DFNB44, a Novel Autosomal Recessive Non-Syndromic Hearing Impairment Locus, Maps to Chromosome 7p14.1-q11.22

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Key Words

7p14.1-q11.22 · DFNB44 · Non-syndromic hearing impairment · Pakistan

Abstract

The genetic etiology for many forms of hearing impairment (HI) is very diverse. Non-syndromic HI (NSHI) is one of the most heterogeneous traits known. Autosomal recessive forms of prelingual HI account for ~75% of hereditary cases. A novel autosomal recessive NSHI locus, DFNB44, was mapped to a 20.9 cM genetic interval on chromosome 7p14.1-q11.22, according to the Marshfield genetic map, in a consanguineous Pakistani family. Multipoint linkage analysis resulted in a maximum LOD score of 5.0 at marker D7S1818. The 3-unit support interval ranged from marker D7S2209 to marker D7S2435, spanning a 30.1 Mb region on the sequence-based physical map.

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Introduction

Hereditary hearing impairment (HI) that is not associated with any other clinical feature, syndrome, or disorder, is termed non-syndromic. Approximately 75% of

non-syndromic HI (NSHI) cases with a genetic etiology display an autosomal recessive mode of inheritance. Autosomal dominant forms are observed in 15% of cases, while mitochondrial and X-linked NSHI are less frequent [1]. Autosomal recessive NSHI is generally due to a sensorineural defect [2], is manifested at the prelingual stage, and is severe to profound, affecting all frequencies. NSHI is an extremely heterogeneous trait, with over 40 loci mapped for autosomal recessive forms and 18 genes isolated [3]. This heterogeneity is largely due to the complexity of the inner ear, and the various mechanisms that can lead to the HI phenotype [4]. A consanguineous Pakistani family segregating a novel autosomal recessive NSHI locus, DFNB44, is described. This locus was mapped to a 20.9 cM region on chromosome 7p14.1-q11.22.

Materials and Methods

Family History

Approval was obtained from the Quaid-I-Azam University Institutional Review Board prior to the onset of the study. Informed consent was obtained from all family members who participated in the study. A consanguineous family from Pakistan was ascertained. Information obtained during interviews with multiple family members was used to construct the pedigree and to clarify consanguineous relationships. HI displayed an autosomal recessive mode of inheritance in pedigree 4009 (fig. 1). Affected individuals present with prelingual profound HI which affects all frequencies and is probably

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Fig. 1. Drawing of pedigree 4009 that segregates DFNB44. The sexes of some of the family members have been changed to protect the anonymity of the family. Black symbols represent individuals with hearing impairment due to DFNB44. Clear symbols represent unaffected individuals. Haplotypes for the most closely linked STRs are shown below each symbol. The haplotype for DFNB44 is displayed in a box.

Table 1. Two-point LOD score results between DFNB44 and chromosome 7 markers

Marker	Marshfield map position ^a	deCode map position ^b	Physical map position ^c	LOD score at $\theta =$						
				0.0	0.02	0.04	0.05	0.1	0.2	0.3
D7S817	50.29	52.16	31 878 592	$-\infty$	-0.06	0.41	0.54	0.81	0.76	0.48
D7S460	–	54.15	34 035 346	$-\infty$	-1.13	-0.62	-0.47	-0.08	0.12	0.10
D7S484	53.50	54.65	35 027 091	$-\infty$	-1.37	-0.84	-0.68	-0.25	0.01	0.04
D7S2250	54.11	54.65	35 120 620	$-\infty$	-0.65	-0.16	-0.02	0.32	0.41	0.27
<i>D7S2209</i>	<i>57.79</i>	<i>58.84</i>	<i>37 300 642</i>	<i>-2.13</i>	<i>0.04</i>	<i>0.27</i>	<i>0.33</i>	<i>0.46</i>	<i>0.42</i>	<i>0.26</i>
D7S2846	57.79	59.12	37 875 515	1.36	1.29	1.22	1.19	1.02	0.69	0.38
D7S2469	61.53	62.34	40 864 239	3.11	2.97	2.83	2.76	2.41	1.70	0.98
D7S670	69.56	69.46	46 909 751	3.11	2.97	2.83	2.76	2.41	1.70	0.98
D7S1818	69.56	70.71	49 133 606	2.52	2.41	2.29	2.24	1.95	1.37	0.81
D7S674	70.64	71.27	49 284 035	2.55	2.44	2.32	2.27	1.98	1.39	0.80
D7S3069	73.31	74.74	52 023 510	3.11	2.97	2.83	2.76	2.41	1.70	0.98
D7S473	76.71	78.13	57 153 928	1.95	1.86	1.77	1.73	1.51	1.08	0.65
D7S3046	78.65	–	67 964 439	4.48	4.32	4.15	4.07	3.64	2.73	1.77
<i>D7S2435</i>	<i>80.42</i>	<i>81.88</i>	<i>68 477 387</i>	$-\infty$	-0.55	-0.30	-0.23	-0.06	0.01	0.01
D7S1870	86.12	86.89	73 538 645	$-\infty$	-1.40	-0.85	-0.68	-0.22	0.08	0.10
D7S669	90.42	89.39	77 484 487	$-\infty$	-0.88	-0.46	-0.32	0.03	0.20	0.15

Genome scan markers are shown in bold. Markers in italics flank the haplotype.

^a Sex-averaged kosambi cM map distance from the Marshfield genetic map [8].

^b Sex-averaged kosambi cM map distance from the deCode genetic map [16].

^c Sequence-based physical map distance in bases according to Human Genome Project – Santa Cruz [12].

congenital. The hearing impaired individuals in this pedigree use sign language for communication. Hearing impaired individuals underwent a physical examination, and no clinical features, including mental retardation, that would indicate that the HI was part of a syndrome, were observed. In addition, no gross vestibular involvement was observed.

Extraction of Genomic DNA and Genotyping

Venous blood samples were collected from a total of 11 individuals, including 5 who are hearing impaired. Genomic DNA was extracted from whole blood following a standard protocol [5], quantified by spectrophotometric readings at optical density 260, and diluted to 40 ng/μl for PCR amplification. A genome scan was carried out on all the DNA samples at the Center for Inherited Disease Research (CIDR). A total of 388 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 [6]. Multipoint linkage analysis was performed using ALLEGRO [7] with map distances from the Marshfield genetic map [8]. Haplotypes were constructed using SIMWALK2 [9, 10]. An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were

used for the analysis. For the genome scan markers, allele frequencies were estimated from the founders and reconstructed genotypes of founders from pedigree 4009 and thirteen additional pedigrees that underwent a genome scan at CIDR at the same time. Equal allele frequencies were used for the fine mapping markers, because it was not possible to estimate allele frequencies from the founders, since these markers were only genotyped in this family. A sensitivity analysis was carried out in order to evaluate whether a false positive result had occurred due to using incorrect allele frequencies [11]. Multipoint linkage analysis was performed by varying the allele frequency for the allele 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 gave a maximum LOD score of 4.5 ($\theta = 0$) at marker D7S3046. The maximum multipoint LOD score for the genome scan markers was 4.9, and it also occurred at marker D7S3046. In order to fine map the region on chromosome 7 and establish linkage, 12 additional microsatellite markers were selected from the Marshfield genetic map [8]. Two-point and multipoint linkage analyses were repeated after genotyping the family members with the

fine mapping markers. The maximum two-point LOD score remained at marker D7S3046 with a value of 4.5 ($\theta = 0$) (table 1). Multipoint linkage analysis resulted in a maximum LOD score of 5.0 at marker D7S1818. When the marker allele frequencies were varied from 0.2 to 0.8 for the fine mapping markers, the maximum multipoint LOD score remained at marker D7S1818 with a value of 5.0. The 3-unit support interval ranged from marker D7S2209 to marker D7S2435, spanning 20.9 cM according to the Marshfield genetic map [8]. The interval corresponds to a 30.1 Mb region on the sequence-based physical map [12]. Haplotypes were then constructed to determine the critical linkage interval. The region of homozygosity was the same as the 3-unit support interval; flanked by markers D7S2209 and D7S2435. The centromeric boundary of the co-segregating interval was defined by a historic recombination event between markers D7S2209 and D7S2846, which was observed in affected individual 20 (fig. 1). Unaffected individual 21 was homozygous at marker D7S2435, and since unaffected individuals 12 and 13 (parents of individual 21) were heterozygous at marker D7S2435, the telomeric boundary of the interval was set at marker D7S3046.

Discussion

The NSHI locus DFNB44 was mapped to a 20.9 cM region on chromosome 7p14.1-q11.22, according to the Marshfield genetic map [8], in a consanguineous Pakistani kindred. Five loci for autosomal recessive NSHI have previously been localized on chromosome 7, DFNB4 (7q31), DFNB13 (7q34-q36), DFNB14 (7q31), DFNB17 (7q31) and DFNB39 (7q11.22-q21.12). Only the gene for DFNB4, SLC26A4, has been identified [3]. The genetic interval for DFNB44 does not overlap with the region for DFNB39 [13]. For many of the known NSHI loci only one pedigree has been ascertained world wide. Although these pedigrees are large enough to independently establish linkage, due to the limited number of meioses within each pedigree the genetic region for the NSHI locus is often large making gene identification difficult due to the large number of genes within a given region.

To date, more than 80 known, and 21 predicted, genes have been localized to the DFNB44 region [12]. Several of these genes are expressed in the cochlea, and are potential candidates for DFNB44. A potential candidate is the β -glucuronidase gene (GUSB, MIM 253220). GUSB codes for the β -glucuronidase enzyme, and maps

to chromosome 7q11.21. Mutations in this gene are associated with mucopolysaccharidosis type VII (MPS VII, MIM 253220), a lysosomal storage disease caused by dysfunction of β -glucuronidase, resulting in the accumulation of incompletely degraded glycosaminoglycans within lysosomes of various cell types [14]. One of the characteristics of MPS VII is mixed (conductive and sensorineural) hearing loss. In addition, affected patients manifest skeletal abnormalities, vision defects, mental retardation and a decreased lifespan [14]. Moreover, MPS VII mice present with severe pathologic abnormalities in many ear structures, which could account for the hearing impairment similar to that observed in affected humans [15]. The exons and promoter region of the GUSB gene were screened for mutations in two hearing impaired and one unaffected family member. The GUSB gene was excluded as being the underlying gene for DFNB44. Identification of the gene for DFNB44 will enhance our understanding of the molecular mechanisms of the auditory system, as well as those involved in the etiology of NSHI.

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