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USH1K, a novel locus for type I Usher syndrome, maps to chromosome 10p11.21-q21.1

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Abstract

We ascertained two large Pakistani consanguineous families (PKDF231 and PKDF608) segregating profound hearing loss, vestibular dysfunction, and retinitis pigmentosa, the defining features of Usher syndrome type 1 (USH1). To date seven *USH1* loci have been reported. Here, we map a novel locus, *USH1K*, on chromosome 10p11.21-q21.1. In family PKDF231 we performed a genome-wide linkage screen and found a region of homozygosity shared among the affected individual at chromosome 10p11.21-q21.1. Meiotic recombination events in family PKDF231 define a critical interval of 11.74 cM (20.20 Mb) bounded by markers *D10S1780* (63.83 cM) and *D10S546* (75.57 cM). Affected individuals of family PKDF608 were also homozygous for chromosome 10p11-21-q21.1 linked STR markers. Of the 85 genes within the linkage interval, *PCDH15*, *GJD4*, *FZD4*, *RET*, and *LRRC18* were sequenced in both families, but no potential pathogenic mutation was identified. The *USH1K* locus overlaps the non-syndromic deafness locus *DFNB33* raising the possibility that the two disorders may be caused by allelic mutations.

Keywords

deafness; DFNB33; retinitis pigmentosa; Usher syndrome; *USH1K*; vestibular dysfunction; 10p11.21-q21.1

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Conflict of Interest: None

Introduction

Usher syndrome causing genes have provided unexpected insights into developmental and biochemical processes shared by the eye and ear.[1,2] Usher syndrome (USH) is neurosensory disorder affecting both hearing and vision in humans.[3,4] A molecular diagnosis study suggested a frequency of 1/6000 individuals afflicted with USH in the U.S.[5] Among the three defined clinical subtypes, USH type I (USH1) is the most genetically heterogeneous. To date, seven loci for USH1, three loci for USH2 and one locus for USH3 and genes for nine of them have been described.[6-17] All of the USH proteins, including myosin VIIa (MIM 276903), cadherin 23 (MIM 605516), protocadherin 15 (MIM 605514), harmonin (MIM 605242), SANS (MIM 607696), usherin (MIM 608400), GPR98 (MIM 602851), whirlin (MIM 607928) and clarin-1 (MIM 606397), are thought to interact to form a large macromolecular complex,[1,18] which is essential for auditory and visual functions. Furthermore, growing evidence suggests that USH proteins are localized in inner ear stereocilia bundles and are part of mechano-transduction machinery, and in the retina, largely within the molecular network tethering cilium to photoreceptor cells.[1,11,19-26] However, the molecular identities of many essential components of these structures are unknown, precluding our understanding of the precise mechanisms of human hearing and vision.

The *USH* gene discovery process requires investigation of large human families segregating for USH, followed by genetic mapping and positional identification of causal mutations. Consanguineous families, plentiful in the Pakistani population, represent a rich resource for gene discovery research. Here we report two consanguineous Pakistani families in which the USH1 phenotype is linked to a novel *USH1* locus, *USH1K*, on chromosome 10p11.21-q21.1.

Material and Methods

Subject enrollment

This study was approved by IRB Committee at the Cincinnati Children's Hospital Research Foundation, USA (2009-0684; 2010-0291), the IRB at the National Centre of Excellence in Molecular Biology (NCEMB), Lahore, Pakistan (FWA00001758) and the Combined Neuroscience IRB at the National Institutes of Health, USA (OH-93-N-016). Written informed consent was obtained from all adult subjects and parents of minor subjects under the age of 18 years. Subjects in this study were ascertained from Punjab province of Pakistan. Probands were initially identified at schools for the deaf in Punjab, Pakistan. National Centre of Excellence in Molecular Biology (NCEMB), Lahore, Pakistan enrolled the families in this study and performed clinical evaluation in collaboration with National Institutes of Health. Genotyping and sequencing of the DNA samples from the participating individuals was carried out at the Cincinnati Children's Hospital Research Foundation, USA.

Clinical evaluation

We performed medical history interviews to find obvious syndromic and environmental causes of hearing loss. For some of the affected individuals, a physical examination was performed to detect signs, symptoms or stigmata of other disorders such as Waardenburg or

Pendred syndromes. Affected subjects underwent a general otological examination, including otoscopic examination and audiometry. Hearing was evaluated in some affected and unaffected subjects by pure-tone air- and bone-conduction audiometry with or without tympanometry. No air-bone gaps were observed in any tested individuals. Vestibular function was assessed by tandem gait and Romberg testing. Funduscopic examinations were performed by an ophthalmologist to confirm the absence or presence of retinitis pigmentosa.

DNA isolation, genotyping and linkage analysis

Genomic DNA was extracted from peripheral blood samples using a standard protocol.[27] We performed a genome-wide scan in family PKDF231 for homozygosity among offspring of consanguineous marriages using 388 STR markers (v2.5 ABI Prism Linkage Mapping Set, Applied Biosystems, Foster City, CA) and an ABI Prism 3730 Genetic Analyzer. Alleles were assigned using Genscan and Genotyper software (Applied Biosystems). Linkage in family PKDF608 was identified by screening with STR markers linked to chromosome 10p11.21-q21.1.

LOD score calculations

Marker order and map distances are from the Marshfield genetic map (http://research.marshfieldclinic.org/). Two-point LOD scores were calculated with Superlink online version 1.5 (http://bioinfo.cs.technion.ac.il/superlink-online/). We assumed a recessive mode of inheritance, with full penetrance of USH in homozyotes and no phenocopies. The disease allele frequency was set at 0.001 with equal meiotic recombination frequencies for males and females. Short tandem repeat allele frequencies were defined by genotype analyses of 100 unaffected Pakistani individuals.

Candidate genes

We identified candidate *USH1K* genes on the UCSC Human Genome Browser (http://genome.ucsc.edu/). *PCDH15*, *GJD4*, *FZD4*, *RET*, and *LRRC18* genes were sequenced in both PKDF231 and PKDF608 families using the primers flanking all of the exonic and adjacent intronic sequences. PCR, sequencing conditions and mutation analysis procedures were performed essentially as described.[28,29]

Results

Clinical description

At the time of examination, the ages of the affected individuals in family PKDF231 (Figure 1a) ranged from 12 to 26 years, while the ages of the affected individuals of family PKDF608 (Figure 1a) ranged from 18 to 45 years. All affected individuals in both families displayed congenital bilateral profound, sensorineural hearing loss (Figure 1b). Both in family PKDF231 and PKDF608, deaf individuals had delayed onset of independent ambulation, consistent with vestibular dysfunction, which was further confirmed by tandem gait ability and by using the Romberg test. Funduscopic examination of two older affected individuals of both families PKDF231 [V:5 (24 yo) and V:7 (26 yo); Figure 1a] and PKDF608 [V:20 (45 yo) and V:21 (18 yo); Figure 1a] revealed signs of retinitis pigmentosa along with narrowing of retinal blood vessel, bone spicules and waxy appearance of disc.

The severity of retinitis pigmentosa was directly related to the age of the patient and ranged from mild to the severe loss of vision.

Linkage mapping

We undertook a genome wide linkage analysis in family PKDF231. It initially showed suggestive evidence of linkage only to markers on chromosome 10p11.21-q21.1. Affected individuals were homozygous for markers in this interval while unaffected obligate carriers were heterozygous (Figure 1a). Additional markers were genotyped and haplotype analysis revealed a 11.74 cM interval of homozygosity delimited by markers *D10S1780* (63.83 cM) and *D10S546* (75.57 cM; Figure 2). A maximum two-point lod score (Z_{max}) of 3.82 at recombination fraction θ=0 was obtained for the marker *D10S539* (Table 1). Chromosome 10p11.21-q21.1-linked STR markers were then used to screen additional families segregating USH or isolated recessive deafness. One additional family, PKDF608, was found to be segregating USH1 linked to markers in this region (Figure 1a). A maximum two-point lod score (Z_{max}) of 3.22 at recombination fraction θ=0 was obtained for the marker *D10S539* (Table 1).

Part of *PCDH15* gene, mutant alleles are responsible for USH1F/DFNB23 phenotype in humans,[7,28-30] is present within the distal breakpoint therefore we considered it a candidate for the USH1 phenotype. Full sequencing of *PCDH15* in the genomic DNA from two affected individuals from each family along with normal hearing sibling did not reveal any functional mutations. Therefore, HUGO nomenclature committee assigned *USH1K* designation for the locus defined by families PKDF231 and PKDF608. Linkage interval of *USH1K* locus is approximately 20.20 Mb delimited by markers *D10S1780* and *D10S546* and harbor 85 candidate genes (Figure 2). The *USH1K* critical interval overlaps *DFNB33* (MIM 607239), a locus for non-syndromic recessively inherited hearing loss that was previously mapped between markers *D10S193* and *D10S1784*.(ref 31) We next considered the possibility that mutations of a single gene might underlie both USH1K and DFNB33. If so, the mutated gene is located between markers *D10S1780* and *D10S1784*, which spans 19.12 Mb (UCSC human genome browser, Figure 2). On this assumption we examined the overlapping linkage interval of *USH1K* and *DFNB33* and found same 85 genes (Figure 2). Analysis of data reported in a massively parallel signature sequencing libraries of mRNA from inner ear tissues[32] and SHIELD database (https://shield.hms.harvard.edu/) reveal 53 of the 85 genes are expressed in the inner ear (Figure 2). The coding exons and flanking intronic sequence of additional four candidate genes *GJD4*, *FZD8*, *RET* and *LRRC18* were sequenced in two affected individuals from each of the two USH1K families analyzed in this study, and no pathogenic sequence variants were found.

Discussion

Haplotype analysis of two families revealed a 11.74 cM region of homozygosity for *USH1K* on chromosome 10p11.21-q21.1. Families PKDF231 and PKDF608 each have unique haplotypes across this region, and therefore probably segregate different mutant USH1 alleles. The *USH1K* locus overlaps the *DFNB33* locus on chromosome 10 and these two hearing disorders may be due to allelic mutations. Mutant alleles of four of the known USH1

genes, *MYO7A*, *USH1C*, *CDH23* and *PCDH15* are responsible for both non-syndromic hearing loss and USH.[2,7-10,13,14,28,33-35] However, it is also plausible that these two loci are non-allelic.

Besides, *USH1K* and *DFNB33* loci, human chromosome 10q also harbor two other loci for Usher syndrome type 1 and nonsyndromic hearing loss, *USH1D/DFNB12* and *USH1F/DFNB23*.(ref 6,7,9,10,33) *USH1D/DFNB12* locus is approximately 17.10 Mb telomeric to *USH1K* locus, while part of the *PCDH15* gene, responsible for USH1F/DFNB23, lies within the distal boundary of *USH1K* (Figure 2). Sequencing of all the known coding and non-coding exons and 100 bp flanking exon-intron junctions of *PCDH15* in both USH1K families did not reveal any pathogenic mutation. Although it is possible that *PCDH15* may harbor cryptic mutations in cochlear-specific regulatory regions leading to USH1 in these two families, however, statistical analysis did not provide significant evidence of linkage of the USH1 phenotype segregating in USH1K family PKDF231 to *PCDH15* intronic STR marker (*D10S546*; Table 1). There have been precedents for two closely associated or partially overlapping deafness loci in human, for example, *DFNB36* and *DFNB96*, *DFNB3* and *DFNB85*, *DFNB35* and 14q23.1-q31.1 loci.[36-39]

In the linkage interval common to *USH1K* and *DFNB33* there appears to be 85 known genes, out of which 53 are expressed in the inner ear. The candidate deafness genes in the critical *USH1K/DFNB33* interval are *GJD4*, *FZD4*, *RET* and *LRRC18* (Figure 2). *GJD4* (MIM 611922) encodes the gap junction protein connexin 40.1. Mutations in several different connexin sub-units have been identified in individuals suffering with either non-syndromic or syndromic deafness.[40] *FZD4* (MIM 604579) encodes a seven-transmembrane domain protein that belongs to frizzled receptor gene family. FZD4 plays a central role in the inner ear vascular development through Wnt signaling pathway.[41] *RET* (MIM 164761), a member of cadherin superfamily, encodes a receptor tyrosine kinase.[42] Mutations in *RET* also cause Hirschsprung disease (MIM 142623), in which hearing loss is sometime detected.[43,44] *LRRC18* encodes a leucine rich repeat containing protein member 18. Mutations in a different family member, *LRTOMT* (also known as *LRRC51*; MIM 612414), have been implicated in non-syndromic hearing loss.[45-47] However, sequencing of these four candidate genes in both USH1K families did not reveal any potential pathogenic variant. All of the 85 candidate genes and conserved sequences in the *USH1J/DFNB33* interval will now need to be screened for mutant alleles. Rather than continuing hierarchical sequencing of candidate genes based on function or expression, future studies will employ massively parallel sequencing of genomic DNA from the affected individuals of these families enriched for the entire *USH1J* critical interval.

USH mutations are estimated to be responsible for more than 50% of deaf-blindness, 8 to 33% of patients thought to have isolated RP, and 3 to 6% of patients thought to have isolated deafness.[48-50] Effects of hearing loss on quality of life include difficulty in understanding speech and social isolation. In routine life, deaf people are strongly dependent on their vision, and blind people on their hearing, while individuals with Usher syndrome (USH) are deficient in both senses and thus suffer exacerbated quality-of-life effects. Ultimately, by limiting one's ability to communicate and interact, hearing and vision impairments impact cognitive, emotional and social development, making the development of intervention

strategies a clinically significant long-term goal of the current research. Mapping of *USH1K* is a first step for understanding the molecular mechanisms resulting in Usher syndrome.

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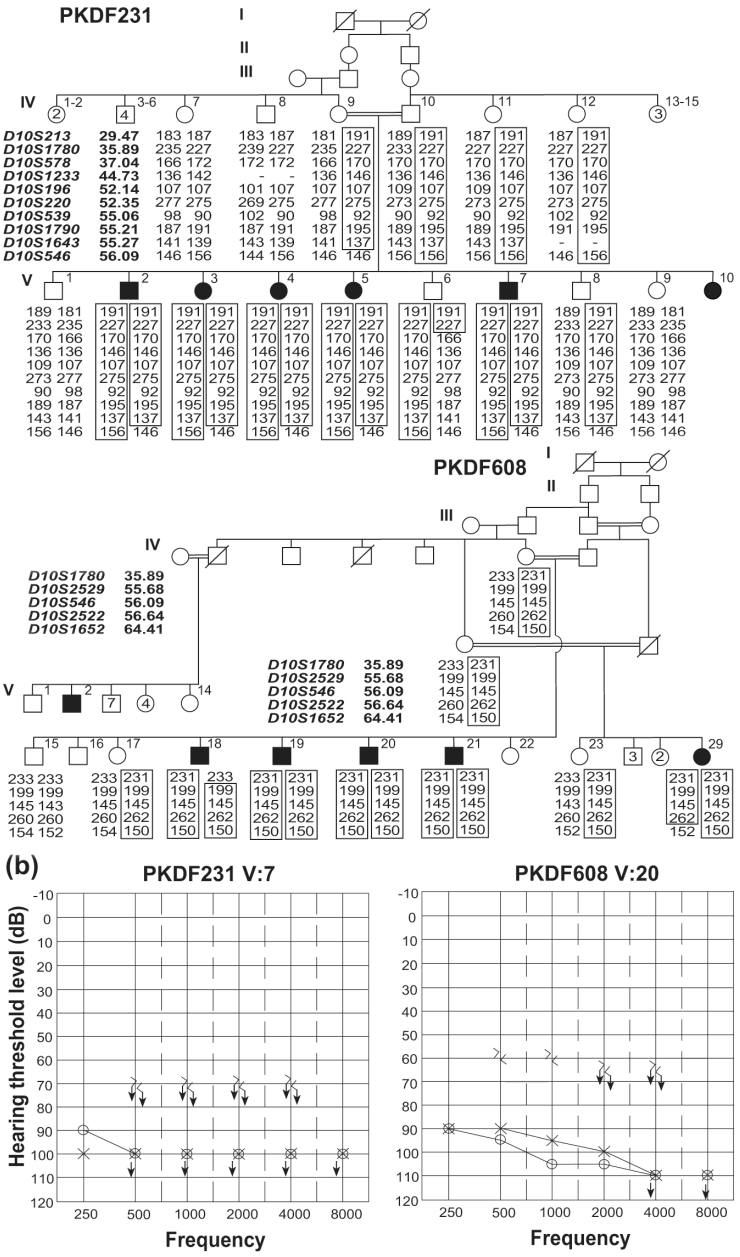

Figure 1.

USH1K families PKDF231 and PKDF608 and their representative audiograms. (**a**) Chromosome 10 haplotypes in family PKDF231. Filled symbols represent deaf individuals. The USH1J-linked haplotype is boxed. The STR markers and physical map positions in megabases (Mb, February 2009 human reference sequence GRCh37, hg19) are shown on the left of the pedigree. Haplotype analysis of PKDF231 shows a linkage region of 20.20 Mb delimited by markers *D10S1780* (35.89 Mb) and *D10S546* (56.09 Mb). Affected individuals V:2, V:3, V:4, V:5 and V:7 provided distal meiotic breakpoint at *D10S546* (56.09 Mb), while the unaffected individual V:6 provided the proximal recombination at *D10S1780* (35.89 Mb). In family PKDF608 affected individual V:18 provided the proximal

meiotic breakpoint at marker *D10S1780* (35.89 Mb). The distal breakpoint at marker *D10S1652* (64.31 Mb) was provided by affected individual V:29 (data not shown). (**b**) Pure tone air and bone conduction thresholds for family PKDF231 V:7 (26 yo male), and family PKDF608 V:20 (45 yo male) revealed profound, bilateral, sensorineural hearing loss. Right ear air conduction: O; Left ear air conduction: X; Right ear bone conduction: >; Left ear bone conduction: <; ↓ indicates the threshold level beyond the measurable range.

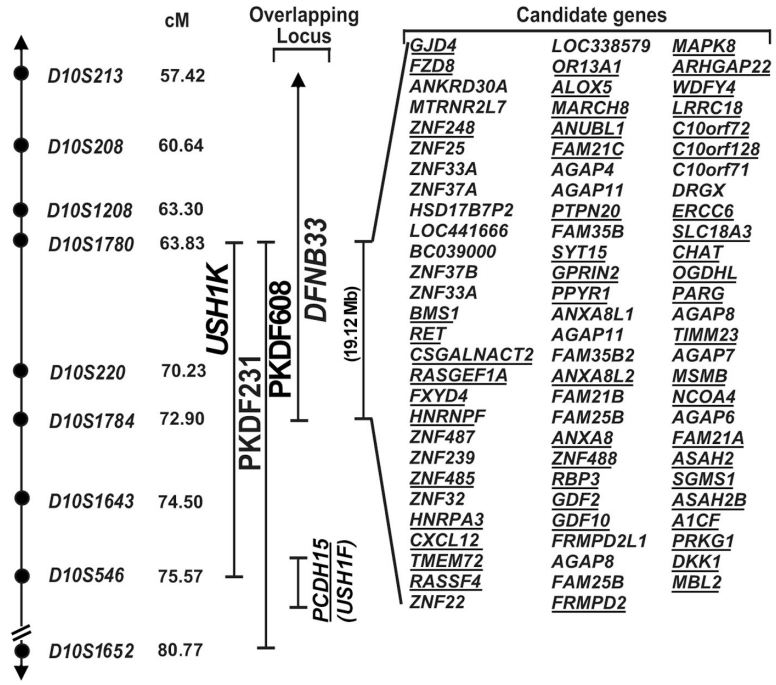

Figure 2.

USH1K linkage intervals in families PKDF231 and PKDF608 on human chromosome 10p11.21-q21.1. STR markers are represented by filled circles. The sex averaged recombination positions in cM are indicated for STR markers. Candidate genes in the *USH1K* interval were identified from the UCSC Human Genome Browser February 2009 assembly (http://genome.ucsc.edu/). Candidate genes expressed in the inner ear are underlined. Previously reported deafness locus *DFNB33* interval and USH1F/DFNB23 causing gene *PCDH15* is also shown.

Table 1

LOD scores for markers on chromosome 10p11.21-q21.1 in two USH1K families

Marker	cM[a]	Mb[b]	Maximum Two-point LOD scores (θ)	
			PKDF231	PKDF608
D10S197	52.10	26,526,881	-	0.66 (.30)
D10S213	57.42	29,473,069	2.52 (.05)	0.38 (.20)
D10S208	60.64	31,680,077	-	-
D10S1780	63.83	35,889,684	2.46 (.05)	1.46 (.10)
D10S578	65.97	37,042,086	0.64 (.20)	3.21 (0)
D10S1233	66.50	44,729,498	3.15 (0)	-
D10S196	70.23	52,142,268	1.98 (0)	0.92 (0)
D10S220	70.23	52,347,295	3.74 (0)	-
D10S539	72.90	55,060,405	4.15 (0)	3.83 (0)
D10S1790	75.57	55,205,311	1.89 (.10)	-
D10S1643	74.50	55,271,473	1.69 (0)	-
D10S546[*]	75.57	56,094,398	0.15 (.25)	0.65 (0)
D10S1652	80.77	64,407,495	-	1.66 (.05)
D10S581	82.50	65,849,304	-	0.35 (.20)

[a] The sex averaged genetic map positions in centiMorgans (cM) are taken from the Marshfield human genetic map (http://research.marshfieldclinic.org/genetics).

[b] Physical map positions in megabases (Mb) are according to February 2009 human reference sequence GRCh37, hg19.

[*] *PCDH15* intronic STR marker.