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Identification and Characterization of a Novel Polycystin Family Member, Polycystin-L2, in Mouse and Human: Sequence, Expression, Alternative Splicing, and Chromosomal Localization

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Polycystins-1, -2, -L, and -REJ are the four known members of the polycystin family of proteins. In this study, we describe a fifth member of the family, polycystin-L2, encoded by PKD2L2 in human and Pkd2l2 in mouse. Full-length cDNA sequences for both mouse and human polycystin-L2 were obtained from testis cDNA. Sequence analysis predicts that the mouse and human polycystin-L2 proteins consist of 621 and 624 amino acid residues, respectively. Polycystin-L2 has significant homology with polycystins-L and -2, with similarities of 58 and 59%, respectively. Both human and murine polycystin-L2 proteins are predicted to have seven putative transmembrane (TM) domains, and, by comparison with transient receptor potential channels, the six carboxyl-terminal TM domains are likely to constitute an ion channel subunit. Northern blot analysis indicated that mouse Pkd2l2 has an abundant ~2.5-kb transcript in testis and an ~2.2-kb transcript in heart. RT-PCR analysis showed that the full-length transcript is expressed in human brain, kidney, testis, and HepG2 cells, and there are three alternatively spliced variants that were differentially expressed. PKD2L2 consists of 17 exons spanning approximately 50 kb of genomic DNA. PKD2L2 was mapped to human chromosome 5q31 and Pkd2l2 to mouse chromosome 18 in band C. © 2000 Academic Press

INTRODUCTION

Polycystins are membrane proteins that share significant sequence homology to one another and some similarity to voltage-gated Ca^{2+} and Na^{+} channel subunits (Nomura *et al.*, 1998; Hughes *et al.*, 1999; Wu *et al.*, 1998b). Polycystins-1, -2, -L, and -REJ are the known members of the family of polycystins. Mutations in polycystin-1 or -2 cause human autosomal dominant polycystic kidney disease (ADPKD), a common (1 in 1000) genetic disease that is characterized by progressive replacement of renal and hepatic tissue by cysts. Polycystin-1, the PKD1 gene product, is a 4303-amino-acid membrane glycoprotein comprising an ~2500-amino acid amino-terminal extracellular domain, 11 membrane-spanning regions, and a short intracellular tail (International PKD Consortium 1995; Hughes *et al.*, 1995). Polycystin-2, encoded by PKD2, is a 968-amino-acid membrane protein with 6 membrane-spanning domains and intracellular amino- and carboxyl-termini (Mochizuki *et al.*, 1996). PKDL or PKD2L1 (HGMW-approved symbol), which encodes an 805-amino-acid membrane protein, polycystin-L, was recently identified (Nomura *et al.*, 1998; Wu *et al.*, 1998b). The mouse orthologue of PKDL, Pkdl, is deleted in a mouse line with renal and retinal defects (Nomura *et al.*, 1998). Polycystin-REJ, a 2253-amino-acid protein encoded by PKDREJ, appears to be specifically expressed in testis (Hughes *et al.*, 1999). Although the overall sequence identity between polycystins-REJ and -1 is low (21–25%), these proteins share homology over a large region (>2000 amino acids) and have similar hydropathy profiles and other key features, suggesting that they have related functions.

Polycystins-1 and -2 are critical in the development of several organs including the kidney and pancreas. Mice homozygous for targeted mutations in either polycystin-1 or -2 develop fetal polycystic kidney and pancreatic disease and die *in utero* (Lu *et al.*, 1997; Wu *et al.*, 1998a). While the predicted domain structure of polycystin-1 suggests its involvement in cell–cell or cell–matrix interactions, the similarity of polycystins-2 and -L to the pore-forming domains of cation channels suggested that these polycystins may form ion channels. Indeed, we have recently shown that polycystin-L is a calcium-regulated cation channel permeable to

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calcium ions (Chen *et al.*, 1999). Polycystin-L may act as a transducer of calcium-mediated signaling *in vivo* as its channel activity was substantially increased when either the extracellular or the intracellular calcium-ion concentration was raised. Polycystin-L is distinguished from other structurally related cation channels by its large single-channel conductance. Taking the channel data together with the known interactions between polycystins-1 and -2 (Qian *et al.*, 1997; Tsiokas *et al.*, 1997), it has been hypothesized that polycystins-1 and -2 together form a regulated or gated channel.

In this study, we report the cloning and characterization of a fifth polycystin gene in mouse (Pkd2l2) and human (PKD2L2), together with data on its genomic structure, chromosomal localization, tissue expression, and alternative splicing.

MATERIALS AND METHODS

Isolation of mouse Pkd2l2 cDNA. Polycystin-L-specific primers mR1, 5′GAGCAAGATGATCACCAGG3′, and AP1, 5′CCATCCTAATACGACTCACTATAGGGC3′ (Clontech, Palo Alto, CA), were used to carry out rapid amplification of cDNA ends (RACE) on mouse testis cDNA. AdvanTaq DNA polymerase (Clontech) was used to perform touchdown PCR with cycling parameters as follows: initial denaturation at 94°C for 30 s; 5 cycles of 94°C for 10 s, 72°C for 4 min; 5 cycles of 94°C for 10 s, 70°C for 4 min; 25 cycles of 94°C for 5 s, 68°C for 4 min; and a final extension at 68°C for 7 min. PCR products were separated on a 1% agarose gel, and all resulting DNA fragments were excised and isolated. The products were purified and sequenced directly. For 3′ RACE amplification to obtain a full-length cDNA sequence, PCR was performed with primers AP1 and mF801, 5′GCATCATCAGGCTGCTGGCAGAGTTC3′. For 5′ RACE amplification, primers AP1 and mR803, 5′AGTGTTGATGACAGCTGCGACATT3′, were used to perform first PCR on mouse testis cDNA. A second amplification was performed using 2 μl of the diluted first PCR product (1:100) with the nested primers AP2, 5′ACTCACTATAGGGCTCGAGCGGC3′ (Clontech), and mR801, 5′GCGAGAAAACAGAGCAGCAGGAGCAG3′. 5′ and 3′ RACE products were cloned, and both strands were sequenced. The sequences were aligned to give an overall consensus sequence.

Isolation of human PKD2L2 cDNA. BLAST analysis of the GenBank database with mouse Pkd2l2 sequence revealed significant hits in one human PAC clone (9C13). Primers specific to the putative exonic sequence in PAC 9C13 were used to perform 5′ and 3′ RACE on human testis cDNA. For 5′ RACE amplification, primers AP1 and hR601, 5′GACTTTGCATGTGTTGTTGCGGACTT3′, were used to perform the first PCR. A second amplification was performed using 2 μl of the diluted first PCR product (1:100) and the nested primers AP2 and hR602, 5′AGTTGGTTCTTTCTTCACCAGGCACA3′. 3′ RACE amplification was carried out similarly with AP1 and hF603, 5′TTGGACACTTCTGTGCCTGGTGAAGA3′, as first PCR primers, and AP2 and hF601, 5′TCAGCTGTCATCAACCTTGTCCCGTTG3′, as nested primers. Prominent bands were excised from the agarose gel, purified, and sequenced. Sequences were compared with the PAC 9C13 sequence.

Sequence analyses. Sequence assembly and analysis were performed using the Genetics Computer Group (GCG) sequence analysis software package. Prediction of coiled-coil structure by Lupas's algorithm (Lupas *et al.*, 1991) was performed with the Coils computer program with and without 2.5-fold weighting of positions a and d. Analysis of transmembrane segments was performed with the Tmpred, SOSUI, and TMHMM programs (Hirokawa *et al.*, 1998; Sonnhammer *et al.*, 1998). Multiple alignments were generated with Pileup software of the GCG package; shading of the figure was performed with the program BOXSHADE. The phylogenetic trees were generated with Pileup software of the GCG package and ClustalX 1.8 (Thompson *et al.*, 1994). Database searches were conducted by using BLAST (Altschul *et al.*, 1990).

Northern analysis. Blots (Clontech) containing ~2 μg of poly(A)$^+$ RNA per lane from eight different adult mouse tissue, were hybridized with the randomly labeled 5′-most 1.1 kb of the mouse polycystin-L2 cDNA probe at 68°C for 1 h, washed three times in 2× SSC, 0.05% SDS at room temperature, and then washed in 0.1× SSC, 0.1% SDS at 68°C for 3× 40 min. Signals were visualized by autoradiography.

RT-PCR analysis. Total RNA from various human cell lines and various human fetal tissues was isolated using the TRIzol protocol (Gibco BRL/Life Technologies, Gaithersburg, MD) and treated with DNase. cDNA templates for amplification were synthesized by reverse transcription of 5 μg total RNA using reverse transcriptase Superscript II (Gibco BRL/Life Technologies) with oligo(dT) primer. PCR was performed with exonic primers (f606, 5′AACGGGCGGTGTAGTGCAGGT, and r602, 5′AGTTGGTTCTTTCTTCACCAGGCACA) by using HotstarTaq DNA polymerase (Qiagen, Hilden, Germany); PCR primers f606 and r602 are located in exon 1 and exon 3, respectively. PCR was performed as follows: initial denaturation and activation of polymerase at 95°C for 15 min; 35 cycles of 94°C for 1 min, 63°C for 1 min, 72°C for 1 min; and a final extension at 72°C for 7 min. The resulting products were electrophoresed on a 1.8% agarose gel, and bands of various sizes were excised and purified with a QIAquick gel extraction kit and sequenced.

Fluorescence in situ hybridization (FISH). As described (Zhao *et al.*, 1995), 2 μg of a 3-kb genomic fragment containing the mouse Pkd2l2 gene and 1 μg of a 2-kb human PKD2L2 cDNA were labeled with digoxigenin-11–dUTP, coprecipitated with a 10-fold excess of mouse Cot-1 DNA (Gibco BRL) and 50 μg of tRNA, respectively. Pellets were resuspended in 1× TE at 100 μg/ml each. The mouse Pkd2l2 gene probe was hybridized at 10 and 20 μg/ml in Hybrisol VII to male mouse embryonic fibroblast metaphase chromosomes. Chromosomes were denatured in 70% formamide in 2× SSC, pH 7.0, for 1.5 min, dehydrated in an ethanol series, and air-dried. Identification of the chromosomes of interest was achieved with subsequent hybridization of the same metaphases with Cy3-labeled mouse chromosome painting probes (Vysis, Inc. Downers Grove, IL). The human PKD2L2 gene was hybridized at 20 μg/ml in Hybrisol VII according to the previously described method (Ney *et al.*, 1993) to peripheral blood metaphase chromosomes obtained from normal human males. Digoxigenin-labeled probes were detected using reagents supplied in the Oncor kit (Oncor, Gaithersburg, MD) according to the manufacturer's recommendations. Metaphase chromosomes were counterstained with 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI).

Map positions of the mouse Pkd2l2 and human PKD2L2 genes were determined by visual inspection of the fluorescent signal in the DAPI-stained metaphase chromosomes. Thirty-three mouse metaphases and 37 human metaphases were assessed for probe localization.

RESULTS

Isolation and Characterization of cDNAs for a Fifth Polycystin Gene

During the course of cloning the mouse Pkdl gene by RACE, we identified in mouse testis a novel sequence that differs from Pkdl. Further 5′ and 3′ RACE of mouse testis cDNA using primers specific to the new cDNA sequence led to the identification of a full-length cDNA of a novel gene, Pkd2l2. Based on its sequence homology with polycystins-2 and -L, we designated the protein encoded by the new gene mouse polycystin-L2 (AF182033). Pkd2l2 is 2486 bp long and encodes a putative 621-amino-acid protein starting at the first

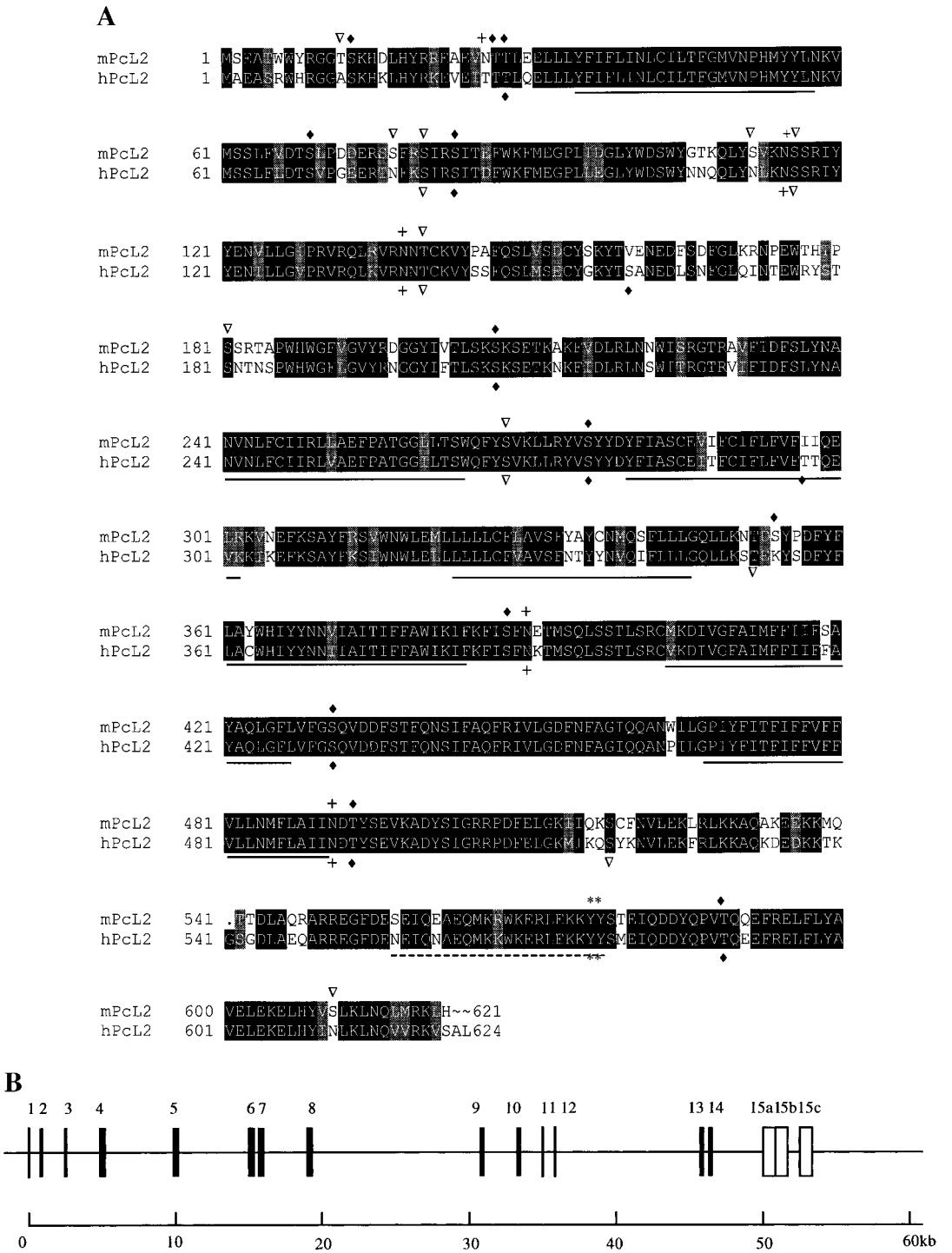


FIG. 1. (**A**) Deduced amino acid sequences of mouse and human polycystin-L2. Identical residues are indicated by black boxes, and similar residues are indicated by gray boxes. Potential transmembrane segments are underlined. The dotted line indicates the putative coiled-coil region. Potential N-glycosylation sites are marked with plus signs. Potential phosphorylation sites are marked as follows: asterisk, tyrosine phosphorylation site; inverted open triangle, protein kinase C phosphorylation sites; and diamond, casein kinase II phosphorylation sites. (**B**) Genomic structure of the human PKD2L2 gene. Open boxes represent noncoding exons, filled boxes represent coding exons.

methionine in the open reading frame (ORF). Blast search with the mouse Pkd2l2 cDNA sequence identified a human genomic clone, PAC9C13 (Accession No. AC006084), which appeared to contain the human orthologue of Pkd2l2. 5′ and 3′ RACE on human testis cDNA revealed that the human PKD2L2 cDNA sequence (AF182034) is 2282 bp long, encoding a 624-amino-acid protein beginning with the first methionine in the ORF. There are three transcripts that differ in their 3′ untranslated regions, each followed by a poly(A) tail. The sequences spanning the first ATG triplet in both mouse and human genes agree with the

consensus sequence for mammalian translation initiation sites (Dalphin *et al.*, 1998; Kozak, 1996). The mouse and human cDNA sequences were 80% identical overall. At the protein level, mouse polycystin-L2 shares 82% identity and 87% similarity with human polycystin-L2 (Fig. 1A).

The exon/intron structure of the human PKD2L2 gene was determined by comparison of genomic and cDNA sequences. We identified 17 exons in a 53-kb region. The first exon contains the start codon while the stop codon lies in exon 14. The three different 3′ untranslated regions identified by 3′ RACE were found to be encoded by the three 3′ exons (15a, 15b, and 15c), each of which contains an untranslated region and a putative polyadenylation signal (PAC9C13; nt 80,100–80,788, nt 80,394–80,788, or nt 82,688–83,051) (Fig. 1B).

Kyte–Doolittle analysis of the putative polycystin-L2 protein identified seven hydrophobic regions (H1–H7) with the potential to form TM segments (Fig. 2A). Further analyses of the TM segments performed with the Tmpred, SOSUI, and TMHMM programs revealed a topology model for polycystin-L2 with seven TM segments. If the topology of polycystin-L2 resembles the topology of transient receptor potential (Trp) channels, the six carboxyl-terminal TM domains are likely to constitute a channel subunit, and the amino-terminus of polycystin-L2 is located extracellularly while the long loop between the first and the second TM segments is intracellular (Fig. 2B). An alternative, six-TM model that resembles the previously proposed topology models for polycystins-2 and -L is postulated on the basis of its homology to polycystin-2 and -L (Fig. 2B).

Amino Acid Sequence Comparison and Phylogenetic Analysis of Polycystins

Polycystin-L2 shares the highest level of homology with polycystin-2 and -L among the polycystin family members. Comparison of all known members of the polycystin family reveals that the highest levels of sequence homology are found in their transmembrane regions. The overall similarities of polycystin-L2 with polycystins-L and -2 are 58 and 59%, respectively. The identities of polycystin-L2 to polycystins-L and -2 are 47 and 48%, respectively. Polycystin-L2 also showed moderate similarities to polycystin-1 over residues 3643 to 4239 (29% similarity, 20% identity) and to polycystin-REJ over residues 1685 to 2253 (34% similarity, 22% identity). Interestingly, polycystin-L2 also possesses a long loop between the first and the second TM segments wherein lies the "polycystin motif" we previously identified (Nomura *et al.*, 1998). The polycystin motif contains a positively charged consensus sequence of LGXPRQL(K/R)X(K/R) (Fig. 3A).

Phylogenetic analysis was performed for polycystin-L2, other members of the polycystin family, and proteins that share the highest level of homology with polycystin-L2 (Fig. 3B). The polycystin family appears to form a significant subbranch distinct from other ion channels. In this subbranch, polycystin-L2 is closely related to polycystins-L and -2, which are more closely related to each other than to polycystin-L2.

Expression, Tissue Distribution, and Alternative Splicing

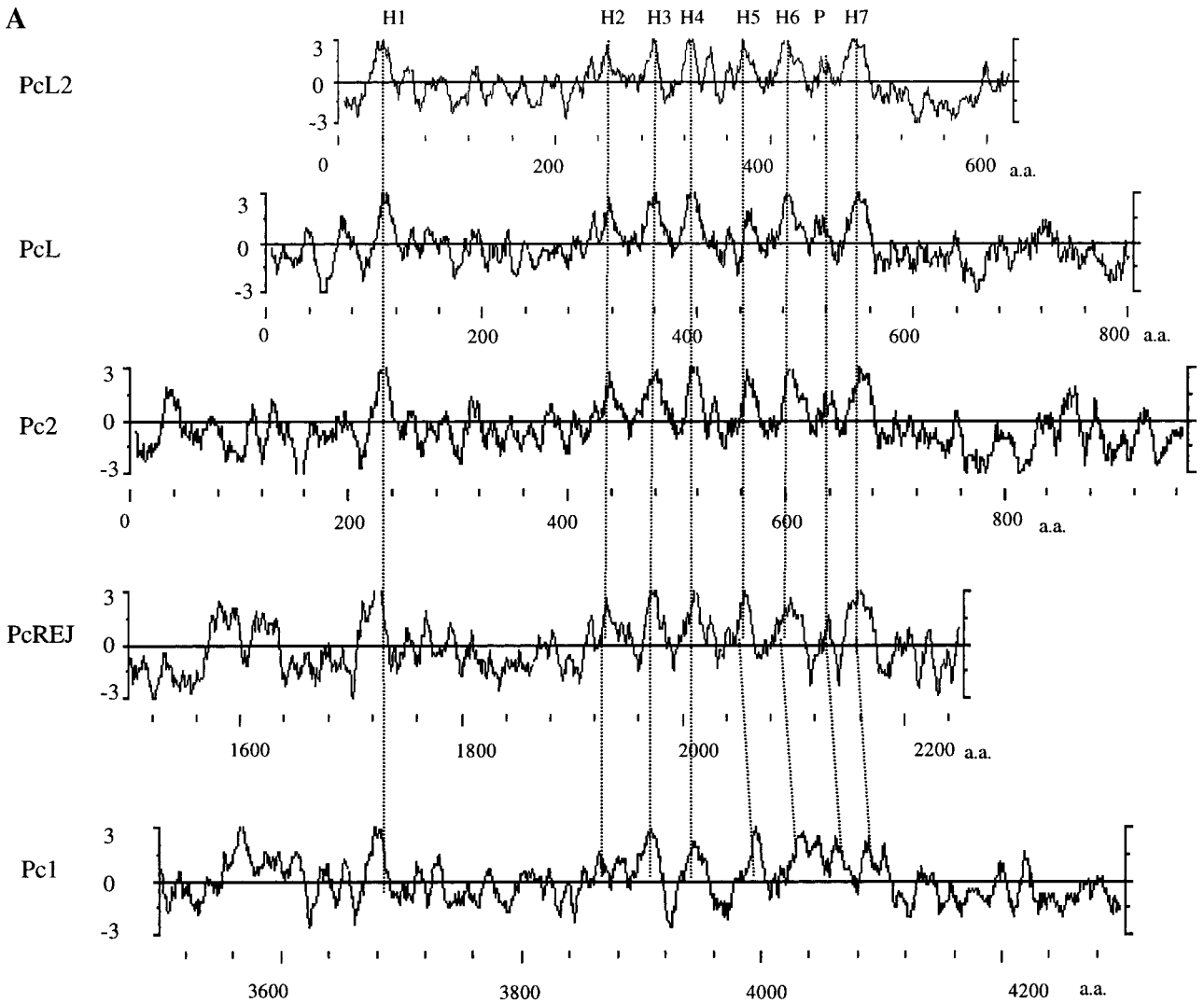
Unlike PKD1 and PKD2, which are widely expressed, Pkd2l2, like PKDL and PKDREJ, has a restricted tissue distribution (Fig. 4A); in adult mouse tissues, Pkd2l2 is expressed only in testis and heart. A Pkd2l2 transcript of ~2.5 kb in length was found in testis but, in the heart an ~2.2-kb form was preponderant. The level of expression in testis is higher than in the heart.

In contrast to mouse, PKD2L2 was expressed at very low levels in adult human tissues. Northern analysis of several human tissues revealed only weak signals of ~2.5 and ~1.9 kb in testis (data not shown), despite attempts to detect signal under a variety of conditions. RT-PCR was thus carried out to detect expression in human tissues. PKD2L2 expression was found in all tissues and cell lines tested (Fig. 4B).

By RT-PCR, three alternatively spliced variants of PKD2L2 were identified in human RNAs (Figs. 4B and 4C). Based on the full-length cDNA of PKD2L2, the predicted product with primers f606 and r602 is 258 bp. A 214-bp splice variant, designated PKD2L2a, uses an alternative splice acceptor in exon 3. In PKD2L2b, a 156-bp variant, exon 2 is spliced out. Exon 2 and part of exon 3 are spliced out in the third variant, PKD2L2c. While PKD2L2b is predicted to produce a protein with a 34-amino-acid in-frame deletion in its long loop region, PKD2L2a and PKD2L2c would result in a frameshift, followed by a premature stop codon, if translated. The predicted peptide for PKD2L2a is 77 amino acids long, containing only one putative transmembrane domain, whereas only a 23-residue-long peptide would be produced by PKD2L2c. The full-length and alternatively spliced PKD2L2 transcripts appear to be expressed in a tissue-specific pattern. The full-length transcript, found in the brain, kidney, testis, and HepG2 cells, is expressed at higher levels than the alternatively spliced transcripts since it can be detected by a single round of PCR. The splice variant PKD2L2a is found in all tissues and cell lines tested except in the HEK 293 cell line. The splice variant PKD2L2b is found only in transformed lymphoblasts. It remains to be seen whether these alternatively spliced transcripts have functional consequences.

Chromosome Localization of PKD2L2 Genes in Human and Mouse

To determine the chromosome assignment of PKD2L2 and Pkd2l2, FISH was performed. In 27 of 37 human metaphase preparations examined, hybridization signals were consistently present on the long arm of chromosome 5 in band q31. In 8 metaphase spreads,



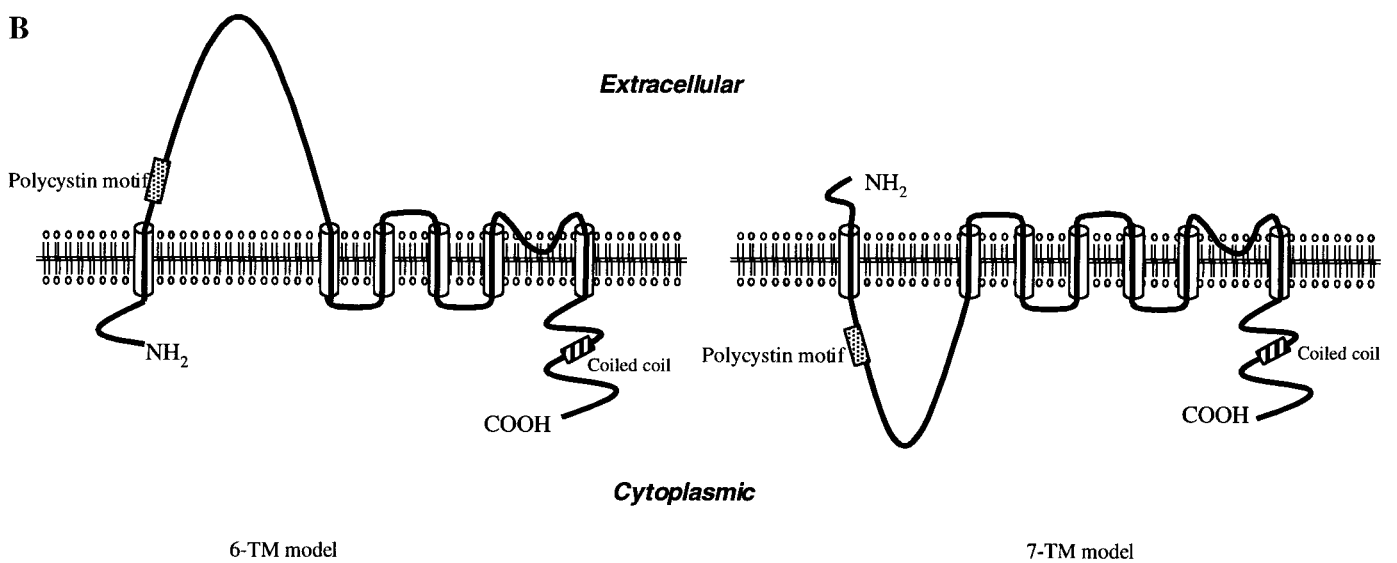


FIG. 2. (**A**) Hydropathy analyses of all five human polycystins. Hydrophobic peaks that are considered to be primary membrane-spanning regions are identified as H1 and H3–H7. Mild hydrophobic peaks indicating secondary transmembrane domains are labeled H2 and P. P also represents a putative pore region. Only the carboxyl-terminal portions of the hydropathy plots for polycystins-1 and -REJ are shown. (**B**) The two predicted transmembrane topology models for polycystin-L2.

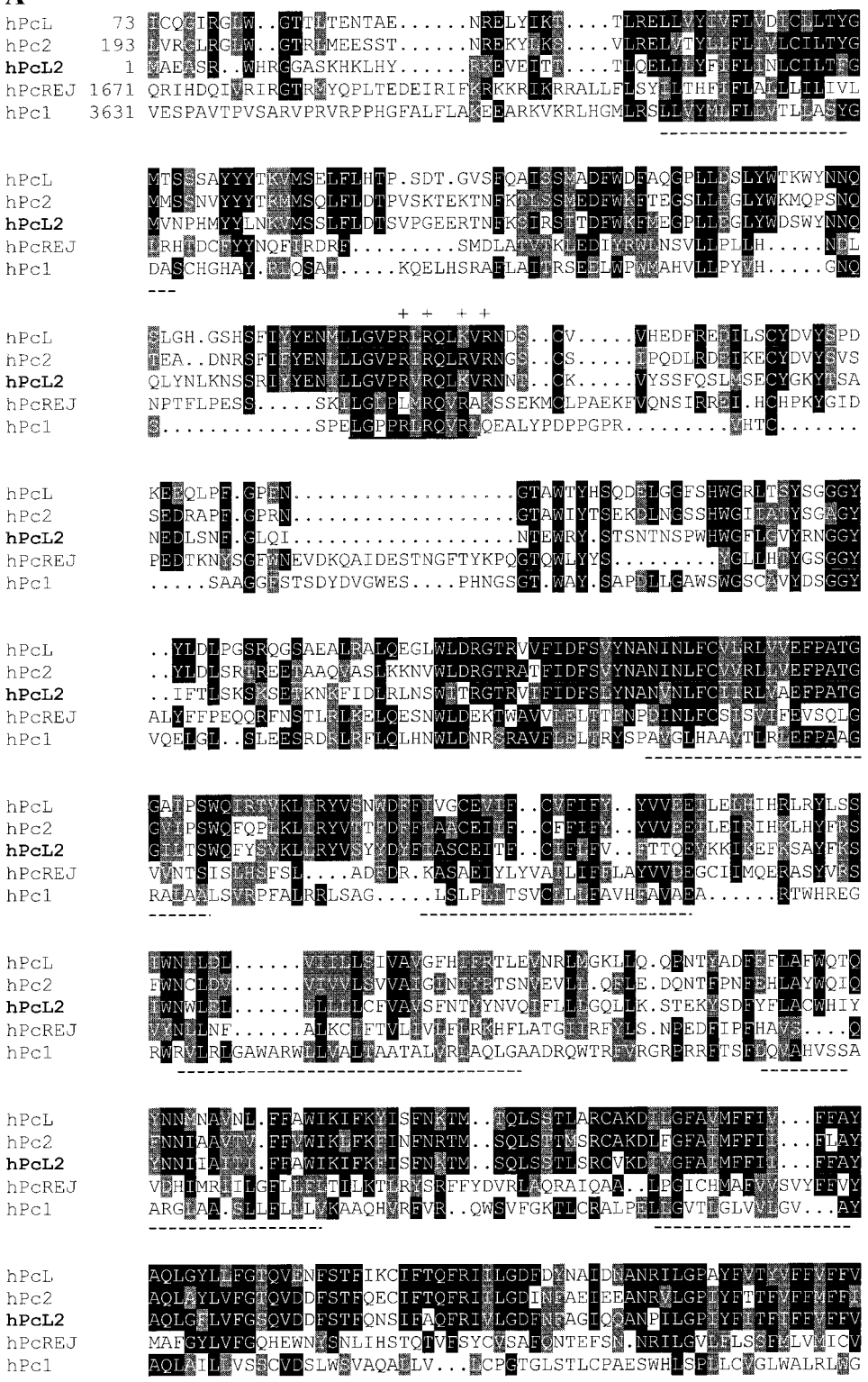
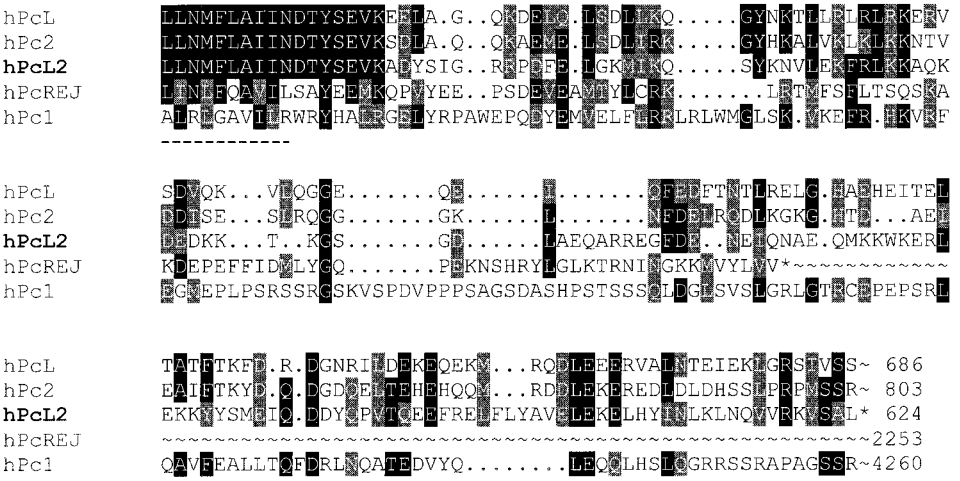


FIG. 3. (**A**) Sequence comparison of human polycystin-L2 and the four other known polycystins (Pcs). Identity is indicated by a black box, and similarity is indicated by a gray box. The putative polycystin motif is underlined. Dotted lines indicate potential transmembrane domains. A plus sign indicates positively charged residues in the polycystin motif. (**B**) The deduced phylogenetic relationship between polycystin-L2, other polycystins, and homologous proteins. The evolutionary tree is calculated by the neighbor-joining method (Saitou and Nei, 1987). Evolutionary distances are shown by the total branch lengths (horizontal lines). The corresponding GenBank accession numbers are: *Borrelia byrgdorferi* cell division protein homologue (bCDPH), A70132; rat calcium channel protein α1 chain isoform A (rCCPA), A41098; fly sodium channel protein (fSCP), A60165; and rat transient receptor potential 3 (rTRP3), JC5807.



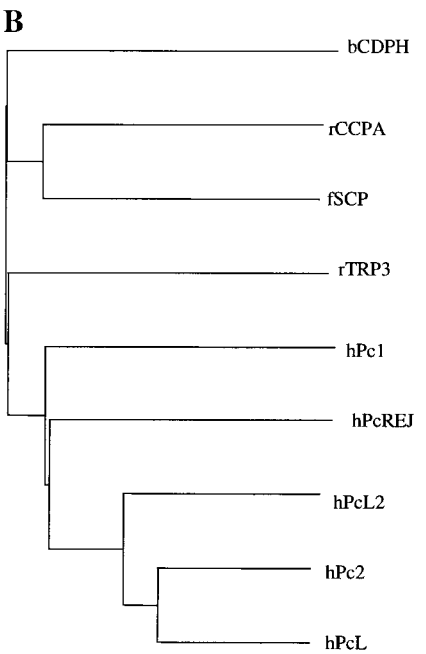


FIG. 3—*Continued*

both chromosomes 5 had hybridization signals at q31, and in 19 metaphase spreads only one chromosome 5 displayed a signal. In 22 of 33 mouse metaphase preparations analyzed, hybridization signal was found on both chromosomes 18 in band C; in 6 metaphase spreads, signal was detected on only one chromosome 18. Thus, we have localized the Pkd2l2 gene to mouse chromosome 18 in band C and the PKD2L2 gene to human chromosome 5 in band q31 (Figs. 5A, 5B, and 5C).

DISCUSSION

Sequence Analysis of a Novel Polycystin, Polycystin-L2—Implication of Polycystin Function

Polycystins show sequence similarity to α1 subunits of voltage-activated Ca^{2+} channel and mammalian Trp channels especially within the carboxyl-terminal transmembrane regions in polycystins-2, -L, and -L2. Human Trp3 protein contains six TM domains (Vannier *et al.*, 1998). However, three separate algorithms, Tmpred, SOSUI, and TMHMM, predict seven membrane-spanning segments for polycystin-L2 (Fig. 2B) of which the six most C-terminal segments are homologous to TMs in Trp channels. These programs suggest seven as the most likely number of TM segments for polycystins-2 and -L as well as polycystin-L2, although previous publications postulated six TM segments for polycystins-2 and -L, relying heavily on homology to the known structures of Trp channels (Mochizuki *et al.*, 1996; Nomura *et al.*, 1998). The seven-TM model, if proven, will have significant functional implications for polycystin function. Independent experimental methods will be needed to determine the actual topologies of these three related polycystins.

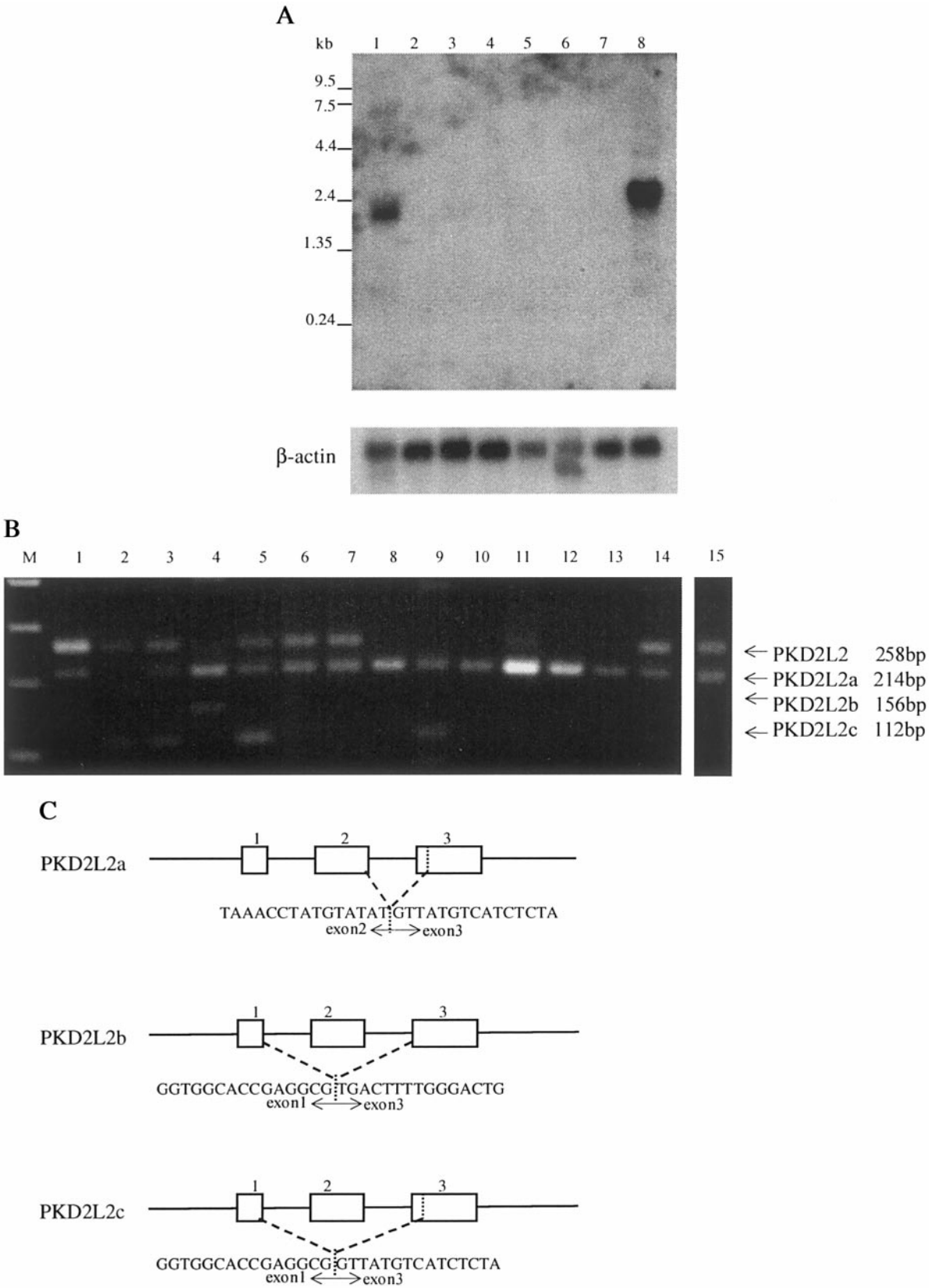


FIG. 4. Tissue distribution and alternative splicing of PKD2L2. (**A**) Northern analysis of mRNA blots containing mouse adult tissues. Lanes **1,** heart; **2,** brain; **3,** spleen; **4,** lung; **5,** liver; **6,** skeletal muscle; **7,** kidney; and **8,** testis. (**B**) RT-PCR analysis of human fetal tissues (unless indicated) and cell lines shows tissue-specific expression of the splicing variants with primers f606 and r602. Lane **M,** 100-bp DNA ladder (NEB). Lanes **1,** HepG2 cell line; **2,** HEK 293 cell line; **3,** SK-N-SH cell line; **4,** transformed lymphoblasts; **5,** brain; **6,** brain cortex; **7,** cerebellum; **8,** lung; **9,** muscle; **10,** eye; **11,** liver; **12,** heart; **13,** kidney; **14,** testis; and **15,** adult kidney. (**C**) Schematic representation of the splicing variants. Boxes indicate exons. Solid lines indicate introns. The letters indicate the nucleotide sequences at the splice junctions.

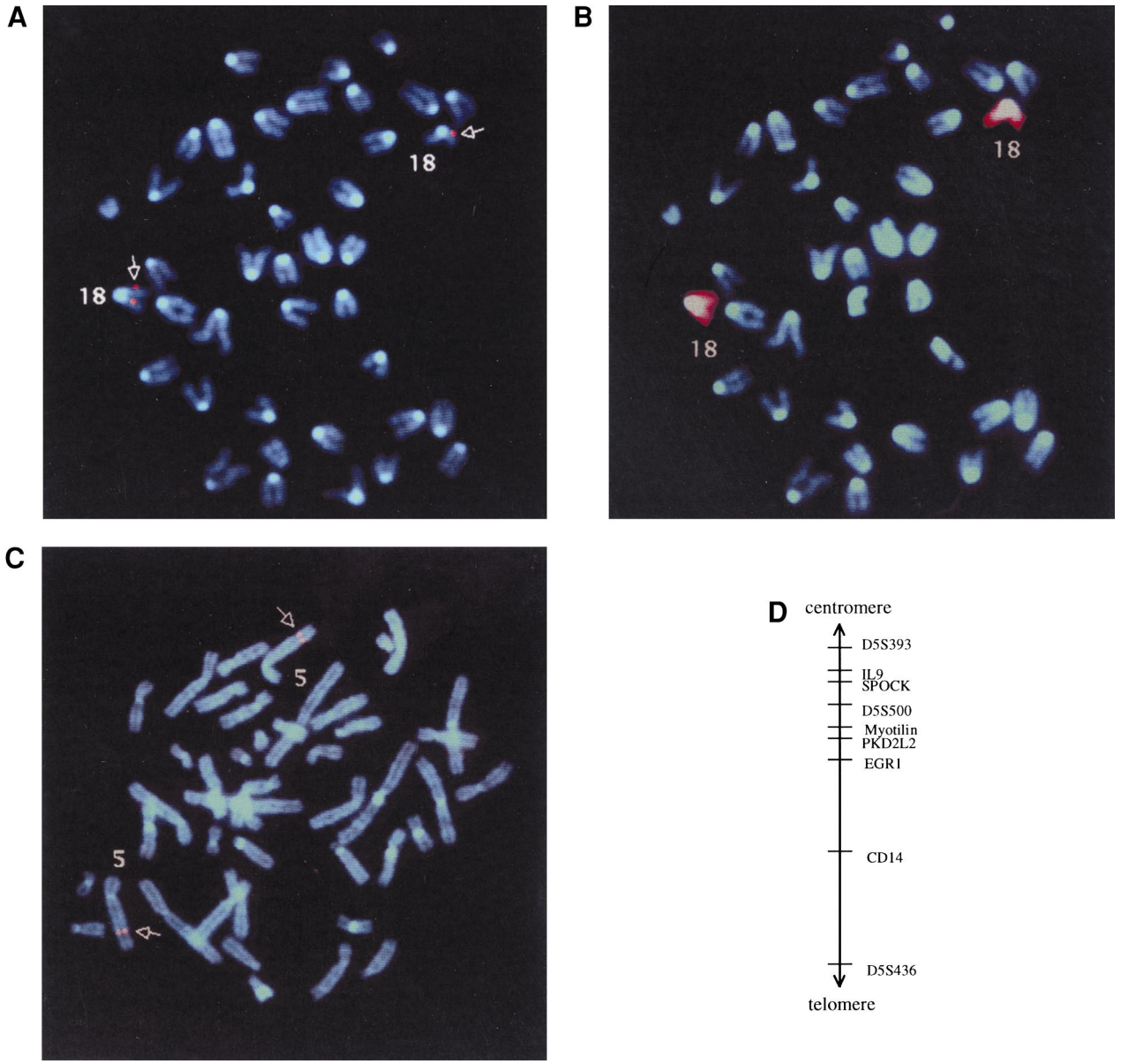


FIG. 5. Chromosomal localization of PKD2L2 in mouse and human. (**A**) Fluorescent signals on mouse metaphase chromosomes hybridized with the mouse Pkd2l2 probe. Arrows point to the site of hybridization of the digoxigenin-labeled probe on both chromosomes 18 in band C. (**B**) Identification of the hybridizing chromosomes as mouse chromosomes 18 using a mouse chromosome 18-specific painting probe. (**C**) Photograph of human metaphase chromosomes countstained with DAPI. The two chromosomes 5 are indicated by numbers. Arrows point to the site of hybridization of the digoxigenin-labeled human PKD2L2 probe on both chromosomes 5 in band q31. Hybridization was observed with a Zeiss Axiophot microscope, and images were captured and printed using the CytoVision Imaging system. (**D**) Position of PKD2L2 on human chromosome 5 in band q31 relative to the positions of other known genes on the long arm of chromosome 5.

Like polycystins-L and -2, polycystin-L2 harbors a coiled-coil motif in its carboxyl-terminus and a putative pore region between the two carboxyl-terminal TM domains. Our previous studies showed that the channel activity of polycystin-L is inhibited in the presence of high intracellular Ca^{2+} concentration (Chen *et al.*, 1999). An EF-hand structure in the carboxyl-terminus of polycystin-L may mediate the effect of cytoplasmic Ca^{2+} concentration on the channel activity of polycystin-L. Binding of Ca^{2+} to the EF-hand structures inactivates several known Ca^{2+} channels (de Leon *et al.*, 1995). Polycystin-2 also has an EF-hand in its carboxyl-terminus but, notably, polycystin-L2 does not. It is thus likely that polycystin-L2 acts as a cation channel subunit but is not regulated by intracellular Ca^{2+} concentration.

Sequence analysis revealed that the five known members of the polycystin family can be divided into polycystin-1-like and polycystin-2-like subgroups. As discussed above, the polycystin-2-like proteins (polycystins-2, -L, and -L2) share structural homology with cation channels such as those of the Trp and voltage-gated Ca^{2+}, Na^{+}, and K^{+} channel families. On the other hand, both polycystin-1-like molecules, polycystins-1

and -REJ, share significant domain and sequence homology to the sea urchin REJ that is believed to be an ion channel regulator.

Accumulating evidence suggests that polycystins are part of large multimeric membrane protein complexes that regulate, in response to a wide range of extracellular signals, gene transcription and transmembrane ion flux. It is likely that polycystins possess multiple cellular, biochemical, and biophysical functions. Polycystin-1 was demonstrated experimentally to be involved in activation of Wnt signaling (Kim *et al.*, 1999) and in interactions with heterotrimeric G-proteins (Parnell *et al.*, 1998). Evidence for a role of polycystins in Ca^{2+} flux was recently demonstrated by immunoprecipitation experiments of polycystin-2 with a member of the Trp channel family, Trp1 (Tsiokas *et al.*, 1999), and the electrophysiological study of polycystin-L, showing it to be a calcium-regulated cation channel (Chen *et al.*, 1999). The structural similarity of polycystin-L2 to polycystin-L, polycystin-2, and other ion channels suggests that polycystin-L2 forms a part of a polycystin channel. It is likely that polycystin-2-like molecules are ion channels regulated by polycystin-1-like molecules (polycystins-1 and -REJ). Depending on the tissue and stage of development, the molecular composition of the channels and their regulators may vary.

Polycystin-REJ, another newly identified member of the polycystin family, is only expressed in testis. Its similarity in sequence and expression pattern to sea urchin REJ (suREJ) suggests that polycystin-REJ is a mammalian equivalent of the suREJ protein and therefore may have a central role in human fertilization. Fertilization is initiated by binding of the intact sperm to the glycoprotein coat of the egg. This event triggers an influx of extracellular Ca^{2+}, which activates the acrosome reaction, an essential step in the fertilization process. Because polycystin-L2 is also highly expressed in testis and has significant homology with polycystin-REJ protein, it is possible that polycystin-L2 interacts with polycystin-REJ and plays a role in fertilization. It is also possible that, akin to polycystins-1 and -2, polycystin-REJ physically associates with polycystin-L2 and/or with other polycystin-2-like molecules or as yet undiscovered subunits and forms a cation channel in testis. Further biochemical and biophysical experiments are required to test this hypothesis.

PKD2L2—A Candidate Gene for Polycystic Kidney Disease or 5q- Syndrome?

The elucidation of the chromosome localization of this new member of the polycystin family both in mouse and in human facilitates the identification of diseases for which these genes may be positional candidates. The chromosomal localization of Pkd2l2 to mouse chromosome 18 excludes it as a positional candidate for the known mouse models for polycystic kidney disease. These include cpk, located on chromosome 12 (Davisson *et al.*, 1991), pcy, located on chromosome 9 (Takahashi *et al.*, 1991), jck, located on chromosome 11 (Atala *et al.*, 1993), kat, located on chromosome 8 (Janaswami *et al.*, 1997), and jcpk, located on chromosome 10 (Bryda *et al.*, 1996). The human chromosome 5q location of PKD2L2 excludes it as a candidate for human autosomal recessive polycystic kidney disease, which was mapped to 6q12–q21.1 (Mucher *et al.*, 1998). Low levels of PKD2L2 expression in the adult kidney make it an unlikely candidate for unmapped ADPKD cases in which all segments of renal tubules are affected, but it remains an excellent candidate for other unmapped cystic kidney diseases that affect only a subset of tubules.

PKD2L2 is situated in a region commonly deleted in 5q- anomaly, a myelodysplastic syndrome, characterized by refractory anemia, morphological abnormalities of megakaryocytes, and an interstitial deletion of the long arm of chromosome 5. Previous studies have defined the proximal and distal ends of the critical region to reside in an ~2.4-Mb region between the genes for IL and EGR1 (Fairman *et al.*, 1995; Neuman *et al.*, 1992). Genomic sequence analysis shows that this region includes PKD2L2 (Fig. 5D). The contribution of PKD2L2 to this syndrome remains to be determined.

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