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Loss of *PKD1* and loss of *Bcl-2* elicit polycystic kidney disease through distinct mechanisms

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Abstract

We have recently demonstrated that ablation of one or both alleles of the proapoptotic gene *Bim* prevents the polycystic kidney disease (PKD) that develops in mice deficient for the prosurvival protein *Bcl-2*. The aim of the present study was to investigate whether loss of *Bim* or *Bcl-2* could influence the disease in the *PKD1^{del34/del34}* mutant mice, a model of autosomal dominant PKD. *PKD1^{del34/del34}* mice were intercrossed with *Bim*-deficient mice and *Bcl-2^{+/-}* mice to generate double mutants. Loss of *Bim* does not prevent the development of PKD in *PKD1^{del34/del34}* mice. On the C57BL/6 genetic background, most older *PKD1^{del34/+}* mice do not develop PKD, but present with liver cysts. Surprisingly, loss of *Bim* completely prevented liver cysts formation in *PKD1^{del34/+}* mice. Loss of one *Bcl-2* allele did not influence the *PKD1^{del34}* phenotype significantly. We conclude that loss of *PKD1* and loss of *Bcl-2* elicit PKD through distinct mechanisms.

Keywords

Polycystic kidney disease; *Bcl-2*; *PKD1*; *Bim*; apoptosis

Introduction

Polycystic kidney diseases (PKD) are a major cause of chronic kidney disease and end-stage renal failure.¹ They form a rather heterogeneous group of diseases, with great variations in the age of onset and severity. Most are hereditary and due to mutations in single genes. Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in either *PKD1* or *PKD2*.^{2–4} Nephronophthisis, an autosomal recessive condition, can be caused by mutation in one of 5 genes, *NPHP1–5*,⁵ and medullary cystic disease, an autosomal dominant disease, is caused by mutations in *MDCK1* or *MDCK2*.^{6,7} Most of these genes code for proteins associated with the primary cilium, a cellular organelle thought to act as a mechano- and chemosensor in the renal epithelium.⁸

Several mouse models of PKD exist.⁹ Some have arisen from spontaneous mutations, while others are the result of genetic manipulations through targeted or random mutagenesis.

Mutations in polycystin, the product of the *PKD1* gene, account for 85–90% of all cases of ADPKD in humans. Both null and hypomorphic alleles of *PKD1* have been generated by gene targeting in mice (reviewed in Guay-Woodford⁹). In all cases, the homozygous mutation leads to the death of the animals around birth, due to the development of polycystic kidneys starting around E15.5. Additional defects are observed in some of these mouse models. For example, *PKD1^{del34/+}* animals may develop cysts not only in the kidney but also in the pancreas and/or liver (as in human ADPKD); this phenotype is influenced by age and genetic background.^{10, 11} Some reports have indicated a role for polycystin in the control of apoptotic cell death,¹² thereby implying that abnormal death of normally long-lived cells in the kidney may be the cause of polycystic disease in PKD1 mutant mice and humans.

The Bcl-2 family of proteins are critical regulators of apoptosis in metazoans.^{13,14} These proteins contain at least one of four Bcl-2 Homology domains (BH1–4). The antiapoptotic members of the family, namely Bcl-2, Bcl-xL, Mcl-1, A1 and Bcl-w all share three or four BH domains. The proapoptotic members Bax, Bak and Bok contain three of the BH domains, BH1–3, whereas a more distantly related group of proapoptotic proteins (including Bim, Bad, Bmf, Bid, Hrk, Bik, Noxa and Puma) contain only the BH3 domain. These so-called BH3-only proteins function as essential initiators of the apoptotic cascade, each sensing a particular subset of developmentally programmed death stimuli and/or cytotoxic insults. The Bax/Bak-like proteins are also essential for apoptotic cell death and they appear to function at a point downstream of the BH3-only proteins. The prosurvival Bcl-2 family members have been shown to bind to both BH3-only proteins and Bax/Bak-like proteins, but it is presently not clear whether they promote cell survival by inhibiting BH3-only proteins, by neutralising Bax/Bak-like proteins or by some other mechanism.

A role for members of the Bcl-2 protein family in PKD has been inferred from studies with gene-targeted mice. Although *Bcl-2^{-/-}* mice appear outwardly normal at birth, they rapidly develop PKD, fail to thrive and die within a few weeks.^{15–17} On the C57BL/6 genetic background, the average age of death is ~4–5 weeks.¹⁸ We have shown that loss of a single allele of the gene encoding the proapoptotic BH3-only protein Bim is sufficient to restore the development of normal kidneys in *Bcl-2^{-/-}* mice and permanently prevent PKD in these animals.¹⁸ We have been able to reproduce this result by downregulating *Bim* *in vivo* by using RNA interference.¹⁹ These observations demonstrated that unopposed proapoptotic activity of Bim causes the fatal PKD in *Bcl-2^{-/-}* mice.

In order to determine whether Bim may be involved in other types of PKD, we have now crossed the *Bim^{-/-}* mice with the *PKD1^{del34}* mice and generated doubly deficient animals (DKO). In contrast to the results obtained with the mice lacking both Bim and Bcl-2, loss of one or both alleles of *Bim* failed to prevent the development of PKD in *PKD1^{del34}* mice. Moreover, detailed histological comparisons suggest that the polycystic diseases seen in *Bcl-2^{-/-}* and *PKD1^{-/-}* mice have a different origin.

Results and discussion

Loss of Bim does not prevent PKD in *PKD1^{del34}* mice

Heterozygous *PKD1^{del34/+}* mice were intercrossed with Bimdeficient mice (266del strain) to determine whether loss of Bim could prevent the death due to the development of PKD in *PKD1^{del34/del34}* homozygous mice. On the C57BL/6 background, *PKD1^{del34/del34}* E18.5 embryos were readily recognizable because they all had massive fluid accumulation and oedema (Figure 1). *Bim^{+/-}/PKD1^{del34/del34}* and *Bim^{-/-}/PKD1^{del34/del34}* animals also died around birth with a phenotype indistinguishable from that of the *PKD1^{del34/del34}* mice. Their kidneys were enlarged and contained numerous cysts at E19.5 (Figure 2, and data not shown). Thus, decreasing or ablating the expression of Bim does not prevent the development of PKD

in $PKD1^{del34/del34}$ mice, although it can prevent that arising in the $Bcl-2^{-/-}$ mice. This observation indicates that the diseases observed in these two mouse models differ in their origin.

$PKD1^{del34/+}$ heterozygous mice have been shown to develop renal and hepatic cysts relatively late in life (~1 year or more).²⁰ In order to determine whether the ablation of *Bim* would delay the time of onset or the severity of the disease in $del34/+$ heterozygous mice, we created and aged cohorts of mice with the following genotypes: $Bim^{+/+}/PKD1^{del34/+}$, $Bim^{-/-}/PKD1^{+/+}$ and $Bim^{-/-}/PKD1^{del34/+}$. To check whether loss of one allele of *Bcl-2* could exacerbate the severity of the $PKD1^{del34/+}$ phenotype, 29 $Bcl-2^{+/-}/PKD1^{del34/+}$ mice were also included in this study. All these mice were killed at 16–22 months of age and their livers and kidneys sectioned to examine for the presence of cysts (Table 1). Only six of 34 $Bim^{+/+}/PKD1^{del34/+}$ mice had cysts or dilated tubules in either kidney. Of the 12 $Bim^{+/+}/PKD1^{del34/+}$ mice that were over 20 months of age, only one had a small cyst in one kidney and none had the large number of cysts reported for mice of that age.²⁰ This lower incidence could reflect a difference in the genetic background of the mice in the two studies. Loss of one or both alleles of *Bim* did not decrease the incidence of kidney cysts in $PKD1^{del34/+}$ mice, as four out of the 22 $Bim/PKD1^{del34/+}$ mice exhibited some cysts or dilated tubules. In addition, loss of one allele of *Bcl-2* did not increase the severity or incidence of PKD significantly, as three of 29 $Bcl-2^{+/-}/PKD1^{del34/+}$ were affected. Most kidneys from older $Bim^{-/-}$ mice contained large lymphocyte infiltrates, a feature only rarely observed in mice of the other genotypes (Figure 3). This feature was independent of *PKD1*, since both $Bim^{-/-}/PKD1^{del34/+}$ and $Bim^{-/-}/PKD1^{+/+}$ kidneys presented it.

Our cohort of compound mutant mice was also analysed for the appearance of liver cysts (Table 1). From 1 to 10 liver cysts were found in 25 of 34 (73.5%) $Bim^{+/+}/PKD1^{del34/+}$ and 14 of 29 (48.3%) $Bcl-2^{+/-}/PKD1^{del34/+}$ mice. The cysts, however, were not as big as those previously described (the biggest cysts in our animals were 7mm in diameter). This apparent reduction of liver cyst incidence found in the absence of one allele of *Bcl-2* was, however, not statistically significant (Fisher's exact test P -value = 0.068). By contrast, none of the 28 $Bim^{-/-}/PKD1^{del34/+}$ mice had liver cysts, suggesting that the absence of *Bim* completely prevents the development of liver cysts in $PKD1^{del34/+}$ mice (FET P = 0.00007).

In summary, the development of renal cysts in heterozygous $PKD1^{del34/+}$ mice appears less severe on the C57BL/6 background than it is on a mixed 129Sv/ C57BL/6 or 129Sv/BALB/c background, and it is not affected by the loss of one or both alleles of *Bim* or loss of one allele of *Bcl-2*. In contrast, the absence of *Bim* prevents the development of liver cysts.

Differences between $Bcl-2^{-/-}$ and $PKD1^{del34/del34}$ kidneys

Since all our mice were on the same genetic background and thus directly comparable, we harvested wt, $Bcl-2^{-/-}$ and $PKD1^{del34/del34}$ kidneys at different stages of development for histological examination. The first differences could be seen at E15.5, when $Bcl-2^{-/-}$ kidneys appeared slightly smaller than wt ones. In contrast, E15.5 $PKD1^{del34/del34}$ kidneys were slightly larger than normal and already contained some cysts. Differences between the different mutant animals became progressively more obvious at later stages of development. At E19.5, $Bcl-2^{-/-}$ kidneys were approximately four times smaller than wt kidneys, but did not display any signs of cysts, whereas $PKD1^{del34/del34}$ kidneys were abnormally large due to the presence of numerous tubular and peri-glomerular cysts (Figure 4). At birth $Bcl-2^{-/-}$ newborn are outwardly indistinguishable from wt pups, and become growth-retarded only 1 week later, while all $PKD1^{del34/del34}$ pups die at birth with a severe polycystic kidney phenotype. These anatomical differences further indicate that the diseases observed in the $Bcl-2^{-/-}$ and the $PKD1^{del34/del34}$ mice are of distinct origin.

Several genes responsible for different forms of PKD have been identified recently.²¹ Interestingly, most of these genes appear to code for proteins which seem to directly interact or form complexes with polycystin, the product of the *PKD1* gene. Polycystin is found in cells at the basal surface in focal adhesion complexes, at the lateral surface in cell adherens junctions, and in the apical primary cilium facing the lumen.⁸ Polycystin-containing complexes are thought to perform a mechano-sensory function in the primary cilia of renal epithelial cells, and possibly at the site of cell–matrix and cell–cell interactions. Although PKDs are heterogenous with regard to their severity and time of onset, it is possible that they are all due to abnormalities in the same signal transduction pathway involving polycystin, as all the proteins involved have been reported to interact with components of this pathway.

The disease in *Bcl-2*^{-/-} mice is clearly due to excessive cell death during kidney development, and appears to be mediated primarily by the un-opposed proapoptotic activity of the BH3-only protein Bim.¹⁸ Indeed, removal of a single allele of *Bim* was sufficient to prevent PKD in the *Bcl-2*^{-/-} mice. None of the *Bcl-2*^{-/-}/*Bim*^{+/-} mice that have lived for 2 years has developed kidney cysts, and their kidneys are normal in size. Although several studies have reported abnormally persistent apoptosis in both autosomal dominant and autosomal recessive PKD,^{22–24} our results indicate that Bim probably has no role in this phenomenon in ADPKD.

However, the apparent involvement of Bim in the development of liver cysts in heterozygous *PKD1*^{del34/+} mice is intriguing. The mechanism of protection offered by the loss of Bim is not clear, but it may be due to abnormally long lifespan of hepatocytes, which might reduce the risk of acquiring mutations in the wt *PKD1* allele (which is probably a prerequisite for cyst development).

It is not clear whether *Bcl-2*^{-/-} mice represent a suitable model for any of the reported human PKDs. Although some of the clinical manifestations appear similar to those of nephronophthisis (bilaterally shrunken kidneys, age of onset), some features are radically different (e.g. position of cysts, absence of fibrosis in *Bcl-2*^{-/-} kidneys).

We observed some differences between our results on *PKD1*^{del34/+} mice and observations reported earlier.²⁰ It appears likely that this is a reflection of a difference in the genetic background of the mice used in the two studies. Genetic background seems to greatly influence the development of PKD. For example, PKD in *Bcl-2*^{-/-} mice is much more severe on an inbred C57BL/6 background than on a mixed C57BL/6/129Sv background.^{15,18} On the contrary, the disease in pcy mice is more severe on a DBA background than on C57BL/6 background.¹⁰

Does ciliary dysfunction underlie all forms of cystic renal disease, as was recently suggested? Although the link between defective apoptosis and ciliary dysfunction does not seem obvious, the cause of development of PKD in *Bcl-2*^{-/-} mice may be the transduction of an abnormal signal through the cilium. Indeed, the main feature of *Bcl-2*^{-/-} kidneys is their small size and their abnormally low number of nephrons. It is possible that such small kidneys may be overwhelmed soon after birth, and that the stress due to overload may be sensed and signalled through the cilium and thereby lead to the formation of cysts.

Materials and Methods

Mice

The generation of *Bim*^{-/-},²⁵ *Bcl-2*^{-/-}¹⁶ and *PKD1*^{del3426} mutant mice has been described. The *del34* mutation is a truncation that mimics many mutations seen in ADPKD patients, but is not equivalent to a complete ablation of the *PKD1* gene.²⁷ All the mice have been backcrossed for more than eight generations onto the C57BL/6 genetic background prior to use in this study. Mice were intercrossed to generate the different genotypes described in this report.

PCR genotyping

Mice were genotyped by polymerase chain reaction (PCR) on tail DNA with the primers listed below.

For *Bim* mice, a three primer PCR reaction was carried out with PB20 5'-GGGATCTGGTAGCAAAGGGCCAG-3', PB65 5'-CTCAGTCCATT CATCAACAG-3', and PB335 5'-GTGCTAACTGAAACCAGATTAG-3'. For *PKD1^{del34}* mice, the mutated allele could be detected by a reaction with primers neo2 5'-CATACTTCAGAAGAAGACTCGTCAAG-3' and neo3 5'-GATTGAACAAGATGGATTGCAC-3', while wild-type (wt) allele was detected with primers PB425 5'-GAATTCAAGGTACCAAGTGT-3' and PB427 5'-CCAGACCAAGTCCTTGAGGT-3'. In *Bcl-2* mice, the wt allele was detected using primers PB191 5'-ATGTATGTACTTCATCAGAT-3' and PB142 5'-GACCCAATCTGGAAACCCTCCTGAT-3', and the mutated allele was detected with PB144 5'-ATTCGTTCTCTTTATACTAC CAAGG-3' and PB 145 5'-TGCTAAAGCGCATGCTCCAGACTG-3'. All the PCR reactions were 30 cycles (94°C 40s, 55°C 30s, 72°C 1 min).

Abbreviations

PKD	polycystic kidney disease
ADPKD	autosomal dominant polycystic kidney disease
PCR	polymerase chain reaction

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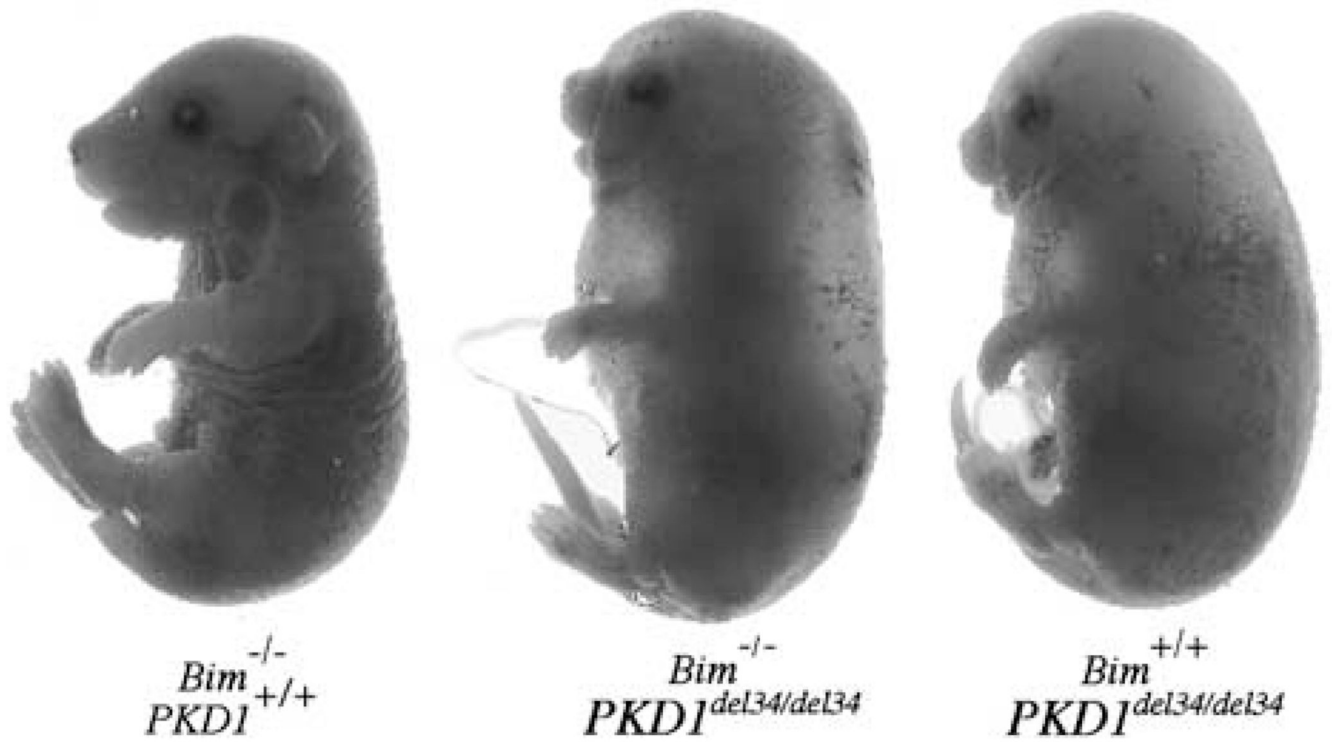


Figure 1.

Loss of *Bim* does not rescue polycystic kidney disease in *PKDI*^{del34/del34} mice. *Bim*^{-/-}/*PKDI*^{del34/del34} newborn mice display the same phenotype as *Bim*^{+/+}/*PKDI*^{del34/del34} pups

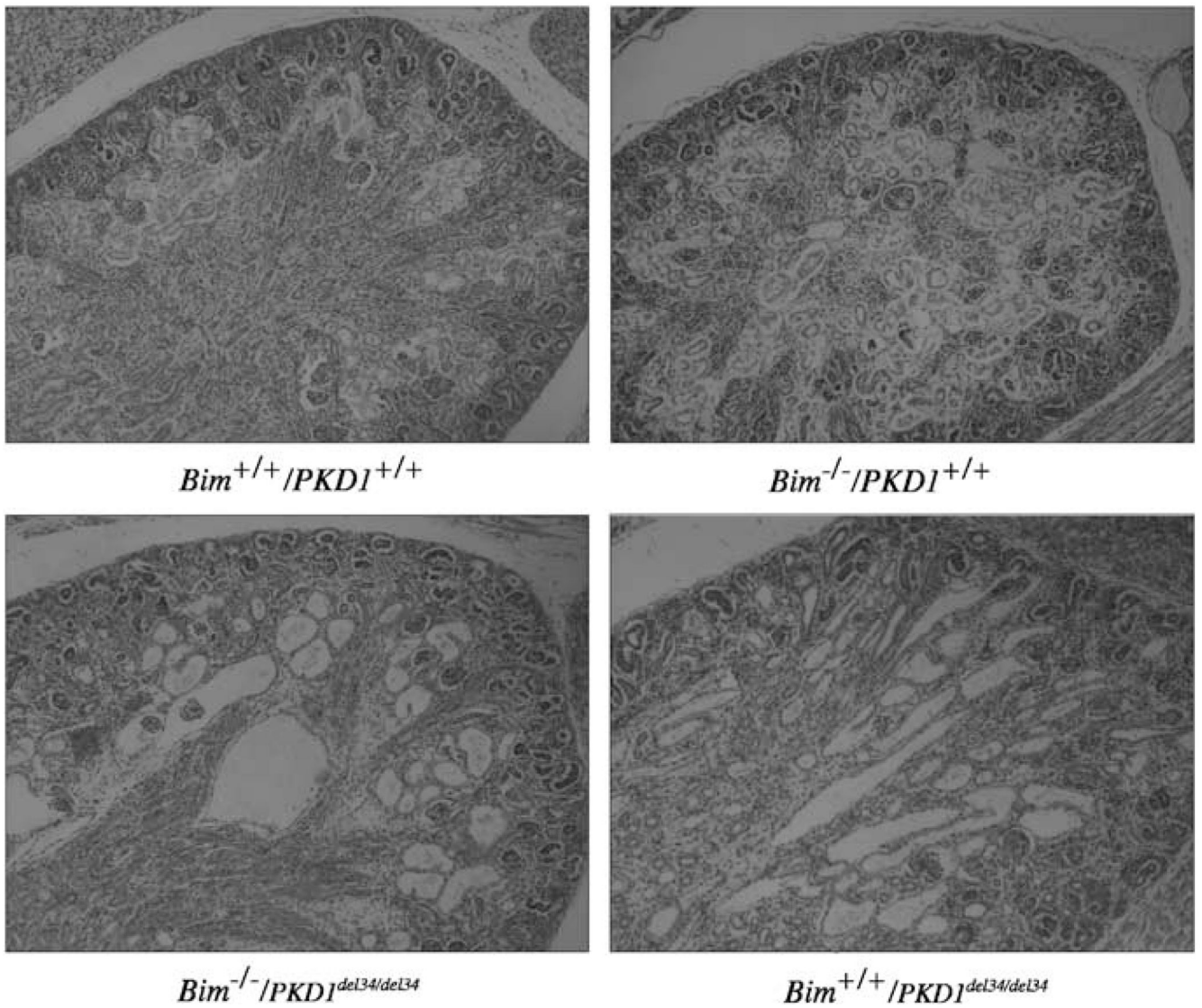


Figure 2. Kidney morphology. Kidneys of wild-type (wt), $Bim^{-/-}/PKD1^{+/+}$, $Bim^{-/-}/PKD1^{del34/+}$ and $Bim^{-/-}/PKD1^{del34/del34}$ newborn mice are compared. Magnification $\times 50$

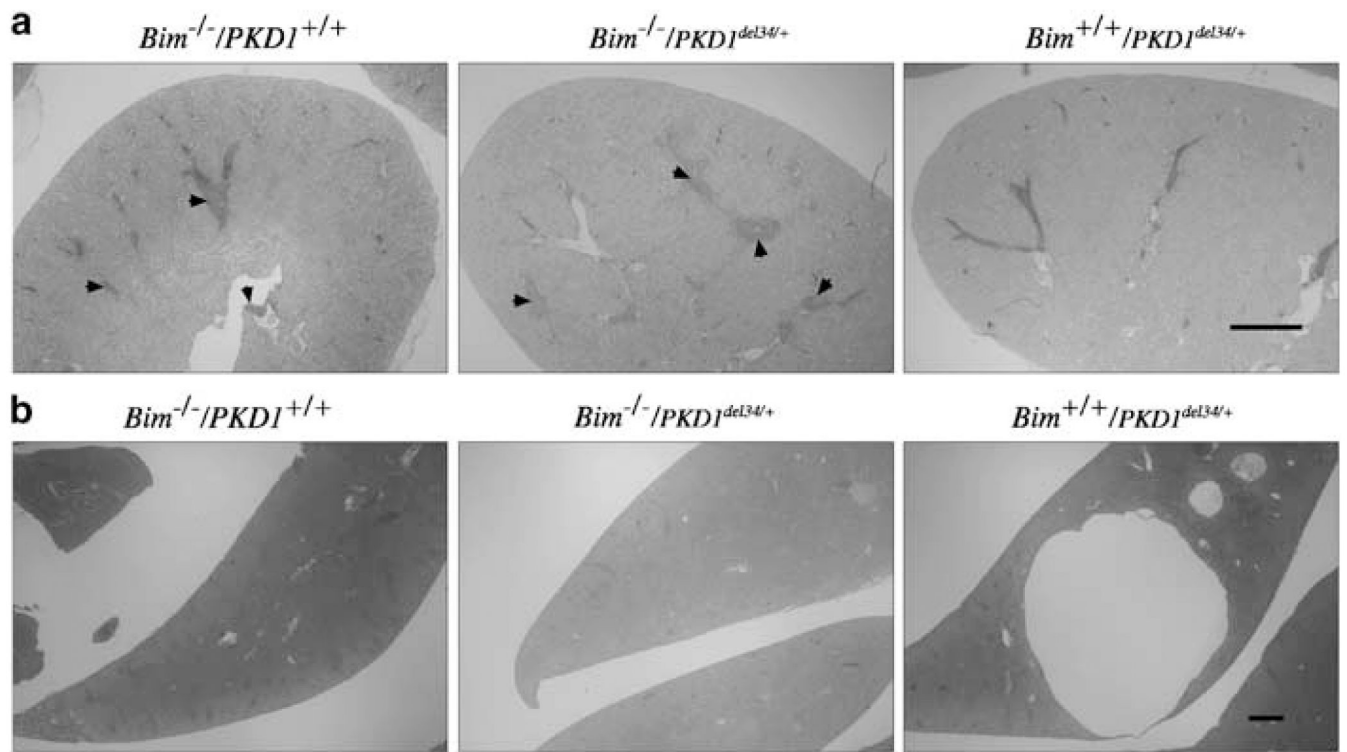


Figure 3.

Older *Bim* and *PKD1*^{del34} mice. (a) Kidneys of all 14–16 month-old *Bim*^{-/-} mice present with lymphocyte infiltrates rarely seen in the other genotypes considered here. Bar, 1 mm. (b) Unlike *Bim*^{+/+}/*PKD1*^{del34/+} mice, *Bim*^{-/-}/*PKD1*^{del34/+} and *Bim*^{-/-}/*PKD1*^{+/+} do not develop liver cysts upon ageing. Bar, 1mm

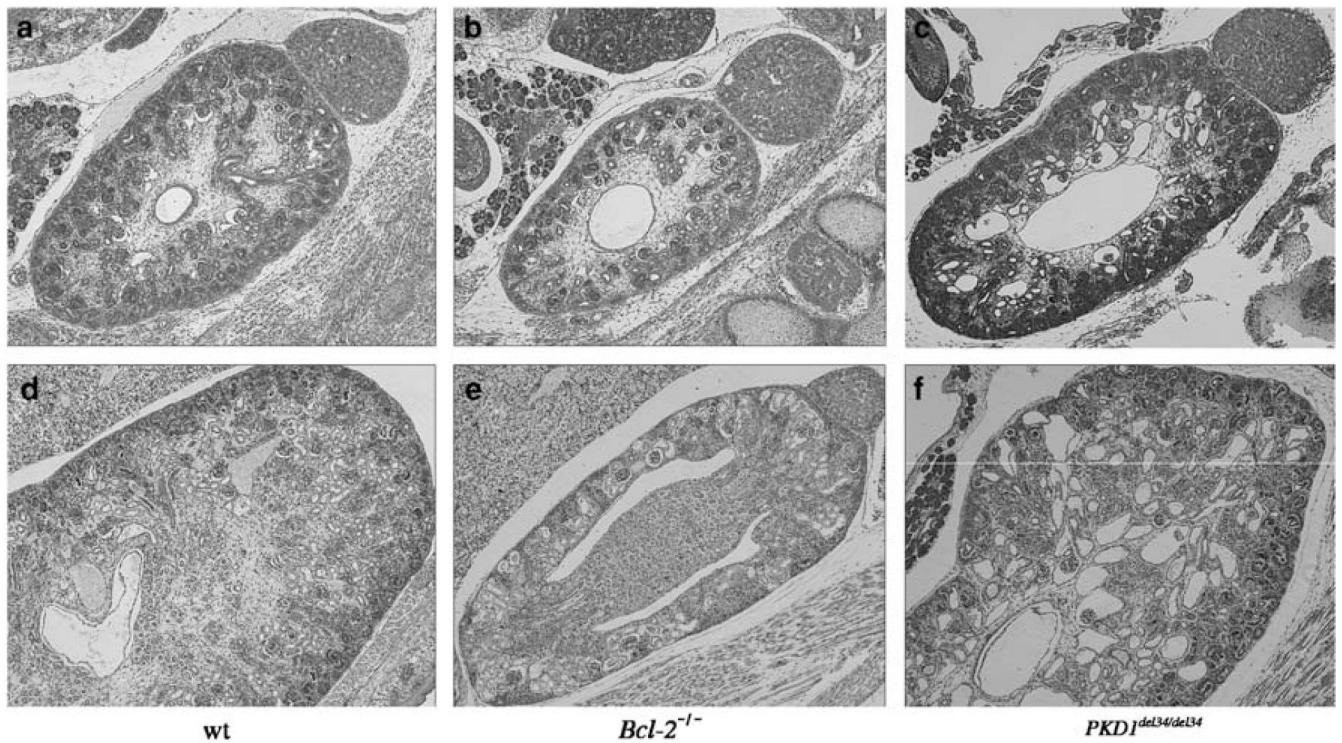


Figure 4. Different manifestations of PKD in *Bcl-2*^{-/-} and *PKD1*^{del34/del34} mice. Kidneys of *Bcl-2*^{-/-} (**b, e**) and *PKD1*^{del34/del34} (**c, f**) were compared with wt (**a, d**). Haematoxylin/eosin-stained kidney sections of E15.5 embryo (**a-c**) and newborn mice (**d-f**). *Bcl-2*^{-/-} kidneys are smaller but do not show any cyst at birth, whereas *PKD1*^{del34/del34} kidneys are larger and show cysts throughout development. Magnification ×50

Table 1

Analysis of renal and liver cyst incidence in older mice of the indicated genotypes

	Kidney cysts		Liver cysts	
	Yes	No	Yes	No
<i>bim</i> ^{+/+} <i>PKDI</i> ^{+/-}	6	28	25	9
<i>bim</i> ^{-/-} <i>PKDI</i> ^{+/-}	4	18	0	22
<i>bim</i> ^{-/-} <i>PKDI</i> ^{+/+}	1	18	0	19
<i>bcl-2</i> ^{+/-} <i>PKDI</i> ^{+/-}	3	26	14	15

Incidence of kidney and liver cysts in themice of the indicated genotypes. All the mice considered here were aged 16–22 months