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Mapping of a Novel Autosomal Recessive Nonsyndromic Deafness Locus (DFNB46) to Chromosome 18p11.32-p11.31

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Abstract

Hereditary nonsyndromic deafness (NSD) is extremely heterogeneous. Autosomal recessive (AR) forms account for ~75% of genetic cases. To date, over 40 ARNSD loci have been mapped. A novel locus (DFNB46) for ARNSD was mapped to chromosome 18p11.32-p11.31 in a five-generation Pakistani family. A 10 cM genome-wide scan and fine mapping was carried out using microsatellite markers. A maximum multipoint LOD score of 3.8 was obtained at two markers, D18S481 and D18S1370. The three-unit support interval is flanked by markers D18S59 and D18S391, corresponds to a 17.6 cM region according to the decode genetic map and spans 5.8 Mb on the sequence-based physical map.

Keywords

18p11.32-p11.31; DFNB19; DFNB46; nonsyndromic deafness; Pakistan

INTRODUCTION

Hearing loss is a common sensory disorder. The incidence of congenital hearing loss is estimated at 1 in 1,000 births, of which approximately 60% of cases are attributed to genetic factors [Morton, 1991; Gorlin et al., 1995]. Seventy percent of deafness due to genetic causes is classified as nonsyndromic. For nonsyndromic deafness (NSD), the defect is generally sensorineural.

Many genes have been localized and identified for NSD; to date over 40 loci for autosomal recessive (AR) NSD have been mapped and 18 genes have been isolated [Van Camp and Smith, 2003]. These genes encode for motor, cell adhesion and transmembrane proteins, extracellular matrix components, and transcription factors [Steel and Bussoli, 1999]. It is not surprising to find such a wide range of genes involved in the etiology of deafness due to the complexity of the inner ear, and the many different pathways that can lead to the deafness phenotype [Steel and Bussoli, 1999]. It is likely that many more genes will be identified that are associated with NSD [Steel and Bussoli, 1999]. This article describes a novel NSD locus (DFNB46) that maps to 18p11.32-p11.31.

MATERIALS AND METHODS

Family History

Before the onset of the study, approval was obtained from the Quaid-I-Azam University Institutional Review Board. Informed consent was obtained from all family members who participated in the study. The pedigree structure is based upon interviews with multiple family members. Pedigree 4046 (Fig. 1) provided convincing evidence for an AR mode of inheritance. The family is from the Punjab province of Pakistan. The hearing individuals from the family speak Sairiki, which belongs to the Aryan language group of which Hindu–Urdu, Gujrati, Punjabi, and Sindhi are also members. Personal interviews with key figures in the kindred clarified consanguineous relationships. Clinical findings in this family are consistent with the diagnosis of ARNSD. All affected individuals have a history of prelingual profound deafness and use sign language for communication. Deaf individuals under-went physical examination for defects in ear morphology, mental retardation and other clinical features that could indicate that the etiology of the deafness was syndromic. There was no evidence in this kindred that deafness belonged to a syndrome or that there was gross vestibular involvement.

Extraction of Genomic DNA and Genotyping

Venous blood samples were obtained from six family members including four individuals who are hearing impaired. Genomic DNA was extracted from whole blood following a standard protocol [Grimberg et al., 1989], quantified by spectrophotometric readings at optical density 260 and diluted to 40 ng/μl for PCR amplification. A genome scan was carried out on six DNA samples at the Center for Inherited Disease Research (CIDR). A total of 396 fluorescently labeled short tandem repeat (STR) markers were genotyped. These markers are spaced ~10 cM apart and are located on the 22 autosomes and the X and Y chromosomes.

Linkage Analysis

Two-point linkage analysis was carried out on all autosomal markers from the genome scan using the MLINK program of the FASTLINK computer package [Cottingham et al., 1993]. Multipoint analysis was performed using ALLEGRO [Gudbjartsson et al., 2002] and haplotypes were constructed using SIMWALK2 [Weeks et al., 1995; Sobel and Lange, 1996]. An AR mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were used for the analysis. Genetic map distances from three sets of genetic maps were used for the analysis: Marshfield genetic map [Broman et al., 1998]; deCode genetic map [Kong et al., 2002] with markers D18S1370 and D18S1138 interpolated onto the deCode genetic map; and a genetic map constructed using MAP-O-MAT [Lander and Green, 1987; Matise and Gitlin, 1999] from the Center d'Etude du Polymorphisme Humain (CEPH) [Dausset et al., 1990] and deCode genotype data.

Pedigree 4046 underwent a genome scan at the CIDR with 43 additional families from Pakistan. In order to ensure that accurate estimates of allele frequencies were obtained for the genome scan markers, allele frequencies were estimated from the founders and reconstructed genotypes of founders from pedigree 4046 and 28 additional families from Punjab whose native language is Sairiki. Equal allele frequencies were used for the fine mapping markers. It was not possible to estimate allele frequencies from the founders, because the fine mapping markers were only genotyped in this family. To evaluate whether a false positive result had occurred due to using incorrect allele frequencies a sensitivity analysis was carried out. Multipoint linkage analysis was performed by varying the allele frequency for the allele that was segregating with the disease allele from 0.2 to 0.8 for the fine mapping markers.

RESULTS

Two-point linkage analysis of the genome scan markers produced a maximum LOD score of 2.1 ($\theta = 0$) at marker D18S976 (see Table I), and multipoint linkage analysis produced a maximum LOD score of 3.6 at two markers on chromosome 18, GATA178F11 and D18S976. In order to fine map the DFNB46 locus, additional markers were selected from the Marshfield [Broman et al., 1998] and deCode genetic maps [Kong et al., 2002]. Fourteen of these markers were informative for linkage. After genotyping these markers, the data was reanalyzed using two-point and multipoint linkage analysis.

A maximum multipoint LOD score of 3.8 was obtained at markers D18S481 and D18S1370. When the marker allele frequencies were varied for the fine mapping markers from 0.2 to 0.6, the maximum multipoint LOD score varied from 3.9 to 3.8 respectively, and remained at markers D18S481 and D18S1370. At allele frequencies of 0.7 and 0.8, the maximum multipoint LOD scores were obtained at marker GATA178F11, with values of 3.6 and 3.3, respectively. The same multipoint LOD score results were obtained regardless whether Marshfield, deCode or the MAP-O-MAT genetic map distances were used in the analysis.

The three-unit support interval ranges from marker D18S59 to D18S391, a region that is 18.7 and 17.6 cM according to the Marshfield and deCode genetic maps, respectively. Based upon the observed haplotype and the three-unit support interval it was not possible to rule out that the telomeric region distal to marker D18S59 contains the deafness gene for DFNB46, thus the physical region contains 5.8 Mb according to the sequence based physical map [International Human Genome Sequence Consortium, 2001]. On the deCode genetic map marker D18S1140 is the most telomeric marker and is distal to D18S59, which is the most telomeric marker on the Marshfield genetic map. However, marker D18S1140 is not informative in family 4046.

The genetic interval for DFNB46 was also determined based upon the region of homozygosity. Haplotypes were constructed using the markers in the 18p11.32-p11.31 region (Fig. 1). Two common haplotypes segregate in all affected individuals. The first haplotype, which begins distal to the most telomeric marker D18S59 and spans to marker GATA178F11, corresponds to 1.4 cM according to the deCode genetic map and contains 0.6 Mb. The second haplotype is flanked by markers GATA178F11 and D18S452 and contains 3.7 Mb in a 12.0 cM region according to the deCode genetic map. Inspection of the haplotype of affected individual 37 identified an ancestral recombination event between markers D18S391 and D18S452 (Fig. 1). The haplotypes of affected individuals 30, 32, 37, and 38 revealed that an historic recombination event must have occurred between markers GATA178F11 and D18S1098. In addition an historic recombination event may have occurred between markers D18S59 and GATA178F11.

DISCUSSION

The majority of families from Pakistan with deafness that this research group is studying are consanguineous. Of the 196 families studied thus far, 94% of the pedigrees display one or more consanguineous matings. Family 4046 (Fig. 1) presented with four consanguinity loops and one marriage loop. The parents (individuals 25 and 26) of deaf individuals 30 and 32 are both first cousins and half-second cousins. The inbreeding coefficient for both individuals 30 and 32 is 0.07031. In this branch of the family there is also a marriage loop where the mother's (individual 26) maternal great aunt is married to her paternal great uncle. The parents (individuals 20 and 27) of deaf individuals 37 and 38 are first cousins once removed. The inbreeding coefficient for individuals 37 and 38 is 0.03125. It is also

interesting to note that for the mother (individual 27) of individuals 37 and 38, her parents are first cousins and the inbreeding coefficient for individual 27 is 0.0625.

DFNB46 maps to a 17.6 cM region on chromosome 18p11.32-31. Another locus, DFNB19, was previously mapped to 18p11 and is flanked by markers D18S62 (starts at 5,816,441 bp) and D18S378 (starts at 10,503,838 bp). The three-unit support interval for DFNB46 is bounded by the telomere and marker D18S391 (starts at 5,771,022 bp). Based on the three-unit support interval for DFNB46 there is no overlap between the genetic intervals for DFNB46 and DFNB19. This is not the case if the region of homozygosity is examined for DFNB46, which is flanked by marker D18S452 (starts at 5,819,473 bp) and is heterozygous in deaf individual 37. Although marker D18S452 is not included in the genetic interval for DFNB46, it does fall within the genetic region for DFNB19. Therefore, the greatest possible region of overlap between the genetic intervals for DFNB46 and DFNB19 is 3 kb; however, it should be noted that since neither marker D18S62 nor D18S452 are included within the genetic interval for their respective loci, it is possible that there is no overlap of the genetic intervals for DFNB46 and DFNB19. Therefore, it is highly unlikely that DFNB46 and DFNB19 are due to the same gene. It is also of interest to note that within the potential 3 kb region of overlap between these two loci there are no known genes.

The DFNB46 locus maps to a region, which contains 5.8 Mb according to the sequence-based physical map. This region corresponds to 17.6 and 18.7 cM on the deCode and Marshfield sex-averaged genetic maps, respectively. The difference between the physical and genetic maps suggests that this region is a recombination hot spot. In order to better evaluate the relationship between the physical and genetic map distances, the natural logarithm of the ratio of the genetic map distances (sex-averaged, female and male) were plotted against physical chromosomal map positions in Mb. This plot revealed that both deserts and jungles of recombination within this region were present (data not shown). The male and female recombination rates in this region vary greatly, with a higher male recombination rate. For the deCode, Marshfield, and MAP-O-MAT genetic maps, the ratio between the male and female sex-specific map distances is 2:1. It is a well-known phenomenon that the rates of male recombination are higher than the rates of female recombination within the human chromosomal telomeric regions.

At present, 24 genes have been mapped to a 5.8 Mb region on chromosome 18p11.32-p11.31. Four candidate genes within the genetic interval of DFNB46 were sequenced: the Zinc finger protein 161 homolog (*ZFP161*), which is expressed in the fetal cochlea [Human Cochlear cDNA library and EST database, 2004 <http://hearing.bwh.harvard.edu>], myosin regulatory light chain-2 (*MRLC2*), myosin regulatory light chain-3 (*MRCL3*), and myomesin 1 (*MYOM1*). *MRLC2* gene product plays a role in the regulation of actin filament assembly [Iwasaki et al., 2001]. The exonic and promoter regions of these genes were sequenced in two deaf individuals and an unaffected pedigree member. No potentially causative variants were identified. The localization of DFNB46 is the first step in identifying the underlying deafness gene.

Acknowledgments

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Fig. 1.

Drawing of pedigree 4046, which segregates DFNB46. The sexes of some of the family members have been changed to protect the anonymity of the family. Black symbols represent individuals with deafness due to DFNB46. Clear symbols represent unaffected individuals. Haplotypes for the most closely linked STRs are shown below each symbol. The haplotypes for DFNB46 are displayed in boxes.

TABLE I
Two-Point LOD Score Results Between the DFNB46 Locus and Chromosome 18 Markers

Marker	Marshfield map position ^a	deCode map position ^b	Combined map (MAP-O-MAT) ^c	Physical map position ^d	LOD score at θ							
					0.0	0.02	0.04	0.05	0.1	0.2	0.3	0.3
D18S59	0.00	1.39	0.00	6,364,60	1.28	1.21	1.14	1.10	0.93	0.60	0.33	0.33
GATA178F11	2.84	5.64	4.30	2,113,098	-1.70	-0.32	0.16	0.30	0.62	0.64	0.43	0.43
D18S1098	5.58	7.60	6.50	2,859,597	1.24	1.17	1.09	1.06	0.88	0.54	0.27	0.27
D18S481	6.94	8.88	8.00	3,056,133	1.24	1.17	1.09	1.06	0.88	0.54	0.27	0.27
D18S1370	6.94	—	8.00	3,147,651	1.72	1.63	1.54	1.49	1.26	0.83	0.44	0.44
D18S459	8.30	9.74	8.70	3,524,819	1.24	1.17	1.09	1.06	0.88	0.54	0.27	0.27
D18S1138	8.30	—	8.70	3,662,022	1.72	1.63	1.54	1.49	1.26	0.83	0.44	0.44
D18S52	9.26	12.56	11.30	4,247,912	1.72	1.63	1.54	1.49	1.26	0.83	0.44	0.44
D18S1132	11.21	14.11	12.80	4,609,674	1.28	1.21	1.14	1.10	0.93	0.60	0.33	0.33
D18S976	12.81	16.16	14.80	5,238,897	2.09	1.98	1.88	1.82	1.55	1.02	0.54	0.54
GATA185C06	16.54	16.79	15.70	5,327,381	1.72	1.63	1.54	1.49	1.26	0.83	0.44	0.44
D18S391	18.70	17.62	16.90	5,771,022	1.28	1.21	1.14	1.10	0.93	0.60	0.33	0.33
D18S452	18.70	17.62	16.90	5,819,473	—	0.02	0.24	0.30	0.41	0.35	0.21	0.21
D18S458	18.70	—	16.90	5,931,028	—	-1.68	-1.13	-0.97	-0.51	-0.17	-0.06	-0.06
D18S967	19.64	21.24	19.20	6,585,263	1.28	1.21	1.14	1.10	0.93	0.60	0.33	0.33
D18S1163	24.08	25.36	22.90	7,462,301	—	-1.87	-1.32	-1.15	-0.65	-0.26	-0.10	-0.10

Genome scan markers are shown in bold.

^a Sex-averaged kosambi cM map distance from the Marshfield genetic map.

^b Sex-averaged kosambi cM map distance from the deCode genetic map.

^c Sex-averaged kosambi cM map distances estimated from the CEPH and deCode data using MAP-O-MAT.

^d Sequence-based physical map distance in bases according to the Human Genome Project—Santa Cruz.