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Linkage of DFNB1 to Non-Syndromic Neurosensory Autosomal-Recessive Deafness in Mediterranean Families

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Key Words

Hearing loss · Non-syndromic neurosensory autosomal-recessive deafness · Linkage analysis · Dinucleotide repeat polymorphism

Abstract

Recent studies show a susceptibility locus (*DFNB1*) responsible for non-syndromic neurosensory autosomal-recessive deafness (NSRD) mapping to the pericentromeric region of chromosome 13q. In order to better understand the frequency with which *DFNB1* is the gene for deafness in our patient population and the role of DFNB1 in Caucasians, we performed a genetic linkage study with four microsatellite markers linked to *DFNB1* in a total of 48 independent Mediterranean families, of which 30 and 18 were of Italian and Spanish descent, respectively. A maximum two-point lod score of 7.28 was found with marker *D13S115* at a recombination frequency of Θ 0.1. Significant lod scores were also obtained for *D13S143, D13S292* and *D13S175.* Genetic heterogeneity was confirmed using the HOMOG program which indicated absence of linkage to *DFNB1* in approximately 21% of the sample. This study clearly demonstrates that *DFNB1* plays an important role in 79% of Mediterranean families with NSRD. Furthermore, results from multipoint analysis predict that the *DFNB1* gene maps between markers *D13S175* and *D13S115* which are separated by approximately 14.2 cM.

Introduction

Hereditary hearing loss comprises a broad spectrum of forms ranging from simple deafness to genetically determined syndromes. Non-syndromic neurosensory autosomal-recessive deafness (NSRD) is the most common form of genetic hearing loss accounting for about half of childhood prelingual deafness [1–3]. Over the past few years several studies of large pedigrees of geographically and/or culturally isolated populations have contributed to the mapping of 12 human candidate genes (DFNB) responsible for NSRD. However, limited clinical differentiation and marked genetic heterogeneity make gene(s) identification a challenging task. *DFNB1* and *DFNB2* were iden-

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tified by homozygosity mapping of deafness in consanguineous families from Tunisia to chromosomes 13q12 and 11q13.5, respectively [4, 5]. *DFNB3* on 17p11.2-q12 was also linked to deafness in consanguineous families from a remote village in Bali [6]. A fourth deafness locus, *DFNB4*, described in Middle Eastern Druze individuals, was positioned at 7q31 [7]. Three additional candidate genes, *DFNB5, DFNB6* and *DFNB7*, mapped to 14q12, 3p14-p21 and 9q13-q21, respectively, by studying multiple inbred families from India [8–10]. *DFNB8* was mapped to the distal arm of chromosome 21 in a family with NSRD from Pakistan [11]; *DFNB9* located at 2p22-23, was described in a consanguineous family living in an isolated region of Lebanon [12]. Finally, *DFNB10* was recently located in a 12 cM region near the telomere of chromosome 21 in a large inbred Palestinian family [13] and *DFNB12* to 10q21–22 in a consanguineous Sunni family from Syria [14].

The *DFNB1* locus was recently studied in 19 families of Celtic ancestry, and those results suggest an important contribution of this locus to deafness in the Caucasian population [15]. In contrast, a study in 15 consanguineous NSRD families from Pakistan showed linkage to chromosome 13 in only one kindred [16]. In order to further investigate the role of *DFNB1* in NSRD families of Mediterranean descent, we have conducted linkage analysis in 48 families of Mediterranean descent with four markers near the *DFNB1* locus. We present here evidence for an important contribution of the *DFNB1* locus to hearing impairment in these populations.

Materials and Methods

Thirty Italian and 18 Spanish families with deafness were included in the study. They were recruited in several clinical genetics services and schools for the deaf from Southern Italy and Spain. Diagnosis of non-syndromic genetic neurosensory deafness was established according to accepted clinical criteria [17]. Only families with at least 2 affected members and a recessive pattern of inheritance were included in the study. The majority of our families included large pedigrees having several healthy and affected individuals. Only a small proportion was characterized as nuclear families with 2 affected sibs (5 of 30 Italian and 6 of 18 Spanish families). In these cases, although formally possible, dominant inheritance seems unlikely given the absence of deafness elsewhere in the pedigree. We therefore assumed recessive inheritance. Five Italian and 5 Spanish consanguineous families were also included. Examples of family pedigrees included in the study are reported in figure 1. In all cases, care was taken to eliminate families in which pharmaceutical treatment, infection or oto-trauma might have been responsible for deafness. Audiometric evaluations were done on affected individuals and their relatives. Hearing loss was severe or profound at the middle and higher frequencies with a few individuals showing some sparing at lower frequencies. Pure-tone audiometry with air and bone conduction at 125, 250, 500, 1,000, 2,000, 4,000 and 8,000 Hz was performed with a Beltone 2000 audiometer. The younger children were tested for auditory brainstem responses (ABR) using a Lindar apparatus. Early onset of the disease was detected in the first year of life. Differential diagnosis of other forms of non-syndromic deafness, such as Usher syndrome, was considered in all cases but excluded on the basis of clinical, audiometric and laboratory findings.

The total number of individuals available for linkage analysis was 382. The 30 Italian and 18 Spanish families included 76 and 45 affected individuals, respectively, leaving 261 unaffecteds for study. Informed consent was obtained from all subjects and from parents of under-aged patients.

Four chromosome 13 microsatellite markers linked to the *DFNB1* locus were investigated in each family using a 373A DNA sequencer and GeneScan software (PE-ABD, Foster City, Calif., USA). High-molecular-weight DNA was extracted from peripheral blood leukocytes using previously described protocols [18] or with a nucleic acid extractor (PE-ABD). PCR primers were synthesized on a 394 DNA Synthesizer (PE-ABD) [19] with fluorochrome-labeled primers prepared by chemically attaching the fluorescent amidite reagent 6-FAM. Deprotection and purification was done using the recommended protocol for dye-labeled oligonucleotides [20]. Primer sequences for *D13S175, D13S143, D13S115* and *D13S292* were as previously described [21–23]. DNA concentration was adjusted to 40 ng/ml and PCR amplification was performed on an automated thermal cycler (Perkin Elmer, Norvalk, Conn., USA) using conditions previously described for each microsatellite marker. Following PCR amplification, samples were multiplexed at the gel loading step. Electrophoresis was done on 4.75% (w/v) polyacrylamide gels in 8 *M* urea containing 1X TBE. Data was collected using GeneScan Data Collection program version 1.1 and analyzed using GeneScan Data Analysis software version 1.2.1b1 or Genotyper version 1.1 (PE-ABD).

Linkage was assessed by analysis of allele segregation of the four different polymorphic markers (*D13S175, D13S143, D13S115* and *D13S292*). Pairwise linkage analysis was performed using MLINK and ILINK from the FASTLINK program package (version 2.2) [24]. Gene frequency was based on NSRD disease prevalence ($q = 0.001$), and full penetrance was also assumed. Calculations were performed at recombination fractions (Θ) of 0, 0.05, 0.10, 0.20, 0.30 and 0.40 between each marker and the disease locus. Markers allele frequencies were as previously reported (Genome Data Database: http://gdbwww.gdb.org/gdb/gdbtop.html).

Multipoint linkage analysis using Linkage LINKMAP software package [25] was performed with the *DFNB1* locus against a fixed genetic map with markers *D13S175, D13S143, D13S115* and *D13S292*. Genetic distance between markers was calculated from data obtained from the chromosome 13 map matched with that obtained in our families. Multipoint analyses were done by recoding the markers to five alleles in each family separately. Recombination frequencies were transformed to map distances by Kosambi's formula and multipoint analyses assumed no interference or sex differences in recombination frequencies [26].

The HOMOG program was used to test for nonallelic heterogeneity using multipoint lod scores calculated for 122 locations of the disease gene at 1-cM intervals within and on either side of the map of markers [26].

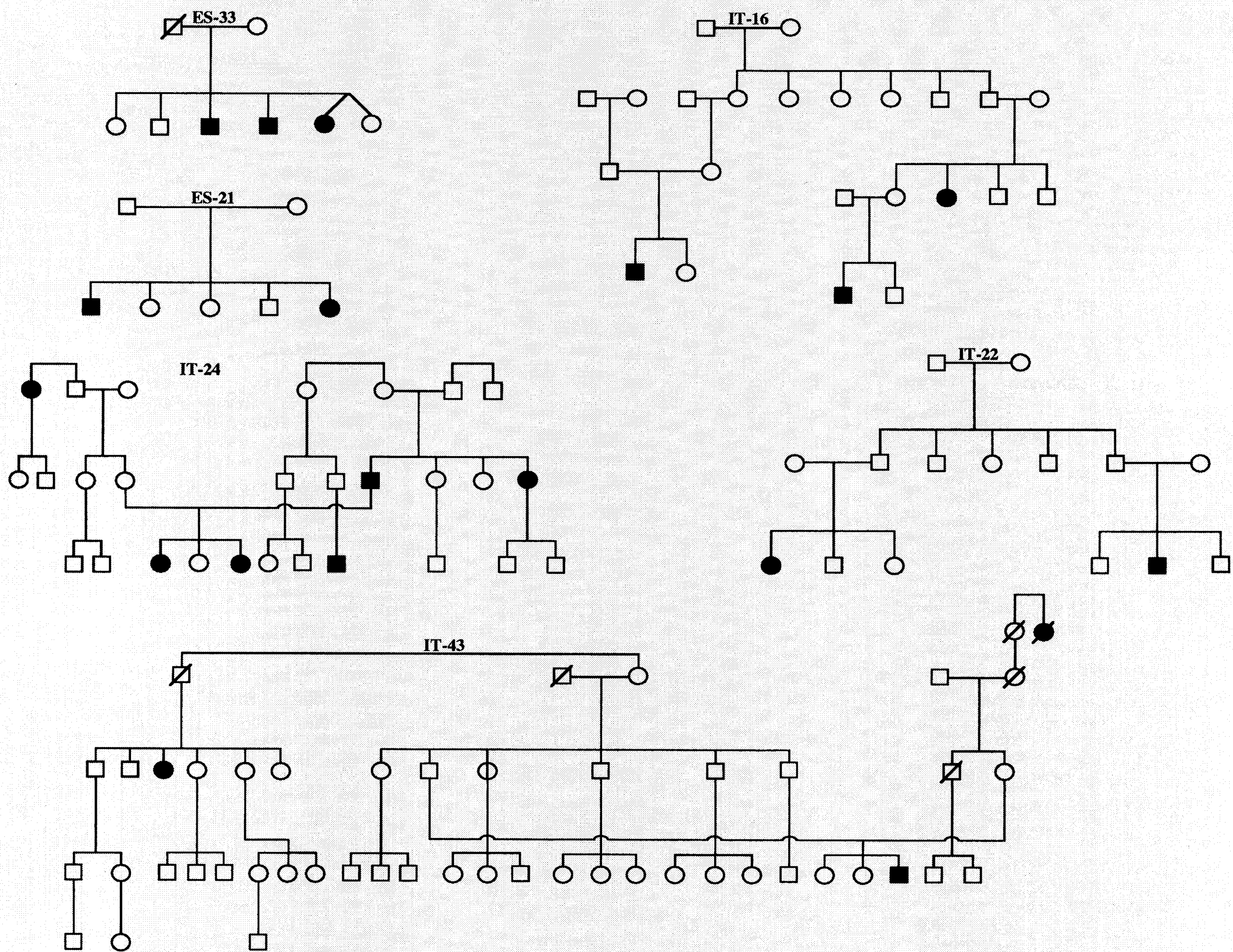

Fig. 1. Examples of pedigrees of some of the families with NSRD. Individuals with deafness are indicated by filled symbols.

Results

To ascertain the frequency with which *DFNB1*, located on chromosome 13, is the gene responsible for deafness in our patient population, we performed linkage analysis with four microsatellite markers which map close to *DFNB1* in 48 large NSRD pedigrees. Pairwise linkage analysis in the 30 Italian families gave positive lod scores with all four markers of chromosome 13 (table 1). The highest linkage was for *D13S115* with a lod score of 5.39 at a recombination frequency (Θ) of 0.09. In addition, positive results were obtained with *D13S143* ($Z = 5.25$ at $\Theta = 0.052$). The same analysis performed on the 18 Spanish families indicated *D13S175* as the most tightly linked marker ($Z = 3.40$ at $\Theta = 0.06$) (table 1B). Positive but not significant lod scores were also obtained with the remaining three markers, particularly with *D13S115* ($Z = 2.02$ at $\Theta = 0.13$). Pooling the two populations under study, a lod score of 7.28 at $\Theta = 0.107$ was detected between *DFNB1* and *D13S115*. Significant lod scores were also obtained with the other three markers. These findings clearly demonstrate that *DFNB1* plays a major role in determining deafness in our populations.

To define the position of *DFNB1* relative to the four markers under study a multipoint linkage analysis was done. The maximum map-specific lod score obtained in multipoint analysis was 8.91, and was found in the interval between *D13S175* and *D13S115*. This location is fur-

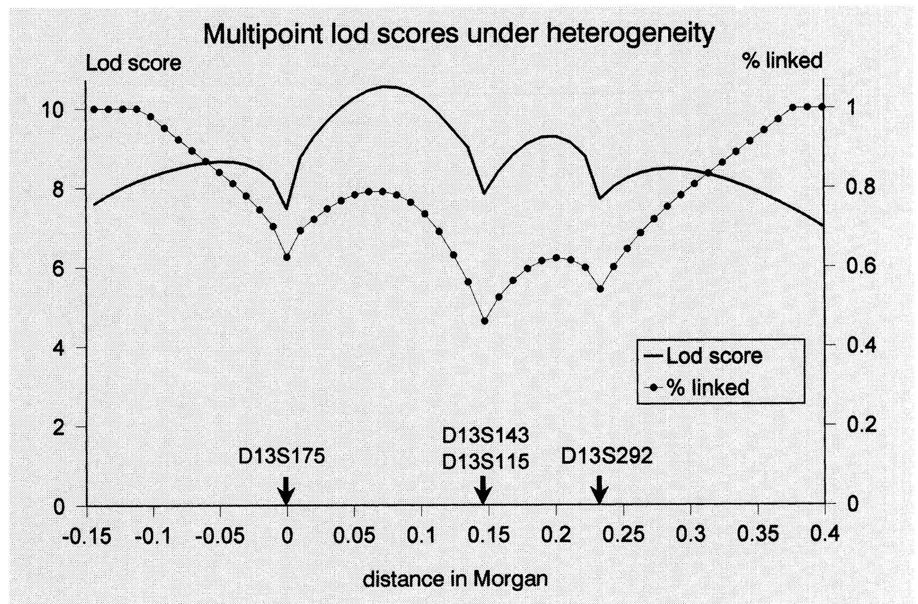

Fig. 2. Multipoint linkage map assuming a fixed genetic map consisting of markers *D13S175, D13S143, D13S115* and *D13S292* with respect to locus trait DFNB1, in Italian and Spanish families affected with NSRD. We placed the 0 cM genetic distance value at *D13S175*. The conversion from recombination fraction values to genetic distances in centi-Morgans has been done according to the Kosambi map function. The peak of the lod score curve favors a location of *DFNB1* between *D13S175* and *D13S115*, which encompasses a genetic region of 14.2 cM.

Table 1. Two-point lod scores between chromosome 13 loci and *DFNB1* obtained for 30 Italian (A), 18 Spanish (B) and combined (C) families, respectively

Locus	Recombination fraction							Z_{max}	Θ
	0.0	0.01	0.05	0.1	0.2	0.3	0.4		
A									
D13S175	∞	−3.80	0.22	1.32	1.43	0.85	0.27	1.55	0.152
D13S143	∞	4.06	5.25	4.86	3.20	1.54	0.43	5.25	0.052
D13S115	∞	0.85	4.89	5.37	4.02	2.11	0.61	5.39	0.09
D13S292	∞	0.01	4.09	4.59	3.36	1.67	0.42	4.61	0.09
B									
D13S175	∞	2.10	3.37	3.25	2.24	1.14	0.37	3.40	0.06
D13S143	∞	−4.50	−0.42	0.71	0.94	0.54	0.15	1.00	0.164
D13S115	∞	−3.66	0.83	1.89	1.76	0.97	0.28	2.02	0.134
D13S292	∞	−3.80	0.19	1.21	1.27	0.73	0.23	1.39	0.148
C									
D13S175	∞	1.77	3.59	4.58	3.68	2.00	0.60	4.59	0.108
D13S143	∞	−0.46	4.83	5.58	4.15	2.09	0.58	5.58	0.095
D13S115	∞	−2.80	5.72	7.26	5.78	3.09	0.89	7.28	0.107
D13S292	∞	−3.78	4.28	5.81	4.63	2.40	0.65	5.84	0.111

Z_{max} is the peak lod score and Θ the corresponding recombination fraction.

ther supported by a recombinational event detected in two affected members in two unrelated families (data not shown). In both cases the recombinations are in agreement with a *DFNB1* gene location centromeric to marker *D13S115*.

For each of the 122 map positions of the disease locus that were evaluated, a maximum multipoint lod score was calculated under non-allelic heterogeneity by varying alpha, the proportion of linked families (fig. 2). Again, lod scores peaked in the interval between *D13S175* and *D13S115* (table 2), with a best estimate of alpha of 0.79. From the maximum heterogeneity lod score of 10.54 and the maximum lod score under homogeneity (8.91), odds in favor of heterogeneity were calculated to be 42.7 to 1. A

Table 2. Heterogeneity test of DFNB1 vs. D13S115 (HOMOG)

Sample	Homogeneity		Nonallelic heterogeneity		
	Z_{max}	at[1]	Z_{max}	at[1]	alpha
Italians	7.77	30	8.31	20	0.71
Spanish	3.21	–7	3.34	–2	0.77
Total	8.91	7	10.54	7	0.79

[1] Locations are given in cM from *D13S175*.
alpha = Proportion of linked families.

one lod-unit down region was constructed jointly for alpha and the map location, and included alpha values between 0.52 and 0.917, and locations from 3 through 11 cM from *D13S175* in the direction of *D13S115*. There was little difference between the Italian and the Spanish data set with respect to the estimated contribution of the *DFNB1* locus: 71 and 77% of families showed linkage, respectively (table 2), when evaluated at the best location for each separate data set, and 80 and 78%, respectively, when evaluated at the best location for the combined data set. Data were analyzed further with the HOMOG2 program, which allows for the presence of two different disease loci within the same map. This analysis yielded only slightly higher lod scores than the previous analyses (Z_{max} = 10.89), and therefore there appears to be little support for the presence of two deafness loci in the 13q12 region.

Discussion

This work represents the first large-scale study on linkage analysis to *DFNB1* of Mediterranean families with NSRD. *DFNB1* was originally mapped in 2 large consanguineous Tunisian kindreds [4]. Its contribution to NSRD in a Pakistani population, from a study of 27 families in the Mirpur region [16], was apparently small, with only 1 family showing linkage. However, a study based on 19 Anglo-Saxon/Celtic families from New Zealand and Australia indicated that a more substantial fraction of NSRD in this population might be due to a mutation at the *DFNB1* locus [15]. While statistically significant linkage was not demonstrated for *D13S175, D13S143* and *D13S115* in this study, nine families showed cosegregation of marker haplotypes with deafness, indicating that the *DFNB1* locus contributes to a fraction of congenital NSRD in individuals of Anglo-Saxon/Celtic ancestry. In this report we clearly demonstrate that *DFNB1* plays an important role in determining deafness in Italian and Spanish patients. In both the Italian and the Spanish NSRD data sets, a large and strikingly similar proportion of families (80 and 78%, respectively) show linkage to DFNB1. The observed odds in favor of nonallelic heterogeneity (42.7 to 1) show that other genes must be involved in determining NSRD in these populations. It is not clear if a single additional gene or multiple genes account for the remaining 21% of the population. Our preliminary data on *DFNB2* and *DFNB4* suggest that *DFNB4* accounts for some of the additional cases of NSRD.

With respect to the location of *DFNB1*, our results suggest that its most likely position maps between *D13S175* and *D13S115*, which were found to be 14.2 cM apart. This location is further supported by two recombinational events detected in affected members from two unrelated families. In both cases the recombinations are in agreement with a *DFNB1* gene location centromeric to the marker *D13S115*.

Work is now focused on analysis of additional chromosome 13 markers in *DFNB1*-linked families to further narrow and refine the location of the *DFNB1* gene. Definition of the two recombinants and identification of additional ones will help in defining the exact location of the *DFNB1* gene. In addition, work continues to determine how many and which loci are linked to NSRD in those families which do not show linkage to *DFNB1*.

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