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Mapping of a new autosomal recessive non-syndromic hearing impairment locus (DFNB45) to chromosome 1q43-q44

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To the Editor

Hearing impairment (HI) is a common sensory disorder that exhibits extensive genetic heterogeneity. Approximately 60% of congenital HI cases can be attributed to genetic factors and approximately 75% are classified as non-syndromic (1,2). In this letter, the mapping of a novel autosomal recessive locus for non-syndromic hearing impairment (NSHI) in a six-generational consanguineous Pakistani family, pedigree 4053 (Fig. 1), is reported. The study was initiated with the prior approval of the Institutional Review Boards of Quaid-i-Azam University and Baylor College of Medicine. Informed consent was obtained from all family members who participated in the study. All affected family members have pre-lingual profound HI and use sign language for communication. There was no evidence that the HI is syndromic or that there is gross vestibular involvement. The locus responsible for NSHI in this family was localized to chromosome 1q43-q44.

In order to carry out a whole genome scan, a total of 396 fluorescently labeled short tandem repeat (STR) markers spaced approximately every 10 cM were genotyped in 10 family members, of which four are hearing impaired. The marker allele frequencies were estimated using genotype data from founders and reconstructed founders from pedigree 4053 and 44 additional families from Pakistan. The occurrence of Mendelian errors and unlikely genotypes were assessed using PEDCHECK and MERLIN (3,4) and none were uncovered. Linkage analysis was carried out under a fully penetrant autosomal recessive model with a disease allele frequency of 0.001. Two-point linkage analysis was performed with MLINK (5) and gave a maximum LOD score of 3.7 ($\theta = 0.04$) at marker D1S547 (Table 1). Multipoint parametric linkage analysis was performed using ALLEGRO (6) and a maximum multipoint LOD score of 4.4 was obtained at marker D1S1609. In order to fine map DFNB45, 12 additional markers were genotyped; of these markers, six were uninformative (D1S321, D1S1634, D1S2842, D1S1590, D1S2679 and D1S102). Two-point and multi-point linkage analyses were carried out using equal allele frequencies for fine mapping markers because they were only genotyped in family 4053 and accurate allele frequencies could not be estimated for these marker loci.

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Electronic database information

The URLs for data presented herein are as follows: University of California Santa Cruz Human Genome Project working draft, March 2006 human reference sequence (National Center for Biotechnology Information Build 36.1), <http://genome.cse.ucsc.edu/> (physical positions of marker loci).

Rutgers Combined Linkage – Physical Map of the Human Genome, <http://compugen.rutgers.edu/maps/index.shtml> (genetic positions of the STR marker loci).

Additionally, in order to evaluate whether false positive results could have been obtained due to using equal and potentially incorrect allele frequencies, a sensitivity analysis (7) was conducted. For the sensitivity analysis, multipoint linkage analyses were carried out by setting the allele frequency for each fine mapping marker's allele that segregated with the hearing impairment phenotype to p and the other allele to $1-p$. Multipoint linkage analyses were then carried out, varying p for each of the fine mapping markers between 0.2 and 0.8 in increments of 0.1. After analyzing the fine mapping markers, the maximum two-point LOD score remained at genome scan marker D1S547 (Table 1). Multipoint linkage analysis was repeated with the genome scan markers within the region, and the six fine mapping markers and a maximum multipoint LOD score of 5.2 was obtained at marker D1S404. For the sensitivity analysis, the highest and lowest maximum multi-point LOD scores of 5.3 and 5.0 were obtained when $p = 0.2$ and 0.8 , respectively. These maximum multipoint LOD scores from the sensitivity analysis also occurred at marker D1S404. Haplotypes were reconstructed using SIMWALK2 (8,9) (Fig. 1). The region that is only homozygous in HI family members spans a 15.3-cM region from markers D1S547 to D1S2836 and contains 5.1 Mb of the genome.

Within the 5.1-Mb (10) region of the DFNB45 locus, there are 15 genes with known function. Three of these genes, *CHML*, *OPN3* and *MAP1LC3C*, are excellent candidates for DFNB45 because of their function and/or expression and thus, were sequenced as previously described (11). The choroideremia-like gene (*CHML*: MIM 118825) encodes an extracellular matrix protein involved in cell adhesion and is also expressed as a transcript in zebrafish hair cells (12). *CHML* lies within intron 1 of the opsonin3 (*OPN3*: MIM 606695) gene (13) and this gene was also sequenced. The microtubule-associated protein 1 light chain 3 gamma (*MAP1LC3C*: MIM 609605) is expressed in zebrafish hair cells. These three genes were excluded as causative because sequencing revealed no potentially functional variants. It should be noted that as only the promoter and exonic regions of these genes were sequenced, it is conceivable one of these genes is involved in the etiology of HI and the functional variant lies outside the sequenced region.

Eight additional loci for NSHI map to chromosome 1 (14-22). The region of homozygosity for DFNB45 overlaps with 2.7 Mb (23) of the physical region for the autosomal dominant NSHI locus DFNA34 (22) that maps between markers D1S102 (first base position 242,232,988) and D1S3739 (last base position 247,115,645). Marker D1S102 lies between markers D1S1609 and D1S423, while marker D1S3739 is telomeric to marker D1S2836 (Table 1). Identification of mutations in the causative gene(s) will determine whether or not DFNA34 and DFNB45 are due to the same gene.

In summary, a whole genome scan was carried out using DNA samples from a consanguineous six-generational Pakistani family with autosomal recessive NSHI. A maximum multipoint LOD score of 5.2 was obtained at marker D1S404. The region of homozygosity maps the novel locus DFNB45 to a 5.1-Mb region on chromosome 1q43-q44 between markers D1S1547 and D1S2836.

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Fig. 1.

Drawing of pedigree 4053. Black symbols represent individuals with hearing impairment and clear symbols represent unaffected individuals. Haplotypes are shown beneath each genotyped individual. The DFNB45 haplotype is highlighted in a box.

Table 1

Two-point LOD score result of STR marker loci^a

Marker name	Physical map position (bp) ^b	Genetic map position (cM) ^c	Two-point LOD score at $\theta =$									
			0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.30
D1S235	233,960,377	261.73	-12.60	-4.50	-3.35	-2.70	-2.25	-1.90	-0.94	-0.23	-0.05	-0.05
D1S1594	238,846,611	276.43	-2.11	-0.42	-0.16	-0.03	0.06	0.12	0.24	0.21	0.12	0.12
D1S304	239,355,103	278.08	1.57	1.53	1.48	1.44	1.39	1.35	1.13	0.74	0.40	0.40
D1S547	239,822,560	278.98	3.46	3.64	3.72	3.74	3.74	3.73	3.51	2.76	1.82	1.82
D1S404	241,101,798	283.40	1.83	1.78	1.72	1.67	1.61	1.56	1.30	0.82	0.42	0.42
D1S1609	242,132,608	284.63	3.14	3.06	2.97	2.89	2.80	2.72	2.30	1.50	0.81	0.81
D1S423	243,612,279	290.89	1.32	1.28	1.24	1.20	1.16	1.12	0.93	0.59	0.31	0.31
<i>D1S2836</i>	244,936,700	294.30	-inf	-2.06	-1.50	-1.19	-0.98	-0.82	-0.41	-0.12	-0.03	-0.03
D1S2215	245,668,734	295.09	-2.43	-0.36	-0.11	0.03	0.11	0.16	0.26	0.20	0.10	0.10
D1S2682	246,197,009	300.63	-8.86	-3.31	-2.46	-1.98	-1.65	-1.40	-0.71	-0.21	-0.05	-0.05

STR, short tandem repeat.

^aMarkers in italic flank the haplotype. Genome scan markers are displayed in bold.

^bNCBI Build 36.1 genome assembly (23).

^cThe Rutgers Combined Linkage – Physical Map of The Human Genome (24).