Adult Inactivation of the Recessive Polycystic Kidney Disease Gene Causes Polycystic Liver Disease

Whitney Besse,1 Charlotte Roosendaal,1 Luigi Tuccillo,1 Sounak Ghosh Roy,1 Anna-Rachel Gallagher,1 and Stefan Somlo1,2

Abstract

Background A major difference between autosomal recessive polycystic kidney disease (ARPKD) and autosomal dominant polycystic kidney disease (ADPKD) lies in the pattern of inheritance, and the resultant timing and focality of cyst formation. In both diseases, cysts form in the kidney and liver as a consequence of the cellular recessive genotype of the respective disease gene, but this occurs by germline inheritance in ARPKD and somatic second hit mutations to the one normal allele in ADPKD. The fibrocystic liver phenotype in ARPKD is attributed to abnormal ductal plate formation because of the absence of PKHD1 expression during embryogenesis and organ development. The finding of polycystic liver disease in a subset of adult PKD1 heterozygous carriers raises the question of whether somatic second hit mutations in PKHD1 in adults may also result in bile duct-derived cyst formation.

Methods We used an adult-inducible Pkd1 mouse model to examine whether Pkd1 has a functional role in maintaining bile duct homeostasis after normal liver development.

Results Inactivation of Pkd1 beginning at 4 weeks of age resulted in a polycystic liver phenotype with minimal fibrosis at 17 weeks. Increased biliary epithelium, which lines these liver cysts, was most pronounced in female mice. We assessed genetic interaction of this phenotype with either reduced or increased copies of Pkd1, and found no significant effects on the Pkd1 phenotype in the liver or kidney from altered Pkd1 expression.

Conclusions Somatic adult inactivation of Pkd1 results in a polycystic liver phenotype. Pkd1 is a required gene in adulthood for biliary structural homeostasis independent of Pkd1. This suggests that PKHD1 heterozygous carrier patients can develop liver cysts after somatic mutations in their normal copy of PKHD1.

Introduction

PKHD1 is the disease gene for autosomal recessive polycystic kidney disease (ARPKD). ARPKD is a pediatric diagnosis with incidence 1:2,000 live births, characterized by diffusely cystic kidneys progressing to renal failure and congenital hepatic fibrosis (CHF) (1). CHF is a fully penetrant disease characterized by liver fibrosis and portal hypertension, whose consequences require liver transplant in 1%–7% of survivors of early childhood (2,3). CHF pathogenesis is attributed to malformation of the ductal plate during liver development, and ARPKD-related CHF can be distinguished from other forms of liver fibrosis by the occurrence of biliary-derived cysts. Patients with nonsense mutations tend to have a milder kidney phenotype compared with those with truncating mutations and in some cases are not diagnosed until liver abnormalities are identified in adulthood (4,5). Mouse models for ARPKD recapitulate the human liver phenotype with fibrosis and cysts (6–10). Pancreas phenotypes are variable. ARPKD mice lack a kidney phenotype at least until late in life when mild tubular dilations have been reported (7,8). The function of fibrocytast is not well understood, and this hinders attempts to identify ARPKD treatments.

The parents of ARPKD children are healthy, and there is no kidney or liver phenotype in heterozygous rodent models (6,9). Monoallelic loss of PKHD1 has been thought to be inconsequential. Nonetheless, 10% of asymptomatic carrier adults were found to have multiple cysts in their livers or kidneys, and a gene discovery analysis of individuals ascertained on the basis of isolated polycystic liver disease (PCLD) identified PKHD1 heterozygous carrier genotype as the causative gene mutation (11,12). Genetic studies and modeling of several other gene causes of PCLD have shown that these occur by somatic second hit mutations akin to the mechanisms underlying autosomal dominant polycystic kidney disease (ADPKD) (12–14). These findings allow for two possible genetic models for cyst pathogenesis in PKHD1 heterozygous mutation carriers. Bile duct cysts may form either because of haploinsufficiency or because of a focal cellular recessive genotype resulting from somatic loss of the single
normal allele. This second possibility can only be true if the PKHD1 gene has an ongoing homeostatic role after liver development. We used an adult-inducible mouse knockout model of Pkd1 to determine whether postdevelopmental inactivation of Pkd1 results in a PCLD phenotype. We found that somatic inactivation of Pkd1 is sufficient to cause bile duct cysts in adult livers.

Materials and Methods

Mouse Lines

All experiments were conducted in accordance with Yale University Institutional Animal Care and Use Committee guidelines and procedures. The Pkd1lox3-4 (Pkd1tm1Ggg [7]) allele was a generous gift from Dr. Gregory G. Germino (National Institute of Diabetes and Digestive and Kidney Diseases/National Institutes of Health). The Pkd1lox, Pkd1F/H-BAC, tamoxifen-inducible UBC-CreER, and doxycycline-inducible Pax8rtTA:TetO-Cre models have been previously described (13,15,16). The Pkd1F/H-BAC contains three copies of a bacterial artificial chromosome modified to express dual-epitope-tagged Pkd1. All mice are predominantly C57BL/6. Littermates were used as controls.

Pharmacological Induction and Sacrifice

Gene inactivation using both the UBC-CreER and Pax8rtTA:TetO-Cre models began at postnatal day 28. Tamoxifen 20 mg/ml in sunflower seed oil was administered as drinking water for 2 weeks. Doxycycline (2 mg/ml in a 5% sucrose solution) was provided in the drinking water for 2 weeks. UBC-CreER mice were sacrificed at age 17 weeks, and Pax8rtTA:TetO-Cre mice were sacrificed at age 26 weeks (6 months). Organs were perfused with 1× PBS then 4% paraformaldehyde, and stored in 4% paraformaldehyde before sectioning for histology.

Histology and Immunohistochemistry

Paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E), Masson Trichrome, Sirius Red, or left unstained. To perform anti-cytokeratin 19 (CK19) immunohistochemistry, unstained slides were treated with xylene and sequential ethanol dilutions to remove paraffin and rehydrate the tissue. Endogenous peroxidase was blocked with 10% hydrogen peroxide in methanol. After water wash, antigen retrieval was achieved with steaming in 1 mM EDTA pH 8 followed by 0.05% Tween 20 in PBS. Samples were then blocked with 1% BSA for 30 minutes before primary antibody application overnight at 4°C. Washes were completed with 0.05% Tween 20. Primary antibody: anti-CK19;TROMA-III (1:100; Developmental Studies Hybridoma Bank, Iowa City, IA); secondary antibody: anti-rat horseradish peroxidase (1:500; Jackson ImmunoResearch, West Grove, PA). Horseradish peroxidase substrate was provided by the 3′-diaminobenzidine peroxidase substrate Kit (Vector Laboratories, Burlingame, CA).

Cystic Parameters

Slides stained with H&E or immunohistochemistry were scanned on a Nikon Eclipse TE2000 microscope using MetaMorph software (Molecular Devices, San Jose, CA) to black and white TIFF files, respectively. For both hepatic cystic index (HCl) and the percentage of the two-dimensional liver area that stained positive for CK19 (CK19+%) calculations, a denominator was set with the manually outlined perimeter of the liver excluding large internal venous structures. Autothresholding within this space was performed by MetaMorph to determine white (cytic) from dark (noncytic) for HCl. HCl is defined as the percentage of cystic/total parenchymal area. CK19+% is defined as the percentage of CK19+ staining over the total parenchymal area.

Plots and Statistics

Two-way comparisons of sample means were calculated using the unpaired two-tailed t test, a parametric test using Prism version 7 (GraphPad Software, Inc., San Diego, CA). Fisher’s exact test was used to make pairwise comparisons of proportions. Sample size calculation was performed using online calculators (http://powerandsamplesize.com) for the Pkd1 dosage experiments to suggest a target of 15 mice of each genotype to have 80% power to detect a difference with 5% risk for type 1 error between a HCl of 12 and 20, with estimated SD of 7.8 from the initial experiment.

Results

Postdevelopmental Inactivation of Pkd1 Results in Liver Cysts

Mouse liver achieves the mature portal triad morphology of bile ducts, hepatocytes, and vascular structures and an adult liver weight/body weight (LW/BW) ratio in the first 2–4 weeks of postnatal life (17,18). We inactivated the Pkd1 gene at age 4 weeks using a tamoxifen-inducible, ubiquitously acting UBC-CreER that expresses in bile ducts and has previously been used to model PCLD with conditional Pkd1 alleles (16). Pkd1lox;UBC-CreER mice sacrificed at age 17 weeks showed bile duct cysts throughout the liver (Figure 1). This cystic phenotype was fully penetrant and, in most cases, grossly visible as a stippled texture of the organ upon perfusion (Supplemental Figure 1). Normal ductal plate formation is reported in mice with the germline heterozygous Pkd1 genotype (6) and, indeed, tamoxifen-induced littermate mice with a monoallelic Pkd1lox;UBC-CreER genotype had normal-appearing livers, including portal triads consisting of artery, vein, and bile duct, without cysts (Figure 1, A and B). Histologic analysis of livers from Pkd1lox;UBC-CreER mice showed clusters of cysts among normal-appearing hepatocytes (Figure 1, A and B). Pkd1lox;UBC-CreER mice (n=11) have an increased LW/BW ratio of 5.7±0.3%, which compared with that of Pkd1lox+/−;UBC-CreER mice (n=10), 4.4±0.2%, P=0.0016 (Figure 1C), without any effect of genotype on body weight (Supplemental Figure 2A). The female subgroup of Pkd1lox;UBC-CreER mice (n=4) had the most significant difference from the noncytic Pkd1lox+/−;UBC-CreER genotype female mice (n=3), P<0.001 (Figure 1C, right panel).

We further characterized the cystic phenotype by two complementary measures. To quantify the area of the cysts as an HCl, we applied MetaMorph software thresholding to scanned H&E-stained liver section images (Figure 1A) to measure the two-dimensional cystic area as a percentage of the total area of the liver section defined within a perimeter that excluded large vascular structures. Mean HCl was 15.2±2.9 in Pkd1lox;UBC-CreER mice (n=7) versus 3.14±0.4 in
Figure 1. | Biallelic loss of Pkhdl with UBC-CreERT results in diffuse liver cysts. (A) Representative liver sections of the indicated genotype at age 17 weeks. The colored boxes denote genotypes throughout the figure. (B) Hematoxylin and eosin-stained histology of Pkhdlfl/fl;UBC-CreERT versus control monoallelic deletion (Pkhdlfl+/UBC-CreERT) at age 17 weeks. Representative structures marked as: v, vein; c, cyst. (C) Liver weight/body weight ratio of Pkhdlfl/fl;UBC-CreERT versus Pkhdlfl+/UBC-CreERT mice. Right panel, sex subgroups visually separated. Blue shapes, male; pink shapes, female. (D) Hepatic cystic index, the percentage of liver section area that is background density (cysts + venous structures) rather than parenchyma on liver lobe sections scanned in greyscale as in (A). (E) Anti-cytokeratin 19 (CK19) immunohistochemistry localizes to the biliary epithelium, which lines all cysts, and not venous structures. (F) Percentage of liver section area positive with CK19 staining. Bottom panel, sex subgroups of Pkhdlfl/fl;UBC-CreERT mice are visually separated. (G) Sirius Red stain at ×10 magnification.
**Phkdl^fl/fl;UBC-Cre^ER** mice (n=7), P=0.0015 (Figure 1D). Immunohistochemical staining of CK19, a marker of biliary epithelium, showed that the liver cysts were uniformly lined with a CK19-positive epithelium, indicating that they were derived from the bile ducts (Figure 1E). In Phkdl^fl/fl;UBC-Cre^ER mice, the CK19 staining highlighted the normal bile ducts, which represent a tiny minority of the cells in the liver (Figure 1E). Additional cells around the central veins were noted to be CK19-positive as has been defined in wild-type rodent liver CK19 immunostaining (19). To quantify the increased burden of biliary epithelium in our Phkdl^fl/fl;UBC-Cre^ER mice, we again applied tissue perimeter outlining and color thresholding, this time to tissue sections stained with CK19. We calculated the CK19+ %, Mean CK19+ % was 5.8 ± 1 in Phkdl^fl/fl;UBC-Cre^ER mice (n=7) versus 0.5 ± 0.1 in Phkdl^fl/fl;UBC-Cre^ER mice (n=7), P=0.0003 (Figure 1F).

Both HCl and CK19+ % calculations were done by blinded to sample genotype on the first seven mice sacrificed for each genotype as only those had histologic sections. Nonzero values in the nonsyntotic Phkdl^fl/fl;UBC-Cre^ER mice were expected because of the inability of the semiautomatic method to distinguish venous versus cystic structures on two-dimensional histology, and the expected presence of CK19 staining of native bile ducts. HCl and CK19+ % were far superior to LW/BW at numerically quantifying the cystic phenotype distinguishing Phkdl^fl/fl;UBC-Cre^ER and Phkdl^fl/fl;UBC-Cre^ER mice. For both HCl and CK19+ %, the comparison between genotypes maintained statistical significance (P<0.05) even in the sex subgroups with small numbers. CK19+ % was the one parameter by which the trend toward increased severity of liver cysts in cystic females versus cystic males met statistical significance (P=0.016) despite the small numbers once split by sex (Figure 1F). There was a robust positive correlation (r^2=0.47), between the CK19+ area and the HCl values for each mouse showing that these measures were congruent with each other (Supplemental Figure 2B).

As the lesion in recessive polycystic kidney disease is CHF, we also sought to assess whether there was any significant fibrosis in our model. Phkd1 null mice develop a significant burden of fibrosis as defined by Sirius Red staining between 3 and 9 months of age (6,20). We stained paraffin sections with Sirius Red stain and Masson’s trichrome stain (data not shown) to localize collagen. Sirius Red stained sections show positive staining outlining liver margins, central veins, and cysts (Figure 1G). There was no additional collagen staining beyond these structures on Sirius Red or Masson’s trichrome-stained sections, suggesting an absence of significant fibrosis in this model.

**Analysis of Postdevelopmental Phkd1 Loss in the Kidney**

Germline Phkd1 null mouse kidneys have no phenotype for at least 6 months of life, or not at all (6,8,9). In keeping with this, the kidneys of the Phkd1^fl/fl;UBC-Cre^ER mice at 17 weeks of age showed no gross, histologic, or kidney weight/body weight ratio abnormalities. Phkd1^fl/fl mouse kidneys are noncystic other than a potential small number of discrete cysts if aged over 9 months (21). The combined Phkd1^fl/fl;Phkd1^fl/fl genotype has diffusely cystic kidneys (7,13). In order to evaluate whether Phkd1 plays a role after development in the kidney, we made use of this established genetic interaction between Phkd1 and Phkd1. To make the conditional equivalent, we used Phkd1^fl/fl;Phkd1^fl/fl;Pax8^TET/TetO-Cre and Phkd1^fl/fl;Phkd1^fl/fl;Pax8^TET/TetO-Cre littermates with doxycycline for a 2-week duration from 4 to 6 weeks of age. We sacrificed the mice at age 6 months. Histologic analysis of the perfusion-fixed kidneys showed no apparent abnormalities in the renal tubules, interstitium, or glomeruli (Figure 2, A and B). There was no difference in the kidney weight/body weight ratio (Figure 2C). This ratio was higher in male mice (n=9) than female mice (n=9), P=0.0039, regardless of genotype. Scanned kidney sections from this experiment are shown in Supplemental Figure 3.

**Phkd1^fl/fl;UBC-Cre^ER Model Liver Cyst Phenotype Is Independent of Pkd1 Dosage**

ARPKD mouse liver is more severely affected when Pkd1 gene dosage is reduced (13). Specifically, the liver phenotype in Phkd1^fl/fl;Pkd1^fl/fl;Pkd1^fl/fl is more severe than in Phkd1^fl/fl, even though the Pkd1^fl/fl genotype itself has no liver phenotype. Increased Pkd1 dosage in the form of a three-copy Pkd1^fl/fl;BAC rescued conditional mouse models of PCLD disease genes PRKCSH and SEC63, but had no effect on germline ARPKD mouse models (Phkd1^fl/fl;Pkd1^fl/fl;Pkd1^fl/fl;BAC) (13). However, given that the Phkd1^fl/fl;BAC mouse develops abnormally, this does not exclude the possibility that the postdevelopmental loss of Phkd1 modeled in this study could be rescued by increased Pkd1 dosage. To assess the effect of Pkd1 dosage on our postdevelopmental Phkd1 model, we generated Phkd1^fl/fl;Pkd1^fl/fl;UBC-Cre^ER and Phkd1^fl/fl;Pkd1^fl/fl;UBC-Cre^ER mice to compare their littermates with the cystic Phkd1^fl/fl;UBC-Cre^ER genotype. We induced these mice with tamoxifen injections at age 4 weeks, identical to our prior experiments, and followed them until 17 weeks of age. There was no appreciable difference in the cystic liver phenotype between these genotypes as assessed grossly by LW/BW or by HCl (Figure 3, A–C, Supplemental Figures 4 and 5). The body weight was also unchanged between these three genotypes (Figure 3D).

**Bile Duct and Extrapancreatic Findings**

We performed gross and histologic analysis of the spleen and pancreas, in addition to the liver and kidney, in a subset of Phkd1^fl/fl;UBC-Cre^ER, Phkd1^fl/fl;UBC-Cre^ER, Phkd1^fl/fl;Phkd1^fl/fl;UBC-Cre^ER, and Phkd1^fl/fl;Pkd1^fl/fl;UBC-Cre^ER mice. Germline Phkd1 null mouse models have an age-dependent penetrance of common bile duct dilation, cholangitis, pancreatic cysts, and splenomegaly in the setting of portal hypertension (7–9). In our postdevelopmental Phkd1^fl/fl;UBC-Cre^ER model, we did not find common bile duct dilation or splenomegaly. Spleen histology appeared normal (Figure 3E). Considering liver histology on 67 Phkd1^fl/fl;UBC-Cre^ER mice with or without concurrent alterations in Pkd1 dosage, we noted findings consistent with varying degrees of cholangitis in four mice. This ranged from mild focal pericystic and intracystic infiltrates including PMNs (n=3) to more diffuse histologic findings.
and grossly visible patchy erythema on the liver surface (n=1, Figure 3F). The four mice in which this was noted each had biallelic Pkh1 loss, but included at least one mouse of each sex and Pkd1 dosage tested.

Histology of the pancreas in Pkh1fl/fl;UBC-CreER mice demonstrated microscopic ductal dilations containing eosinophilic material, albeit with incomplete penetrance. The gross appearance of the pancreas was unremarkable. Figure 3G shows representative histologic images of mouse pancreatic tissue with or without pancreatic ductal dilations. This phenotype was either notable throughout the sampled tissue or it was absent. This pancreatic ductal dilation was seen in 17 of 27 (63%) of Pkh1fl/fl;UBC-CreER mice, but was never seen in Pkh1fl/+;UBC-CreER mice (n=7), P=0.007 (Figure 3H). The pancreatic ductal dilations were seen in a significantly higher percentage of the mice with reduced Pkd1 compared with those who had extra copies of Pkd1: 12 of 13 (92%) Pkh1fl/fl;Pkd1fl/+;UBC-CreER mice versus 7 of 15 (47%) Pkh1fl/fl;Pkd1fl/fl;UBC-CreER mice, P=0.016.

**Discussion**

This study is the first to evaluate whether Pkh1, and thus its protein product fibrocystin, continue to play a critical role in biliary homeostasis in the developed liver. We show that grown mice that lose Pkh1 after normal liver development have a dramatic and fully penetrant cystic liver phenotype. Whereas ductal plate malformation is known to be involved in the pathogenesis of CHF, we now show that it is not required for the Pkh1-related liver cysts themselves. This finding could serve to distinguish the pathogenesis of cysts that are seen in variable degrees in patients with ARPKD from the fibrotic phenotype of CHF. CHF accompanied by cysts is a distinguishing feature of ARPKD unique from other genetic causes of such hepatic fibrosis. Further, this
Figure 3. Liver phenotype does not change with alterations of Pkd1 in Pkhd1fl/fl;UBC-CreER mice. (A) Liver section scan and hematoxylin and eosin-stained histology for the indicated genotypes. Representative structures marked as: *, vein; c, cyst. Colors indicated for each genotype are used throughout the figure. (B) Liver weight/body weight ratio. (C) Hepatic cystic index as defined in Figure 1. (D) Body weight. (E) Hematoxylin and eosin-stained histology of spleen at ×2 magnification. (F) Patchy appearance of liver in one Pkhd1fl/fl;Pkd1fl/;UBC-CreER mouse affected by cholangitis. Middle and right panels, hematoxylin and eosin-stained histology shows cystic biliary structures full of polymononuclear lymphocytes (arrows). (G) Pancreatic ductal dilations were seen in a subset of mice with Pkhd1fl/fl;UBC-CreER with or without alterations in Pkd1 genotype. Hematoxylin and eosin-stained histology of pancreas at ×10 magnification representative of presence or absence of ductal dilations. d, ductal dilation. (H) Quantification of number of mice of each genotype with pancreatic ductal dilations present or absent.
finding demonstrates that the development of the recessive
genotype in adulthood, as would be the case in a heterozy-
gous individual who has a somatic mutation to the normal allele, likely results in cysts as is known to occur in PCLD. One limitation to conditional mouse models of dominantly inherited polycystic kidney disease and PCLD is that all biliary epithelial cells lose the disease gene simultaneously, in contrast to human organs in which somatic mutations occur in individual cells. Given this, we cannot yet definitively conclude that a single cell affected with PKHD1 loss is sufficient to initiate a cyst as is known to be the case in patients with PKD1 and PKD2. Nonetheless, a recent study followed Pkhd1 heterozygous mice by magnetic resonance imaging and although they showed no abnormalities at 10 months of age, by 1.5 years when somatic mutations could have occurred with time for subsequent cyst growth, the mice had cysts analogous to those found in human heterozygotes (22). This finding in mice, together with the focal nature of some cysts seen in PKHD1 mutation carriers, supports the idea that recessive loss in individual cells is sufficient to form cysts.

Our discovery of PKHD1 as an autosomal dominant PCLD disease gene prompted the investigations in this study. Unlike all other established PCLD disease genes, Pkhd1 does not cause PCLD by affecting polycystin-1 (PC1) maturation (12,23). Biallelic loss of Pkrsksh, Sec63, Alg8, Gana1, Sec61b, DnaJb11, or Alg8 results in significant reduction of the expression of mature PC1, whereas biallelic loss of Pkhd1 does not (12,13,24–26). Instead, we asked the question of whether Pkhd1 loss affected the PC1 functional dosage without affecting PC1 protein levels of trafficking. We found that in contrast to mouse models of Pkrsksh and Sec63, in which the presence of extra genomic copies of Pkd1 in the form of the Pkd1fl/fl;BAC rescues the liver cystic phenotype, the extra copies of PC1 had no effect on bile duct cysts because of loss of Pkhd1. Similarly, whereas biallelic Pkd1 dosage worsens PCLD because of Pkrsksh and Sec63, it has no effect on Pkhd1 liver cysts resulting from adult inactivation. This shows that the Pkhd1-related liver cystic phenotype is independent of PC1 dosage and thus likely occurs via a non-PC1-dependent mechanism.

Adult-inducible inactivation of Pkhd1 does not recapitulate the genetic interaction between Pkd1 and Pkd1 that occurs with germline Pkd1+/−;Pkd1+/− mice. The loss of one Pkd1 allele was not able to bring out a kidney phenotype in our Pkd1fl/fl;Pkd1fl+/−;Pax8fl+/−;TetO-Cre system, and the cystic liver phenotype was no worse in Pkd1fl/fl;Pkd1fl+/−;UBC-CreE3×E3× mice than those with two functional copies of Pkd1. This suggests that the previously described genetic interaction between the Pkd1fl/fl and Pkd1+/− genotypes in both the kidney and liver is not involved in adult tissue homeostasis, but rather requires abnormal organogenesis as likely occurs with the germline null Pkd1 alleles. Both patients with ADPKD and mice, and ARPKD mice, develop pancreatic cysts with incomplete penetrance (7–9,27,28). Pancreatic ductal dilation in our postdevelopmental Pkd1 model occurred with significantly higher frequency in mice with Pkd1fl/fl;Pkd1fl+/−;UBC-Cre versus Pkd1fl/fl/Pkd1fl+/−;BAC UBC-CreE3× mice, even though there was no appreciable difference in severity. This was the only parameter in the Pkd1fl/fl;UBC-CreE3× mice that differed with PC1 dosage in adult inactivation of Pkhd1.

We describe a novel mouse model of Pkhd1/fibrocystin inactivation after liver development. We learn that Pkhd1 plays a crucial ongoing role in biliary homeostasis. This may suggest that the pathogenesis of ARPKD liver cysts is not intertwined with that of CHF. Further, our findings support the possibility that the polycystic liver phenotype in PKHD1 heterozygous carriers could occur through somatic mutation, and the lack of Pkhd1/Pkd1 genetic interaction in this model lends support to a hypothesis that liver cyst formation in ARPKD and ADPKD occur through different mechanistic pathways. Such investigations are relevant to understanding whether future targeted treatments for ADPKD should be expected to be relevant to ARPKD. The robust liver cystic phenotype in our Pkd1fl/fl;UBC-CreE3×E3× mice makes this a valuable model for future studies to compare the functional pathways of Pkhd1 and Pkd1 and their treatment.

Disclosures
S. Somlo is a founder, shareholder, and consultant for Goldfinch Bio, and reports personal fees from Otsuka Pharmaceuticals and Goldfinch Bio, outside the submitted work. All remaining authors have nothing to disclose.

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Author Contributions
W. Besse, S. Ghosh Roy, C. Roosendaal, and L. Tuccillo were responsible for investigation; W. Besse was responsible for formal analysis, funding acquisition, methodology, visualization, and wrote the original draft; A. Gallagher was responsible for data curation; S. Somlo was responsible for funding acquisition and methodology; W. Besse, A. Gallagher, and S. Somlo conceptualized the study, and reviewed and edited the manuscript.

Supplemental Material
This article contains the following supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0002522020/-/DCSupplemental.

Supplemental Figure 1. Liver histology of all Pkd1fl/fl;UBC-CreE3×E3× and Pkd1fl/fl;UBC-CreE3×E3× mice evaluated by this method.

Supplemental Figure 2. Additional information for the UBC-CreE3× model.

Supplemental Figure 3. Kidney histology of all Pkd1fl/fl;Pkd1fl/fl;Pax8fl+/−;TetO-Cre mice in the study.

Supplemental Figure 4. Males from contemporaneous mouse cohort of Pkd1fl/fl;UBC-CreE3×E3× Pkd1fl/fl;Pkd1fl/fl;UBC-CreE3×E3× and Pkd1fl/fl;Pkd1fl/+;BAC UBC-CreE3×E3×.

Supplemental Figure 5. Females from contemporaneous mouse cohort of Pkd1fl/fl;UBC-CreE3×E3× Pkd1fl/fl/Pkd1fl/+;UBC-CreE3×E3× and Pkd1fl/fl;Pkd1fl/+;BAC UBC-CreE3×E3×.

References
1. Guay-Woodford LM: Autosomal recessive polycystic kidney disease: The prototype of the hepato-renal fibrocystic diseases. J Pediatr Genet 3: 89–101, 2014
Genetics Center: ALG9 mutation carriers develop kidney and liver cysts. *J Am Soc Nephrol* 30: 2091–2102, 2019. Available at: https://doi.org/10.1681/ASN.2019030298

27. Lu W, Peissel B, Babakhanlou H, Pavlova A, Geng L, Fan X, Larson C, Brent G, Zhou J: Perinatal lethality with kidney and pancreas defects in mice with a targeted Pkd1 mutation. *Nat Genet* 17: 179–181, 1997. Available at: https://doi.org/10.1038/ng1097-179

28. McNicholas BA, Kotaro Y, Martin W, Sharma A, Kamath PS, Edwards ME, Kremers WK, Chari ST, Torres VE, Harris PC, Takahashi N, Hogan MC: Pancreatic cysts and intraductal papillary mucinous neoplasm in autosomal dominant polycystic kidney disease. *Pancreas* 48: 698–705, 2019. Available at: https://doi.org/10.1097/MPA.0000000000001306

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Supplemental Figure 1: Liver histology of all Pkhd1$^{fl/fl}$,UBC-Cre$^{ER}$ and Pkhd1$^{fl/+}$,UBC-Cre$^{ER}$ mice evaluated by this method

Supplemental Figure 2: Additional information for UBC-Cre$^{ER}$ model

Supplemental Figure 3: Kidney histology of all Pkhd1$^{fl/fl}$,Pkd1$^{fl/fl}$,Pax8$^{rtTA}$TetO-Cre mice in the study

Supplemental Figure 4: Males from contemporaneous mouse cohort of Pkhd1$^{fl/fl}$,UBC-Cre$^{ER}$ Pkhd1$^{fl/fl}$,Pkd1$^{fl/fl}$,UBC-Cre$^{ER}$ and Pkhd1$^{fl/fl}$,Pkd1$^{F/H}$-BAC UBC-Cre$^{ER}$

Supplemental Figure 5: Females from contemporaneous mouse cohort of Pkhd1$^{fl/fl}$,UBC-Cre$^{ER}$ Pkhd1$^{fl/fl}$,Pkd1$^{fl/fl}$,UBC-Cre$^{ER}$ and Pkhd1$^{fl/fl}$,Pkd1$^{F/H}$-BAC UBC-Cre$^{ER}$
Supplemental Figure 2

Panel A: Bar graph showing body weight (grams) comparison between two groups: "Pkhd1^fl/+;UBC-Cre^ER" (black) and "Pkhd1^fl/fl;UBC-Cre^ER" (green).

Panel B: Scatter plot showing the relationship between Hepatic Cystic Index (%) and CK19+ Liver Area (%).
Supplemental Figure 4

Males

*Pkhd1*^fl/fl^;UBC-Cre*ER*

*Pkhd1*^fl/fl^;*Pkd1*^fl/+^;UBC-Cre*ER*

*Pkhd1*^fl/fl^;*Pkd1*^fl/H-BA;UBC-Cre*ER*
