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A new locus for nonsyndromic deafness *DFNB49* maps to chromosome 5q12.3-q14.1

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Abstract Cosegregation of markers on chromosome 5q12.3-q14.1 with profound congenital deafness in two Pakistani families (PKDF041 and PKDF141) defines a new recessive deafness locus, *DFNB49*. A maximum two-point lod score of 4.44 and 5.94 at recombination fraction $\theta=0$ was obtained for markers D5S2055 and D5S424 in families PKDF041 and PKDF141, respectively. Haplotype analysis revealed an 11 cM linkage region flanked by markers D5S647 (74.07 cM) and D5S1501 (85.25 cM). Candidate deafness genes in this region include *SLC30A5*, *OCLN*, *GTF2H2*, and *BTF3*, encoding solute carrier family 30 (zinc transporter) member 5, occludin, RNA polymerase II transcription initiation factor, and basic transcription factor 3, respectively. Sequence analysis of the coding exons of *SLC30A5* in DNA samples from two affected individuals of families PKDF041 and PKDF141 revealed no mutation. The mapping of *DFNB49* further confirms the heterogeneity underlying autosomal recessive forms of nonsyndromic deafness.

Introduction

Nonsyndromic forms of deafness transmitted as a recessive trait are the most frequent cause of hereditary hearing loss and often exhibit the most severe hearing

phenotype (Cohen et al. 1995). Nonsyndromic autosomal recessive deafness is usually clinically homogeneous and is nonprogressive in nature (Van Camp et al. 1997). As expected from the structural and functional complexity of the inner ear, sensorineural deafness exhibits a high degree of genetic heterogeneity. It has been estimated that at least 1% of human protein-coding genes are involved in the hearing process (Friedman and Griffith 2003). Presently, there are 36 published recessive deafness loci (*DFNB*), and 20 of the corresponding genes have been identified (Hereditary Hearing Loss Homepage, <http://www.uia.ac.be/dnalab/hhh/>).

Based on our ongoing genetic studies, a large repertoire of human genes associated with deafness remains to be mapped and identified, and these genes can be used to elucidate the molecular bases of hearing impairment. Because of the high degree of consanguinity, the Pakistani population represents a genetic resource for the identification of these novel deafness loci/genes (Hussain and Bittles 1998). Consanguineous families have contributed significantly to the identification of mutated genes associated with hearing loss (Friedman and Griffith 2003). This communication, describes two such large families segregating recessively inherited, profound congenital deafness, which is linked to markers that lie on chromosome 5q12.3-q14.1 and that define a novel locus, *DFNB49* (Fig. 1).

Materials and methods

Family enrollment

Approval for this study was obtained from the IRB at the National Centre of Excellence in Molecular Biology, Lahore, Pakistan (FWA00001758) and the NINDS/NIDCD IRB at the National Institutes of Health, USA (OH-93-N-016). Signed informed consent was obtained from all participants. Genomic DNA was extracted from 10 ml blood samples by using a standard protocol (Grimberg et al. 1989).

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of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Results

Regardless of age, all affected individuals in families PKDF041 and PKDF141 displayed congenital bilateral profound sensorineural hearing loss, whereas characteristic audiograms of unaffected individuals of both families showed normal hearing (Fig. 2). The unaffected

individual of family PKDF141 (IV:5) had a mild conductive hearing loss at lower frequencies. Audiometric evaluations of two other normal hearing individuals IV:3 and IV:4 (data not shown) also showed the same hearing profile, which was different from the profound sensorineural hearing loss observed in the affected individuals. Vestibular function was normal in affected individuals from both families, and clinical evaluations suggested no features of ophthalmologic, skin, or renal anomalies. Funduscopy examinations of affected individuals of PKDF041, viz., V:5 (42 years), VI:3 (16 years), VI:4 (10 years), VI:5 (20 years), and of PKDF141, viz., V:5 (22 years), showed no signs of retinitis pigmentosa (RP). Moreover, ERGs were normal for affected members of PKDF041, viz., V:5 (42 years) and V:7 (30 years), thus excluding a novel Usher syndrome gene segregating in these two families.

The deafness phenotype segregating in families PKDF041 and PKDF141 was not found to be linked to the published deafness loci, thus indicating the involvement of at least one new locus. For deafness segregating in family PKDF041, evidence of linkage was detected on chromosome 5q12.1-q23.3 following a genome-wide scan. Additional markers were genotyped in the *DFNB49* interval for all participating members. Haplotype analysis revealed a region of homozygosity of approximately 66 cM (Fig. 1a) flanked by markers D5S427 (69.23 cM) and D5S2110 (135.25 cM). A maximum two-point lod score (Z_{\max}) of 4.44 at recombination fraction $\theta=0$ was obtained for marker D5S2055 (Table 1).

Genome-wide linkage analysis of family PKDF141 segregating nonsyndromic deafness also showed linkage to markers at chromosome 5q12.3-q14.1, and haplotype analysis refined the linked region to 11 cM (Fig. 1b) delimited by markers D5S647 (74.07 cM) and D5S1501 (85.25 cM). Individual IV:5 of PKDF141 had normal hearing and was homozygous for markers in the proximal portion of the linked haplotype, with a recombination at D5S647 defining the proximal breakpoint of

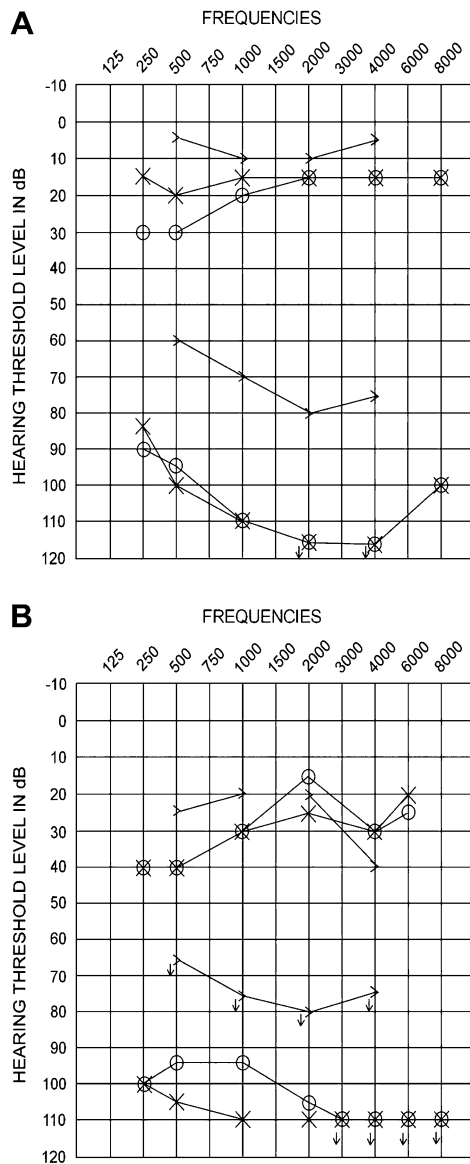


Fig. 2 Pure tone air (right and left ear) and bone conduction (right ear) audiograms (O, X right and left ear, respectively). **a** Normal hearing individual VI:2 (9 years) and affected individual V:5 (42 years) of PKDF041. **b** Normal hearing individual IV:5 (35 years) and affected individual V:5 (22 years) of PKDF141. Unaffected individuals have normal hearing, whereas affected individuals are profoundly deaf with hearing thresholds at or above 90 dB at all tested frequencies.

Table 1 Two-point lod scores

Family	Marker	Z_{\max}	θ	
PKDF041	D5S427	1.77	0.05	
	D5S647	4.29	0	
	D5S672	3.98	0	
	D5S641	4.27	0	
	D5S495	4.39	0	
	D5S2055	4.44	0	
	D5S471	3.21	0	
	D5S2110	2.44	0.05	
	PKDF141	D5S426	0.84	0.2
		D5S418	1.52	0.2
D5S407		1.69	0.1	
D5S427		2.25	0.04	
D5S647		3.21	0.1	
D5S1977		3.51	0	
D5S424		5.94	0	
D5S1501	1.61	0.1		

the linked region at 74.07 cM. Individuals IV:6, IV:7, V:3, V:4, and V:5 provided the distal breakpoint of the critical linked region at D5S1501. The parents of affected individuals V:6 and V:7 were unrelated. The deafness phenotype in these individuals may have been the result of compound heterozygosity for allelic mutations. A Z_{\max} of 5.94 at $\theta=0$ was found for D5S424 (Table 1).

Discussion

The region of homozygosity on 5q12.3-q14.1 for affected individuals of family PKDF041 overlaps with the reported interval for Usher syndrome (USH2C; Pieke-Dahl et al. 2000). Usher syndrome is an autosomal recessive disorder characterized by sensorineural hearing loss, RP and variable vestibular dysfunction (Ahmed et al. 2003b). Mutations of the gene encoding *VLGR1* (MIM 602851), the very large G-protein-coupled receptor-1, have recently been reported in affected individuals of several USH2C-linked families (Weston et al. 2004). Although affected members of families PKDF041 do not have RP, *VLGR1* cannot be excluded as a candidate gene. Mutant alleles of several Usher syndrome genes are reported to be associated with nonsyndromic hearing loss (Weil et al. 1997). We have previously shown that mutations of *CDH23*, *USH1C*, and *PCDH15* can cause nonsyndromic hearing loss (Bork et al. 2001; Ahmed et al. 2002, 2003a).

Assuming the deafness segregating in these two families is caused by allelic mutations, the linkage region for *DFNB49* is 11 cM in length and is delimited by D5S647 (74.07 cM) and D5S1501 (85.25 cM), which excludes *VLGR1* as a candidate gene. Family PKDF141 delineates this smallest linkage region, which contains a number of candidate genes (Fig. 3). As the haplotypes of both families differ from each other, most likely the two mutant alleles are not the same.

Three deafness loci (*DFNA42*, *DFNA1*, and *DFNA15*) have been previously mapped to chromosome 5q, but their genetic intervals do not overlap with the larger interval for *DFNB49* defined by family PKDF041 (Xia et al. 2002; Leon et al. 1992; Vahava et al. 1998).

One of the plausible candidate genes in the region of *DFNB49* locus is solute carrier family member *SLC30A5*. Mutations in a solute carrier family member, *SLC26A4*, are responsible for *DFNB4* (Li et al. 1998), Pendred syndrome (Everett et al. 1997), and Enlarged Vestibular Aqueduct syndrome (Usami et al. 1999). *SLC26A4* mutations account for approximately 10% of hereditary deafness in diverse populations that include eastern and southern Asians (Park et al. 2003). However, all 16 protein-coding exons of *SLC30A5* have been sequenced by using DNA samples from two affected individuals of families PKDF041 and PKDF141, and no mutation has been found.

Other interesting candidate genes within the region of the *DFNB49* locus include *OCN*, *GTF2H2*, and *BTF3*,

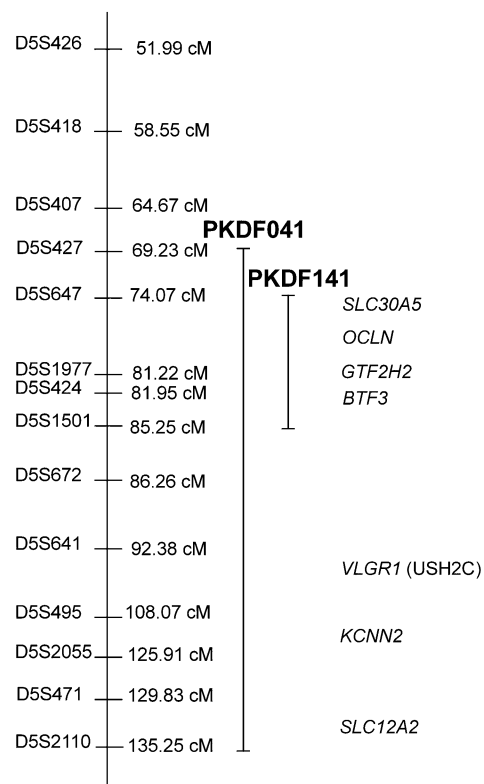


Fig. 3 Deafness segregating in families PKDF041 and PKDF141 show linkage to a region of human chromosome 5q12.3-q14.1. Candidate genes for *DFNB49* deafness are derived from data available on the UCSC Genome Browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>, July 2003 assembly).

encoding the tight-junction protein occludin, RNA polymerase II transcription initiation factor, and basic transcription factor 3, respectively. Tight-junction proteins contribute to ion-selective paracellular barriers, and mutant alleles of the claudin 14 gene *CLDN14* are responsible for nonsyndromic deafness in *DFNB29* linked families (Wilcox et al. 2001; Ben-Yosef et al. 2003). *OCN* is, therefore, a good candidate for the deafness gene segregating in our two families. The occludin knock-out mouse has not been reported to have hearing loss, but its hearing status does not seem to have been evaluated (Saitou et al. 2000). Genes encoding transcription factors *EYA4*, *POU3F4*, *POU4F3*, and *TFCP2L3* are reported to be mutated in *DFNA10* (Wayne et al. 2001), *DFN3* (De Kok et al. 1995), *DFNA15* (Vahava et al. 1998), and *DFNA28* (Peters et al. 2002), respectively. *TFCP2L3* is expressed in the epithelial cells of many tissues, and yet the only phenotype of a dominant mutant allele of *TFCP2L3* is progressive loss of hearing (Peters et al. 2002).

An alternative to a one-locus hypothesis explaining the segregation of deafness in our two families is that there are two different, closely linked, deafness loci. In the linkage interval defined by family PKDF041, there are more than 100 genes including *KCNN2*, a member of the potassium channel genes. *KCNN2* is an attractive

candidate, because *KCNQ4*, another potassium channel gene, is mutated in dominant deafness *DFNA2* (Kubisch et al. 1999). However, *SLC12A2* is perhaps the most interesting candidate gene in this interval, because there is a deaf mouse model for *SLC12A2* (Delpire et al. 1999). The 27 protein-coding exons of *SLC12A2* have been sequenced by using DNA from affected individuals of family PKDF041, but no mutation has been found.

Rather than continuing to screen the two families for mutations that define *DFNB49*, we propose that it would be more efficient to identify additional families segregating recessive hearing loss mapping to this interval and to use these families to refine the critical region(s). Localization of locus *DFNB49* is a first step toward the identification of a novel gene that is necessary for the development and/or maintenance of normal hearing.

Electronic database information

URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation: <http://research.marshfieldclinic.org/genetics/>

Hereditary Hearing Loss Homepage: <http://www.uia.ac.be/dnalab/hhh/>

Online Mendelian Inheritance in Man (OMIM): <http://www.ncbi.nlm.nih.gov/entrez/OMIM>

Primer3 Web-Based Server (primer3_www.cgi v 0.2): http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

The Human Genome Organization (HUGO): <http://www.gene.ucl.ac.uk/hugo/>

UCSC Genome Bioinformatics: <http://genome.ucsc.edu/>

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