The Sequence, Expression, and Chromosomal Localization of a Novel Polycystic Kidney Disease 1–Like Gene, *PKD1L1*, in Human

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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. Since the first two polycystins were cloned, three new members, polycystin-L, -2L2, and -REJ, have been identified. In this study, we describe a sixth member of the family, polycystin-1L1, encoded by PKD1L1 in human. The full-length cDNA sequence of PKD1L1, determined from human testis cDNA, encodes a 2849-amino-acid protein and 58 exons in a 187-kb genomic region. The deduced amino acid sequence of polycystin-1L1 has significant homology with all known polycystins, but the longest stretches of homology were found with polycystin-1 and -REJ over the 1453- and 932-amino-acid residues, respectively. Polycystin-1L1 is predicted to have two Ig-like PKD, a REJ, a GPS, a LH2/PLAT, a coiled-coil, and 11 putative transmembrane domains. Several rhodopsin-like G-protein-coupled receptor (GPCR) signatures are also found in polycystin-1L1. Dot-blot analysis and RT-PCR revealed that human PKD1L1 is expressed in testis and in fetal and adult heart. In situ hybridization analysis showed that the most abundant and specific expression of *Pkd1l1* was found in Leydig cells, a known source of testosterone production, in mouse testis. We have assigned PKD1L1 to the short arm of human chromosome 7 in bands p12-p13 and Pkd1l1 to mouse chromosome 11 in band A2 by fluorescence in situ hybridization. We hypothesize a role for polycystin-1L1 in the heart and in the male reproductive system.

INTRODUCTION

Polycystins are a family of transmembrane proteins. Polycystin-1 and -2 are widely expressed [1,2], whereas polycystin-L, -2L2, and -REJ have limited expression [3–6]. Mutations in *PKD1* and *PKD2*, the respective genes encoding polycystin-1 and -2, cause autosomal dominant polycystic kidney disease (ADPKD), one of the most common monogenic diseases (estimated frequency of occurrence, 1 case per 1000 births), characterized by progressive cystic development in the kidney and eventual renal failure [7]. The involvement of other polycystins in disease states is currently unknown. Polycystin-L was the third polycystin isolated, but the first whose function was demonstrated as a Ca²⁺-regulated, Ca²⁺-permeable cation channel [8]. Recently, polycystin-2 has also been shown to function similarly as a cation channel, both intracellularly [9] and on cell membranes with [10] or without [11,12] the coexpression of polycystin-1. Advances with regard to polycystin channels have recently been reviewed [13]. Polycystin-2L2 and -REJ are likely to play a role in fertilization because of their specific expression in testis, although additional experimental evidence is required.

Polycystin-1 is a large plasma membrane protein with a number of adhesive domains. Most of its extracellular segment is made up of 16 copies of an 80- to 90-amino-acid immunoglobulin (Ig)-like repeat called the PKD domain [1,14]. One of the PKD domains is situated between a leucine-rich repeat domain and the C-type lectin domain; the rest are arrayed in tandem between an LDL-1A module and an REJ domain. There is a putative GPCR proteolytic site (GPS) and a putative lipid-binding site (LH2/PLAT) between the REJ domain and the first transmembrane region, and in the first intracellular loop, respectively [15,16]. Following a number of transmembrane regions is a

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human	1	MAEEAAQNISDDQERCLQAACCLSPGGELSVSTDKSWGLHLCSCSPPGGGLWVEVYANHVLLMSDGKCGCPWCALNGKAEDRESQSPSSSASRQKNIWKT
human	101	${\tt TSEAALSVVNEKTOAVVNEKTOAPLDCDNSADRIPHKPFIIIARAWSS0GPRFHHRRLCAT0TADSTFSALLQLQGTTSAAAPCSLKMEASCCVLRLLCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$
human	201	$\verb+Aedvatgllpgtvtmetptkvarptotscorvplwpishfptsprsshglppgiprtpsptascosgseilypptohppvailarnsdnfmnpvlncsleversetration and an antipation and antipation antipation and antipation antipatio$
human	301	earappnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqldmstykeaetqnttlmvylcqsenscledsdpsnlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphllhfqdssgvemrlhnmseamavtayhqyskgipphlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphlgfrvhmseamavtayhqyskgipphlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphlgfrvhmseamavtayhqyskgipphlgfrvhmasgealclmmdfgdssgvemrlhnmseamavtayhqyskgipphlgfrvhmseamavtayhqyskgipphlgfr
human	401	YELISAFVTKGVYMLKAVIYNEFHGTEVELGPYYVEIGHEAVSAFMNSSSVHEDEVLVFADSQVNQKSTVVIHHFPSIPSYNVSFISQTQVGDSQANHSM Ig-like PKD domain 1
human	501	¢ TVWYMOSVSVYTNGTVPATOTDITFTAVTKETIPLEFEMYPGEDPPVRTTSRSIKKRLSIPGWYRVMVKASNRMSSVVSEPHVIRVQKKIVANRLTSPS Ig-like PKD domain 2
human	601	salvnasvapecwinpgtdvaylmdpddgtvslasssshvysregeptvevlapmvsastlroolpivcepcopplvxmmgpdkvoiwrsopvrlgvtregetdessastlroolpivcepcopplastlroolpivcepcdessastlroolpivcepcopplastlroolpivcepcdessastlroolpiv
human	701	FEAAVFCDISQUSYTWNLMDSEGLPVSLPAAVDTHRQTLILPSHTLEYGNYTALAKVQIEGSVVYSNYCVGLEVRAQAPVSVISEGTHLFFSRTTSSPI
human	801	// // VLRGTQSFDPDDPGATLRYHWECATAGSPANPCFDSSTANQLDAAAPTVSFEAQWLSDSYDQFLVMLRVSSGGRNSSETRVFLSPYPDSAFRPVHISWVS
human	901	FKDTFVNWNDELSLQAMCEDCSEIPNLSYSWDLFLVNATEKNRIEVPFCRVVGLLGSLGLGAISESSQLNLLPTEPGTADPDATTTPFSREPSPVTLGQP
human	1001	* + + + ATSAPRGTPTEPMTGVYWI PPAGDSAVLGEAPEEGSLDLEPGPQSKGSLMTGRSERSQPTHSPDPHLSDPEAYYSDIQEAIPSGGRQPAKDTSPPGSGPS
human	1101	LSAEESPGDGDNLVDPSLSAGRAEPVLMIDWPKALLGRAVPQGYSSSGITEQTVTIKPYSLSSGETYVLQVSVASKHGLLGKAQLYLTVNPAPRDMACQV
human	1201	QPHHGLEAHTVPSVPCMSGKPDFHYEPSYQIGNTSKHTLYHGRDTQYYFVLPAGEHLDNYKVMVSTEITDGKGSKVQPCTVVVTVLPRYHGNDCLGEDLY
human	1301	NSSLKNLSTLQLMGSYTEIRNYITVITRILSRLSKEDKTASCNQWSRIQDALISSVCRLAFVDQEEMIGSVLMLRDLVSFSNKLGFMSAVLILKYTRALL
human	1401	AQGQFSGPFVIDKSVRLELIGLISRVWEVSEQENSKEEVYRHEEGITVISDLLLGCLSLNHVSTGQMEPRTLLHYNLQSSVQSLGSVQVHLPGDLAGHSP
human	1501	AGAETQSPCYISQLILFKKNPYPGSQAPGQIGGVVGLNLYTCSSRRPINRQMLRKPVMVEFGEEDGLDNRRNKTTFVLLRDKVNLHQFTELSENPQESLQ
human	1601	IEIEFSKPVTRAFPVMLLVRPSEKPTPSDFLVKQIYPMDESIVQIYIPAASKAASVGYLSLLDADYDRKPPNRYLAKAVNYTVHPGWIRCLPMDKREWK
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human mouse	2098 371	
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barren er.	2208	• • •
numan	2298	LCVIYGRPSQDETSLNQAIRKEPTRNARNCLGGLRNIADHWDWSLTTLLDGLYPGGTPSARVPGAQPGALGGKCYLIGSSVIRQLKVPPRHLCKPPRPPS
numan	2398	ALIEDSIPTCSPEVGGPENPTLIDPENQNVTLNGPGGCGTREDCVLSLGRTRTEAHTALSRLRASMWIDRSTRAVSVHPTLYNPPTGLFTSVSLRVEILP tm7tm8
human	2498	* TGSLVPSSLVESPSIFRSDSALQYHLMLPQLVFLALSLIHLCVQLYRMMDKGVLSYWRKPRNWLELSVVGVSLTYYAVSGHLVTLAGDVTNQPHRGLCRA
human PE	011.1#	
human	2598	tm9t tm10* // f FMDLTIMASWNQRARWLRGILLPLFTLKCVYLPGIQNTMASCSSMMRHSLPSIFVAGLVGALMLAALSHLHRPLLSMWVLPPDTPTDAFPGLLPHPPRS
		tml1 0 1 <u>Coiled-coil domain</u>
human	2698	QKDCLLGLSKSDQRAMACYFGILLIVSATLCPGMLRGPLMTLPQKRKSPQSKSPVRLKDVTAYMWEKVLTFLRLETPKLBEAEMVENHRYYLDEFANLLD
human	2798	ELLMKINGLSDSLOLPLLEKTSNNTGEARTEESPLVDISSYQAAEENTTLSGLSLYEPADIKDF

FIG. 1. Deduced amino acid sequences of human polycystin-1L1 and a portion of mouse *Pkd111*. ClustalW1.7 and Boxshade 3.21 were used for amino acid sequence alignment. Black and gray shading indicate amino acid identities and similarities, respectively. Various domains are overlined. Potential *N*-glycosylation sites are marked with an asterisk (*). Potential phosphorylation sites are marked as follows: (+) cAMP phosphorylation sites; (◊) protein kinase C phosphorylation sites; (¶) crosine kinase phosphorylation sites.

short carboxy-terminal cytoplasmic tail that contains a coiled-coil domain shown to interact *in vitro* with a coiled-coil domain in the C-terminal cytoplasmic region of polycystin-2 [17], and with G-proteins [18] and regulators of Gprotein signaling (RGS) proteins [19]. The large size of polycystin-1 has hindered its analysis, and its function remains unknown. Identification of novel proteins closely related to polycystin-1 would not only contribute to a broader understanding of the role of this family of proteins in organ morphogenesis and function, but also would help us to predict the function of polycystin-1 and facilitate our understanding of the pathogenesis of ADPKD.

In this study, we report the identification, chromosomal localization, and expression of a novel gene encoding a

	TA	ABLE 1: Sequer polycystin-	nce compariso 1, -REJ, 1L1, -	ons betweer -2, -L, and 2	n human L2	
	PC1	PCREJ	PC1L1	PC2	PCL	PC2L2
PC1	Х	21%	26%	32%	33%	28%
	Х	38%	42%	48%	73%	45%
	Х	1371	856	150	150	149
PCREJ	20%	Х	22%	21%	24%	22%
	35%	Х	39%	39%	41%	41%
	2016	Х	836	497	395	431
PC1L1	23%	22%	x	23%	21%	23%
	38%	38%	х	40%	36%	39%
	1453	932	х	376	536	374
PC2	24%	21%	26%	Х	52%	48%
	42%	42%	42%	Х	70%	68%
	425	514	295	Х	685	579
PCL	25%	25%	21%	54%	Х	50%
	42%	40%	40%	73%	Х	68%
	408	253	490	685	Х	547
PC2L2	24%	22%	23%	47%	52%	х
	41%	41%	40%	67%	71%	х
	256	497	355	606	509	Х

lop, middle, and bottom rows indicate the percentage of identity and similarity, and the number of residues of the proteins in the left column that share with the proteins in the top row, respectively. Bold text represents the comparison between PCIL1 and other PCs.

polycystin-1-like protein, polycystin-1L1, in humans. Polycystin-1L1 possesses a topology similar to that of polycystin-1 and contains two Ig-like PKD domains that are followed by a small REJ, a GPS, a LH2/PLAT, a coiled-coil, and 11 putative transmembrane domains.

Results

Isolation and Characterization of cDNAs for a Sixth Polycystin, Polycystin-1L1

Through database searches we identified two genomic sequences of chromosome 7 (EMBL/GenBank acc. nos. AC069282 and AC019066) with similarity to polycystin-2. The deduced amino acid sequence of these clones showed 43% similarity and 25% identity over 282 residues (235–516) with polycystin-2. We tentatively assumed that these genomic sequences encoded a novel polycystin.

We carried out 5'- and 3'-RACE with a human testis cDNA library and obtained a 4.5-kb and a 3-kb fragment. Direct sequencing of the PCR products revealed a 7.3-kb consensus cDNA sequence with an open reading frame, which encodes a protein of 2400 amino acids with termination codons and a consensus polyadenylation signal (5'-AATAAA-3'), but without an ideal initiation codon. We then continued 5'-RACE using the gene-specific primers hP9R4 and AP2 and obtained 2 kb more cDNA. The sequence spanning the first ATG triplet of this cDNA has a G residue at position +4 and a G residue at position -3; this is in complete agreement with the consensus sequence for a mammalian translation initiation site [20].

Thus, this novel cDNA encodes a 2849-amino-acid protein (AB061683; Fig. 1) with homology to all known polycystins (Table 1). Although the sequence homology between this novel polycystin and polycystin-1 or polycystin-REJ is not higher than that with polycystin-2, they share 38% homology over a large region (1453 and 932 amino acids, respectively; Fig. 2A). Phylogenetic analysis of this novel polycystin with the other five known polycystins shows that this novel polycystin belongs to the polycystin-1-like subfamily (Fig. 2B); we thus named it polycystin-1L1.

We determined the exon/intron structure of human *PKD1L1* by comparison of genomic and cDNA sequences (Table 2). In the 187-kb genomic region, we found 58 exons varying in size from 26 bp to 369 bp; the intron size varies from 0.1 kb to 17.0 kb.

Kyte-Doolittle hydropathy analysis of polycystin-1L1 revealed 11 highly hydrophobic regions, suggesting the

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FIG. 2. Protein family classification of polycystin-1L1. (A) (previous page) Comparison among human polycystin-1, polycystin-REJ, and polycystin-1L1. The C-terminal ends of the proteins are indicated by an asterisk. The predicted transmembrane domains are overlined. (B) The deduced phylogenetic relationship among human polycystin-1L1, other polycystins, and homologous proteins. Evolutionary distances (0.1 million years) are shown by the total branch lengths (horizontal lines).

presence of 11 transmembrane segments (Fig. 3A). After further analysis with two more transmembrane prediction programs (TMHMM and TMpred) and comparison of the results with those obtained from the analysis of polycystin-1 and -REJ, we postulated a topology model with 11 transmembrane segments for polycystin-1L1. Analysis with the protein secondary-structure prediction programs SMART and Pfam revealed two Ig-like PKD domains, a small REJ domain, a GPS domain, and an LH2/PLAT domain that are all found in polycystin-1 (Fig. 3B). A coiled-coil structure was predicted in the C-terminal cytoplasmic tail (between residues 2776 and 2811) by the Lupas coiled-coil prediction program (Fig. 3C), as in the other polycystins. The domain structure of polycystin-1L1 also indicates that it is a member of the polycystin-1 subfamily. Notably, polycystin-1L1 has a number of rhodopsin-like GPCR superfamily signatures throughout its sequence.

We identified two smaller bands (2.4 and 2.1 kb) that encode alternatively spliced transcripts of *PKD1L1* during 3'-RACE. Each transcript contained a partial coding sequence and termination codons with a consensus polyadenylation signal (5'-AATAAA-3') 10 nucleotides upstream from the poly(A) tail. These spliced variants, *PKD1L1a* and *PKD1L1b*, are predicted to contain seven and six transmembrane domains, respectively (Fig. 3D). Although the significance of these alternatively spliced products is unclear, they may provide one way of regulating the abundance of the long transcript.

Determination of Partial cDNA Sequence of Mouse *Pkd1l1*

Through database searches, we identified one genomic sequence (AC024116) of mouse chromosome 11 that is syntenic to human chromosome 7p with similarity to human *PKD1L1*. The DNA sequence of AC024116 showed > 70% identity to human *PKD1L1* (nucleotide sequence from 4956 to 6552 of human *PKD1L1*). We tentatively assumed that this genomic clone contains the mouse ortholog of the *Pkd1l1* gene and designed RT-PCR primers based on the genomic sequence information. We obtained a cDNA fragment of 1594 bp and confirmed it to be the mouse *Pkd1l1* (AB061684) with 72.3% nucleotide identity and 61% and 69% amino acid identity and similarity to its human counterpart, respectively, by DNA sequencing (Fig. 1). This fragment was used as a probe for further northern blot and *in situ* hybridization analysis.

Tissue-Specific Expression of Human and Mouse *PKD1L1* and Their Alternatively Spliced Transcripts

We analyzed the tissue-specific expression of both the human and mouse genes by northern blot analysis using cDNA probes (1.9 kb and 1.6 kb, respectively) amplified by RT-PCR. Neither the human *PKD1L1* nor the mouse *Pkd111*, however, was detected by northern blot analysis of multiple tissues (data not shown). We then examined the expression of human *PKD1L1* by dot-blot analysis using the same cDNA probe, and detected *PKD1L1* expression in adult and fetal heart and in testis (Fig. 4A). The expression pattern was further confirmed with RT-PCR from multiple tissues using two sets of primers that can detect all three *PKD1L1* transcripts. Both splicing variants, *PKD1L1a* and *PKD1L1b*, were found in adult testis, but only *PKD1L1a* was found in fetal or adult heart (Fig. 4B). These *PKD1L1* transcripts can be detected by one-round RT-PCR (35 cycles).

Cellular Localization of Mouse *Pkd1l1* by *in Situ* Hybridization

We carried out *in situ* hybridization in various normal mouse tissues, including brain, heart, kidney, and testis, using a 1.6-kb ³⁵S-UTP-labeled *Pkd1l1* antisense riboprobe. Testis showed more widespread expression than did other tissues (Figs. 5A–5D). It is interesting that Leydig cells, which are known to produce testosterone, showed strong expression of *Pkd1l1*. A low level of expression was noticed in seminal ducts, myoid cells, and tunica vaginalis (spindle-shaped cells that surround the basal cell layer of germinal cells and act as a support system). Other tissues, including adrenal gland and heart myocardium, also showed weak

exon	size	3' splice site	5' splice site	intron	interrupted
no.	(bp)	(acceptor)	(donor)	(KD)	amino acid
1	102	++	CTAAG gt atc	15.9	A /
2	105	(initiation sites GAG	GAAAG gt gag	4.9	Ar/g
	116	(Initiation site: GAC	ATGG)	2.0	$C/l_{\rm H}$
) 1	110	CTT ag GTGTC	GGTCG gt aag	5.0	G/IU Laur (A arr
± =	125	tetagAGGTC	AGAAG gt att	5.5 1.2	Lys/ Asn
,	115	tacagAACAT	GATAG gt atg	1.5	Se/r
7	121 218	tacagAATTC	TCCAG gt tgg	0.0	So/r
, >	210		AGAAGGEacg	12.6	C/1
י ג	168	tatagCTCCC	CACCA gt ata	12.0	G/ Iy
7 10	100		CARCAGE	2.8	L/ys T/br
10	1/4	tttagAAGGA	GAAAAgtaag	2.8	1/111 E/or
12	140		GLAATGEatg	0.5	5/er
12	240		CAAIG ge cag	2.0	11/p
1.3	122	LGCAGGIAIC	MGTAG ge gae	2.0	Cln/Ilo
.4	207	CCCagGGAAG	CCACGEagg	4.2	L vs /Val
6	182	taaaacmmch	CTCACgtcag	4.0	Lys/ vai
7	202	aacagGTTCA	TTCAG gt gag	2.0	Ar/g
19	161			2.4	V/al
10	360	ttagmmccc	CTCTC at 22 a	1.9	V/ai
20	60		CCCC	2.6	A/5p
20	177	catagAIIII	GCCAG g tgac	2.0	C/lu
21	78		TGTGGgtatg	3.0	G/1y A/1a
	143	tttagCTTCC	ACCCotacg	13	Pro/Asp
24	191	Catagence	Chacgeacy	2.0	V/al
	1121	CacagerCATC	GACCTetag	73	V/ai
-5	196	ttcagGTATA	ATCAGet at g	11	Cln/Clu
-0 07	57	gtcagGAAGA	ATAAGetaag	68	Leu/Glv
28	213		TGTTG gt aag	1.0	Gln/Ile
20	213	ttcagGGTTG	GCAGetaca	2.3	Leu/Asp
	111	CCARGATTGG	GCCTGgtaag	0.1	Ar/o
31	158	gacagGATAA	GTAAGatata	17	A/sp
32	101	CCT agATTCT	GAAAGatcag	59	Val/His
33	193	tttagATGCC	TGCAGetata	18	Se/r
34	84	cctagCTACC	CAGAGataag	1.8	Se/r
35	208	tatagCCACC	CAAAGatago	2.4	Lys/Val
36	107	ttgagGTGTA	CTGAG gt att	0.8	Se/r
37	217		GGAAGetagg	23	Lvs/Leu
38	174	ttcagCTTTT	AGCAGgtgag	1.7	Gln/Pro
39	111	tgcagCCCCA	GCAAGgtacc	0.2	Lys/Gln
10	73	tgcagGAAGC	CCATGetaaa	0.6	G/lv
11	47	atcagGTCCT	GCAAGetaaa	11	Lys/Gln
12	163	tctagCAACC	TCAAGgtaac	1.8	A/la
13	136	cgcagCACCC	TACAGgtgag	1.1	Ar/g
14	85	ggcagGTTTG	TTATGgtaag	0.4	Cv/s
15	177	tccagGTATG	AAAAAgtaaa	2.0	Lvs/Val
16	119	gacagGTCTT	CTGAGataga	6.2	Ar/g
7	111	tacagAGACA	ACAAGgtagg	5.6	Ar/g
8	124	tta ag AAATG	CTCAGgtgag	1.3	Gln/Pro
9	84	ttcagCCTGG	GCAAGgtaag	0.6	Lvs/Pro
50	173	tgcagCCTCC	ACAAGgtcag	1.0	Ar/g
51	235	tacagGACTG	CCCAGataaa	2.2	Gln/Leu
52	105	accagCTGGT	TGGAGatage	1.9	Glu/Leu
53	141	tccaqCTCTC	ATCAGataca	4.9	Gln/Arg
54	135	ctcagAGGGC	CAGGGataag	2.3	Glv/Gln
55	231	tgcagCTGGT	GAATGataag	4.5	Met/Leu
56	162	cctagCTGAG	ATCACataag	3.2	His/Asn
57	171	ttcagAATTA	CTGAGataag	5.2	Glu/Pro
58			termination site:		
	26	tttagCCAGC	GACTTC TGA		

FIG. 3. Peptide sequence analysis and alternative splicing of PKD1L1. (A) Hydropathy analysis of human polycystin family. Hydrophobic peaks are indicated as H1-H11. (B) (opposite page) Alignment analysis of PKD, GPS, and LH2/PLAT domains. The following key represents consensus amino acid residues or respective amino acid classes: (o) alcohol; (l) aliphatic; (.) any; (a) aromatic; (c) charged; (h) hydrophobic; (-) negative; (p) polar; (+) positive; (s) small; (u) tiny; (t) turnlike. (C) (opposite page) Predicted coiled-coil domain of polycystin-1L1. (D) (opposite page) Schematic representation of the splicing variants. Boxes indicate exons. Solid lines indicate introns.

expression for *Pkd1l1*. In addition, we have localized *PKD1L1* to human chromosome 7p12–p13 and *Pkd1l1* to mouse chromosome 11 in band A2 by FISH (data not shown).

DISCUSSION

Polycystin-1L1 has two Ig-like PKD domains, a small REJ domain, a GPS domain in the N-terminal extracellular region, a LH2/PLAT domain in the first intracellular loop, and 11 transmembrane domains. The Ig-like PKD domains of polycystin-1 mediate specific homophilic interactions in vitro and are involved directly in the cellular adhesive process in culture [21]. Application of an anti-PKD domain antibody to cultured MDCK cells perturbs their normal epithelial architecture. These results suggest that the PKD domains of polycystin-1L1 play important roles for cell adhesion and signal transduction through homophilic or heterophilic interactions mediated by these domains. By contrast to the REJ domains of polycystin-1 and -REJ (525 and 544 residues, respectively), the REJ domain in polycystin-1L1 is smaller, with only 111 amino acids (between residues 726 and 836). This domain is believed to be associated with calcium transport during fertilization in sea urchins [22].

The GPS domain in polycystin-1L1 is homologous to those in members of the GPCR subfamily, which includes the calcium-independent latrotoxin receptor (CL-1/latrophilin) and flamingo, a newly identified protein of the cadherin superfamily [15,23,24]. The GPS domains of several such genes are shown in Fig. 3B. Lectomedin is a human homolog of CIRL/latrophilin; cadherin, EGF LAG seven-pass G-type receptor is a seven-transmembrane protein first described in *Drosophila*, where it was found in cell/cell boundaries and was believed to play specific roles in protein–protein interactions [24]. All the above evidence supports our hypothesis that polycystin-1L1 has several important functions in protein–protein and/or cell-cell



interactions. It is interesting to note that one form of *PKD1L1*, *PKD1L1a*, has seven transmembrane domains like most GPCRs, and also has a number of rhodopsin-like GPCR motifs in its sequence, indicating that polycystin-1L1 or one of its isoforms functions as a GPCR. Indeed, two interesting studies have linked the C-terminal cytoplasmic domain of polycystin-1 with G-protein-coupled signaling pathways by direct binding and activating of G-protein [18], and by directly binding to RGS proteins and terminating this signaling pathway [19]. Very recently, it has been shown that polycystin-1 acts as a G-protein coupled receptor [34].

The LH2/PLAT domain of polycystin-1L1 contains a β -barrel LH2 domain homologous to a noncatalytic domain of lipoxygenases. This domain in lipoprotein lipase is proposed to possess a lipid-binding function and may have an internal conserved ligand-binding site [16,25]. Many lipoprotein lipase family proteins possess the LH2/PLAT

Accession nm AL13395 AF104256 AF104935 AL157903 AF307009 AF102403 U76764 AF23403 U76764 AF23403 AF114491 AF234034 AF234887 D12638 AJ204499 J05125 M93283

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eptor 1 (CELS1)

ase (ALOX128)

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nkaa

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cadherin, EGF LAG aeven-pass G-type n CEL52

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efial lipase (LIPG) taman bapatic lipuse (LIPC)

human arachidonare lipoxygenase (ALOX12B) human arachidonare lipoxygenase-3 (ALOXE3) human puncreatic lipase (PALIP) human puncreatic lipase related protein 1 (PNLIPRP1) PNLIPRP2

B PKD domain

Pc1L1(R1)	1 -	SIKIRLSIPSIKIRLSIP
Pc1L1(R2)	1 -	VANRLTSPSSALVNASAAFECWINP#TDAALATENTVSLGSSSSEVIJSRE
Pc1(R1)	1 -	PGATLVGPHGP ASGOLAAFHIAAPLP-VTDTP DIGUS SAEVDAAG PAA POVLPPAA POVLPPAAHATAVLALGAGSALLGTD OVEAA-
Pc1(R2)	1 -	RATPSPEAR LOGVIERYSPUSEASD WERTING COLTON
Pc1(R3)	1 -	GLOVSTVPAVLSPNATLATAGVLDS_VPAALLATABHE COALNOFOPPYNES_PVPDPSVAQVLVENNVTHTYAAPECLITELOSAFENLTQVPSSPAS-
Pc1(R4)	1 -	SVAVGVSDGVL_AGRPVTFYPHPPSPGGLATED_HESPVLTOSQPAANTT_ASR
Pc1(R5)	1 -	SVEMSLA_EQGAPEVVS_AQTEON_TT_DOUT_TVLSGPEATVEEVULRAQNCT#TGGSPAGHLARSLHELEPVL
Pc1(R6)	1 -	VLRVEPAAC PTOPDARLT YST-GNPAHYL COTONESSNTTVRGCPTV/DETTS
Pc1(R7)	1 -	VGNVTLQPEROP/QLGDEAMLV_CAWPFFPYRTEDATTEEAAPTRARGPEVB/IJRDP
Pc1(R8)	1 -	VTSIKVNGSLGLELQOPYLFSAGPERPASELOLATEGNLEGPEVTAANST
Pc1(R9)	1 -	GLVVNASRTV_PLAGSSFSTSEASDARGSVLCRCTPIPOGPTI_TT_RSVTI_NLITTARE_GGAQDSIPATALQL-
Pc1(R10)	1 -	GRYFPTNHTBOLO_VRDFTNSSSTANRDRGPALAGSGKGFSLTVLEA
Pc1(R11)	1 -	KVAASPNPAAVNTSTLS EAGSGNVTTSLELSWETSEPFT ES PTPLHLAT TOOP GSANATVEGDOVP-
Pc1(R12)	1 V	SGLSIRASEPOGSP AAGSS PFW O ATTING CAVPGESSKRGPHVWV PDA
Pc1(R13)	1 -	VGLVLWASSKV_APOOLAHFOILAASAAT_RLOVGONPEVLPG PRF_BSJPRV
Pc1(R14)	1 -	SGLOVPHCCEPGATGTERNPT_RORSRAAATYSLOKVOGDSLVILSGRDVTYTPVAALLEOORPTAAGSENRTLVIDODA-
Pc1(R15)	1 -	QYVALQSGPCPTNRSAQFEATSPSPRRAAPHOCKCSPGQDTDEPRAESSLRP
VPS10(R1)	1 -	GLRIVTADGKLTAEQGHNVTLMVQ EEGDVQRTLIQVD BE IAVSYVNLSSHEDGIKTVQQNV
VPS10(R2)	1 -	LPFVTTKNKERNAT V MPSQVGT TV M NTEPLITLEGSI FR TSE
CONSENSUS/80	۱.	h.h.th.hps htt.Wshsstt.tshta.psG.aphplts.st sph h.l
CONSENSUS/65	1	ssthssssts.slpapspssst.hsapWshGDupstpttsssasasps
CONSENSUS/50	i .	sushsssssshsspsVsFsssssGsslsapWsFGDGssssusssoNoYscs

GPS domain

Pc1L1	1	-WIREL DKREKERFSPOPGTSPE VIESYH
Pc1	1	-TSLOWSEEDHVERBELLPEETSPROAVELTR
PCREJ	1	FSVC LDRYGI QSE RECY I GEKTSWYEVH I KNVVRARRQLGTIGLTGI HHTHY AKVIV
BUREJ	1	HTLOWNNEDQOEDOTTER GPLS PTTEL N
Lectomedinla	1	FNAMSSERVYS-ERTHOLYNERCERER D
Lectomedin1b	1	FNAMESTARYS-ERTHOGYNSYCHARE D
Lectomedinlg	1	PNAMESTERYS-ERTHOGYNSTCREED DIE - THTTA-
Lectomedin2	1	FNAMESTERNYS-ERSHLEY COLORER E
Lectomedin3	1	FNPMSSPISYS-KRTMTRYNORCIAPS T
ETL	1	YRSLAADINYS-PDTHNESIN FERENTY RE-I ISEEN
CD97	1	QELLANDKSDSDROMM HER OV GIKN-GST CO
EMR2	1	OKVLAVERENGONOCIME TTREST GURD-ISITER
ENR3	1	KKVPEVIEKSTOODSO REFERENCE HVE REFERENCE
CELS1	1	TKPVEVENHSLAVQGTEGERARIES SREI- VA OF TASEA MDISRR
CELS2	1	SKAIEVOEDPPGLAEOHEV ARDEE HREG- AREF
CONSENSUS/80%		.pshCs.as.tts.WsspsC.hh.tst.spshC.Cs+hssFulhht
CONSENSUS/65%	÷	.pshCshWs.sputWsspGCplltpst.spspCpCsHLosFullhthst.
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LH2/PLAT domain

Pc1L1	1 -LAAVID -FRAPARITSKIYIV CENTERSCREEPERENERIEIISAPACELENE IISAPACELENE IISAPACELENE IISAPACELENE	
Pc1	1 - KREILARIN-WERGSOTT H.G.M. VDSREGHRH DODR APHRNELDIR ATPHS SMILL- BHONKGL-SPA 201 IROLQTARSAFELVND K ETEANOGLVEK	
PCREJ	1 LCBL THEM - SRIGSOTR N. FOOT WITS DVHCES PHETTYRG IN LTTKS WITH THE - DINNEGR-SPS WEST RENLESCRET BOTTORTEWYT	
ALOX12B	1 GRV AND SAND SAND STRUCTURE BELLOU PA RGEEEE DHDVAP CLOPY - RUBBALY - DDA. CD. TOOPGACAEVA PCYR VOGEL SLPEG	
ALOX3	1 AVT COTT - PYLRAGTLENISTEVE CEEPKORD NG-RDPAPOVOK K RCTA C 2LL VYKERYAFFRKDS CSHOTEP-DOSVSH PCYOE EGYCT ELREG	
PNLIP	1 TY SVT-LSGK-KUTHULSE ENGENKOVEP GT KPDEHMEPDSDV E OKUP VIBNON-PTLPHUGASKI IVETNVG-KOMPCSPE BEELLTLTPC	
PNLIPRPLIP1	1 TYORSIT-LSGR-TAT OFFAR ENGENHOUSS F GI KROSH SEPARLE FT S OF SUBNY N-PTLPK GATK TYOKGEEKTV SPCSED BODTLLTLTPC	
PNLIPRPLIP2	1 W SVT-LSGKEK NEY PRIA & NEXX YEFF GS KPD HICA DVDP BOOT PENKRON-LSEPK GASO TVOSGED TE NFCSSD SON LOSLYPC	
LIPG	1 YHO ARVF SYKNIGE EPTFYET THE DOUTLINE IVER BOM IN BL YTERE I AUGUT EGASOSHYNLEKEF SY SOPRINGRELNIRRIP.KSOPTORKLTPCTEDPENTSISPGRELAF	RKC
LIPC	1 YHO JOFI-NOTETP OTTPT SLIPKEKHOKIPTLGKG ASNK Y LITLDY INTERNA WANNED OT IPSTGPRESGLULKTIR KAGTOORNTPCSENTDULLAPTOEKIF	VKC
CONSENSUS/80	plhhhshl.ltLhutpt.sttttst.phphphs.s.hGhhhlpt.thhhp.lplps.stt.h.Fss.pWl.thh	h.s
CONSENSUS/65	<pre>t pVshhshphsshtstlplpLlusptt.uptstpstaphphphsts.hGttlhlppttpspaalpplplcsh.stsphpFsspsWlhsptpplh</pre>	hss
CONSENSUS/50	oValtstchsusssthh.p.,slslpLluuchstpscpuct.hssssashcF-sDps.lGsltAlcl+NPcps-aaLcslolcsssspuslpFsCsSWVtsstph.scRlF	Fas





domain (Fig. 3B). When we consider that the lipoprotein metabolism plays an important role in the male reproductive system, it is interesting to note the relatively high levels of Pkd1l1 expression in testis and that lov1, a Caenorhabditis elegans homolog of PKD genes, is required for male mating behavior in these worms [26]. Polycystin-1L1 has a short C-terminal cytoplasmic tail with a coiledcoil domain that is also found in polycystin-1 and is believed to be the domain interacting with a coiled-coil domain in the C-terminal cytoplasmic region of polycystin-2 [16]. Polycystin-1L1 also has strong motif sequences for putative phosphorylation sites in the C-terminal cytoplasmic region: one for protein kinase C, one for casein kinase, and one for tyrosine kinase. Phosphorylation of these motif sequences may be involved in the regulation of downstream signaling mediated by polycystin-1L1.

Our FISH analyses have assigned *PKD1L1* to chromosome 7p12-p13 in humans and *Pkd1l1* to mouse chromosome 11 in band A2, which is linked to the mouse mutation *jck*, a naturally occurring mouse line with recessive polycystic kidney disease [27]. However, it is unlikely that *Pkd1l1* is the gene for polycystic kidney disease in these mice, as we could not detect any expression of *Pkd1l1* in mouse kidney. The specific, albeit low, levels of *PKD1L1* expression in human fetal and adult heart raise the possibility of its involvement in heart diseases. Indeed, gene targeting experiments have recently shown that homozygous mice with mutations in *Pkd1* develop not only kidney, pancreas, and skeletal defects but also cardiovascular abnormalities [28–30].

MATERIALS AND METHODS

Isolation of human PKD1L1 and mouse Pkd1l1. We used primers specific to the putative exonic sequence in genomic clone RP11-61L9 (AC069282) to carry out 5'- and 3'-RACE on human testis Marathon-Ready cDNA (Clontech, Palo Alto, CA). AdvanTaq DNA polymerase (Clontech) was used to perform touchdown PCR with cycling parameters as follows: initial denaturation of 94°C for 2 minutes; 5 cycles of 94°C for 30 seconds, 72°C for 4 minutes; 5 cycles of 94°C for 30 seconds, 70°C for 4 minutes; 25 cycles of 94°C for 30 seconds, 68°C for 4 minutes, and a final extension at 68°C for 7 minutes, as described [5]. The primer sequences used in this study are listed in Table 3. For 5'-RACE, primers AP1 (Clontech) and hP9R1 were used to do the first PCR. Nested PCR was carried out using 1 μ l of the diluted first PCR product (1:100) and the nested primers AP2 (Clontech) and hP9R2. For the second 5'-RACE, primers AP1 and hP9R3, and nested primers AP2 and hP9R4 were used. 3'-RACE was carried out similarly with primers AP1 and hP9F1, and nested primers AP2 and hP9F2. Prominent bands were excised from the agarose gel, purified, and sequenced.



FIG. 5. Localization of *Pkd111* expression in mice testis by RNA *in situ* hybridization. Bright- (A, C) and darkfield (B, D) photographs show the *Pkd111* expression. Arrows indicate sites of *Pkd111* expression. (B) and (D) Single seminiferous tubule and its surrounding Leydig cells, showing strong expression of *Pkd111*. Magnification: (A, B) ×100; (C, D) ×200.

The sequences obtained were compared with the sequences of the clones RP11-61L9 and RP11-164L4 (AC019066).

Primers specific to the putative exonic sequence in mouse genomic clone RP23-298M16 (mP9F1 and mP9R1) were used to perform RT-PCR on mouse testis cDNA similarly as described above.

Sequence analysis. We aligned DNA sequences obtained by 5'-RACE and 3'-RACE to get an overall consensus sequence. Alignment analysis was performed with ClustalW (http://clustalw.genome.ad.jp/) and Boxshade3.21 (http://www.ch.embnet.org/software/BOX_form.html). The programs MOTIF (http://motif.genome.ad.jp/), SMART (Simple Modular

Architecture Research Tool) (http://smart.embl-heidelberg.de/), and Pfam (Protein families database) (http://www.sanger.ac.uk/Software/ Pfam/search.shtml) were used to predict protein function and localization. The programs TMHMM (http://genome.cbs.dtu.dk/services/TMHMM/) and TMpred (Prediction of Transmembrane Regions and Orientation) (http://www.ch.embnet.org/software/TMPRED_form.html) were used to predict the transmembrane helices. Prediction of coiled-coil structure by Lupas algorithm was performed with Coils (http://www.ch.embnet.org/ software/COILS_form.html) [31]. The phylogenetic trees were plotted using Treeview [32].

	TABLE 3: Primer sequences used in this study	
name	sequence	position
		(exon: nucleotides)
human PKD1L1		
hP9F1 hP9F2 hP9F3 hP9R1 hP9R2 hP9R3 hP9R4 hP9R5	GAATTTTCCAAACCTGTTACAAGGGC CACGGGACAGGGCTGGTTCTTCCCTGC CCCGACTCAGGGCCAGCATGTGGATTG GAGGGGACGAGACTCCCCGTAGGGAGGA CGGCGTGTGCAGGTAGCGGCTGGAGGA TCACTCCCAGCCTCACAGGCTGAGAACCTCC CTGGCATTTACCAGAGCTGAGGAGGGGGA GACTCTCTTCTGTCCTTGCCTCCCCTGTGT	31: 4810-4836 37: 5656-5684 51: 7370-7395 51: 7484-7631 38: 5839-5864 15: 2069-2098 13: 1792-1820 57: 8462-8491
human PKD1L1a		
hP9R6 mouse <i>Pkd1l1</i>	TTTTTCAAAAGAAAATGACACAAGAGAGGCT	52: 7615-7639
mP9F1 mP9R1	TGTCCTAAGAAGAAAGCTGAATGCCA CCAGCGCAGGTGGCGTTCTCGTTGTCGG	3572-3600 3703-3730
human GAPDH		
GAPDH/F GAPDH/R	GACCACAGTCCATGCCATCACT TCCACCACCCTGTTGCTGTAG	8: 525-546 9: 957-977
adapter primers (CLC	DNTECH)	
AP1 AP2	CCATCCTAATACGACTCACTATAGGGC ACTCACTATAGGGCTCGAGCGGC	

RNA dot blot, northern blot hybridization, and RT-PCR. We hybridized human Multiple Tissue Array (Clontech) and Multiple Tissue Northern Blot (Clontech) with a randomly labeled 1.9-kb coding sequence of *PKD1L1* (nucleotide sequence 5658–7511) amplified by RT-PCR using hP9F2 and hP9R1, in ExpressHyb Hybridization Solution (Clontech) at 65°C and 50°C overnight, respectively, as described [5]. Mouse Multiple Tissue Northern Blot (Clontech) was hybridized with a randomly labeled 1.5-kb coding cDNA probe of mouse *Pkd111* (mP9F1-mP9R1) in ExpressHyb Hybridization Solution (Clontech) at 50°C overnight. The radiolabeled membranes were washed twice in 2× SSC, 0.05% SDS at 50°C and twice in 0.1× SSC, 0.1% SDS at 50°C.

Total cellular RNA of human adult and fetal tissues for RT-PCR was prepared as described [5], and first-strand cDNA was synthesized from 5 mg of total RNA using Superscript II (GIBCO, Gaithersburg, MD). Two primer sets (hP9F3-hP9R5 and hP9F3-hP9R6) were synthesized according to the nucleotide sequence of human *PKD1L1* or *PKD1L1a*, respectively. Human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene-specific primers, *GAPDH/F* and *GAPDH/R* were used as controls. Portions (1 μ I) of the cDNA were amplified by PCR with cycling parameters as follows: 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 minute, with 35 cycles for *PKD1L1* and 25 cycles for *GAPDH* [5].

In situ hybridization. We carried out in situ hybridization of mouse Pkd111 gene as described [33]. The following materials were obtained from the indicated sources: T7 RNA polymerase, RNasin, and DNase I (Promega, Madison, WI); dithiothreitol (DTT), formamide, and restriction endonuclease (Roche Molecular Biochemicals, Indianapolis, IN). The 1.6-kb RT-PCR fragment of mouse Pkd1l1 (mP9F1-mP9R1) was cloned into Bluescript SK vector (Stratagene, La Jolla, CA), which was linearized with XbaI for sense and XhoI for antisense riboprobe preparation using T7 and T3 promoter, respectively. Transcription was carried out essentially as recommended by the manufacturer with 200-600 ng of linearized DNA as template. ³⁵S-UTP (DuPont NEN, Boston, MA)-labeled sense and antisense riboprobe (5 imes 10⁴ cpm) was hydrolyzed in 0.1 M DTT to an average size of 100 bases, and hybridization was carried out at 55°C overnight. The slides were then washed in 5× SSC containing 10 mM DTT at 55°C for 30 minutes, and in 2× SSC containing 50% formamide and 10 mM DTT at 65°C for 30 minutes. Finally, the slides were dipped in NTB-2 autoradiographic emulsion (Eastman Kodak, Rochester, NY) and stored in complete darkness at 4°C for 2 weeks. The slides were developed with Kodak D-19 developer (Eastman Kodak) and counterstained with hematoxylin/eosin. The experiment was repeated once to validate expression and localization. Control sense RNA probes were used to verify the specificity of the signal.

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Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries under accession numbers AB061683 and AB061684.