markers generated by the Cooperative Human Linkage Center and made available through Research Genetics Inc., was used to screen the remainder of the genome (Sheffield et al. 1995).

PCR was performed using 30 ng of DNA in a 15.0-μl reaction mixture containing 1.5 μl buffer (100 mM TRIS-HCl pH 8.8, 500 mM KCl, 15 mM $MgCl_2$, and 0.01% w/v gelatin); 1 μl each of 10 mM dCTP, dGTP, and dTTP; 1 μl of unlabeled dATP (0.1 mM) supple-

Received June 4, 1997; accepted for publication July 22, 1997.

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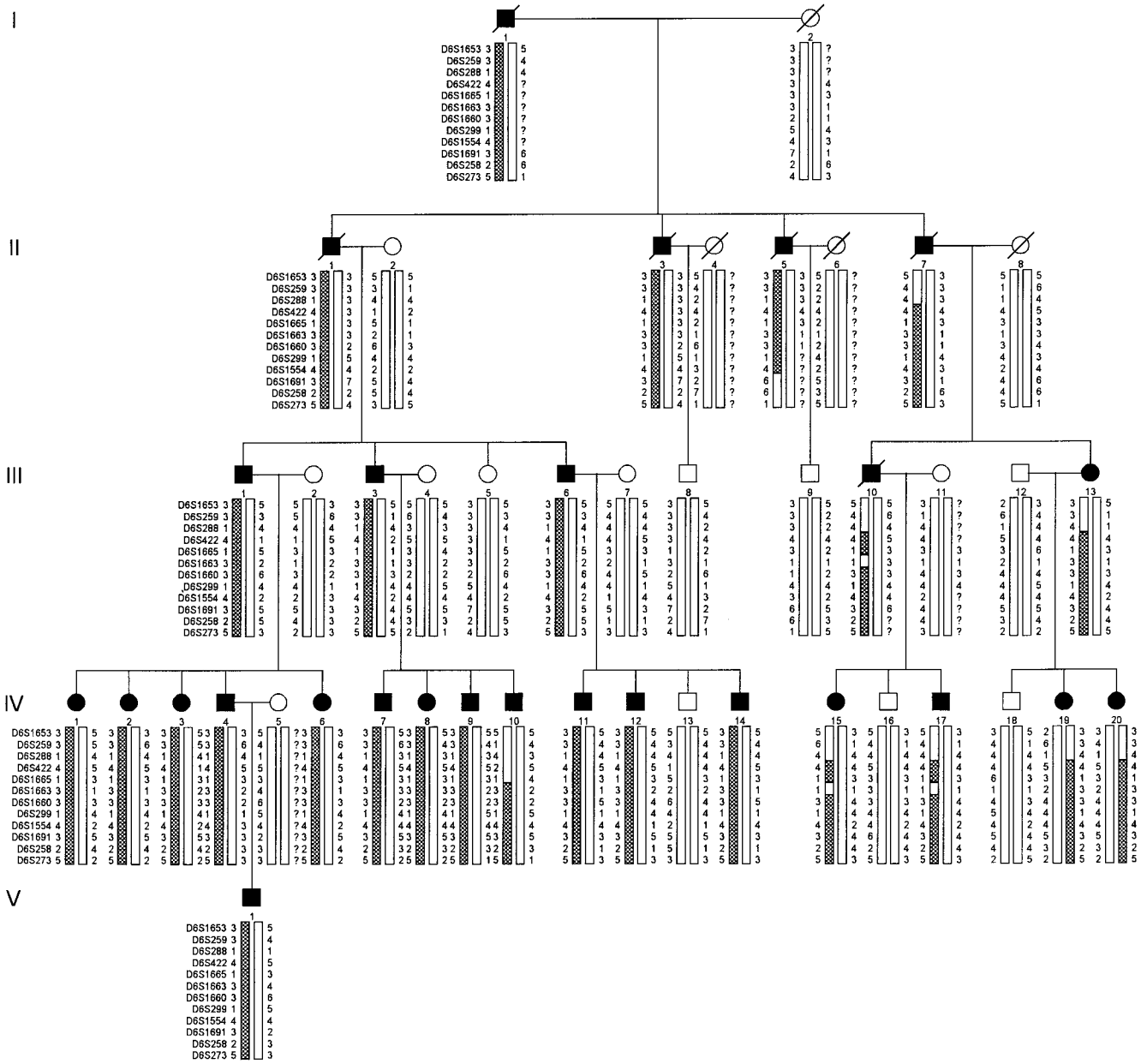

Figure 1 Pedigree of DFNA13 family demonstrating the segregation of STRPs on 6p. Flanking markers are D6S1663 and D6S1691, as defined by crossovers in individuals IV-10 and II-5. A double crossover at D6S1663 occurred in individual IV-10 and has been inherited by his affected offspring. (For individuals I-1, I-2, II-1 and II-3 to II-8, haplotypes are reconstructed; □ = male; ○ = female; ■ = affected male).

mented with 1 μl S35-dATP; 7.5 pmol of forward and reverse primers; 0.2 U *Taq* polymerase; and the remainder, ddH2O. Twenty-five cycles of amplification were completed at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Reaction products were resolved on 6% denaturing polyacrylamide gels (7.7 M urea) and visualized by autoradiography.

LOD Score Calculations

LOD scores were calculated using FASTLINK (Cottingham et al. 1993). The frequency of the DFNA13 gene was set at 0.001, and the disease was assumed to be fully penetrant and dominant.

Results

Disease Phenotype

Persons with hearing impairment in this kindred typically have a bilateral moderate-to-severe sensorineural loss. With the exception of individual V-1, affected persons are >20 years of age; all are otherwise healthy, with

Table 1

Two-Point Linkage Results Showing Z_{max} and θ

STRP	Z_{max}	θ
D6S1653	.140	2.154
D6S259	.304	.411
D6S288	.111	3.753
D6S422	.041	4.917
D6S1665	.040	5.431
D6S1663	.040	4.699
D6S1660	.001	5.400
D6S299	.001	6.409
D6S1554	.001	5.970
D6S1691	.075	4.840
D6S258	.000	4.911
D6S273	.053	3.808

normal life spans and no dystrophic features, learning disorders, or other abnormal findings.

Exclusion Analysis

Linkage to the known ADNSHL and ARNSHL loci was excluded (LOD scores <-2.0) by two-point analysis using the MLINK program package. Approximately 180 markers then were tested before a significant LOD score (>3.0) was obtained with D6S422. Using STRPs tightly linked to this locus, the DFNA13 interval was shown to be flanked by D6S1663 and D6S1691 and to span a 4-cM interval. A maximum two-point LOD score of 6.409 was obtained with D6S299 (table 1).

Discussion

The 4-cM interval to which the DFNA13 locus has been mapped is flanked by STRPs D6S1663 and D6S1691 and is tightly linked to the HLA class II region on chromosome 6p (Hanson et al. 1989) (fig. 1). This region has been intensely studied, with >40 human diseases reported to share a genetic predisposition with specific HLA II alleles (Cao et al. 1996). Relevant within this group of diseases, by virtue of an association with hearing impairment, are otosclerosis, Ménière disease, sudden sensorineural hearing loss, and progressive sensorineural hearing loss (Cao et al. 1996), suggesting that there may be important genes within the HLA II region that affect hearing. This hypothesis is made more attractive by the localization of DFNA13 to the region.

Also of interest, because of the possible interrelationship between auditory perception and reading, is the linkage of a quantitative trait locus for reading disability to chromosome 6p21.3 (Cardon et al. 1994), a genomic region originally targeted because of a hypothesized association between autoimmune disease and dyslexia (Geschwind and Galaburda 1985). Further work on dyslexic families has placed the dyslexia gene within the 10–16-cM region flanked by D6S109 and D6S306 (Grigorenko et al. 1997), completely encompassing the smaller 4-cM DFNA13 interval.

Two genes have been mapped to the region that may play a role in auditory function. The first, *COL11A2,* localizes to 6p21.3 (Hanson et al. 1989) near the proximal border of the HLA II region and is associated with a unique Stickler syndrome phenotype characterized by hearing impairment and cartilaginous defects of the face and extremities (Brunner et al. 1994). The other two chains of the type XI collagen heterotrimer, *COL11A1* and *COL11A3,* map to 1p21 and 12q14.3, respectively (*COL11A3* is not encoded by a separate gene but is believed to be a heavily glycosylated form of *COL2A1*) (Hanson et al. 1989). In contrast to the classic Stickler phenotype, lack of ophthalmological involvement in persons with the *COL11A2* defect reflects the absence of *COL11A2* in the vitreous (Brunner et al. 1994). Hearing impairment is most commonly a mild-to-severe sensorineural loss that slowly progresses to involve all frequencies (Brunner et al. 1994). Just as mutations in the genes for collagen types I and II cause a spectrum of phenotypes, allelic mutations in the *COL11A2* gene may cause hearing impairment in individuals with DFNA13. A possible animal model of the disease is the mouse mutant *chondrodysplasia* (*cho*). The homozygote phenotype is characterized by disproportionately shortened limbs, shortened head, protruding tongue, cleft palate, short mandible, and neonatal lethality caused by asphyxia due to defective tracheal rings (Lyon and Searle 1990).

The second gene mapped to 6p21, *OTF3,* is a transcription factor containing a *POU* domain (Guillaudeux et al. 1993). At least five *POU*-domain genes are expressed in the cochlea of the developing rat, and in the human *POU3F4* is responsible for the X-linked form of congenital hearing impairment, DFN3 (de Kok et al. 1995). The DFN3 phenotype is characterized by a progressive mixed loss, the conductive component of which is caused by stapes fixation (de Kok et al. 1996).

Also relevant as a positional candidate is a cochlear-derived expressed sequence tag (EST) that has been mapped to this region (http://www.ncbi.nlm.nih.gov/). This sequence can be extended to ~2,000 bp by cyberscreening of genetic databases such as dBest using BLAST. Although this EST must be characterized and screened for mutations, previous work suggests that there are likely to be several genes expressed in the cochlea within the currently defined region (Greinwald et al., in press). Because of the magnitude of work involved in identifying, characterizing, and screening these candidates, an alternative approach is to refine the candidate region by extending the pedigree. The large size of the DFNA13 kindred will permit us to adopt this strategy to reduce the 4-cM DFNA13 region to a more manageable interval.

Acknowledgments

We are grateful to family 1100, who made this research possible. This study was supported by a grant from the Deafness Research Foundation (to M.R.B.).

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