A Functional Floxed Allele of Pkd1 that Can Be Conditionally Inactivated In Vivo

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Abstract. Gene targeting has been used to create a variety of lines of mice with Pkd1 mutations that share many common features. Homozygous Pkd1 mutants invariably develop pancreatic and renal cysts if they survive to day 15.5 post coitum and die in either the fetal or the perinatal period. In contrast, mice with heterozygous mutations of Pkd1 are generally normal and have few if any renal cysts. These features have limited the utility of these models as tools to study the pathogenesis of cyst formation and the effect of various therapeutic interventions on disease progression. This report describes a new line of mice with a floxed allele of Pkd1 (Pkd1cond) that has an FRT-flanked neomycin cassette inserted into intron 1 and lox P sites inserted into intron 1 and intron 4. The Pkd1cond allele is fully functional, and homozygotes are viable and healthy. It is shown that the lox P and FRT sites can be selectively induced to recombine to produce two new alleles, Pkd1del2–4 and Pkd1AO, by crossing to animals that express either the cre or FLPe recombinase, respectively. It is found that Pkd1del2–4 allele functions as a true null, whereas presence or absence of the neomycin gene has no functional effects. It also is shown that somatic loss of Pkd1 results in renal and hepatic cysts. This new line of mice will be invaluable in the study of Pkd1 biology and serve as a powerful new tool that can be used to study the pathogenesis of autosomal dominant polycystic kidney disease.

Autosomal dominant polycystic kidney disease (ADPKD) in humans is characterized by a slowly progressive course in which cysts increase in size and number throughout the lifetime of an individual (1,2). Molecular genetic studies of human cystic epithelial cells suggest that the disease is recessive on a molecular level (3). However, it is not presently known whether somatic mutations are acquired during the lifetime of an individual or during the relatively brief period of fetal development, at a time of relatively rapid growth and cellular proliferation. Distinguishing between these two possibilities has important implications for understanding the pathobiology of the disease and for the development of future therapeutic approaches. The results also have important implications for our understanding of the role of polycystins in renal tubulogenesis.

A number of mouse lines with targeted mutations of either Pkd1 or Pkd2 have been described (4–8). Regardless of which gene is inactivated, the different mouse lines share many common features. Homozygous inactivation of either gene results in bilateral renal cyst formation beginning at approximately embryonic day 15.5 (E15.5; the presence of a vaginal plug being defined as E0.5d) and invariably results in fetal or perinatal demise. In contrast, mice with heterozygous mutations of Pkd1 or Pkd2 are generally normal and have few if any renal cysts, although hepatic cysts often are a late presentation. Although these studies have shown conclusively that Pkd1 and Pkd2 are required for the establishment of normal renal tubular morphology, the severity of the presentation associated with the homozygous null state coupled with the relatively benign status of heterozygosity has limited their utility.

Wu et al. (9) reported an exceptionally useful line of animals that had a local duplication of the 5’ end of Pkd2 that resulted from gene targeting. This fortuitous event resulted in an unstable allele that when combined with a Pkd2 null allele resulted in animals with renal and hepatic disease of variable severity that closely mimics human ADPKD. Despite its unique properties, this model has several features that limit its usefulness to explore the pathobiology of Pkd2: Somatic mutation is random and unregulated, the targeted allele with the local duplication can revert to either a normal or a mutant allele by postmitotic recombination, and one cannot establish with certainty when the somatic events have occurred. The last point is essential if one seeks to determine whether polycystin-2 is necessary for the maintenance of tubular morphology or whether its postdevelopmental loss is an important factor in disease progression because the precise time of gene inactiva-
tion cannot be defined. In this report, we describe a new line of mice with a floxed allele of Pkd1 (Pkd1cond/cond) that functions normally in the undeleted state and results in a null allele after cre-mediated deletion of the intervening sequence.

**Materials and Methods**

**Generation of Pkd1cond/cond Targeting Construct**

A probe that contained exons 2 to 4 of human PKD1 was used to screen a lambda bacteriophage library constructed using genomic DNA from the 129sv strain of mice. One clone was found to contain an insert of ~10 kb that included part of intron 1 through exon 15. Detailed genomic mapping and sequencing confirmed that it was colinear with the corresponding segment of mouse genomic DNA. Two adjacent EcoRI fragments (3 and 7 kb, respectively) were selected for the backbone of the Pkd1cond targeting construct. A neomycin cassette flanked by two FRT sites and a single loxP site at its 3' end (10) (gift of Dr. Gail R. Martin, University of California at San Francisco, San Francisco, CA) was inserted in a BglII site of intron 1 (Figure 1A) 1 kb from the 5' end of the targeting vector. Another loxP site was placed in an XbaI site in intron 4 in the same orientation. The correct orientation and integration of all fragments in the final targeting construct was confirmed by DNA sequence analysis. 129SV-J1 cells were electroporated and subjected to selection of neomycin.

**Mouse Lines**

The Pkd1floxed-null allele has been briefly described elsewhere (11). Briefly, in this line, the bacterial β-galactosidase gene was inserted in place of most of mouse Pkd1 exon 2 and all of exon 3 by gene targeting. This allele is a true null with transcriptional termination after the β-galactosidase gene but with β-galactosidase expression driven by the native Pkd1 promoter. Mice homozygous for the Pkd1floxed-null allele are embryonic lethal and have hemorrhages and massive edema with polyhydramnios as described previously for other Pkd1 mutants (5–7). The human β-actin FLPdeleter strain (B6;J-strain TgN[ACTFLPdeleter]925Dym) expresses a thermostable variant of the yeast FLP1 recombinase gene under the direction of the human ACTB promoter and was a gift of Dr. S.M. Dymecki (Harvard Medical School, Boston, MA) (12). The mouse mamillary tumor virus (MMTV)-Cre (B6;129BalSwTgN[MMTV-Cre]4Mam line) express P1 Cre recombinase under the control of the MMTV LTR promoter and was provided by Dr. K.U. Wagner (Eppliey Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE) (13,14). Meox-cre (B6;129S4-Meox2tm1[cre/Sor]) transgenic mice were obtained from Jackson Laboratory (15). In this line, the Cre recombinase had been “knocked-in” to the Meox2 gene (mesenchyme homeobox 2 gene) locus. Heterozygotes for Cre are phenotypically normal with Cre expression regulated by the native promoter of Meox2. All studies were performed using approved protocols, and animals were housed and cared for in pathogen-free facilities that are accredited by the American Association for the Accreditation of Laboratory Animal Care and meet federal (National Institutes of Health) guidelines for the humane and appropriate care of laboratory animals.

**Genotyping**

Genomic DNA was prepared from tail specimens using standard procedures. PCR genotyping was performed using the following primers (Figure 2A): F1, AAT AGG GGT GGG GCT TGT GGG TCG; R3: TGG CGA AAG GGG GAT GTG CTC C; R1, TAC TCA CAC CTC CAG TGC; F2, CTA TAG GAC AGG GAT GAC A; R2, CCC TTA CCA ACC CTC TTT T; Pkd1 wild-type (and Pkd1floxed-null) and Pkd1cond alleles were identified in 2% 3:1 NuSieve agarose gels of 220- and 250-bp bands, respectively. The Pkd1cond allele was detected as a 0.85-kb band versus a 1.9-kb nondeleted band in 1% agarose gels (Figure 2A; F3, CGA CCA CCA ACC GAA ACA ACA TC; R4, TCG TGT TCC TTT ACC AAC CTC). The Pkd1cond allele was detected by PCR amplification using the primer set shown in Figure 3A (F4, CCT GCC TTG CTC TAC TTT CC; R5, AGG GCT TTT CTT GCT GGT CT). Details regarding PCR conditions are available upon request. Genomic Southern blots of mouse-tail DNA (5 μg/sample) were prepared using standard techniques and probed with P32-labeled probes generated using gel-purified fragments of cloned DNA as template.
Reverse Transcription–PCR
Total RNA was extracted from fetal tissues using the Qiagen RNA extraction kit according to the manufacturer’s protocol. First-strand cDNA was synthesized using Superscript II (Invitrogen) from 100 ng of total RNA and then used as template for PCR amplification of \( \text{Pkd1} \) transcripts (Platinum Taq; Invitrogen). Primers F5 (CAG ACG CTA GGG CCG AGT), R6 (CCC TAT GTC CAG CGT CTG AAG TAG), and R7 (TCC AAA GTT CCA GCG TGT TGA) were used to amplify exon 1 to 2 and exon 1 to 5 products, respectively.

Histopathology and Immunohistochemistry
Kidney and liver specimens from adult animals were collected and immediately fixed in 10% buffered formalin at 4°C overnight. Embryos at day 14.5 post coitum were fixed with ice-cold 4% paraformaldehyde in PBS via cardiac perfusion of the mother, placed in 4% paraformaldehyde overnight at 4°C, and then transferred to 70% ethanol before embedding in low-melting paraffin. Sections (5 μm) were deparaffinized with xylene and rehydrated in a graded alcohol series. For immunohistochemistry studies, sections were microwaved for 10 min in citrate buffer solution (pH 6.0) to enhance antigen retrieval, preincubated with monoclonal blocker solution (Zymed Lab), and then incubated overnight at 4°C with 1:500 dilution of α-LRR, a monoclonal raised against recombinant human LRR domain. The samples were washed twice with PBST (5 min), and signal cDNA was synthesized using Superscript II (Invitrogen) from 100 ng of total RNA and then used as template for PCR amplification of \( \text{Pkd1} \) transcripts (Platinum Taq; Invitrogen). Primers F5 (CAG ACG CTA GGG CCG AGT), R6 (CCC TAT GTC CAG CGT CTG AAG TAG), and R7 (TCC AAA GTT CCA GCG TGT TGA) were used to amplify exon 1 to 2 and exon 1 to 5 products, respectively.

Figure 2. \( \text{Pkd1}_{del2–4} \) homozygotes develop cystic kidneys and pancreas. (A through D) Hematoxylin- and eosin-stained sections of two different E15.5 \( \text{Pkd1}_{del2–4} \) embryos (B and D) and wild-type littermates (A and C) showing cystic kidneys (B and D) and pancreas (D) of the mutant animals (A and B, bar = 50 μm; C and D, bar = 100 μm). (E) Schematic drawing showing the genomic map of the wild-type and \( \text{Pkd1}_{del2–4} \) alleles (middle) and their transcripts (top) and the position of primers used to amplify exons 1 to 5 or 1 to 2 (bottom). The colored box indicates the relative position of the neomycin gene (Figure 1A). (F) Genomic PCR of tissues from \( \text{Pkd1}_{del2–4} \) (lanes 2 and 5), \( \text{Pkd1}_{del2–4} \) (lanes 3 and 6), and \( \text{Pkd1}^{\text{homo}} \) (lane 4) embryos using primers F1-R1 (Figure 4A) to link intron 1 to exon 2. Lane 1 contains the 1-kb Plus marker lane (Invitrogen). \( \text{Pkd1}_{del2–4} \) mice lack exon 2 and thus have no product, although other genomic products can be amplified from the same sample with primers F3-R4 (lanes 5 and 6). (G) Reverse transcription–PCR linking exon 1 to more distal exons (exon 2, F5-R6, lanes 2 to 4; exon 5, F5-R7, lanes 5 to 8) in transcripts of \( \text{Pkd1}_{del2–4} \) (lanes 2 and 5), \( \text{Pkd1}_{del2–4} \) (lanes 3 and 6), and \( \text{Pkd1}^{\text{homo}} \) (lanes 4 and 7). \( \text{Pkd1}_{del2–4} \) transcripts lack exons 2 to 4. Lane 1 contains 1-kb Plus marker. The \( \text{Pkd1}_{del2–4} \), \( \text{Pkd1}_{del2–4} \), and \( \text{Pkd1}^{\text{homo}} \) samples are the same used for lanes 2 to 4 in F. Magnification: ×20 in A and B; ×10 in C and D.

Figure 3. Cre-mediated somatic loss of \( \text{Pkd1} \) results in liver cysts. (A) Schematic illustration of the \( \text{Pkd1}^{\text{cond}} \) and \( \text{Pkd1}_{del2–4} \) alleles. The positions of the primer pairs, probe, and restriction enzyme used for Southern blot experiments are as indicated. The predicted sizes for the various products are illustrated in the schematic gel drawing on the right. Primer pair F3-R4 is predicted to yield 1.9- and 0.85-kb fragments from the \( \text{Pkd1}^{\text{cond}} \) and \( \text{Pkd1}_{del2–4} \) alleles, respectively, whereas F3-R5 is predicted to yield a 0.9-kb product from both. (B) Ethidium bromide–stained agarose gel with PCR products amplified by the primer pairs shown in A using DNA isolated from paraffin-embedded liver sections of a mouse with the \( \text{Pkd1}^{\text{cond}} \) and \( \text{Pkd1}_{del2–4} \) (cond/cond, cre+) or \( \text{Pkd1}^{\text{cond}} \) (cond/cond) genotype as template. The products in the first three lanes for each sample were amplified using increasing amounts of DNA as template and primer pair F3-R4. The prominent product in the last lane of each sample was amplified using primer pair F3-R5 as a positive control to test for DNA quality (cond). A 0.85-kb product (del2–4) was present only in the cystic sample, whereas a 1.9-kb product derived from the undeleted \( \text{Pkd1}^{\text{cond}} \) allele was present in the noncystic sample (arrow). (C) Liver specimens used for the PCR reactions in B.
TABLE 1. Genotypes of progeny that result from $Pkd1^{cond}$ : $Meox2^{cre/w}$ × $Pkd1^βgal-null/w$ mating

<table>
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<th>Genotype</th>
<th>Observed</th>
<th>Expected</th>
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<tr>
<td>PKD1$^w/w$</td>
<td>28</td>
<td>19.5</td>
</tr>
<tr>
<td>PKD1$^{del2-4}/w$</td>
<td>27</td>
<td>19.5</td>
</tr>
<tr>
<td>PKD1$^βgal-null/w$</td>
<td>23</td>
<td>19.5</td>
</tr>
<tr>
<td>PKD1$^{cond-del2-4}$/w</td>
<td>0</td>
<td>19.5</td>
</tr>
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low levels of expression in multiple organs, including the kidney (13). This yielded offspring with genotypes of the expected Mendelian ratio. Mice heterozygous for both MMTV-cre and Pkd1cond were phenotypically normal in all respects. Brother–sister matings were arranged, and animals of the F2 generation were obtained with genotypes in expected Mendel-

Table 2. Genotypes of progeny from mating of Pkd1del2–4/w with Pkd1del2–4/w mice

<table>
<thead>
<tr>
<th>PKD1w/w</th>
<th>PKD1del2–4/w</th>
<th>PKD1del2–4/del2–4</th>
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<tbody>
<tr>
<td>Observed</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Expected</td>
<td>20</td>
<td>40</td>
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Figure 4. Pkd1del2–4 is a functional null allele of Pkd1. (A) Schematic drawing of four different alleles that can result from a cross of Pkd1gal-null/w:Meox2/w × Pkd1cond/w:Meox2cre/w. WT, wild type; βgal-null, Pkd1gal-null; cond, Pkd1cond; del2–4, Pkd1del2–4. The positions of the various primer pairs used to confirm the genotypes are as indicated. Black bands are products amplified only from wild-type sequence; blue and red boxes are the β-gal and neomycin genes, respectively. The gray box identifies the slightly longer 3' end for the neomycin gene used in the conditional “ko” construct. The open arrows represent the lox P sites. The long red line with hatches is too long to be amplified under the conditions used. The dashes indicate deleted segments. (B) Pkd1gal-null/del2–4 embryos have the null phenotype. #5 had polyhydramnios and was grossly edematous (arrow in the top picture with a line demonstrating the expected body boundary). #9 had bled into the yolk sac cavity (red haze), and #7 had a cerebral hemorrhage (arrow). (C) (Top) Schema of breeding strategy and possible allelic combinations that might result. (Bottom) Predicted PCR patterns for each allelic combination. (D) Results obtained for 11 offspring harvested at E14.5. The cre used in this experiment is highly active in the male germline; thus, most offspring have the Pkd1del2–4 allele. Three animals (red arrows) were compound heterozygotes for null alleles (Pkd1gal-null/del2–4). No embryos of the Pkd1cond/cond or Pkd1cond/gal-null genotype were present. (F) Autoradiograph of a Southern blot of tail DNA from offspring of a Pkd1gal-null/w:Meox2cre/w × Pkd1cond/w:Meox2/w cross-digested with EcoRI and probed with the fragment shown in Figure 7A. The DNA samples in lanes 1 and 2 were isolated from animals with the Pkd1 null phenotype. A very small amount of undeleted Pkd1cond allele is barely visible in lanes 1 and 2.
lian ratios. Mice with the $Pkd1^{cond/cond}:MMTV^{cre}$ genotype were healthy and reproduced normally.

We selected a set of five animals with the $Pkd1^{cond/cond}:MMTV^{cre}$ genotype and five littermate controls ($Pkd1^{cond/cond}$) for detailed histopathologic analysis at 10 wk of age. Given that ultrasound had previously been used to assess renal cystic disease in vivo, we performed a pilot study using a 15-MHz Sequoia linear array transducer on the two sets of animals (16). A single ~3-mm kidney cyst was seen in only one animal ($Pkd1^{cond/cond}:MMTV^{cre}$). We then killed the mice and performed post mortem examinations of the kidneys and livers. The cyst that was identified by ultrasound and several smaller cysts were visible on the surface of the organ (Figure 6A). Several small (~1 mm) renal cysts were also visible on the surface of kidneys from two additional mice of the same genotype. The two animals that lacked visible cysts on gross examination also did not have any microscopic cysts detected in a histopathologic survey of 100-μm slices. One of the animals with kidney cysts also had one visible liver cyst of ~4 mm (Figure 6B). None of the control animals, composed of genotypes $Pkd1^{cond/w}:MMTV^{cre}$, $Pkd1^{w/w}:MMTV^{cre}$, or $Pkd1^{cond/cond}$, had any visible or microscopic cysts (Table 3).

To investigate whether a change in phenotype is observed after a longer time period, we analyzed three additional pairs of mice with the $Pkd1^{cond/cond}:MMTV^{cre}$ genotype and littermate controls at the age of 20 wk. The kidneys of the first two mice with the $Pkd1^{cond/cond}:MMTV^{cre}$ genotype were found on post mortem examination to have bilateral cyst formation with a few cysts in each kidney (Figure 6C). Remarkably, their livers had a marked increase in total cyst number, with >15 cysts in one mouse and >40 in the other (Figure 6D). Most of the cysts were ≥2 mm and of a relatively uniform size. In contrast, none of the kidneys or livers of control animals, composed of genotypes $Pkd1^{cond/w}:MMTV^{cre}$, $Pkd1^{w/w}:MMTV^{cre}$, or $Pkd1^{cond/cond}$, had any cysts (Table 3).

The last of the pairs was also examined by ultrasound imaging with a recently acquired higher resolution apparatus (40 MHz) before tissue harvesting. Ultrasound detected a cyst of ~0.5 mm on the right kidney and several hepatic cysts, the largest of which was ~3 mm, in the mouse with the $Pkd1^{cond/cond}:MMTV^{cre}$ genotype and none in the control (Figure 6, E and G). The initial ultrasound findings were confirmed by the post mortem examination. The liver cysts were immediately visible, and their position and size matched the ultrasound findings. Although the right kidney did not have any visible cysts, the single cyst identified by ultrasound was confirmed by histopathologic analysis of the sectioned tissue (Figure 6, E through H).
Table 3. Overview of liver and kidney phenotypes at 10 and 20 weeks of age in \( Pkd1^{\text{cond/cond}; \text{MMTV-cre/w}} \) and control mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Kidney</th>
<th>Liver</th>
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<tbody>
<tr>
<td></td>
<td>10 Weeks (( n ) [cysts(^{-1})])</td>
<td>20 Weeks (( n ) [cysts(^{-1})])</td>
</tr>
<tr>
<td>( Pkd1^{\text{cond/cond}} ) MMTV-cre/w</td>
<td>60% (3/5)</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>All other</td>
<td>0% (0/10)</td>
<td>0% (0/10)</td>
</tr>
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One possible explanation for the higher rate of cyst formation associated with the \( Pkd1^{\text{cond}} \) allele is that the latter is associated with a higher rate of cyst development by mechanisms other than cre-recombinase–mediated deletion. We tested for this possibility by generating compound heterozygous \( Pkd1^{\text{cond}/\text{gal-null}} \) mice and examining tissues of 6-mo-old animals. None of the organs of eight mice, ranging from 10 to 33 wk of age, had signs of cyst development in either kidney or liver. These results are consistent with what we had previously observed in analyses of \( >100 \) heterozygous \( Pkd1^{\beta\text{gal-null}} \) animals. In this latter set, renal and hepatic cysts were rarely observed in animals 6 mo or younger (unpublished observation).

The clear association between the formation of cysts and the \( Pkd1^{\text{cond/cond}; \text{MMTV-cre}} \) genotype suggested that cre-mediated somatic deletion of \( Pkd1 \) was likely the underlying mechanism. To test this hypothesis, we examined cystic and noncystic tissues for the presence of the \( Pkd1^{\text{del2–4}} \) allele. We prepared DNA from sections of cystic liver and from comparable sections of normal liver from control animals and used this as the template for PCR amplification. Two sets of primers were designed for this study (Figure 3A). The first pair flank the floxed segment and produce a 1.9-kb fragment from control specimens and an \( \sim0.85\)-kb fragment after cre-mediated deletion of exons 2 to 4. Given that we were using paraffin-embedded specimens on glass slides as source material for DNA isolation, we were concerned that the quality of DNA might be suboptimal for reliable amplification of the 1.9-kb fragment. Therefore, we used a second primer (F3-R5; Figure 3A) that was positioned nearby and could amplify a fragment of slightly larger size (0.9 kb) than the deleted fragment from undeleted alleles that could be used as a control for PCR. As shown in Figure 3B, primer pair F3-R4 amplified a 0.85-kb deleted product solely from the cystic specimens, whereas adding primer R5 amplified the 0.9-kb fragment from both cystic and control samples. In contrast, primer pair F3-R4 weakly amplified the 1.9-kb undeleted product from control samples. The lack of 1.9-kb product in the cystic sample likely is the result of preferential amplification of the smaller product.

**FRT-Flanked Neomycin Gene of \( Pkd1^{\text{cond}} \) Can Be Removed In Vivo**

Most gene-targeting constructs include one or more marker genes that allow selection for the relatively small number of cells that are successfully transfected. In standard “gene knock-out” strategies, retention of the selectable marker in the targeted locus after recombination often introduces few problems and may even help ensure successful inactivation of the gene of interest. The risks of untoward effects resulting from a retained marker are far higher when one seeks to develop an allele that can be conditionally inactivated. Even when strategically placed in large introns far from sequences thought essential for proper splicing, the consequences can be unpredictable. Therefore, it is common to flank the selectable marker with additional DNA recombination signals that can be used to delete the marker once it is no longer required. Meyers et al. (10) described a novel way of using both FRT and \( \text{loxp} \) sites that would allow selective deletion of either the selectable marker or one’s targeted gene segment. An advantage of this approach is that it allows for the possible generation of three different alleles (Figure 7A). In situations in which retention of the selectable marker results in a hypomorphic allele, one can potentially use one targeting event to produce a normal, hypomorphic, and completely null allele. Therefore, we had incorporated into the design of our targeting construct FRT sites that flanked the neomycin gene (Figure 1). We had crossed the original \( Pkd1^{\text{cond}} \) chimeric male mice to \( \text{FLPe} \) recombinase female mice to produce \( Pkd1^{\text{cond}\text{-ne}}:\text{FLPe}^{+} \) offspring. Southern blot and PCR-based analysis confirmed that the neomycin gene was successfully deleted only in mice that were positive for the \( \text{FLPe} \) transgene, producing a new \( Pkd1 \) allele (\( Pkd1^{\text{cond}\Delta\text{neo}} \), Figure 7, B and C). However, deletion of the neomycin gene seemed to have no effect on \( Pkd1 \) activity in that the phenotypes of homozygotes for the \( Pkd1^{\text{cond}} \) and \( Pkd1^{\text{cond}\Delta\text{neo}} \) alleles were identical.

**Discussion**

In this report, we describe the first line of mice with a floxed allele of a PKD gene (\( Pkd1^{\text{cond}} \)) that functions as a wild-type allele in the undeleted state and as a complete null after cre-mediated deletion. We have shown that mice homozygous for the \( Pkd1^{\text{cond}} \) allele are viable, fertile, and born at the expected Mendelian ratio. In contrast, all previously described lines of animals with targeted mutations of \( Pkd1 \), including our \( Pkd1^{\beta\text{gal-null}} \), were either embryonic lethal or had perinatal demise. By breeding \( Pkd1^{\text{cond}\text{-ne}} \) mice that also were positive for a cre-recombinase to \( Pkd1^{\beta\text{gal-null}} \) heterozygotes, we could evaluate quickly the functional impact of cre-mediated loss of \( Pkd1 \) exons 2 to 4. As expected, embryos with the \( Pkd1^{\text{del2–4}\beta\text{gal-null}} \) genotype were present in normal Mendelian ratios and recapitulated the range of phenotypes (edema, polyhydramnios, and hemorrhage) associated with homozygous loss of \( Pkd1 \). These results show conclusively that the...
floxed allele functions as a null allele after cre-mediated deletion. Similar results were obtained using mice from different strains, excluding strain differences as a trivial explanation for our findings. In the studies described, all female mice were BlackSwiss/129SVeV F1 hybrids. The male mice were either C57/Bl6/129SVeV (Pkd1cond) or BlackSwiss/129SVeV (Pkd1/H9252 gal-null).

An important limitation of the currently available lines of mice with targeted mutations of Pkd1 is that heterozygous animals rarely have cystic disease below 6 mo of age. For example, <10% of our Pkd1βgal-null heterozygotes (n > 100) had macroscopic renal cysts at 3 mo of age, and none had liver cysts at <6 mo of age. It is interesting that mice of this genotype universally develop cystic livers if aged 18 mo or more (unpublished observations). These results are in striking contrast with what is observed in humans, in whom renal cysts are a universal and early finding and liver cysts are acquired later and generally are less abundant. These are important differences that if explained might provide insights into the mechanism of disease. Likewise, it is important that one understand the pathophysiology of disease in the mice models if one seeks to use them to test various therapeutic interventions.

We propose several possible explanations for these species differences. One possibility is that the somatic mutation rate of PKD1 in human renal and biliary epithelial cells is significantly higher and lower, respectively, than in the corresponding cell types of mice. An alternative explanation is that species differences in the rate of cyst growth in the two organs account for the observations. In this schema, the rate of somatic mutation may not differ, but instead the rates of proliferation or fluid secretion may significantly affect outcome. Finally, it is possible that the molecular mechanisms that govern cyst formation may differ subtly between organisms. The prevailing view regarding the initiation step of cyst formation is that this process begins when some threshold level of PC1/PC2 receptor/channel activity is breached. This can occur as a result of a decrease in the level of activity of the receptor/channel complex and/or an increase in the threshold level required to establish and maintain normal tubule morphology. In heterozy-
gotes, this may result when acquired mutations disrupt the activity of the previously normal allele. It is also possible, however, that the activity of the PC1/PC2 complex normally decreases with age and that in some cell types of heterozygotes it falls below the minimal level. Either the relative level of activity of the complex or the threshold level required to suppress cyst formation in various organs may differ between the species.

The floxed allele of Pkd1 described in this report can be used to distinguish between these various possibilities. Our studies show that somatic expression of cre recombinase in Pkd1cond/cond mice can result in a null allele and cyst formation. Using this line of animals, one can now examine the consequences of temporally and spatially regulated inactivation of Pkd1. By fixing the time and location of gene inactivation, one can examine what other factors affect the relative rate of cyst growth in the kidney and liver. Similarly, by removing the unpredictability of the timing of somatic mutation, this model offers a uniquely powerful way of testing various interventions on the rate of cyst growth. Our pilot study suggests that high-resolution ultrasound imaging may be a useful, noninvasive method for screening for cysts.

This new line of animals also will be invaluable in the study of numerous other aspects of Pkd1 biology. By controlling the timing of Pkd1 inactivation, one can show in a definitive way that Pkd1 is, in fact, necessary for both formation and maintenance of tubules. Likewise, one can use this model to determine the cause of fetal demise. Using this information, one can selectively inactivate the gene in a manner that bypasses the embryonic lethality and then assess the role of PC1 in the development of various other tissues that are altered in Pkd1 nulls. Finally, one can use this novel model to screen for as-of-yet unidentified postdevelopmental functions of Pkd1 that are not manifest in humans because of the two-hit nature of disease and not observed in mice because of the early lethality associated with its homozygous loss.

Acknowledgments
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