Identification of PKDL, a Novel Polycystic Kidney Disease 2-Like Gene Whose Murine Homologue Is Deleted in Mice with Kidney and Retinal Defects

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Hideki Nomura*, Alberto E. Turco‡, York Pei§, Luba Kalaydjieva, Tina Schiavolo, Stanisława Weronowicz**, Weizhen Ji††, Cynthia C. Morton**†‡, Miriam Meisler‡‡, Stephen T. Reeder‡‡, and Jing Zhou†††

From the Renal Division, Department of Medicine, and Departments of Pathology and Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02115, Institute of Genetics, University of Verona School of Medicine, 37134 Verona, Italy, Division of Nephrology, Department of Medicine, Toronto Hospital and University of Toronto, Toronto, Ontario, Canada, Department of Human Biology, Edith Cowan University, Joondalup Campus Perth, Western Australia 6027, Australia, and Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109

Polycystin-1 and polycystin-2 are the products of PKD1 and PKD2 genes, that are mutated in most cases of autosomal dominant polycystic kidney disease. Polycystin-2 shares ~46% homology with pore-forming domains of a number of cation channels. It has been suggested that polycystin-2 may function as a subunit of an ion channel whose activity is regulated by polycystin-1. Here we report the identification of a human gene, PKDL, which encodes a new member of the polycystin protein family designated polycystin-L. Polycystin-L has 50% amino acid sequence identity and 71% homology to polycystin-2 and has striking sequence and structural resemblance to the pore-forming α1 subunits of Ca2+ channels, suggesting that polycystin-L may function as a subunit of an ion channel. The full-length transcript of PKDL is expressed at high levels in fetal tissues, including kidney and liver, and down-regulated in adult tissues. PKDL was assigned to 10q24 by fluorescence in situ hybridization and is linked to D10S603 by radiation hybrid mapping. There is no evidence of linkage to PKDL in six ADPKD families that are unlinked to PKD1 or PKD2. The mouse homologue of PKDL is deleted in Krd mice, a deletion mutant with defects in the kidney and eye. We propose that PKDL is an excellent candidate for as yet unmapped cystic diseases in man and animals.

Polycystin-1 and polycystin-2 are the respective gene products of PKD1 and PKD2, mutations in which account for ~95% of cases of ADPKD. ADPKD affects up to 1/1,000 individuals and is associated with a 50% incidence of end-stage renal failure by the sixth decade of life (1). At least one additional gene is known to be mutated in the ADPKD population (2, 3) but has yet to be identified.

Polycystin-1 encodes a 4,303-amino acid plasma membrane protein with a large extracellular N-terminal domain that contains leucine-rich repeats, a C-type lectin domain, and an LDL-A-like domain, all three of which are involved in cell-cell or cell-matrix interactions in other proteins (4–6). These domains are followed by 16 repeats of the so-called PKD domain and by an REJ (receptor for egg jelly in sea urchin sperm)-like domain. Polycystin-1 has 7 to 11 transmembrane domains. The short cytoplasmic tail (197 amino acids) of polycystin-1 contains a coiled-coil domain, which appears to interact with other proteins containing similar structures (7, 8).

The predicted amino acid sequence of the PKD2 gene is homologous to the C terminus of polycystin-1 (9, 10). Polycystin-2 is a 968-amino acid protein with ~46% sequence similarity to each domain of the pore-forming α1 subunits of Ca2+ and other cation channels, and like these channel subunits, it is predicted to have six transmembrane domains. Polycystin-2 has a putative Ca2+ binding structure (EF-hand) in its C-terminal cytoplasmic domain. It interacts biochemically with polycystin-1 and with itself (7, 8).

Here we report the identification, chromosomal localization, and expression of a third gene encoding a protein of the polycystin family. The product of this gene is an excellent candidate for a component of the pore-forming subunit of a polycystin-related channel and is also a candidate for various human and murine cystic diseases.

EXPERIMENTAL PROCEDURES

Isolation of PKDL cDNAs—Overlapping EST sequences W27963 and W28231, derived from a retina cDNA library, were identified by their gene product homology to polycystin-2 (gb 189 US50926). A 940-base pair fragment in the overlap region of both ESTs was amplified from human adult kidney and brain poly(A)-selected RNA by reverse transcription-PCR using primers 5′-TCTTTCTGTTCACTTTAAGATG-3′ and 5′-CCTGTGGATTTCTCCTGTT-3′. 5′- and 3′-rapid amplification of cDNA ends were performed with human skeletal muscle and kidney rapid amplification of cDNA ends kits (CLONTECH, Palo Alto, CA), respectively. Primers were designed based on PKDL reverse transcription-PCR products. Nested amplification was performed following manufacturer’s instructions. The 5′-rapid amplification of cDNA ends product was random-labeled with [32P]dCTP and used to screen a human retina cDNA library (CLONTECH). Hybridization was performed in a buffer containing 5 × SSC (1 × SSC, 0.15 M NaCl and 0.015 M sodium citrate), 50% formamide, 1% SDS, and 5 × Denhardt’s solution at 42 °C, overnight. Filters were washed three times in buffer (0.1 × SSC and 0.1% SDS) at 65 °C. Positive signals were purified, and inserts were subcloned into pBlueScript II (Stratagene, La Jolla, CA) and sequenced.

Sequence Analysis—Clones were sequenced from both strands, and the sequences were aligned to give an overall consensus sequence. The
A Novel Member of the Polycystin Family

RESULTS

Through data base searches we identified two EST sequences of ~500 nucleotides, W27963 and W28231, with similarity to polycystin-2. The deduced amino acid sequences of W27963 and W28231 showed 78% homology and 56% identity (over residues 649 to 749) and 65% homology and 39% identity (over residues 678 to 786 with a single three-residue gap) to polycystin-2, respectively. The two EST sequences shared 94% identity over 421 base pairs. We tentatively concluded that these ESTs arose from the same gene.

Using primer sets based on these overlapping EST sequences, we amplified the same reverse transcription-PCR product from adult kidney and brain RNA whose translated amino acid sequence shows 67% homology and 46% identity to residues 670 to 779 of human polycystin-2. We further performed 5' and 3'-rapid amplification of cDNA ends with SKAT and kidney poly(A) RNA, respectively, and obtained 0.8 kb (5MR1) and 0.9 kb cDNAs (3MR20), respectively. Using 5MR1 as a probe, we screened a human retina library. Three clones, PKDL-6, PKDL-7 and PKDL-8, were obtained and sequenced. The consensus 3,044-base pair sequence revealed an open reading frame of 2,415 base pairs, which encodes a protein of 805 amino acids (Fig. 1). The putative translation start site at cDNA position 384 (5'TTCCCATGA-3') is not accompanied by a typical Kozak sequence. A single in-frame stop codon is found in the putative 5'-untranslated region. The open reading frame is followed by several in-frame stop codons, and the 3'-untranslated region contains a consensus polyadenylation signal (5'-AATAAA-3') 10 nucleotides upstream from the poly(A) tail.

The deduced amino acid sequence of PKDL is shown in Fig. 1. Hydrophy analysis of the polycystin-L sequence showed five highly hydrophobic regions predicted to be transmembrane segments (Fig. 2A). Three additional relatively hydrophobic peaks were identified. Polycystin-L showed significant homology to polycystin-2 as expected (71% homologous, 50% identical). This homology is generally higher in predicted transmembrane segments and in the loops between transmembrane segments (Fig. 2B). Polycystin-L also showed a moderate similarity (similarity 45%, identity 22%) to polycystin-1 over residues 1 to 797. This similarity is slightly higher in transmembrane segments, but there is one conserved positively charged short amino acid stretch in the first loop between transmembrane segments (Fig. 2B).

Polycystin-L, like polycystin-2, shows homology (similarity ~47%, identity ~21% overall) to each of the four domains of various Ca$^{2+}$ channel α1 subunits and other cation channels. Regions of homology are clustered in the last four transmembrane segments and the pore region of each domain of the Ca$^{2+}$ channel α1 subunits (Fig. 2B). In polycystin-L and polycystin-2, the regions corresponding to this pore region include the last of the three relatively hydrophobic peaks. The first two-thirds of this region is predicted to form a helical structure, which is characteristic for various cation channels.

Two algorithms (12, 13) predict that polycystin-L has a coiled-coil domain in its C-terminal cytoplasmic tail (Fig. 1). Polycystin-L also has a putative Ca$^{2+}$ binding structure or EF-hand (25) that generally consists of two helices and a loop between them (Fig. 1). The C-terminal helix in the EF-hand of polycystin-L overlaps with the predicted coiled-coil region. Polycystin-L has a putative cAMP phosphorylation site in its C terminus. Putative protein kinase C phosphorylation sites are all in regions predicted to be cytoplasmic. Four of five putative casein kinase II phosphorylation sites with strong motif sequences (positions 249, 563, 674, 703, 719) are also found in the C-terminal cytoplasmic domain (Fig. 1).
Multiple tissue RNA dot blot analysis using the 5' 1.5-kb of the coding sequence as a probe revealed highest expression in adult heart and kidney (Fig. 3A). Northern blot analysis showed the presence of 5- and 1.5-kb bands in fetal tissues including kidney, liver, and brain (Fig. 3, B and C). This result suggests the presence of alternatively spliced forms. The abundance of the two splice variants is ~1:1 in fetal tissues. In adult tissues, however, the long transcript is only detected after prolonged autoradiography.

Chromosomal assignment of PKDL to 10q24 was achieved by fluorescent in situ hybridization on 4,6-diamidino-2-phenylindole-dihydrochloride-stained metaphase human chromosomes using a 1.7-kb genomic probe (Fig. 4). In 17 of 21 metaphase preparations analyzed, a hybridization signal was found to be present on the long arm of chromosome 10 in band q24. In six spreads, both copies of chromosome 10 were labeled, and in 11 metaphase spreads, a signal was detected on a single chromosome 10. No signals were observed on other chromosomes. With the Stanford G3 radiation hybrid panel, PKDL was found to have an identical distribution pattern as polymorphic marker D10S603 (lod score greater than 1,000). Linkage analysis of the PKDL locus using flanking markers D10S198, and D10S192 gave negative lod scores in six ADPKD families previously documented to be unlinked to PKD1 and PKD2 loci (Table I).

The human PKDL gene is located within a linkage group that is conserved on the distal portion of mouse chromosome 19 (26) (Fig. 5A). A 7-centimorgan deletion of this region has been described in Kr# mice (27). To determine whether the mouse homologue, Pkdl, is located within the Kr# deletion, we analyzed genomic DNA from F1 animals obtained from a cross of C57BL/6J-Krd X SPRET/Ei (Fig. 5B). Strain C57BL/6J-Krd-derived allele in the F1 DNA, we utilized restriction fragment-length polymorphisms detected by hybridization with a 1.5-kb human PKDL cDNA probe (Fig. 5B). Strain C57BL/6J contains three hybridizing TaqI fragments of 5.5, 5.0, and 1.9 kb. Strain C3H, in which the Kr# mutation originally arose, contains three hybridizing TaqI fragments of 5.5, 5.0, and 1.6 kb. Strain SPRET/Ei contains two hybridizing fragments of 8 and 4.5 kb. The (C57BL/6J-Krd X SPRET/Ei) F1 mouse inherited the hybridizing fragments contributed by the SPRET/Ei parent but did not inherit the fragments from C57BL/6J or C3H (Fig. 5B). This result indicates that the mouse Pkdl locus is located within the region that is deleted by the Kr# mutation.

**DISCUSSION**

**A Novel PKD2-like Gene**—The manifestations of PKD1- and PKD2-linked ADPKD are generally similar, raising the likelihood that the gene products function in the same or parallel biological pathways. Homology between polycystin-2 and the pore-forming a1 subunits of voltage-activated Ca2+ channel proteins, combined with evidence of interaction between polycystin-1 and polycystin-2 (7, 8), has led to the proposal that polycystin-2 forms homo- or heteromultimeric complexes with itself, with polycystin-1, or with another protein to function as an ion channel (9). Inasmuch as a small fraction of ADPKD families are not accounted for by PKD1 and PKD2 mutations and the function of polycystin family members may be cooperative, we postulated the existence of additional polycystin family members.

Here we report the identification and cloning of a third gene encoding a member of the polycystin superfamily, polycystin-L. Its gene, PKDL, is therefore an excellent candidate gene for human and murine cystic diseases. The temporal expression pattern of PKDL is similar to that of PKD1.

**Sequence Analysis: Implications for Polycystin Function**—The hydrophathy patterns of polycystin-L and polycystin-2 are similar except in the region corresponding to the S4 segment of polycystin-2 where polycystin-L has a much lower hydrophobicity score, suggesting that this is a secondary membrane-spanning region. Polycystin-L and polycystin-2 both have putative EF-hand structures in their C-terminal cytoplasmic domains, suggesting that their functions are influenced by cytoplasmic Ca2+ concentration. In several Ca2+ channels, binding of Ca2+ to EF-hand structures inactivates the channels (28).

Polycystin-L and polycystin-2 show moderate but significant sequence similarity to Ca2+ and other cation channels, especially within their S3-S6 segments and the loop between the S5 and S6 segments. In addition, the last two membrane-spanning segments of polycystin-L, polycystin-2, and Ca2+ channel a1 subunits share structural characteristics with the Streptomyces lividans K+ channel (KcsA) whose structure has been determined by crystallography (29). The common structural features include: lining residues of the last membrane-spanning segments that are mostly hydrophobic except for the negatively charged acidic amino acid near the end of these segments; loops between the last two membrane-spanning regions (pore region) that are mildly hydrophobic (Fig. 2A); first 8% of pore regions
that are predicted to form short helical structures (pore-helix); and finally, last 1/3 of pore regions that begins with negatively charged residues which have been considered to determine the selectivity to Ca²⁺ in known Ca²⁺ channels (30).

Polycystin-L differs from polycystin-2 most significantly in the N-terminal cytoplasmic domain where it lacks a 100-amino acid segment. In the C-terminal cytoplasmic domain, polycystin-L is strongly predicted to have a coiled-coil structure, which has the potential to tightly interact with molecules with a similar structure like polycystin-1. Lupas' algorithm (12) also predicts a coiled-coil structure in polycystin-2, but it is not supported by Berger's algorithm (13).

Polycystin-L and polycystin-2 have three positively charged residues in S4 as opposed to five to eight in voltage-gated Ca²⁺ channels. Whereas the S4 region in voltage-gated Ca²⁺ channels is considered to be a voltage sensor (31), it is not clear whether a membrane-spanning region with only three basic residues could act as a voltage sensor. Polycystin-L also has several putative phosphorylation sites: one cyclic nucleotide, two protein kinase C, and four casein kinase II phosphorylation sites.

![Image showing hydropathy analysis and alignment of polycystin-L and polycystin-2](image-url)

**FIG. 2.** A, hydropathy analysis of polycystin-L (Pc-L) and polycystin-2 (Pc-2). Hydrophobic peaks that are considered to be primary membrane-spanning regions are described as S1, S2, S3, S5, and S6. Mild hydrophobic peaks indicating secondary transmembrane domains are labeled S1/2, S4, and p. a.a., amino acids. B, alignment of polycystin-L with polycystin-2 (gb 189 U50928), polycystin-1 (Pc-1) (gb 189 U24497), voltage-activated Ca²⁺ channel α1G, α1C, and α1E (31), and transient receptor potential related channel 3, trpc3 (EMBL 189 Y13758). Roman numbers indicate domains of voltage-activated Ca²⁺ channel α1 subunits. Positively charged residues in polycystin-shared motif and S4 segment are marked with a plus sign; negatively charged residues in pore-loop and S6 segment are marked with a minus sign.
sites with strong motif sequences in the C-terminal cytoplasmic domain. Two other putative protein kinase C phosphorylation sites are also found in the N-terminal cytoplasmic domain. Phosphorylation of these motif sequences may be involved in the gating process of the channel. Another scenario is that the channel is gated by a direct or indirect signal from associating proteins, e.g., polycystin-1. Given that polycystin-1 has domains that may be involved in cell-cell or cell-matrix interaction and is known to interact with polycystin-2 (7, 8), we hypothesize that the binding of ligand(s) to polycystin-1 may be associated with the gating of a polycystin-related channel.

Sequence analysis and comparison to other channels support the six or seven membrane-spanning plus one pore-region topology of polycystin-2 and polycystin-L. In addition to the five putative transmembrane segments, the middle of the three relatively hydrophobic peaks, which corresponds to S4 in α subunits of cation channels, is likely to be another transmembrane segment. Whether the N-terminal peak (S1/2) forms a membrane-spanning region is not clear.

One common feature of the polycystin-L/polycystin-2 structure that is rarely observed in known ion channels is that they both have relatively long extracellular loops between the first}

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**FIG. 3. Expression of PKDL gene.** The first 1.5 kb of coding sequence was used as a probe. A, RNA dot blot hybridization analysis. Dot A1, whole brain; A2, amygdala; A3, caudate nucleus; A4, cerebellum; A5, cerebral cortex; A6, frontal lobe; A7, hippocampus; A8, medulla oblongata; B1, occipital lobe; B2, putamen; B3, substantia nigra; B4, temporal lobe; B5, thalamus; B6, subthalamic nucleus; B7, spinal cord; C1, heart; C2, aorta; C3, skeletal muscle; C4, colon; C5, bladder; C6, uterus; C7, prostate; C8, stomach; D1, testis; D2, ovary; D3, pancreas; D4, pituitary gland; D5, adrenal gland; D6, thyroid gland; D7, salivary gland; D8, mammary gland; E1, kidney; E2, liver; E3, small intestine; E4, spleen; E5, thymus; E6, peripheral leukocyte; E7, lymph node; E8, bone marrow; F1, appendix; F2, lung; F3, trachea; F4, placenta; G1, fetal brain; G2, fetal heart; G3, fetal kidney; G4, fetal liver; G5, fetal spleen; G6, fetal thymus; G7, fetal lung; H1, yeast total RNA 100 ng; H2, yeast tRNA 100 ng; H3, Escherichia coli rRNA 100 ng; H4, E. coli DNA 100 ng; H5, poly(rA) 100 ng; H6, human Cot-1 DNA 100 ng; H7, human DNA 100 ng; H8, human DNA 500 ng. Northern analysis with mRNA blots containing human adult (B) and human fetal tissues (C).

**FIG. 4. Chromosomal localization of the human PKDL gene.** A, ideogram of human chromosome 10 showing the map location of human PKDL at 10q24 (arrow). B, photograph of human metaphase chromosome counterstained with 4,6-diamidino-2-phenylindole dihydrochloride. The two chromosomes are indicated by numbers. Arrows point to the site of hybridization of the digoxigenin-labeled human PKDL on both chromosomes 10 in band q24.
and the second putative transmembrane segments. Although this loop region does not show high homology to any known ion channels, polycystin-2 and polycystin-L maintain a high level of homology with each other in this region. Moreover, this region contains a 13-amino acid stretch with 3 to 4 basic residues that is conserved not only between polycystin-2 and polycystin-L but also with polycystin-1. The function of this polycystin-shared motif is not clear.

Chromosomal Assignment and Linkage Studies—Studies using D10S603, which maps to the same interval as PKDL by radiation hybrid mapping, and two adjacent markers, D10S192 and D10S198, did not reveal linkage in six non-PKD1, non-PKD2 families, making it unlikely that mutations in PKDL cause the disease in these families. Among other as yet unexplained human cystic kidney diseases, it is unlikely that mutations in PKDL play a role in autosomal recessive polycystic kidney disease, as mutations in most autosomal recessive polycystic kidney disease families have been mapped to chromosome 6 (32). The PKDL locus can, however, be considered as a candidate for unmapped human genetic cystic disorders such as dominantly transmitted glomerulocystic kidney disease of postinfantile onset (33), isolated polycystic liver disease (34), and Hajdu-Cheney syndrome/serpentine fibula syndrome (35, 36).

The region syntenic to the human PKDL locus is located on chromosome 19 in mice (26). This region is partially deleted in mice with the mutation Krd (Kidney and retinal defects) (27). Our Southern analysis demonstrates that the mouse homologue of PKDL is deleted in Krd mice.

Chromosomal assignment and linkage studies—

| Family    | Theta | 0   | 0.05 | 0.1  | 0.2  | 0.3  |
|-----------|-------|-----|------|------|------|------|
| F431      | D10S198 | 0   | 0    | 0    | 0    | 0    |
|           | D10S603 | -0.721 | -0.463 | -0.317 | -0.149 | -0.060 |
|           | D10S192 | -0.721 | -0.463 | -0.317 | -0.149 | -0.060 |
| F432      | D10S198 | -∞   | -1.184 | -0.761 | -0.343 | -0.136 |
|           | D10S603 | 0    | 0    | 0    | 0    | 0    |
|           | D10S192 | -2.698 | -1.376 | -0.862 | -0.380 | -0.148 |
| TOR1      | D10S198 | 0.700 | 0.599 | 0.500 | 0.300 | 0.160 |
|           | D10S603 | -0.770 | -0.510 | -0.390 | -0.260 | -0.190 |
|           | D10S192 | -1.110 | -0.850 | -0.690 | -0.430 | -0.250 |
| TOR2      | D10S198 | 0.700 | 0.599 | 0.500 | 0.300 | 0.160 |
|           | D10S603 | -0.770 | -0.510 | -0.390 | -0.260 | -0.190 |
|           | D10S192 | -1.110 | -0.850 | -0.690 | -0.430 | -0.250 |
| Singa 1   | D10S198 | 0    | 0    | 0    | 0    | 0    |
|           | D10S603 | 0    | 0    | 0    | 0    | 0    |
|           | D10S192 | 0.602 | 0.535 | 0.465 | 0.318 | 0.170 |
| Cumulative| D10S198 | -∞   | 0.014 | 0.239 | 0.257 | 0.184 |
|           | D10S603 | -2.261 | -1.483 | -1.097 | -0.669 | -0.440 |
|           | D10S192 | -5.037 | -3.004 | -2.094 | -1.071 | -0.538 |

The region syntenic to the human PKDL locus is located on chromosome 19 in mice (26). This region is partially deleted in mice with the mutation Krd (Kidney and retinal defects) (27). The 7 centimorgans Krd deletion is located between Tdt and Cyp17 and includes the paired box gene Pax2. Mice heterozygous for a null mutation of Pax2 frequently demonstrate reduction in kidney weight, which ranges from 10 to 100% normal (37). The reduced size is due mainly to calyceal and proximal ureteral diminution as well as cortical thinning, with a reduced number of developing nephrons (37). In contrast, the phenotype of Krd/+ heterozygotes includes aplastic, hypoplastic, and cystic kidneys, as well as reduced viability on strain C57BL/6J (27). Our Southern analysis demonstrates that the mouse ortholog of PKDL is deleted in Krd mice. Further study is needed to clarify the contribution of PKDL to the Krd phenotype.

Several other congenital murine and rat models with polycystic kidney disease are also known to exist, although the genetic defects in these models are as yet to be identified (38, 39). Among mouse PKD models, loci for cpk, bpk, pcy, jck, jcpk, kd have been mapped to mouse chromosomes 12, 10, 9, 11, 10, and 10, respectively, and are unlikely to involve the mouse.
homologue of PKD1. In Han:SPRD cy/+ rat, the disease gene was mapped to rat chromosome 5, whose human syntenic region resides on human chromosome 8 (18).

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