Cyst expansion and regression in a mouse model of polycystic kidney disease

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Autosomal-dominant polycystic kidney disease is characterized by progressive cyst formation and fibrosis in the kidneys. Here we describe an orthologous *Pkd1*^{nl,nl} mouse model, with reduced expression of the normal *Pkd1* transcript, on a fixed genetic background of equal parts C57Bl/6 and 129Ola/Hsd mice (*B6Ola-Pkd1*^{nl,nl}). In these mice, the first cysts develop from mature proximal tubules around birth. Subsequently, larger cysts become visible at day 7, followed by distal tubule and collecting duct cyst formation, and progressive cystic enlargement to develop into large cystic kidneys within 4 weeks. Interestingly, cyst expansion was followed by renal volume regression due to cyst collapse. This was accompanied by focal formation of fibrotic areas, an increased expression of genes involved in matrix remodeling and subsequently an increase in infiltrating immune cells. After an initial increase in blood urea within the first 4 weeks, renal function remained stable over time and the mice were able to survive up to a year. Also, in kidneys of ADPKD patients collapsed cysts were observed, in addition to massive fibrosis and immune infiltrates. Thus, *B6Ola-Pkd1*^{nl,nl} mice show regression of cysts and renal volume that is not accompanied by a reduction in blood urea levels.

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Autosomal-dominant polycystic kidney disease (ADPKD) is a disease characterized by the formation of large fluid-filled cysts in both kidneys that ultimately leads to end-stage renal disease and the requirement for renal replacement therapy at the age of 50–60 years. In addition to the renal pathology, extra-renal manifestations can develop, such as cysts in the liver and pancreas, hypertension, cardiac valvular abnormalities, and cerebral aneurysms.[1] Usually, in young adults, only a small number of cysts can be detected, whereas at middle age thousands of cysts surrounded by fibrotic tissue have replaced almost all normal renal parenchyma, leading to a decline in renal function.

In most ADPKD patients, one *PKD1* or *PKD2* allele carries a germline mutation while the other allele is normal. Renal cysts are hypothesized to develop after somatic inactivation of the 'healthy' allele of *PKD1*, or *PKD2* by a 'second hit' mechanism.[2] In addition, haploinsufficiency, stochastic fluctuations in *PKD1* or *PKD2* gene dosage below a tissue-specific threshold, may suffice to cause cyst formation.[3] A more complex picture was proposed in recent studies reporting several families with incompletely penetrant alleles of *PKD1* inherited at a homozygous state, or together with an inactivating mutation in the other allele.[4–6]

Many mouse and rat models exist for studying early phases of ADPKD, the formation and growth of cysts. However, few models have been described representing the phenotype observed in ADPKD patients with advanced stage disease characterized by cystic tissue, fibrotic areas with inflammation, but also regions with relative normal renal morphology.[7–9] Clearly, animal models closely resembling this phenotype are needed to evaluate accurately the *in vivo* effect of potential therapeutics on ADPKD disease progression in patients.

Previously, the orthologous *Pkd1*^{nl,nl} mouse model with reduced expression of the normal *Pkd1* transcript had a variable mixed genetic background.[10] These *Pkd1*^{nl,nl} mice were crossed back to full C57BL6/J (B6) and full 129Ola/Hsd (Ola) genetic background. Here we study *Pkd1*^{nl,nl} mice on a 50%/50% genetic background of B6 and Ola, (*B6Ola-Pkd1*^{nl,nl} mice). These mice showed no developmental delay, in contrast

to the *B6-Pkd1*^{nl,nl} mice on a full B6 genetic background.[11,12] Here, we demonstrate that *B6Ola-Pkd1*^{nl,nl} mice showed great similarities with the human renal phenotype regarding fibrosis and inflammation. Interestingly, *B6Ola-Pkd1*^{nl,nl} mice showed an initial increase in renal volume, followed by a partial regression due to collapse of cysts. This was accompanied by the focal formation of fibrotic areas. Similar features were found in human kidneys with ADPKD.

RESULTS

B6Ola-Pkd1^{nl,nl} mice show reduced levels of *Pkd1* gene expression and survive without growth retardation

Pkd1^{nl,nl} mice carry a neomycin selection cassette in intron 1, resulting in alternative splicing of the majority of the *Pkd1* transcripts.[10] In *B6Ola-Pkd1*^{nl,nl} mice, expression levels of the normal *Pkd1* transcript was reduced to 10% compared with 4-week-old wild-type mice (Supplementary Figure S1A online). Although expression of full-length *Pkd1* is strongly reduced, a 3′-transcript is still produced (Supplementary Figure S1B online).[10]

In wild-type mice, normal *Pkd1* expression decreased with age to 20% of the expression at 4 weeks, while normal *Pkd1* expression in mutants remained at the same low level (Supplementary Figure S1A online). *Pkd2* gene expression was significantly reduced in mutants (25–30%) compared with wild types at the age of 4 and 16 weeks (Supplementary Figure S1C online). As the tuberous sclerosis 2 (*Tsc2*) and *Pkd1* gene are located tail-to-tail, *Tsc2* expression was analyzed.[13] Expression in 4-week-old mice was significantly reduced in *B6Ola-Pkd1*^{nl,nl} mice, while at older ages expression was not significantly altered (Supplementary Figure S1D online).

In order to determine whether hypomorphic *Pkd1*-mutant mice show grow retardation as previously reported,[10] body weight was monitored. *B6Ola-Pkd1*^{nl,nl} mice, however, showed similar growth curves compared to wild-type littermates, both for males and females (Figure 1a and b). Only after 35 weeks, *B6Ola-Pkd1*^{nl,nl} male mice did not increase in weight, while wild-type males still did. Mice could live up to 52 weeks of age, although from 24 weeks onward occasional dropout of mice was observed ($n = 3/12$ before 32 weeks), and between 43 and 52 weeks 3/4 mice died (at 43, 48, and 50 weeks).

Renal volume declined after 4 weeks without improvement of renal function

At different time-points mice were killed and kidney-to-body weight ratios (2KW/BW) were determined as a measure for renal volume. Figure 1c and d show 2KW/BW and kidney weights, respectively. 2KW/BW strongly increased during the first 4 weeks of age (12.6%) due to cystic expansion as shown by renal histology (Figures 1c and 2a). Strikingly, after 4 weeks 2KW/BW started to decline and stabilized after 12 weeks (~2.5%). This phenomenon was confirmed by the cystic index, showing a maximum at 4 weeks (~70%) followed by a decline (~35% at 12 weeks; Figure 1e).

Renal function was impaired at 4 weeks compared with 1 week, as indicated by increased blood urea (BU) levels (Figure 1f). However, BU levels did not decrease significantly as renal volume decreased, indicating no improvement of renal function at later stage.

Cyst formation and origin of cysts

The origin of cyst formation was evaluated at postnatal days 1, 3, 7, 14, and 28 and subsequently at 24 weeks, using nephron segment-specific markers ($n = 2$–3 per age). In newborn mice, at days 1 and 3, many dilated proximal tubules and small cysts positive for megalin were present in the cortex. In addition, although less numerous, dilated aquaporin-2-positive collecting ducts were observed (Supplementary Figure S2A and C online). This indicates that first the majority of cysts arise in proximal tubules in the cortex.

At day 7, more cysts of proximal tubular origin were found in the cortex (Figure 2J). Also the aquaporin-2-positive dilated collecting ducts became more numerous (Supplementary Figure S2D–F online).

At day 14, epithelial cysts occupied the entire cortex and medulla, which were mostly derived from the collecting ducts (Figure 2k). Although uromodulin-positive cysts were also present, many of these cysts showed staining for aquaporin-2 as well. Only few cysts were megalin positive (Supplementary Figure S2G–I online). In addition, normal-appearing tubules were still present.

From day 14 onward the number and size of cysts increased until 21–28 days, while only few normal-appearing tubules were present. Four-week-old *B6Ola-Pkd1*^{nl,nl} mice showed cysts derived from the proximal tubules. However, the majority of cysts with marker staining were derived from the distal tubules (or loops of Henle) and collecting ducts as indicated by staining for uromodulin and aquaporin-2 (Figure 2l and Supplementary Figure S2J–L online). A small number of cysts were megalin positive, whereas most cysts showed partial, or no marker staining at all.

At later age, when renal volume declined, cysts derived from all segments were still observed. For example, at 24 weeks small cysts and dilated tubules of proximal tubular origin, distal tubular origin/loop of Henle and originating from collecting ducts were seen. Large cysts often had lost expression of segmental markers. In addition, glomerular cysts could be observed from 8 weeks onward.

Renal volume regression is accompanied by collapsing cysts, extensive fibrosis, and inflammation

Histological analysis (Figure 2a) and cystic index (Figure 1e) revealed that the cysts were the largest at 4 weeks. Subsequently, cysts partially regressed, became fibrotic, and the kidneys revealed relatively normal tissue (Figure 2b and c).

Although wild-type kidneys showed proliferating epithelial cells at the age of 4 weeks, the kidneys of *B6Ola-Pkd1*^{nl,nl} mice showed significantly more proliferating cells as illustrated by the proliferation index (Figure 3a), that is, multiple Ki-67-positive nuclei and stretches of positive nuclei were present in the epithelia of dilated tubules and cysts (Figure 2r). At 8 weeks of age, proliferation in wild