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LETTERS

An Autosomal Recessive Nonsyndromic Form of Sensorineural Hearing Loss Maps to 3p–*DFNB6*

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Autosomal recessive nonsyndromic hearing loss (ARNSHL) is the most common form of congenitally acquired inherited hearing impairment. Although numerous loci are believed to exist, only five have been identified. Using a pooled genomic DNA screening strategy, we have identified a sixth locus, *DFNB6*, on 3p in the interval bounded by *D3S1619* and *D3S1766*.

Autosomal recessive nonsyndromic hearing loss (ARNSHL) is the most common type of inherited hearing impairment (Bergstrom et al. 1971). Although almost exclusively monogenic, it is highly heterogeneous with some estimates of the number of gene loci exceeding 100 (Chung and Brown 1970). Only five loci have been identified (Guilford et al. 1994a,b; Baldwin et al. 1995; Friedman et al. 1995; Fukushima et al. 1995), however, as affected families cannot be pooled and single nonconsanguineous families rarely are informative enough to obtain linkage. Recently, we proposed simultaneous analysis of multiplex sibships of consanguineous unions as a mapping strategy to exploit the heterogeneity of ARNSHL for gene localization (Fukushima et al. 1995). The hearing-impaired progeny of a consanguineous union are expected to share a region of many centimorgans (cM) around the disease locus that is homozygous by descent—other genomic regions also are homozygous by descent but vary from one child to next. For this strategy to be successful, highly informative polymorphic markers must be used to minimize the false-positive rate (estimated at 5%; Fukushima et al. 1995), and powerful algorithms and software must be available to perform linkage calculations within reasonable time periods (Kruglyak et al. 1995).

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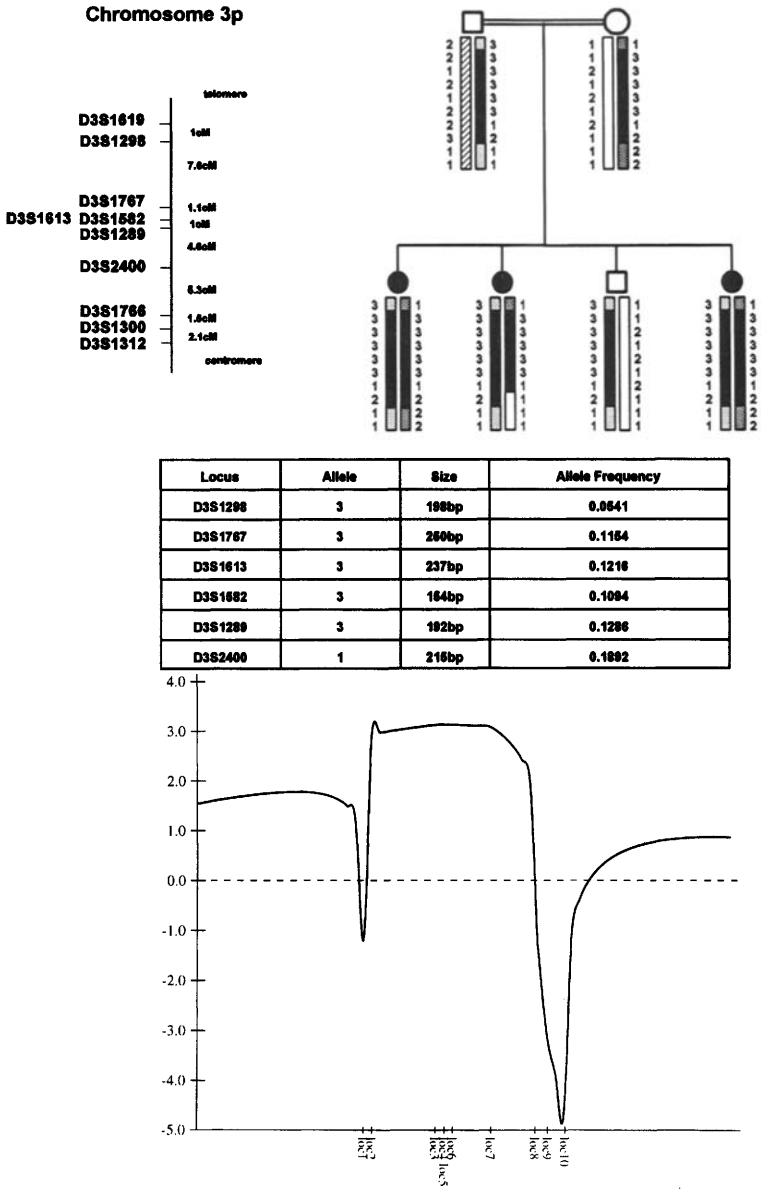
RESULTS

Single alleles were demonstrated in two of nine families using *D3S1767*. Two other markers tightly linked to *D3S1767* (*D3S1619* and *D3S1289*) did not shown allelic homozygosity in one of these families. In the remaining family, homozygosity by descent was demonstrated in the parents over an interval of 19–22 cM. From the affected progeny, the location of *DFNB6* was mapped to the 14- to 20-cM interval bounded by *D3S1619* and *D3S1766* ($\hat{Z} = 3.3$; Fig. 1).

DISCUSSION

This region is the site of several genes that could be essential for normal auditory function, including zinc finger protein 35 (Calabro et al. 1995), β-catenin (CTNNB1; Bailey et al. 1995), guanine



Locus	Allele	Size	Allele Frequency
D3S1298	3	198bp	0.0541
D3S1767	3	260bp	0.1154
D3S1613	3	237bp	0.1216
D3S1582	3	164bp	0.1094
D3S1289	3	192bp	0.1286
D3S2400	1	216bp	0.1892

Figure 1 Homozygosity by descent in a second cousin marriage localizing a gene for ARNSHL (*DFNB6*) to the interval flanked by *D3S1619* and *D3S1766* (□) male; (◯) female; (■) affected male. Allele frequencies are calculated in the population under study.

nucleotide-binding protein α-transducing polypeptide-1 (GNAT-1; Wilkie et al. 1992), laminin S (LAMS; R. Vuolteenaho, M. Nissinen, R.L. Eddy, T.B. Shows, and K. Tryggvason, pers. comm.), dystrophin-associated glycoprotein-1 (DAG; Ibraghimov-Beskrovnaya et al. 1992), long (electrocardiographic) QT syndrome-3 (LQT-3; Jiang et al. 1994), and SYNII (Li et al. 1995). Although the last two are intriguing as candidate genes for *DFNB6*, we have not been able to confirm expression of LQT-3 in a fetal-derived cochlear-specific cDNA library (R.J.H. Smith, unpubl.). A possible animal model of *DFNB6* is the *spinner* (*sr*) mouse mutant (Fox et al. 1978), which as a homozygote exhibits signs of defective hearing and shows the typical head tossing, circling, deafness, and hyperactivity of the Shaker–Waltzer mutants. Inner ear abnormalities in the *sr/sr* homozygote include degeneration of the organ of Corti and spiral ganglion, reduction in size of the stria vascularis, and degeneration of the saccular macula (Deol and Robbins 1962).

METHODS

A total of 54 unrelated simplex and multiplex families with probable ARNSHL have been ascertained by identifying probands from four schools for the deaf (St. Louis Institute for the Deaf and the Blind, CSI School for the Deaf, Little Flower Covenant School for the Deaf and Bala Vidyalaya School for the Deaf) and from the Institute of Basic Medical Sciences in Madras, India, the capital city of Tamil Nadu. Audiograms and data on age at onset of hearing loss were gathered through repeated household visits. Affected persons had congenital prelingual severe to profound hearing loss, and in most instances, demonstrated no response to auditory testing at equipment limits (Maico Pure Tone Audiometer). Conditions such as rubella, prematurity, drug use during pregnancy, perinatal trauma, sudden infant death syndrome, and meningitis were eliminated by history.

The biological relationship between spouses was determined by extensive questioning and verified by elderly members of the household. A subset of 26 families with two or more affected siblings was considered for possible homozygosity mapping. Within this subset, there were 11 first cousin unions, 7 uncle–niece unions, and 8 second cousin unions. The calculated maximal LOD score for individual families ranged from 1.8 to 4.53, nine families generated LOD scores of >3, and in two other families, LOD scores were 2.8 and 3.0 (calculation assumptions: an infinitely rare disease allele, an infinitely polymorphic marker, zero recombination between the marker and the disease allele). Of these 11 families, one was demonstrated to map to *DFNB2* and a second identified another locus for ARNSHL, *DFNB5* (Fukushima et al. 1995). The remaining nine families were used in this study.

Genomic DNA was prepared from blood samples (Grimberg et al. 1989), and pooled aliquots of DNA from all affected persons in each nuclear family were screened for allelic homozygosity with highly polymorphic markers (Fukushima et al. 1995). Markers were selected by focusing

on regions of high GC content (Antonarakis 1994). When allelic homozygosity was demonstrated with a particular marker, parents and nonaffected siblings were genotyped and haplotypes were reconstructed by typing other polymorphic markers in the region. If results suggested homozygosity by descent, more markers were typed to determine the size of the homozygous interval. Allele sizes were assigned in reference to an M13mp18 sequencing ladder, and genomic DNA from CEPH individual 1347-O2 with known alleles for each marker was typed with all DNA samples.

LOD scores were calculated using the MAPMAKER/HOMOZ computer package (Kruglyak et al. 1995), setting the frequency of each ARNSHL gene at 0.005 (Morton 1991) and coding the disease as fully penetrant. To guard against inflated LOD scores, the frequency of all homozygous alleles was determined by screening 35 unrelated individuals from the same geographic region and cultural background. A sensitivity analysis also was calculated by imposing a lower band of 10% on allele frequencies.

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