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Localization of a novel autosomal recessive non-syndromic hearing impairment locus *DFNB55* to chromosome 4q12-q13.2

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Abstract

Hereditary hearing impairment (HI) is the most genetically heterogeneous trait known in humans. So far, 54 autosomal recessive non-syndromic hearing impairment (ARNSHI) loci have been mapped, and 21 ARNSHI genes have been identified. Here is reported the mapping of a novel ARNSHI locus, *DFNB55*, to chromosome 4q12-q13.2 in a consanguineous Pakistani family. A maximum multipoint LOD score of 3.5 was obtained at marker D4S2638. The region of homozygosity and the 3-unit support interval are flanked by markers D4S2978 and D4S2367. The region spans 8.2 cM on the Rutgers combined linkage-physical map and contains 11.5 Mb. *DFNB55* represents the third ARNSHI locus mapped to chromosome 4.

Keywords

4q12-q13.2; autosomal recessive non-syndromic hearing impairment; *DFNB55*; Pakistan

Hearing disorders produce significant health problems in the world population. The vast majority of genetic hearing impairment (HI) is designated as non-syndromic, which is further categorized by mode of inheritance: approximately 77% of cases are autosomal recessive; 22% are autosomal dominant; 1% are X-linked and <1% are due to mitochondrial inheritance (1). Autosomal recessive non-syndromic HI (ARNSHI) is usually clinically homogeneous, is non-progressive in nature and exhibits a high degree of genetic heterogeneity. To date, 54 loci have been mapped for ARNSHI and 21 genes have been identified (2). The majority of these genes were mapped and identified by studying large consanguineous families. Identification of genetic defects causing HI contributes to the understanding of the molecular basis that is important to hearing function. This article describes a new ARNSHI locus, *DFNB55*, which maps to 4q12-q13.2 and was localized in a consanguineous Pakistani family.

Materials and methods

Family history

Before the start of the study, approval was obtained from the Quaid-i-Azam University and Baylor College of Medicine Institutional Review Boards. Signed informed consent was obtained from all family members who participated in the study. The pedigree structure is based upon interviews with multiple family members. Personal interviews with key figures

in the kindred clarified the consanguineous relationships. For the analysis, correct specification of consanguineous relationships is extremely important, as misspecification of familial consanguineous relations will increase type I and II error. Family 4153 is from the Sind province of Pakistan. This consanguineous pedigree provides convincing evidence of autosomal recessive mode of inheritance (Fig. 1). Clinical findings in this family are consistent with the diagnosis of ARNSHI. Medical history and physical examination of the affected individuals were performed by trained otolaryngologists affiliated with government hospitals. All affected individuals have a history of prelingual profound HI involving all frequencies and use sign language for communication. The hearing-impaired family members underwent a clinical examination for mental retardation, defects in ear morphology, dysmorphic facial features, eye disorders including night blindness and tunnel vision, and other clinical features that could indicate that HI was syndromic. There was no evidence in this kindred that HI belonged to a syndrome or that there was gross vestibular involvement.

Extraction of genomic DNA and genotyping

Venous blood samples were obtained from seven family members including four individuals who are hearing-impaired. Genomic DNA was extracted from whole blood following a standard protocol (3). As the *GJB2* gene is the most frequent cause of ARNSHI, all non-syndromic HI families are screened for mutations in this gene before undergoing a genome scan. Those families, which are positive for functional *GJB2* variants, are excluded from the genome scan. In family 4153, the *GJB2* gene was sequenced in two hearing-impaired family members, V-5. and V-6. (4), and no functional variants or benign polymorphisms were observed. A genome scan was carried out on seven DNA samples at the National Heart, Lung and Blood Institute (NHLBI) Mammalian Genotyping Service (Center for Medical Genetics, Marshfield, WI). A total of 410 short tandem repeat polymorphism markers with an average heterozygosity of 0.75 were genotyped. These markers are spaced approximately 10 cM apart and are located on the 22 autosomes and the X and Y chromosomes. After the completion of the genome scan, three additional unaffected (V-6., V-7. and V-8.) family members were ascertained and their DNA samples were used for fine mapping of the *DFNB55* locus.

For fine mapping, polymerase chain reactions (PCR) for microsatellite markers were performed according to standard procedure in a total volume of 25 μ l with 40 ng of genomic DNA, 0.3 μ l of each primer, 200 μ M of dNTP and 1 \times PCR buffer (Fermentas Life Sciences, Burlington, ON, Canada). PCR was carried out for 35 cycles: 95 $^{\circ}$ C for 1 min, 57 $^{\circ}$ C for 1 min and 72 $^{\circ}$ C for 1 min in a thermal cycler (PerkinElmer Life and Analytical Sciences, Boston, MA). PCR products were resolved on 8% non-denaturing polyacrylamide gel and genotypes were assigned by visual inspection. Alleles were numbered according to the CEPH Genotype database V10.0 (5).

Linkage analysis

The National Center for Biotechnology Information (NCBI) Build 34 sequence-based physical map was used to determine the order of the genome scan markers and fine mapping markers (6). Several of the genome scan markers were not found on the NCBI sequence-based physical map and were placed on the sequence-based physical map using e-PCR (7). Genetic map distances according to the Rutgers combined linkage-physical map of the human genome were used to carry out the multipoint linkage analysis for the fine map and genome scan markers (8). For those genome scan markers for which no genetic map position was available, interpolation was used to place these markers on the Rutgers combined linkage-physical map. PEDCHECK (9) was used to identify Mendelian inconsistencies, while the MERLIN (10) program was used to detect potential genotyping errors that did not

produce a Mendelian inconsistency. Haplotypes were constructed using SIMWALK2 (11,12). Two-point linkage analysis was carried out using the MLINK program of the FASTLINK computer package for the genome scan and fine mapping marker loci (13). Multipoint linkage analysis was performed using ALLEGRO (14). An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were used. For the genome scan, marker allele frequencies were estimated from the founders and reconstructed genotypes of founders from this family and 34 additional families from Pakistan that underwent a genome scan at the same time at the NHLBI Mammalian Genotyping Service. For the fine mapping markers, it was not possible to estimate allele frequencies from the founders because these markers were genotyped only in this family. False-positive results can be obtained when analysing the data using too low of an allele frequency for the allele segregating with the disease locus (15). Therefore, a sensitivity analysis was carried out for the multipoint linkage analysis by varying the allele frequency of the marker alleles that are segregating with the disease locus from 0.2 to 0.8 for the fine mapping markers. This was performed in order to determine whether the multipoint LOD scores are robust to allele frequency misspecification.

Results

From the genome scan data, a maximum two-point LOD score of 1.8 ($\theta = 0$) was obtained at marker D4S3248 and a maximum multipoint LOD score of 2.6 was obtained at marker D4S3248. In order to establish linkage and fine-map the *DFNB55* locus on chromosome 4, fifteen additional polymorphic microsatellite markers were selected from the Marshfield genetic map (16). Eight markers are proximal to D4S3248 (D4S2632, D4S1627, D4S2996, D4S428, D4S2916, D4S2978, D4S2638 and D4S1569) and seven of the markers distal to D4S3248 (D4S189, D4S1645, D4S1600, D4S409, D4S2387, D4S2389 and D4S1517). These markers and the genome scan markers in the region were genotyped in the seven family members who were originally included in the genome scan and in three additional family members that were ascertained after completion of the genome scan. Analysis of the marker genotypes within this region with PEDCHECK and MERLIN did not elucidate any genotyping errors. For the genotype data on the additional marker loci and family members, a maximum two-point LOD score of 2.8 ($\theta = 0$) was obtained with marker D4S1645 (Table 1). A maximum multipoint LOD score of 3.5 was obtained at marker D4S2638. The 3-unit multipoint support interval is an 8.2-cM region according to the Rutgers combined linkage-physical map of the human genome and spans from marker D4S2978 to marker D4S2367 (8). Haplotypes were then constructed to determine the critical recombination events (Fig. 1). The region of homozygosity in HI individuals was also flanked by markers D4S2978 and D4S2367. This region corresponds to a physical map distance of 11.5 Mb (6).

When the marker allele frequencies for the alleles segregating with HI were varied for the fine mapping markers from 0.2 to 0.4, the maximum multi-point LOD score remained to be 3.5 at marker D4S2638. When the allele frequencies were varied between 0.6 and 0.8, the maximum LOD score occurred at marker D4S2638 but decreased to 3.4.

Discussion

In this study, a novel ARNSHI locus, *DFNB55*, was mapped to an 8.2-cM interval on chromosome 4q12-q13.2. Seven non-syndromic hearing impairment (NSHI) loci, *DFNA6* [4p16.3] (17), *DFNA14* [4p16] (17), *DFNA24* [4q35] (18), *DFNA27* [4q12] (19), *DFNA39* [4q21.3] (20), *DFNB25* [4p15.3-q12] (21) and *DFNB26* [4q31] (22), have been previously mapped to chromosome 4. For these seven loci, two genes, *WFS1* for *DNA6/DFNA14* (17) and *DSPP* for *DFNA39* (20), have been identified.

The genetic region for *DFNB55* overlaps with the autosomal dominant NSHI locus *DFNA27* (19). The *DFNA27* locus maps between markers D4S428 (71.42 cM) and D4S392 (83.58 cM) and thus the genetic interval for *DFNB55* is within the genetic interval for *DFNA27*. Although two genes in close proximity might cause *DFNB55* and *DFNA27*, it is also possible that NSHI in *DFNB55* and *DFNA27* families is caused by different mutations in the same gene. It has been observed that different mutations in the same gene can cause both autosomal dominant and recessive NSHI (e.g. *GJB2*, *MYO7A*, *TECTA* and *TMCI*) (2). It should also be noted that the *DFNB55* locus does not overlap with the *DFNB25* locus (Richard Smith, personal communication).

The *DFNB55* interval contains 14 known genes and a large number of hypothetical genes and expressed sequenced tags. Several of the known genes in this region are expressed in the inner ear (23,24), namely ephrin receptor *EPHA5* [MIM 600004], *PPAT* [MIM 172450], *POLR2B* [MIM 180661] and *IGFBP7* [MIM 602867]. In particular, *EPHA5*, a member of a family of ligands that are implicated in the development and spatial patterning of cortical pathways to target organs such as the retina and the olfactory bulb (25,26), has been immunolocalized to the apical and basal portions of supporting cells which flank utricular hair cells in the rat (27). Likewise, *EPHA5* was detected in lateral wall fibrocytes and interdental cells in the spiral limbus of postnatal and adult gerbil cochlea (28). All exons of *EPHA5* were sequenced in one hearing and two hearing-impaired members of family 4153 and were found to be negative for functional sequence variants.

An additional strong candidate gene found within the *DFNB55* region is RE1-silencing transcription factor (*REST*) [MIM 600571], which is a transcriptional repressor of neural genes in non-neural cells (29). The *REST* gene was found to be expressed in supporting cells but not in hair cells of chick auditory epithelium (30). Additionally, *REST* mRNA is upregulated in both supporting cells and hair cells following gentamicin damage to the inner ear. The coding regions of the *REST* gene were sequenced in one unaffected and two hearing-impaired family members and no functional variant was identified.

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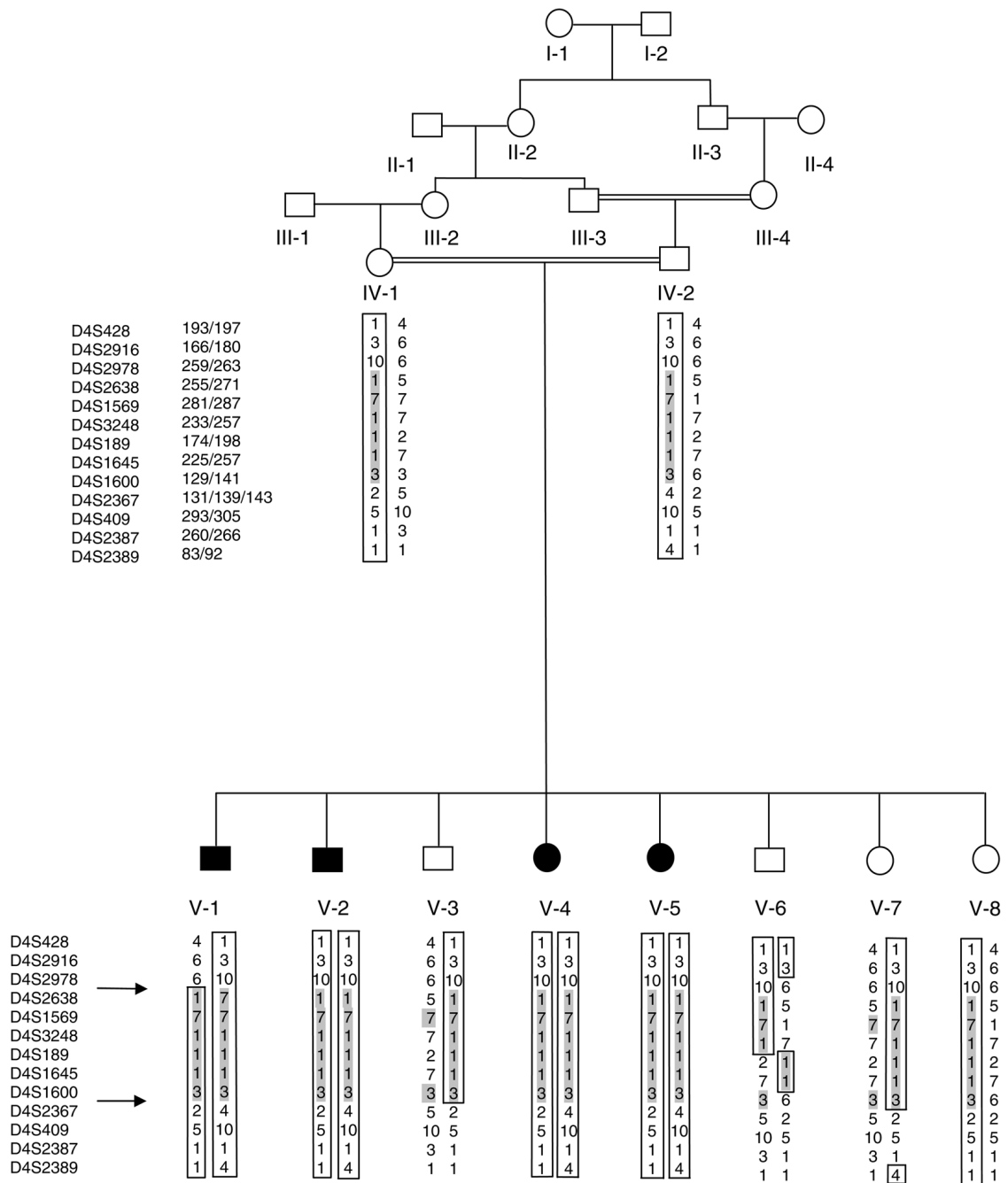


Fig. 1. Pedigree drawing of family 4153. Black symbols represent individuals with hearing impairment. Clear symbols represent unaffected individuals. The sex of some of the family members was changed to protect the anonymity of the family. Allele sizes in kilobases are indicated beside the upper column of microsatellite markers. Alleles are numbered according to the CEPH Genotype database V10.0 (5). Haplotypes are shown below each individual for whom genotypes are available. For individuals in generation V, maternal haplotypes are displayed on the left-hand side and paternal haplotypes on the right-hand side. Arrows and shaded areas indicate the region of homozygosity.

Table 1

Two-point LOD score results between the *DFNB55* locus and chromosome 4 markers

Marker ^a	Genetic map position ^b	Physical map position ^c	LOD score at =									
			0.0	0.01	0.02	0.04	0.05	0.1	0.2	0.3		
D4S428	71.42	55,549,948	-∞	-1.12	-0.58	-0.09	0.05	0.05	0.37	0.42	0.26	
D4S2916	72.35	55,728,673	-∞	-1.12	-0.58	-0.09	0.05	0.05	0.37	0.42	0.26	
D4S2978	73.97	56,831,962	-∞	0.57	0.82	1.00	1.04	1.04	1.04	0.77	0.42	
D4S2638	75.85	58,503,365	2.63	2.57	2.51	2.38	2.32	2.32	2.00	1.37	0.76	
D4S1569	76.81	59,564,470	1.13	1.10	1.08	1.03	1.00	1.00	0.88	0.62	0.37	
D4S3248	76.81	60,024,835	2.63	2.57	2.51	2.38	2.32	2.32	2.00	1.37	0.76	
D4S189	77.32	60,668,220	2.63	2.57	2.51	2.38	2.32	2.32	2.00	1.37	0.76	
D4S1645	77.37	61,986,341	2.75	2.68	2.62	2.48	2.42	2.42	2.08	1.43	0.79	
D4S1600	78.57	62,900,873	1.13	1.10	1.08	1.03	1.00	1.00	0.88	0.62	0.37	
D4S2367	82.19	68,286,182	0.19	1.04	1.27	1.46	1.50	1.54	1.27	0.82		
D4S409	82.19	68,327,249	-1.65	-0.79	-0.54	-0.30	-0.24	-0.24	-0.07	-0.01	-0.02	
D4S2387	82.60	69,105,934	1.37	1.35	1.32	1.27	1.24	1.24	1.10	0.82	0.52	
D4S2389	85.86	73,110,824	-0.93	-0.18	0.06	0.28	0.34	0.34	0.49	0.46	0.29	

^aMarkers displayed in italics flank the haplotype. Genome scan markers are shown in bold type.

^bCumulative sex-averaged Kosambi cM genetic map distances from the Rutgers combined linkage-physical map of the human genome (8).

^cSequence-based physical map distance in bases according to Build 34 of the human reference sequence (6).