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Mutations of the Protocadherin Gene *PCDH15* Cause Usher Syndrome Type 1F

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Human chromosome 10q21-22 harbors *USH1F* in a region of conserved synteny to mouse chromosome 10. This region of mouse chromosome 10 contains *Pcdh15*, encoding a protocadherin gene that is mutated in *ames waltzer* and causes deafness and vestibular dysfunction. Here we report two mutations of *protocadherin 15* (*PCDH15*) found in two families segregating Usher syndrome type 1F. A Northern blot probed with the *PCDH15* cytoplasmic domain showed expression in the retina, consistent with its pathogenetic role in the retinitis pigmentosa associated with *USH1F*.

Introduction

Usher syndrome is estimated to be the most frequent cause of deaf-blindness in the United States (Kimberling et al. 1991). Usher syndrome type I (USH1) is the most common of the three clinical subtypes (Astuto et al. 2000). It is characterized by congenital profound sensorineural hearing loss, vestibular areflexia, and retinitis pigmentosa, with onset near puberty (Smith et al. 1994). Six loci (A–F) for USH1 are known (Kaplan et al. 1992; Kimberling et al. 1992; Smith et al. 1992; Wayne et al. 1996, 1997; Chaib et al. 1997). Genes for three of these loci have been identified: Usher syndrome type 1B (*USH1B* [MIM 276903]; Weil et al. 1995), Usher syndrome type 1C (*USH1C* [MIM 276904]; Bitner-Glindzicz et al. 2000; Verpy et al. 2000), and Usher syndrome type 1D (*USH1D* [MIM 601067]; Bolz et al. 2001; Bork et al. 2001).

Usher syndrome type 1F (*USH1F* [MIM 602083]) was first reported by Wayne et al. (1997). The flanking markers, D10S199 and D10S596 at 10q21-22, span a 15-cM interval centromeric to *DFNB12*/*USH1D* (Wayne et al. 1997). *USH1D* and *USH1F*, both on chromosome 10, are the second-most-common cause of Usher syndrome, following mutations in myosin VIIa (*MYO7A* or *USH1B* [MIM 276903]; Astuto et al. 2000). The frequency of Usher syndrome was estimated to be 3.5 in 100,000 individuals living in Scandinavia (Hallgren 1959; Nuutila 1970; Grondahl 1987) and 4.4 in 100,000 individuals living in the United States (Boughman et al. 1983). Of the three Usher syndromes, type 1 is the most common in families of European extraction. Among families with USH1, mutations in *MYO7A* (*USH1B*) account for >40% of incidences of deaf-blindness (Liu et al. 1998*b*; Friedman et al. 1999; Astuto et al. 2000). In our sampling of 120 families, ascertained in Pakistan, who have inherited profound, congenital deafness (Friedman et al. 2000), 8 families showed linkage to *USH1D/DFNB12* (Bork et al. 2001) and 3 families showed linkage to *USH1F/DFNB23*.

Mutations in mouse orthologues of known Usher genes are excellent animal models of this phenotype. Mutations in *MYO7A/Myo7a* are found in patients with *DFNB2*, *USH1B*, *DFNA11*, and in *shaker-1* (*sh1*) mice (Gibson et al. 1995; Weil et al. 1995; Liu et al. 1997*a*, 1997*b*; Weil et al. 1997). The phenotype of *sh1* mice is characterized by hyperactivity, head tossing, and circling behavior due to vestibular and cochlear dysfunction (Gibson et al. 1995). Moreover, it has recently been shown that recessive mutations of *cadherin 23* (*CDH23* [MIM 605516]) are associated with nonsyndromic deafness DFNB12 (Bork et al. 2001) and USH1D (Bolz et al. 2001; Bork et al. 2001) and that mutations of the mouse orthologue, *Cdh23*, are the cause of the *waltzer* phenotype (Di Palma et al. 2001).

In the present study, we refined the map location of *USH1F* to an interval of 1 Mb of DNA at 10q21.1. Mutations in *protocadherin 15* (*PCDH15* [MIM 605514; GenBank accession number AY029237])—the human orthologue of mouse *Pcdh15*, which is respon-

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sible for the *Ames waltzer* (*av*) phenotype (Alagramam et al. 2001)—were found in two families with USH1F.

Subjects, Material, and Methods

Subjects, Sample Collection, and Genomic DNA Isolation

Institutional review board approval (OH93-N-016) and written informed consent were obtained for all subjects in this study. Peripheral blood samples were obtained from participating subjects. DNA was extracted from peripheral blood (Grimberg et al. 1989) or from buccal swabs (BuccalAmp™ DNA-extraction kit, Epicentre Technologies). Pure-tone audiometry, fundoscopy, and electroretinogram (ERG) exams were performed on selected affected individuals aged >13 years from each family.

Linkage Analysis

DNA samples were PCR amplified with fluorescently labeled primers surrounding microsatellite repeats at known Usher syndrome and recessive deafness loci. PCR products were visualized by gel electrophoresis on an ABI 377 DNA sequencer, and genotypes were determined by Genescan and Genotyper (PE Biosystems). All genetic distances herein are based on published data (Broman et al. 1998). Additional primers in the *USH1F* linkage region were end-labeled with [γ-^{32}P] through use of T4 polynucleotide kinase. PCR products were separated by electrophoresis on a 6% denaturing urea/polyacrylamide gel and were visualized by autoradiography.

LOD scores were calculated by FASTLINK (Schaffer 1996). The disease was coded as fully penetrant, and the disease-allele frequency was set at .001. Meiotic recombination frequencies were assumed to be equal for males and females. The allele frequencies of the simple-tandem-repeat polymorphism (STRP) markers were calculated by genotyping of 96 randomly selected unaffected individuals from the same population.

Physical Mapping

A physical map of the refined linkage region was constructed through use of information from the Washington University Genome Sequencing Center FPC database Web site and the Sanger Centre Web site (fig. 2*A*). Sequence homology searches of available BAC sequences were performed, using the National Center for Biotechnology Information Web database and the Celera Human Genome Publication Web site, to find additional sequence information. Sequence data were confirmed to belong to the linkage interval through the discovery and typing of additional STRPs found among BACs or of sequences that pertained to the linkage region. Known genes and expressed-sequence tags (ESTs) present in the refined region were obtained by BLAST searches using BLASTn, BLASTx, and tBLASTn (Zhang et al. 1998) against the available "nt" and "nr" databases downloaded from the National Center for Biotechnology Information Web database to our local computer. The public Human Genome Project assembly confirms the minimal tiling path and EST hits (Lander et al. 2001). Gene prediction was carried out by GENSCAN (Burge and Karlin 1997; GENSCAN Web server at MIT), FGENES (Solovyev and Salamov 1997; Salamov and Solovyev 2000; CGG Nucleotide Sequence Analysis Web site), and MZEF (Zhang 1997; Gene Finder Web site).

Sequence Analysis

The 33 coding and noncoding exons of *PCDH15* were PCR amplified from genomic DNA, using touchdown PCR in a 20-μl reaction volume. Primers were designed (using software at the Primer3 Web site) to flank the exon-intron boundaries (see Appendix). PCR reactions were performed with 50 ng genomic DNA in the presence of 5 pmol each of forward and reverse primers, 4.8% DMSO, 200 mM each dNTP, 0.75 M Betane, 1 × PCR buffer (PE Biosystems), 1.5–2.5 mM $MgCl_2$ (PE Biosystems), and 0.5 U of a thermostable DNA polymerase. The thermal cycling conditions (PTC-225 DNA Engine, MJ Research) were 95°C for 2 min, 64°C–69°C for 45 s, 72°C for 45 s, and then nine cycles of touchdown PCR, with annealing temperatures of 69°C–54°C, followed by 35–38 cycles of annealing at 54°C–60°C, and final extension at 72°C for 15 min. Fifteen microliters of this PCR product were then treated with 0.3 U of shrimp alkaline phosphatase and 3 U of exonuclease I at 37°C for 1 h, followed by incubation at 80°C for 15 min. This was diluted with an equal volume of dH_2O, and 6 μl was used for the final sequencing reaction. In the sequencing reaction, we added 3.2 pmol of primer, 2 μl of Big Dye Terminator Ready Reaction Mix (PE Biosystems), and 1 μl of 5 × dilution buffer (400 mM Tris-HCl pH 9 and 10 mM $MgCl_2$). Cycling conditions were 96°C for 2 min and 45–49 cycles of 96°C for 10 s, 55°C for 10 s, and 60°C for 4 min. Sequencing reaction products were ethanol precipitated, and the pellets were resuspended in 3 μl of formamide loading dye. An ABI 377 DNA sequencer was used to resolve the products, and ABI prism software was used to analyze the results. PHRED/PHRAP/CONSED/POLYPHRED software was used for mutational analysis (Nickerson et al. 1997; Ewing and Green 1998; Ewing et al. 1998; Gordon et al. 1998).

5′ RACE and cDNA Analysis

The genomic structure of *PCDH15* was predicted on the basis of "tblastn" homologies to *Pcdh15* found in the available human genomic DNA sequence. Fourteen

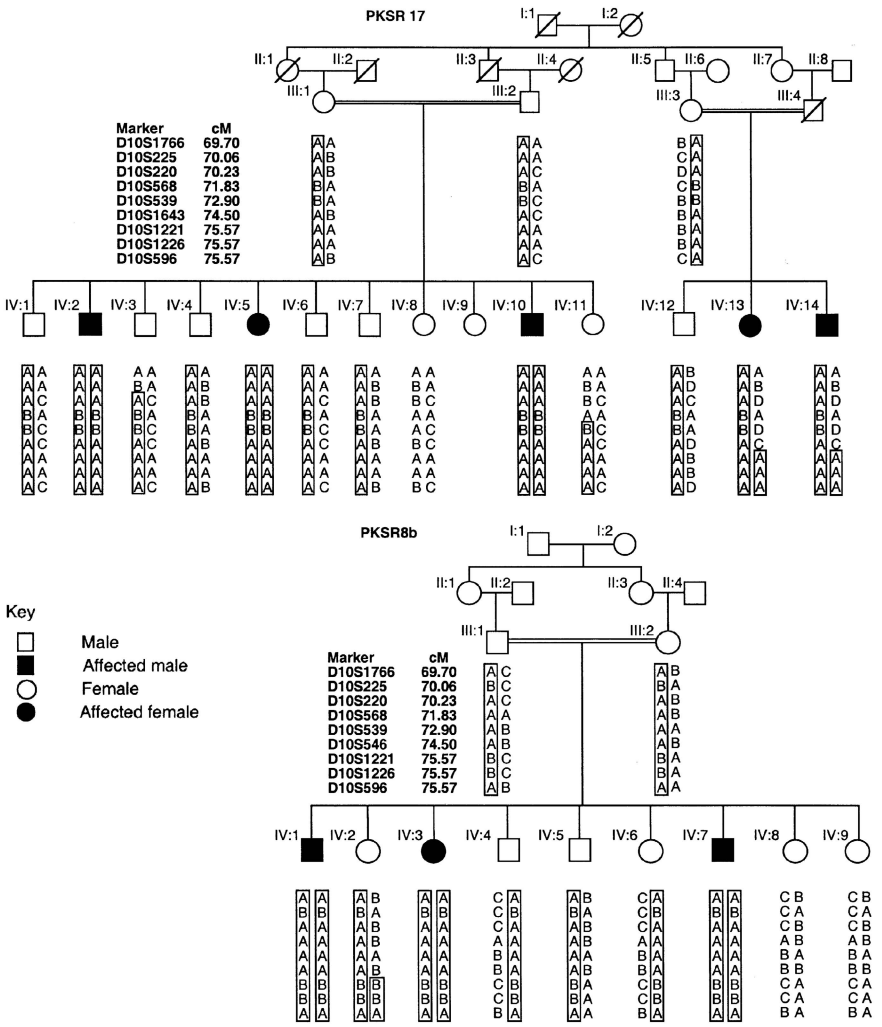

Figure 1 Haplotypes of two Pakistani families demonstrating linkage to *USH1F* at 10q21-22. Two affected individuals, IV:13 and IV:14 in family PKSR17, provided the proximal recombination at marker D10S1643 (74.5 cM). The distal recombination is provided by unaffected individual IV:2 of family PKSR8b, at marker D10S1226 (75.5 cM). Fundoscopic and ERG examination revealed early signs of retinitis pigmentosa in affected members of both families. The genetic linkage distances are from the Center for Medical Genetics, Marshfield Medical Research Foundation Web site (Broman et al. 1998).

primer pairs, designed from the predicted genomic homology, were used to amplify the *PCDH15* in overlapping segments from human brain Marathon-Ready cDNA (Clontech). Primers from exon 9 (5′-GGAATAGAGGATTCCTGGCCTATCTG-3′), exon 8 (5′-TATGGCAGCTTGATAAGTGAGTGGAC-3′), exon 6 (5′-TCCATTTGGTCCATCATCTATATCT-3′), exon 5 (5′-GTCATTCCTGTCTCTCACCACTATT-3′), exon 4 (5′-ATCAACACCCAGTAATCCACATTAT-3′), and exon 3 (5′-CGACTTTCTTCATCAATAGCAACTA-3′) were used for a 5′ RACE PCR, using human brain Marathon-Ready cDNA as template (Clontech). The thermal cycling conditions used were 94°C for 1 min, 25 cycles at 94°C for 30 s, and 72°C for 5 min. Five microliters of each sample were analyzed on a 1.2% agarose/ethidium bromide gel, were extracted with a QIAquick gel extraction kit (Qiagen), and were cloned into *Escherichia coli* through use of pGEM®–T Easy Vector (Promega). DNA was purified from minipreps using QIAquick miniprep kit (Qiagen), and inserts were sequenced using T7 and SP6 primers.

Northern Blot Analysis

Human donor eyes (ages 54 and 60 years) and human occipital cortex (age 74 years) were obtained from the National Disease Research Interchange. Total RNA was isolated from dissected ocular tissues and brain, through use of RNAzolB (Tel-Test). Ten micrograms each of total RNA from retina, ciliary body, retinal pigmented epi-

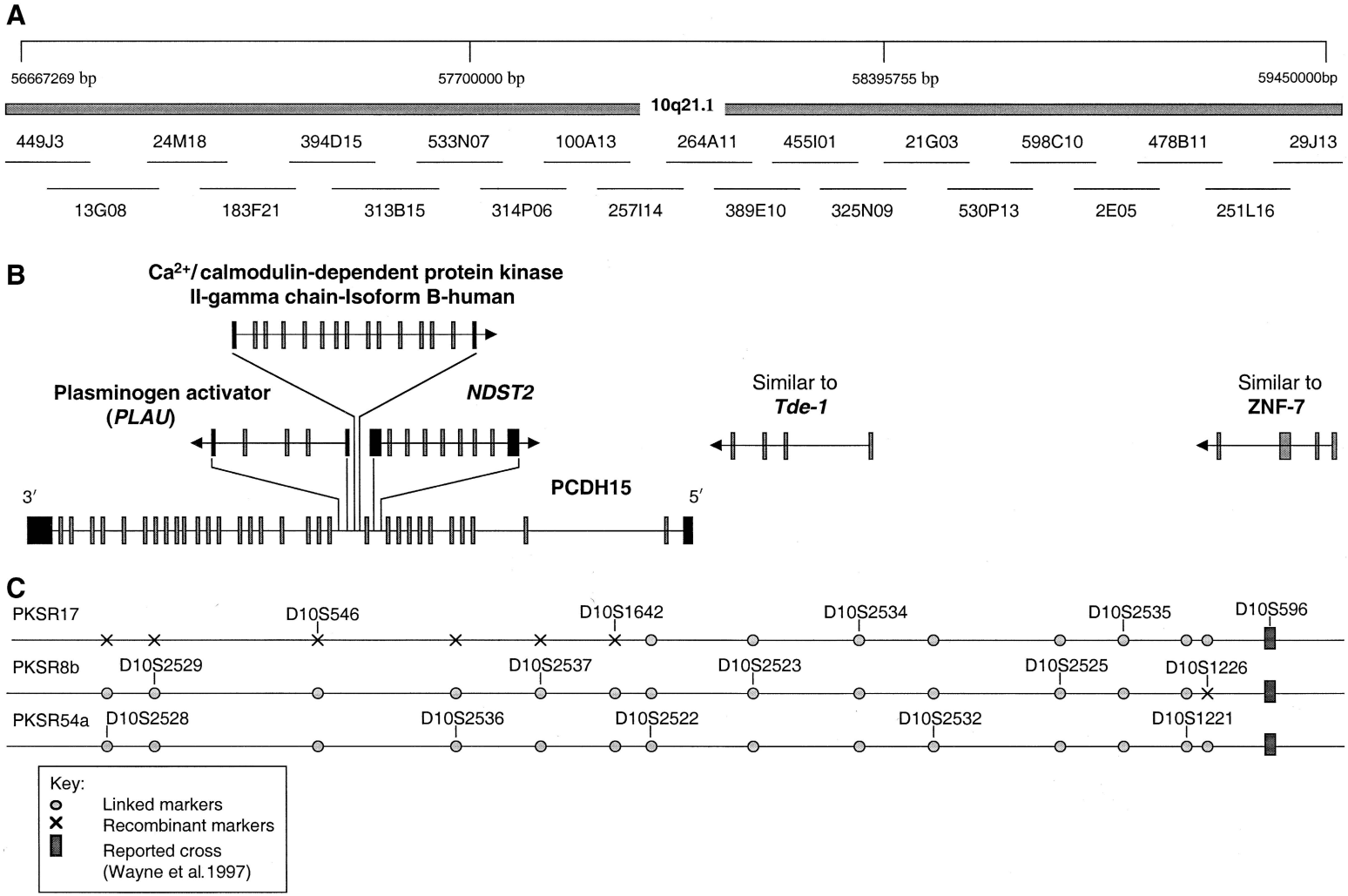

Figure 2 *A*, Genetic and underlying physical map of *USH1F* at 10q21.1 and 21 overlapping BACs covering this interval. The bp distances are from the Human Genome Project Working Draft Web site. *B*, Exons of *PCDH15*, spanning ~1.6 Mb on the physical map, denoted by horizontal lines. There are three genes found in the introns of *PCDH15*: Ca^{2+}/calmodulin-dependent protein kinase type II, *NDST2*, and *PLAU*. Also shown are two genes predicted by GENSCAN. One is similar to mouse tumor differentially expressed transcript-1 (Tde-1, a transmembrane protein). The other is similar to zinc finger protein-7. *C*, Linked markers in each family (denoted as shaded circles on the horizontal line), and recombinant markers (denoted with an " × "). The previously reported distal recombination D10S596 (Wayne et al. 1997) for *USH1F* is denoted by a black block.

Table 1

Two-Point LOD Scores

	LOD Score (Recombination Fraction) for Family		
Marker	PKSR17	PKSR54a	PKSR8b
D10S539	2.34 (.05)	2.73 (0)	1.05 (0)
D10S1643	2.64 (.05)	3.48 (0)	.35 (0)
D10S1221	1.94 (0)	4.22 (0)	2.37 (0)
D10S1226	1.94 (0)	3.69 (0)	1.17 (.08)
D10S596	4.94 (0)	2.97 (0)	.94 (0)

thelium (RPE)-choroid, iris, human brain (Clontech), and human liver (Clontech), along with RNA size standards (Life Technologies), were electrophoresed on an agarose-formaldehyde denaturing gel and were transferred to GeneScreen Plus membrane (Dupont-NEN). RNA loading per lane was initially monitored using the 18S rRNA band (Correa-Rotter et al. 1992). The northern blot was hybridized with a random primed, triple-labeled ([α^{32}P]-dATP, -dCTP, and -dGTP) 590-bp cDNA probe, which had been PCR amplified through the use of primers 5′-AGCAGAACTTGAAAAATCAGTAGC-3′ and 5′-CCTTATGTCTGTCATCTCCTACAATC-3′ from the unique carboxy-terminal domain of *PCDH15*. The northern blot was hybridized at 63°C in Hybrisol II (Intergen) for 18 h and was washed at 63°C in 0.2 × SSC and 0.1% SDS.

Results

Family and Clinical Data

The disease phenotype in three Pakistani families demonstrated linkage to STRP alleles in the reported *USH1F* and *DFNB23* interval (figs. 1 and 2). These consanguineous families segregate autosomal recessive severe-to-profound congenital deafness, determined by pure-tone audiometry. Affected individuals from families PKSR17 and PKSR8b had frank signs of retinitis pigmentosa, determined by fundoscopy or ERG examinations.

Family PKSR8b had three affected individuals for whom polymorphic markers were found linked from D10S1766 (69.7 cM) through D10S596 (75.6 cM), with a LOD score of 2.37 (table 1). One individual with normal hearing, IV:2 (fig. 1), was homozygous for markers D10S1226 (75.6 cM) and D10S596, providing the distal recombination and further refining the linkage region. Nine additional Usher loci (for types IA, 1B, 1C, 1D, 1E, IIA, IIB, IIC, and III) were excluded in this family by genotype analysis of four STRPs at each locus (data not shown).

Family PKSR17 had five affected individuals in two sibships cosegregating retinitis pigmentosa and deafness. Individuals IV:13 and IV:14 (figs. 1 and 2*C*) provided the proximal recombination at marker D10S1642 (74.5 cM). Family PKSR54a had five affected individuals in two sibships who were homozygous for seven markers from D10S220 (70.23 cM) to D10S596 (75.6 cM). The published distal recombination for *USH1F* is D10S596 (Wayne et al. 1997). LOD scores for all three families are shown in table 1.

Identification of the USH1F *Gene*

The *av* mutation is a recessive allele associated with deafness in the mouse (Schaible 1956). The gene mutated in *av* is *Pcdh15* (Alagramam et al. 2001). Conserved synteny between human and mouse chromosome 10 suggested that *Pcdh15* may be the murine model for *USH1F* and/or *DFNB23* (Alagramam et al. 2001). A protein-sequence–similarity search indicated that the human orthologue of *Pcdh15* spanned ~1.6 Mb of genomic DNA represented by 11 overlapping BACs on human 10q21.1. There are three genes nested in the introns of *PCDH15*: Ca^{2+}/calmodulin-dependent protein kinase type II, N-

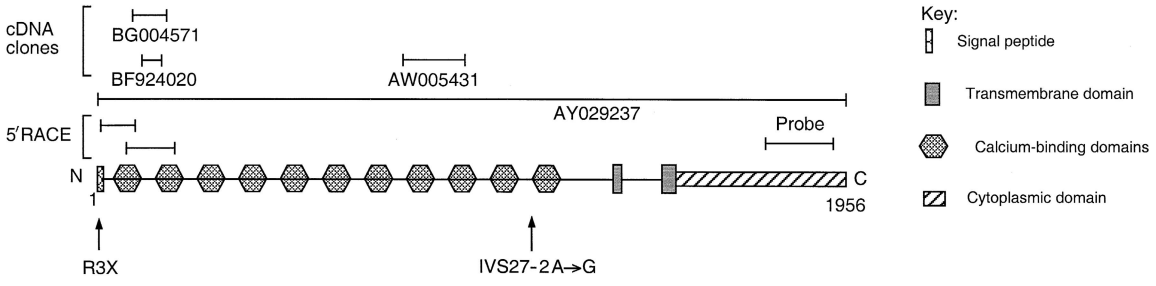

Figure 3 Domains of *PCDH15*, predicted from 1,955 amino acids. *PCDH15* has a signal peptide at the amino terminus, 11 extracellular calcium-binding domains, 1 predicted transmembrane domain, and a unique carboxy terminus (GenBank). TMpred and SMART predicted two transmembrane domains (1054–1072 and 1376–1397), TMHMM predicted only one transmembrane domain, at 1376–1397 (SMART Web site; TMHMM Server, version 2; TMpred Prediction of Transmembrane Regions and Orientation Server). The mutations found in two families with USH1F are also annotated. RACE products obtained and three ESTs found in the NCBI nucleotide database are shown, along with the 590-bp probe used for the northern blot (fig. 5).

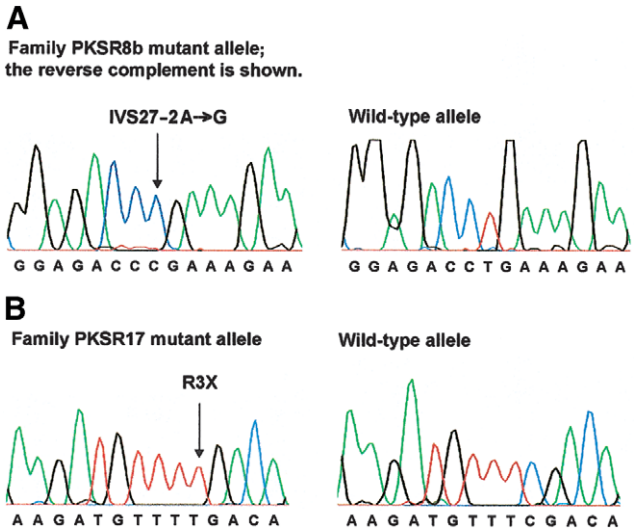

Figure 4 *PCDH15* mutations in two Pakistani families. *A*, Sequence chromatograms for the splice-site mutation and wild-type allele of family PKSR8b. An arrow (↓) indicates the IVS27−2A→G replacement in intron 27. The sequence trace file for the antisense strand is shown. This transition mutation results in an alteration in the acceptor splice site of exon 28. For genomic DNA with this mutation, GENSCAN predicted the skipping of exon 28 and the use of an alternate exon in intron 27. *B*, Sense strand–sequence chromatograms for +7C→T, found in family PKSR17, and for the wild-type allele. This mutation results in a transition from an arginine to a termination codon (R3X).

deacetylase/N-sulfotransferase (heparin glucosaminyl) 2 (*NDST2*), and plasminogen activator, urokinase (*PLAU*) (fig. 2*B*). Only the first 2 of the 33 exons of *PCDH15* are in the *USH1F* critical interval defined in family PKSR17 (fig. 2*B*). Primer pairs from the predicted human *PCDH15* exons, in combination with 5′ RACE, allowed us to sequence *PCDH15* from human brain cDNA. The start codon is found in the middle of the second exon, at 396 bp (GenBank). The stop codon is in exon 33, at 6,263 bp, with additional downstream termination codons in all three reading frames. *PCDH15* encodes a predicted protein of 1,955 amino acids with a molecular weight of ~216 kD. The predicted protein has 83% identity and 88% similarity with *Pcdh15* (Alagramam et al. 2001). The sequence-analysis program SMART predicted a protein (fig. 3) with a signal peptide at the amino terminus, 11 extracellular calcium-binding domains, a transmembrane domain, and a unique cytoplasmic domain in human protocadherin-15 (Schultz et al. 2000; SMART Web site).

Sequence analysis of all the exons of *PCDH15* (fig. 2*B*) in three Pakistani families revealed a splice-acceptor–site mutation (IVS27−2A→G) in intron 27 of family PKSR8b (figs. 2*B* and 4). This mutation alters the invariant "A" of the acceptor splice site "AG" of exon 28, which encodes the 11th extracellular calcium-binding domain of protocadherin-15. This mutation was not present among 240 chromosomes sequenced from 120 randomly selected unaffected control subjects of the same ethnic group, nor was it found in 100 chromosomes from 50 control samples of the human diversity panel (Coriell Cell Repositories). The GENSCAN predicted effect of IVS27−2A→G, when included in the otherwise wild-type genomic sequence, is inclusion of a putative exon in intron 27 and skipping of exon 28. The use of this predicted additional exonic sequence would maintain an open reading frame with the replacement of the previous 29 amino acids encoded by exon 28 with 33 incorrect amino acids. The predicted protein would have 10 extracellular calcium-binding domains, rather than 11.

In family PKSR17, only the first coding exon (exon 2) of *PCDH15* is in the *USH1F* critical interval (fig. 2). In exon 2 we found a substitution at nucleotide +7 (7C→T) resulting in the replacement of an arginine codon by a stop codon in the predicted signal peptide sequence of protocadherin-15 (R3X, figs. 3 and 4). If it is assumed that there are no downstream cryptic translation-initiation codons, the predicted result of this nonsense mutation is the complete loss of protocadherin-15 in the affected individuals of family PKSR17. This mutation was not found in 580 chromosomes sequenced from a total of 290 randomly selected unaffected control

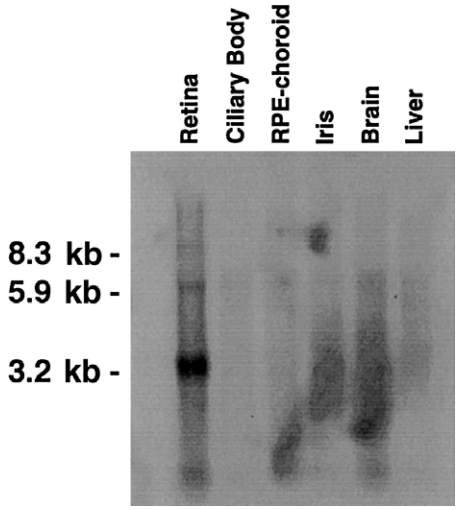

Figure 5 Northern blot for several human tissues. Lanes 1–6 contain 10 μg each of total RNA from human retina, ciliary body, RPE-choroid, iris, brain, and liver. Positions of bands from an RNA ladder are indicated along the right margin. A 6-d exposure to autoradiographic film is shown. In the retina, bands are apparent at ~3.3, 6.0, and 8.6 kb, with the larger transcript possibly being a doublet.

DNA samples from Pakistan and the Human Diversity Panel. No other *PCDH15* mutations were found in affected individuals of family PKSR54a.

A northern blot was performed using a probe designed from the unique cytoplasmic domain of *PCDH15* (fig. 5). *PCDH15* hybridizes to a major transcript from retina at ~3.3 kb, with additional lower-abundance transcripts at ~6.0 kb and ~8.6 kb. These transcripts appear smaller than those found in mouse brain. The longest *PCDH15* open reading frame so far identified is 5.87 kb (fig. 3). We also observed an ~0.4-kb 5′ UTR and an ~0.67-kb 3′ UTR. There are three ESTs found for *PCDH15* in the NCBI database (fig. 2), two from brain tissues and one from placenta cDNA (fig. 3). In contrast, the five mouse *Pcdh15* ESTs in GenBank are all from retina cDNA.

Discussion

In affected individuals from two Pakistani families with USH1F, we identified two mutant alleles of *PCDH15*, demonstrating that PCDH15 function is necessary in the sensory tissues of the human eye and ear. Affected members of these two families with USH1F are profoundly deaf at birth and show progressive retinopathy. In contrast to USH1F, the *av* mice have hearing loss but no reported retinopathy. The *av* mice have a normal endocochlear potential, ruling out the involvement of stria vascularis, but the hair cells and supporting cells are abnormal (Raphael et al. 2001). The *av* phenotype may not be a good model for the retinopathy of USH1F, but it may be a model for nonsyndromic recessive deafness if mutant alleles of *PCDH15* are found in affected members of families linked to *DFNB23*. *DFNB23* is listed on the Hereditary Hearing Loss Homepage and may be an allelic variant of *PCDH15*.

It is not unusual to observe phenotypic differences between species carrying a mutation in the orthologous gene; however, allelic differences can also underlie the variation in phenotype (Libby and Steel 2001). Nevertheless, retinal pathology may be present in *av* mice. The mouse model for *USH1B, sh1,* was initially thought to have a fully wild-type eye; however, an abnormal accumulation of opsin occurs in the connecting cilium of *sh1* mice, and there is a failure of melanosome transport to the apical processes in retinal epithelium (Liu et al. 1998*a*, 1999; Liu and Williams 2001). In addition, a more sensitive evaluation revealed that five of nine mutant *Myo7a* alleles cause a reduction in ERG amplitude with normal thresholds (Libby and Steel 2001).

There are six major subclassifications of the cadherin superfamily, including protocadherins, almost all of which are expressed in neuronal tissues (Nollet et al. 2000; Angst et al. 2001). A typical protocadherin will have up to seven extracellular calcium-binding domains, one transmembrane domain, and a unique intracellular domain (Angst et al. 2001). There are 11 extracellular calcium-binding domains predicted in the deduced amino acid sequence of human and mouse brain *PCDH15* (fig. 3). On a northern blot containing human RNA from several tissues, including brain, we find—in addition to the expected full-length 7.0-kb *PCDH15* message in human retina—a variety of putative *PCDH15* transcripts, some of which are too small to contain all 11 calcium-binding domains, suggesting the possibility of *PCDH15* splice variants with various numbers of predicted calcium-binding domains.

Examination of the regrowth patterns of neurons after surgical intervention led to the idea that neurons recognize their synaptic partners through "lock-and-key" interactions between molecular "specifiers" displayed on neuronal surfaces (reviewed by Shapiro and Colman [1999]). Cadherins—protocadherins in particular—are hypothesized to be part of the lock-and-key molecular mechanism involved in synaptic sorting (reviewed by Shapiro and Colman [1999]). Some protocadherin genes occur in clusters, which may allow the generation of many isoforms, by rearrangement in a manner similar to immunoglobulin genes (Shapiro and Colman 1999; Wu and Maniatis 1999); however, *PCDH15* is not part of a protocadherin gene cluster on 10q21.1, and the stereocilia of both outer and inner hair cells are abnormal in *av* mice. *PCDH15* is hypothesized to be involved in the development or maintenance of the stereocilia bundles on the apical surface of hair cells (Alagramam et al. 2001).

In summary, we report that mutant *PCDH15* alleles are responsible for USH1F. We do not yet understand the pathophysiology occurring in individuals with USH1F or the normal function of the cadherin-like protein encoded by *PCDH15*. This knowledge would be expected to form the keystone for the development of a therapy.

Acknowledgments

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Appendix

Table A1

Primer Pairs for Sequencing the Exons of *PCDH15*

Exon	Primer		Product Length (bp)
	Forward	Reverse	
1	ttgtcttttgggctgtagca	tcatgacagagatcattgtactgaaa	612
2	ccagtgcattaaaccagtgtgt	ggacatctgatttcacttttctaagc	452
3	attaattagattgcaagattttcctt	tccatggatttgggttgaaa	300
4	gcagatactttggccttttgg	gaaattgacaagcttccaggat	563
5	tgattgtcttctcttgtccttaaaa	ttttcccatagttaaatgaattcctaa	518
6	cacttcagtttgtggttgtgg	ccatcaaaaattagcttctagcaata	446
7	tcattctattttcagatgataagca	ggtcaaagaatacagtgaataaaaaca	437
8	tgcctaatttctataaactacctgttg	ccctgaaaataatttcggaca	367
9	tgctccccctaacattcaa	ttgaattgctttgcttcttcc	520
10	gtctgctttccctgaactgc	tgggcttcgcttattttctt	418
11	gcctcactggattttcatttc	ctttttaaaatatccttgttttgga	551
12	ttccacgatataggtcaccatc	ttcttctgtgaggaagaatgttaat	557
13	tgcttcttaccatattagaacacttttt	tgcttttatttgaaaagtaacagaaat	515
14	ttgtgattgctttagtattgtaacgg	tgcctctgatattgtccttttc	587
15	tcctgtaaacccaacaactgaata	atccatattaccctttgctttccttt	371
16	tttccacatcaggtaggattaca	aaaatgttaaaaataaacagtctcctg	487
17	gccttaaaagctgttttcaatttt	aaggttgcagcacaatagca	471
18	cagatagacaaatgccagaatga	aaatgtaaatctgtatccttgaaagtt	408
19	tcctccttagagggccaaat	cacaaaccctaatagcaaatctcc	495
20	gcactaacgcatgacttcca	tgttatgggtctggtaccactg	437
21	tgaggtgccagtctgaaatg	tgcattctagaaaaacaaaaagca	500
22	tgatctggctacatttcagctc	tccaagatgtgagataccaagtg	488
23	ccttctggaaccaggcaat	cgcctaaaccaaaattggaa	337
24	cctgcagacctcctccagta	tcactttagctaacatcactggattt	302
25	caaaagccagcattttgtca	atcactccctgccttgtcat	465
26	ggcatggcatctagaaatgg	aaaatgaaactcaagaagttgct	463
27	cagacatttaggagggcttt	agtgactaacaatctgagtgaaaa	520
28	gaatttgaaaatcagaaaccactg	cagttaaaaatcatggtcatataaaaa	267
29	tgggagctaaatgctgaaaaa	cctgaaaactttaaggcatgaga	532
30	tgaattctacccctattcaagaga	tcagagttcctgaacggtctactta	417
31	caagccatcctttactttatccaa	tgcaattgtctcctgaagttg	428
32	tgtcattaactctgtcattatgaattt	aaactattacaaacgcaaaacaagg	270
33a	catttctcattcttctcacaactc	cctcctgggtaagctgactg	595
33b	ttcagacatttcacagagaacaga	gagagtgaagaatgtaaaacacaagg	522
33c	caagaactgtggaactcaaatcag	cctgttatacagacacactctgtgg	502
33d	ctacctccatttccaactcctct	atttcattgaatttggggtaaaat	553
33e	gcaaacctcttgttgatcatagtc	ccaaatacataggcttcaagaaaaa	444
33f	tgtggatgcagtaacatttacatt	aaatgccttacaaaagtcaacgac	446

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Celera–Human Genome Publication Site, http://publication.celera.com/ (for sequence homology searches of BAC sequences)

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ (for genetic linkage distances)

CGG Nucleotide Sequence Analysis, http://genomic.sanger.ac.uk/gf/gf.html (for gene prediction)

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/index.html (for *PCDH15* [accession number AY029237])

Gene Finder, http://argon.cshl.org/genefinder/ (for the MZEF program)

Genome Sequencing Center, Human Genome Project BAC and Accession Maps (FPC), http://genome.wustl.edu/gsc/human/Mapping/index.shtml (for construction of the physical map)

GENSCAN web server at MIT, http://genes.mit.edu/GENSCAN.html (for gene prediction)

Hereditary Hearing Loss Homepage, http://dnalab-www.uia.ac.be/dnalab/hhh/ (for a complete listing of nonsyndromic deafness and Usher syndrome loci)

Human Genome Project Working Draft at UCSC, http://genome.cse.ucsc.edu/ (for bp distances)

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/ (for sequence homology searches of BAC sequences)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *CDH23* [MIM 605516], *MYO7A* [MIM 276903], *PCDH15* [MIM 605514], *USH1B* [MIM 276903], *USH1C* [MIM 276904], *USH1D* [MIM 601067], *USH1F* [MIM 602083])

Primer3, http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi (for the design of primers used in PCR and DNA sequencing)

Sanger Centre, The, http://www.sanger.ac.uk/ (for construction of the physical map)

SMART, http://smart.embl-heidelberg.de/ (for transmembrane domain prediction)

TMHMM Server, Version 2, http://www.cbs.dtu.dk/services/TMHMM/ (for prediction of transmembrane spanning amino acids)

TMpred Prediction of Transmembrane Regions and Orientation Server, http://www.ch.embnet.org/software/TMPRED_form.html (for prediction of transmembrane spanning amino acids)

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