

Localization of A Novel Autosomal Recessive Non-Syndromic Hearing Impairment Locus (DFNB38) to 6q26-q27 in a Consanguineous Kindred from Pakistan

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Key Words

Autosomal recessive hearing impairment · DFNB38 ·
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Abstract

For autosomal recessive nonsyndromic hearing impairment over 30 loci have been mapped and 19 genes have been identified. DFNB38, a novel locus for autosomal recessive nonsyndromic hearing impairment, was localized in a consanguineous Pakistani kindred to 6q26-q27. The affected family members present with profound pre-lingual sensorineural hearing impairment and use sign language for communications. Linkage was established to microsatellite markers located on chromosome 6q26-q27 (Multipoint lod score 3.6). The genetic region for DFNB38 spans 10.1 cM according to the Marshfield genetic map and is bounded by markers D6S980 and D6S1719. This genetic region corresponds to 3.4 MB on the sequence-based physical map.

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Introduction

Hearing loss is a common sensory disorder in the human population. 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 [1, 2]. Approximately, 70% of hearing impairments which are due to genetic factors are classified as nonsyndromic. For syndromic hearing loss the pathology varies widely in contrast to nonsyndromic hearing loss where the defect is usually sensorineural.

Nonsyndromal hearing impairment in humans is genetically heterogeneous. To date over 30 loci for autosomal recessive nonsyndromic hearing loss have been mapped and 19 genes have been identified [3]. This extreme genetic heterogeneity suggests that there are many different processes that can malfunction within the inner ear to cause hearing loss [4].

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

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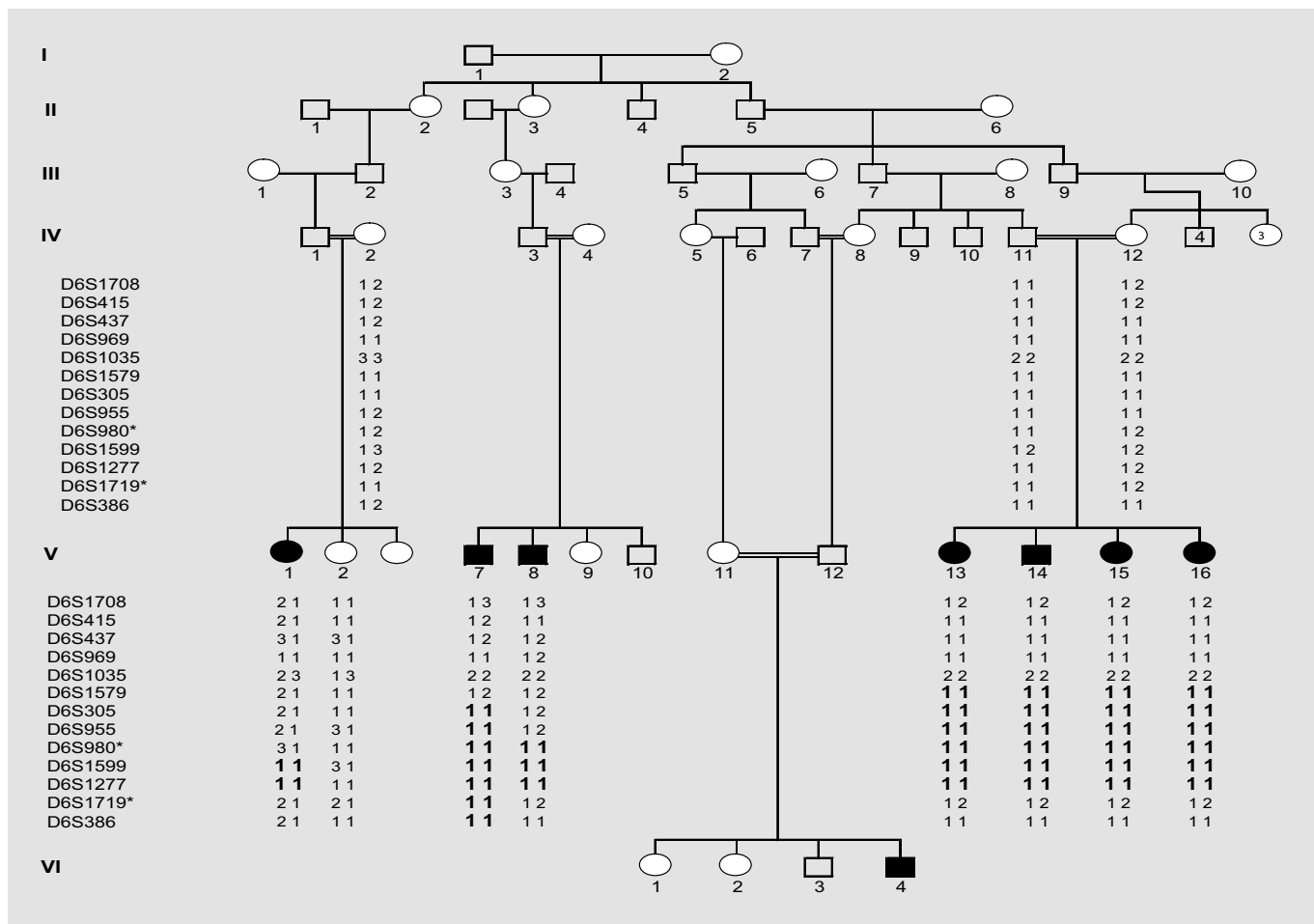


Fig. 1. Drawing of pedigree 4004 that segregates the DFNB38 locus. The sexes of some of the family members have been changed to protect the anonymity of the family. Black symbols represent individuals with hearing impairment and clear symbols represent unaffected individuals. Haplotypes for the most closely linked STRPs are shown below each symbol. When it is possible to distinguish the first haplotype displayed is the paternal haplotype. The region of homozygosity for hearing impaired individuals is shown in bold. The markers D6S980 and D6S1719 which flank the critical region are denoted by a star.

the study. The pedigree structure is based upon interviews with multiple family members. Pedigree 4004 (fig. 1) from Pakistan provided convincing evidence of an autosomal recessive mode of inheritance. Personal interviews with various family members clarified consanguineous relationships. Spouses IV.1 and IV.2 and IV.3 and IV.4 are distantly related cousins; however their exact relationship could not be confirmed. All affected individuals have prelingual profound hearing impairment that affects all frequencies and use sign language as their means of communication. All hearing impaired family members underwent a physical examination for defects in ear morphology, vision, mental retardation and other clinical features that could indicate that the hearing impairment belonged to a syndrome. There was no evidence that the hearing impairment in this kindred is syndromal or that there is gross vestibular involvement.

Genotyping

Venous blood samples were obtained from 11 family members including 7 individuals who are hearing impaired. Genomic DNA was extracted from whole blood following a standard protocol [5]. A genome scan was carried out on 11 DNA samples at the Center for Inherited Disease Research (CIDR). A total of 390 fluorescently labeled simple tandem repeat (STRP) 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 by means of the MLINK program of the FASTLINK computer package [6]. Multipoint analysis was performed using ALLEGRO [7] utilizing map distances from the

Table 1. Two-point lod score results between the DFNB38 locus and chromosome 6 markers; also displayed are the genetic and sequence-based physical map distances

Markers	Marshfield map position ¹	deCode map position ²	Physical map position ³	Lod score at $\theta =$								
				0.0	0.01	0.02	0.03	0.04	0.05	0.1	0.2	0.3
D6S1708	157.84	not on map	156072864	-infini	-1.76	-1.21	-0.91	-0.71	-0.57	-0.20	-0.02	0.01
D6S415	159.98	166.42	157748153	1.49	1.45	1.41	1.38	1.34	1.30	1.12	0.77	0.46
D6S437	161.59	167.04	158637457	-2.64	-0.88	-0.61	-0.45	-0.35	-0.27	-0.07	0.02	0.01
D6S969	161.55	not on map	159156005	-infini	-0.72	-0.45	-0.30	-0.21	-0.14	0.02	0.07	0.05
D6S1035	164.78	169.76	159900564	1.11	1.07	1.03	1.00	0.96	0.92	0.75	0.44	0.21
D6S1579	166.39	170.25	160897683	-2.56	-0.98	-0.70	-0.55	-0.44	-0.37	-0.16	-0.03	-0.01
D6S305	166.39	NA	162068740	-2.56	-0.98	-0.70	-0.55	-0.44	-0.37	-0.16	-0.03	-0.01
D6S955	167.78	173.00	162417178	1.05	1.02	0.98	0.95	0.91	0.88	0.71	0.41	0.19
<i>D6S980</i>	<i>167.78</i>	<i>not on map</i>	<i>162530186</i>	<i>-1.44</i>	<i>0.03</i>	<i>0.29</i>	<i>0.43</i>	<i>0.52</i>	<i>0.58</i>	<i>0.70</i>	<i>0.62</i>	<i>0.42</i>
D6S1599	169.95	174.48	162713158	3.62	3.53	3.45	3.36	3.27	3.19	2.75	1.90	1.10
D6S1277	173.71	176.12	164171287	2.29	2.23	2.17	2.12	2.06	2.00	1.72	1.17	0.67
<i>D6S1719</i>	<i>177.88</i>	<i>178.6</i>	<i>165943229</i>	<i>-infini</i>	<i>-4.09</i>	<i>-3.20</i>	<i>-2.67</i>	<i>-2.30</i>	<i>-2.02</i>	<i>-1.19</i>	<i>-0.49</i>	<i>-0.20</i>
D6S386	182.11	not on map	168673727	1.00	0.96	0.92	0.89	0.85	0.82	0.65	0.37	0.17
D6S1027	187.27	not on map	168930026	-infini	-3.42	-2.57	-2.09	-1.76	-1.50	-0.79	-0.27	-0.10

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

¹ Sex-average Kosambi cM map distance from the Marshfield genetic map.

² Sex-average Kosambi cM map distance from the deCode genetic map.

³ Sequence-based physical map distance in bases according to the Human Genome Project – Santa Cruz.

Marshfield genetic map [8]. For the analysis an autosomal recessive mode of inheritance with complete penetrance and no phenocopies was used. A disease allele frequency of 0.001 was assumed. The marker-allele frequencies for the genome scan markers were estimated from the data by means of both observed and reconstructed genotypes of founders from this pedigree and 6 additional large pedigrees from Pakistan which underwent a genome scan at the same time pedigree 4004 was genotyped.

Results

Analysis of the results obtained from genome search identified an area of interest on chromosome 6q26-q27. Two-point analysis gave a LOD score of 2.3 for marker D6S1277 at recombination fraction 0. A flanking marker D6S1035 spaced at 10 cM proximal to D6S1277 yielded LOD score of 1.1 also at recombination fraction 0.

In order to fine map the DFNB38 locus, eleven additional markers were selected from the Marshfield map [8]; nine markers are proximal to D6S1277 (D6S1599, D6S980, D6S955, D6S305, D6S1579, D6S969, D6S437, D6S415 and D6S1708) and two are distal (D6S1719 and D6S386). The Marshfield [8] genetic map order for markers D6S437 and D6S969 did not agree with the Human Genome Project-Santa Cruz sequence-based

physical map order [9]. Marker D6S969 was not included on the deCode [10] genetic map. In order to confirm that the position of marker D6S437 and D6S969 on the Marshfield map was potentially incorrect, Centre d'Etude du Polymorphisme Humain (CEPH) genotype data was analyzed using MAP-O-MAT [11, 12]. The support for the order of D6S437 and D6S969 on the Marshfield genetic map was low, therefore for multipoint linkage analysis marker D6S437 was removed.

After genotyping the family members with these additional markers, the data was reanalyzed using two-point linkage analysis (table 1) and multipoint linkage analysis. The maximum two-point LOD score occurred at marker D6S1599 with a LOD score of 3.6 ($\theta = 0$). Multipoint analysis for the family derived a maximum LOD score of 3.6 also at marker D6S1599. The 1-unit support interval ranges from marker D6S980 to D6S1719, a region which is 10.1 cM according to the Marshfield map. In addition, haplotypes were examined to determine the region which contains the DFNB38 locus. A common haplotype that spans a 10.1-cM region between markers D6S980 and D6S1719 segregated in all affected members. The critical recombination events that define the co-segregating interval were observed in hearing impaired individuals. A recombination event can be observed in individual V-1

that occurred between markers D6S980 and D6S1599. Hearing impaired individuals V-1, V-8, V-13, V-14, V-15 and V-16 are all heterozygous for marker D6S1719, which bounds the telomeric region of the haplotype. The 6q haplotype for pedigree 4004 is presented in figure 1. No additional markers are available from either the deCode [10] or the Marshfield [8] genetic maps to further refine the genetic region for the DFNB38 locus.

Discussion

Large consanguineous families from isolated populations have been instrumental in mapping autosomal recessive hearing impairment loci. In the present study we present evidence for linkage of a novel hearing impairment locus DFNB38 to a 10.1-cM region on chromosome 6q26-q27. It is interesting to note that the 10.1-cM genetic region corresponds to region which is only 3.4 contains MB (table 1) on the Human Genome Project – Santa Cruz sequence-based physical map [9].

In this region of 3.4 MB there are only three known genes which include PARK2 [MIM 602544], PACRG [13] and human phosphodiesterase PDE10A [14]. There is no evidence that these genes would be involved in a hearing impairment phenotype. In addition there are a large number of hypothetical genes within this region.

A number of genes have been identified on chromosome 6 that have been implicated in the etiology of non-syndromic hearing impairment. Three of these genes are responsible for autosomal dominant hearing loss: EYA4 [MIM 603550], COL11A2 [MIM 120290] and MYO6 [MIM 600970]. Mutations within the MYO6 gene are also responsible for autosomal recessive nonsyndromic hearing impairment. A fourth gene, GJA1 [MIM 121014] on chromosome 6 is also responsible for autosomal recessive nonsyndromic hearing impairment [3]. Neither the region of homozygosity nor the 1-unit support interval for DFNB38 overlaps with any of these genes. The identification of a gene for DFNB38 is anticipated to broaden our understanding of the molecular basis of hearing.

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