Linkage of Congenital Recessive Deafness (Gene DFNB10) to Chromosome 21q22.3

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Summary

Deafness is a heterogeneous trait affecting ~1/1,000 newborns. Genetic linkage studies have already implicated more than a dozen distinct loci causing deafness. We conducted a genome search for linkage in a large Palestinian family segregating an autosomal recessive form of nonsyndromic deafness. Our results indicate that in this family the defective gene, DFNB10, is located in a 12-cM region near the telomere of chromosome 21. This genetic distance corresponds to <2.4 Mbp. Five marker loci typed from this region gave maximum LOD scores ≥3. Homozygosity of marker alleles was evident for only the most telomeric marker, D21S1259, suggesting that DFNB10 is closest to this locus. To our knowledge, this is the first evidence, at this location, for a gene that is involved in the development or maintenance of hearing. As candidate genes at these and other deafness loci are isolated and characterized, their roles in hearing will be revealed and may lead to development of mechanisms to prevent deafness.

Introduction

It is estimated that ~50% of congenital deafness is caused by genetic defects. Most cases of deafness are not accompanied by additional obvious abnormalities and are therefore considered to be nonsyndromic. It is estimated that ~80% of inherited nonsyndromic deafness is caused by autosomal recessive defects, while 18% is caused by autosomal dominant defects and 2% is caused by defects in either mitochondrial genes or genes

on the X chromosome (Konigsmark and Gorlin 1976; Bodurtha and Nance 1988; Arslan et al. 1991; Beighton et al. 1991; Brunner et al. 1991; Cremers et al. 1991; Morton 1991; Arnos et al. 1992; Grundfast and Lalwani 1992; Reardon 1992; Liu et al. 1993; Marazita et al. 1993; Gold and Rapin 1994; Spillmann 1994). Recessive nonsyndromic deafness has been linked to chromosomal locations 13q12 (DFNB1), 11q13.5 (DFNB2), 17p11.2 (DFNB3), 7q22-31 (DFNB4), 14q12 (DFNB5), 3p14-21 (DFNB6), and 9q11-13 (DFNB7) (Guilford et al. 1994a, 1994b; Baldwin et al. 1995; Friedman et al. 1995; Fukushima et al. 1995, and in press; Jain et al. 1995). However, no genes involved in nonsyndromic recessive deafness have been cloned yet.

In this study, we present evidence for linkage between a gene for nonsyndromic recessive deafness (gene designation DFNB10) in a large Palestinian family and several markers located near the telomere of chromosome 21q. Most of the affected members of this family resulted from consanguineous unions, suggesting that they are homozygous for the same genetic defect. The high degree of inbreeding in this kindred composed of multiple large sibships facilitated the search for linkage.

Subjects and Methods

Subjects

Peripheral blood was obtained from members of an inbred Palestinian family. The pedigree shown in figure 1 constitutes a portion of a much larger kindred from a Muslim tribe consisting of several hundred individuals who reside in one small town in central Israel. Personal interviews with key figures in the kindred clarified consanguineous relationships throughout the past seven generations. More than 40 nonhearing members, probably affected with nonsyndromic autosomal recessive deafness, were identified in the last three generations (not all are shown in pedigree). There was one mating between a deaf person (individual 23) and a hearing carrier (individual 41), resulting in five hearing and four deaf children, and there was one mating between two
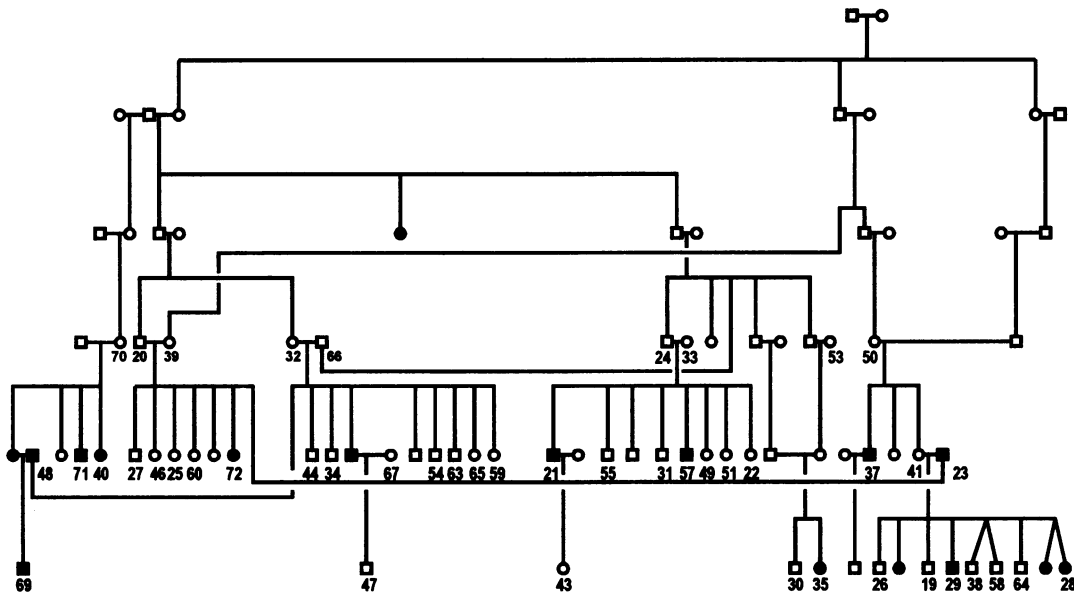
Figure 1   Pedigree of Palestinian kindred segregating nonsyndromic sensorineural deafness. Identification numbers are given below the symbols for members who were genotyped.

deaf members (individual 48 and untyped spouse), which produced one deaf child (individual 69).

Hearing evaluations of affected and unaffected members in the pedigree consisted of pure-tone audiometric tests, which showed severe deafness without any hearing remnants, at a level of ≥75–80 decibels. All affected individuals from various age groups showed the same level of severe hearing loss, implying that deafness was not progressive. None of the patients showed signs of vestibular involvement. At 1 wk after birth the twin girls (individual 28 and her co-twin; see fig. 1) underwent a BERA (brain stem–evoked potential) test, the results of which confirmed the diagnosis of sensorineural deafness. All of the affected individuals were also examined for defects in ear morphology, mental retardation, and any other defects that could indicate that deafness was part of a syndrome. All deaf individuals showed an otherwise normal phenotype and normal intelligence.

Typing Microsatellite Markers

Peripheral blood was obtained from all subjects, in accordance with institutional guidelines for human subjects, and DNA was isolated from blood leukocytes by the proteinase K-SDS method (Sambrook et al. 1989). Oligonucleotides for typing microsatellite markers were synthesized on a Milligen 7500 DNA synthesizer. A standard amplification reaction (10 μl) was performed with 40 ng genomic DNA and 1.0 pmol each primer (Sakai et al. 1988). Cycling parameters consisted of 95°C for 5 min, followed by 35 cycles of 94°C (1 min), 60°C (30 s), and 72°C (30 s). Prior to amplification, one primer was end-labeled with 32-P with polynucleotide

kinase (New England Biolabs), in order that the amplification product could be visualized by autoradiography after application to a standard sequencing gel. Highly polymorphic markers generated by the Cooperative Human Linkage Center (Murray et al. 1994) and Généthon (Gyapay et al. 1994) were used. To facilitate the genome search, screening was limited to a subset of the family, including all affected individuals and their parents. All subjects for whom we had a DNA sample (see fig. 1) were typed for markers in regions showing suggestive evidence for linkage.

Statistical Analysis

Two-point linkage analysis was performed by using the computer program LIPED (Ott 1991). The disease was modeled as an autosomal recessive trait with complete penetrance at birth. Computation of two-point LOD scores (Z) in this complex pedigree was facilitated by reducing the number of alleles for each marker system to no more than five, by using the scheme of Braverman (1985). Four inbreeding loops (focused at the matings of individuals 39, 50, and 66 and at the parents of individual 35) were preserved in the pedigree. A disease-allele frequency of .005 and equal allele frequencies within each marker system were assumed initially. These assumptions were challenged by repeating the analysis with a disease-allele frequency of .01 and varying the frequency of linked marker alleles from .1 to .5. Conclusions about linkage or the maximum-likelihood estimates of recombination (θ) were unchanged (results not shown).

Support for linkage in various intervals of the linkage

map of 21q22-qter was evaluated by multilocus linkage analysis using FASTLINK version 2.3 (Cottingham et al. 1993). This analysis was computationally intractable in this large kindred composed of numerous inbreeding loops and several generations of individuals providing relationship ties but no marker genotype information. To make this problem computational feasible, only a single inbreeding loop (doubled individual 66) was preserved, and the number of alleles was further reduced to two, three, or four alleles for each marker system. The effects of reconfiguring the pedigree and allele reduction were assessed for pairwise comparisons, and conclusions about linkage or the maximum-likelihood estimates of θ were unchanged (results not shown). The following sex-averaged genetic map, which was derived from the ones presented by Buetow et al. (1994) and Gyapay et al. (1994), was utilized for markers in the candidate region: cen–D21S259–(1 cM)–D21S1255–(3 cM)–D21S168–(5 cM)–D21S1260–(4 cM)–D21S1259–tel. Two overlapping five-locus (i.e., four markers plus the disease locus) analyses were carried out in a manner described elsewhere (Bowcock et al. 1987). Odds for order over all intervals were obtained by scaling the end intervals on the basis of the overlapping region.

Haplotypes were constructed for all persons in the pedigree by using the chromosome 21 markers included in the multilocus analysis. The most parsimonious interpretation of linkage phase (i.e., the one minimizing the number of recombination events or new haplotypes) was accepted for the few individuals showing ambiguous haplotype data. The assignment of some haplotypes to the pool of deaf or nondeaf chromosomes could not be made in several hearing sibs of deaf individuals who had inherited one intact nondeaf chromosome and one recombinant chromosome that could possibly harbor the deaf allele.

Results

A search for linkage of DFNB10 in family BT117 to >250 markers (initially by using RFLPs and, subsequently, di-, tri-, and tetranucleotide repeat polymorphisms) distributed on all autosomes excluded ~40% of the genome. Suggestive evidence for linkage to marker D21S1270 (maximum $Z = 1.07$ at $\theta = .10$) was obtained. Analyses of additional markers near D21S1270 then established linkage to the telomeric region of chromosome 21. Five markers showed significant evidence for linkage ($Z \geq 3.0$) (table 1). Obligate recombinants were detected with D21S1255 and the two markers proximal to this locus. Single recombination events were inferred for D21S168 and D21S1260. A maximum Z was obtained at $\theta = 0$ for only one marker (D21S1259).

A multilocus Z analysis, using five markers in this region, was performed to determine odds for order over all intervals in this region. This analysis showed maximum support for linkage at D21S1260. However, the disease gene could not be excluded from the region between D21S1255 and D21S1259, since the odds for linkage to any location within this region were only 1.5 times less than odds for linkage to the location with maximum support. The inability to localize DFNB10 to a smaller interval—and, specifically, to the region adjacent to D21S1259—as suggested by the pairwise analysis, is probably related to the reduction in the number of inbreeding loops and to a concomitant loss of meiotic information, which was necessary to perform the multilocus analysis. The interval between D21S259 and D21S1255 was excluded, with odds of ~200,000:1.

Evaluation of haplotypes constructed from the markers included in the multilocus analysis revealed critical recombination events above D21S168. Recombination in the maternal chromosome of individual 19 places DFNB10 within a 12-cM region between D21S1255 and the telomere of 21q. This conclusion is supported by the LOD-score analyses. Three disease core haplotypes, based on markers below D21S1255 (D21S168–D21S1260–D21S1259), were evident in this pedigree and are as follows: 5-5-4, 3-1-4, and 5-2-4 (see haplotypes a–d in table 2). These haplotypes were not present on any nondeaf chromosomes. It is noteworthy that all affected members were homozygous for the 4 allele of D21S1259, which is located at the telomere of chromosome 21q, and that none of the marker alleles for loci proximal to D21S1259 were homozygous in all deaf persons. This observation and the trend toward increasing recombination frequency with markers located proximally to D21S1259 suggest that DFNB10 is closest to D21S1259. The finding of higher Z values for D21S1260 and D21S168 than for D21S1259 is probably misleading, because the 4 allele is relatively frequent in this pedigree. Four of the 10 nondeaf haplotypes (e–h) and 1 deaf unknown haplotype (s) also have the 4 allele (table 2).

Discussion

Large consanguineous families from isolated populations have been instrumental in mapping recessive deafness loci (Guilford et al. 1994*a*, 1994*b*; Baldwin et al. 1995; Fukushima et al. 1995, and in press; Jain et al. 1995). Here we have presented evidence for linkage of nonsyndromic recessive deafness, in a large Palestinian family, to a 12-cM region at the telomere of chromosome 21q. This is the first step in cloning and characterization, at this chromosomal location, of a gene that is involved in development or maintenance of hearing.

We have assumed that deafness in family BT117 is due to a single genetic defect, because this family is from a relatively small mating population and practices an

Table 1

LOD Scores for DFNB10 and Chromosome 21q Markers

MARKER	Z AT θ =							θ̂	MAXIMUM Z
	0	.01	.05	.1	.2	.3	.4		
D21S1270	−.27	.31	.94	1.07	.85	.48	.14	.10	1.07
D21S259	−∞	2.29	2.96	2.92	2.26	1.34	.46	.07	3.00
D21S1255	−∞	3.1	3.76	3.65	2.79	1.67	.64	.06	3.77
D21S168	4.29	4.41	4.45	4.06	3.08	1.94	.79	.03	4.46
D21S1260	4.86	4.94	4.74	4.22	2.99	1.76	.67	.01	4.93
D21S1259	4.15	4.06	3.69	3.21	2.26	1.36	.58	0	4.15

endogamous marriage system. However, we found four haplotypes associated with the deafness allele (table 2). One of these haplotypes can be accounted for by recombination occurring among persons whose DNA was studied. Two of the other three haplotypes may represent historical recombinants that occurred in persons from the older generations. A second explanation for the large number of haplotypes in table 2 is mutation at the microsatellite loci. Although markers having mutation rates as high as 1/250 have been reported (Hastbacka et al. 1992), current estimates are 10^{-4} (Bowcock et al. 1994). Alternatively, lack of homozygosity for most markers may be interpreted as evidence for multi-

ple ancestral mutations in the same gene (i.e., DFNB10). Our finding of multiple deafness loci in another consanguineous family from the Middle East demonstrates the plausibility of this hypothesis (Baldwin et al. 1995).

This precedent notwithstanding, our data are consistent with a single mutation identical by descent. Lack of homozygosity may be attributed to high recombination rates associated with telomeric regions of most chromosomes, including chromosome 21 (Tanzi et al. 1988; Burmeister et al. 1991; Petersen et al. 1991). Excessive recombination may have led to the occurrence of the large number of haplotypes observed in a single inbred family (table 2). The 12-cM region between D21S1255

Table 2

Distribution of Marker Haplotypes on Deaf and Nondeaf Chromosomes

HAPLOTYPE	MARKER[a]				CHROMOSOME[b]	
	D21S1255	D21S168	D21S1260	D21S1259	Deaf	Nondeaf
a	1	5	5	4	+	−
b	3	5	5	4	+	−
c	6	3	1	4	+	−
d	5	5	2	4	+	−
e	1	4	3	4	−	+
f	4	4	3	4	−	+
g	5	4	3	4	−	+
h	6	3	2	4	−	+
i	1	6	2	3	−	+
j	5	4	2	2	−	+
k	4	5	2	2	−	+
l	5	4	5	X	−	+
m	2	X	5	1	−	+
n	3	3	3	3	−	+
o	6	3	1	1	?	?
p	6	3	1	2	?	?
q	1	5	3	3	?	?
r	1	5	5	2	?	?
s	4	X	1	4	?	?

[a] X = genotype unknown.
[b] + = Present; − = absent; and ? = unknown.

and D21S1259 corresponds to a physical region of ~2.4 Mbp (Wang and Smith 1994). This ratio of 200,000 bp/ cM is approximately five times less than the purported genome average of 1 Mbp/cM. These facts suggest that homozygosity would be observed only for markers within several hundred thousand base pairs of DFNB10. Furthermore, if the deafness-associated allele for marker D21S1259 in all affected members is identical by descent, then DFNB10 is located within the 4-cM region between D21S1260 and the telomere. Thus, D21S1259 may be a very important landmark for cloning the deafness gene in this family.

Since the affected individuals in family BT117 do not have any visible abnormalities other than deafness, one might predict that the gene defect affects a protein that has a fundamental role in hearing. This is in contrast to syndromic forms of deafness such as Waardenburg syndrome, which is caused by defects in proteins regulating early development (Baldwin et al. 1992; Tassabehji et al. 1995), or Alport syndrome, which is associated with defects in proteins common to multiple anatomic structures (Barker et al. 1990). Thus, nonsyndromic genetic disorders offer the opportunity for identifying genes with specific structural or physiologic roles in complex and fragile tissues, such as the inner ear, that are difficult to isolate and study. We expect that positional cloning of DFNB10 will lead to the identification and characterization of a single gene that has a critical role in the development or maintenance of hearing.

Note added in proof.—In a recent independent study by Veske et al. (1996), linkage of deafness (locus DFNB8) to the same chromosomal region has been established in an inbred Pakistani family.

Acknowledgments

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