Cystic Gene Dosage Influences Kidney Lesions After Nephron Reduction

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Abstract

Cystic kidney disease is characterized by the progressive development of multiple fluid-filled cysts. Cysts can be acquired, or they may appear during development or in postnatal life due to specific gene defects and lead to renal failure. The most frequent form of this disease is the inherited polycystic kidney disease (PKD). Experimental models of PKD showed that an increase of cellular proliferation and apoptosis as well as defects in apico-basal and planar cell polarity or cilia play a critical role in cyst development. However, little is known about the mechanisms and the mediators involved in acquired cystic kidney diseases (ACKD). In this study, we used the nephron reduction as a model to study the mechanisms underlying cyst development in ACKD. We found that tubular dilations after nephron reduction recapitulated most of the morphological features of ACKD. The development of tubular dilations was associated with a dramatic increase of cell proliferation. In contrast, the apico-basal polarity and cilia did not seem to be affected. Interestingly, polycystin 1 and fibrocystin were markedly increased and polycystin 2 was decreased in cells lining the dilated tubules, whereas the expression of several other cystic genes did not change. More importantly, *Pkd1* haploinsufficiency accelerated the development of tubular dilations after nephron reduction, a phenotype that was associated to a further increase of cell proliferation. These data were relevant to humans ACKD, as cystic genes expression and the rate of cell proliferation were also increased. In conclusion, our study suggests that the nephron reduction can be considered a suitable model to study ACKD and that dosage of genes involved in PKD is also important in ACKD.

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Introduction

Cystic kidney diseases are characterized by the progressive development of multiple fluid-filled cysts in renal parenchyma leading to end-stage renal disease. Cystic kidney diseases may arise sporadically, be acquired, or inherited. Acquired cystic kidney diseases (ACKD) have been described for the first time by Dunnill in 1977 [1]. They often result from chronic renal failure or aging, and appear frequently in patients who are on long-term dialysis and who have no history of hereditary cystic disease. The most severe complication of ACKD is renal cell carcinoma, developed by 2–7% of individuals with ACKD

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[2]. The mechanisms underlying ACKD are still unknown.

The majority of cystic kidney diseases are inherited and among them, the most frequent forms are polycystic kidney diseases (PKD). PKD can be inherited either as a dominant or as a recessive trait. The autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited cystic disease and the most frequent monogenic disorder with an estimated incidence between 1:500 and 1:1,000. ADPKD results from mutations in two genes: *PKD1*, encoding polycystin-1 (PC1), a large membrane-associated protein [3] and *PKD2*, encoding polycystin-2 (PC2), a calcium channel of the Transient Receptor Potential (TRP) family [4]. The autosomal recessive polycystic kidney disease (ARPKD) occurs in about 1:20,000 individuals and results from mutation in the *PKHD1* gene. *PKHD1* encodes for fibrocystin, a large transmembrane protein whose function is still unknown [5]. Despite differences in the age of onset, the severity of the disease or the localization of the cysts, it has been suggested that common mechanisms are involved in cyst formation, including cell proliferation and apoptosis [6], disturbance of cell polarity and cell-matrix interaction [7], enhanced fluid secretion, or loss of oriented cell division [8]. On the other hand, the primary cilium as well as calcium signaling have been suggested to play a role [9–12].

Interestingly, cysts arising from acquired or hereditary diseases appear morphologically similar. In this context, it is tempting to speculate that molecules and events involved in inherited PKD might participate in cyst formation in ACKD. In particular, one could wonder if polycystins play a role in the pathogenesis of ACKD. To test this hypothesis, we used the remnant kidney model as a model of severe and progressive tubular dilations [13] to unravel the contribution of cystic genes to cystogenesis. We found that the expression of PC1 and fibrocystin increased in cystic epithelium following nephron reduction, whereas that of PC2 was decreased. More importantly, we observed that *Pkd1* haploinsufficiency worsens the development of tubular dilations and interstitial fibrosis after nephron reduction, suggesting that the increase of PC1 in remaining nephrons could be linked to a compensatory event.

Methods

Animals

Mice used for these studies were FVB/N (Charles River) and mutant *Pkd1*$^{+/-}$ mice [14]. *Pkd1*$^{+/-}$ G1 mice were generated by breeding *Pkd1*$^{+/-}$ on a mixed C57BL/6X129/Sv genetic background with FVB/N mice. All experiments were performed on 9-week-old

Table 1. Demographic and clinical characteristics of ACKD patients

Patient	Gender	Age	Pathology
1	M	32	Alport syndrome
2	M	48	Chronic glomerulonephritis
3	M	56	Chronic glomerulonephritis
4	F	61	Chronic interstitial nephritis
5	F	43	Chronic interstitial nephritis
6	M	53	Diabetic nephropathy
7	M	57	Diabetic nephropathy
8	M	49	FSGS
9	F	60	FSGS
10	F	70	FSGS
11	F	40	Hemolytic uremic syndrome
12	M	52	IgA nephropathy
13	F	40	Reflux nephropathy
14	M	25	Renal dysplasia

FSGS = Focal segmental glomerular sclerosis. All ACKD patients had end stage renal disease. The age is the age at the time of transplantation, when kidney was removed.

females, except for *Pkd1*$^{+/-}$ G1 mice that were studied only in 9-week-old males. Animals were fed ad libitum and housed at constant ambient temperature in a 12-hour light cycle. Animal procedures were approved by the Departmental Director of 'Services Vétérinaires de la Préfecture de Police de Paris' and by the ethical committee of the Paris Descartes University.

Protocol

Mice were subjected to 75% nephrectomy (Nx) or sham-operation (controls), as previously described [15]. After surgery, mice were fed a defined diet containing 30% casein and 0.5% sodium. Several groups of mice were investigated in complementary studies. For time-course study of nephron reduction model, 6 and 10 FVB/N mice for each time point were subjected to either sham-operation or Nx, respectively. Mice were sacrificed 30, 42, or 60 days after surgery. For transgenic studies, 4–6 mice were subjected to sham-operation and 9–11 mice to Nx for both *Pkd1*$^{+/-}$ G1 and wild-type (WT) littermates. Mice were sacrificed 4 months after surgery and kidneys were harvested for morphological, protein, and mRNA studies.

Human Tissue Samples

Kidneys from patients with end-stage renal failure (n = 14; table 1), removed at time of transplantation were analyzed for PC1, PC2, fibrocystin, and Ki-67 expression. Kidneys not used for transplantation or tumor-free pole of kidneys removed for carcinoma were used as controls (n = 7).

Morphological Analysis

For morphological analysis, kidneys were fixed in 4% paraformaldehyde, paraffin embedded, and 4-µm sections were stained with PAS, Masson's trichrome, or Picrosirius Red. The degree of tubular, glomerular, and interstitial lesions was evaluated using the semiquantitative score methodology as previously described [16]. Briefly, ten to fifteen randomly selected microscopic fields were

scored using a Nikon digital camera Dx/m/1,200 and Lucia software (Laboratory Imaging Ltd.). For tubular lesions, the area of tubular lumen was measured on PAS-stained sections and the results were expressed as a percentage of the total area of the selected fields. For interstitial fibrosis, the area covered with red staining was quantified on picrosirius-stained sections and the results were expressed as a percentage of the total area of the selected fields. The degree of glomerular lesions was measured according to a semi-quantitative method and graded from 0 to 3+ depending on the severity of the lesions.

Immunohistochemistry

Four-μm sections of paraffin-embedded kidneys were incubated overnight at 4°C with biotinylated Lotus Tetragonolobus Lectin (Vector), or primary antibodies (sheep anti-Tamm-Horsfall Biogenesis), rabbit anti-aquaporin 2 (Sigma), mouse anti-megalin (a gift of Pierre Verroust), mouse anti-NaKATPase (Upstate biotechnology), mouse anti-acetylated tubulin (Sigma), mouse anti-PC1, mouse anti-fibrocystin 1 (kindly provided by Christopher J. Ward), rabbit anti-PC2 (kindly provided by Peter Harris), and rabbit anti-Ki67 (Novus biological). The sections were then incubated with a secondary antibody for 1 h at room temperature and revealed by 3,3′-diaminobenzidine (Dako). The proliferation index was calculated as the number of tubular Ki-67-positive nuclei for the total number of tubular nuclei. Ten randomly selected microscopic fields (×400) were scored using a Nikon digital camera Dx/m/1200 and Lucia software (Laboratory Imaging Ltd.).

Real-Time Quantitative PCR

Total RNAs were obtained from kidneys using RNeasy Midi kit (Qiagen) according to the manufacturer's protocol. RNAs were reverse transcribed (oligo dT primer) according to manufacturer's protocols (Invitrogen). Quantitative RT-PCR was carried out using an ABI PRISM® 7700 sequence detection system using SYBR® Green. Primers (Eurogentec) were as follows: *Pkd1*: forward 5′-TTTTAAAGTGCAGAAGCCCCA-3′ and reverse 5′-GCTGCATGCCAGTTCTTTTG-3′; *Pkd2*: forward 5′-CATGTCTCGATGTGCCAAAGA-3′ and reverse 5′-ATGGAGAACATTATGGTGAAGCC-3′; *Pkhd1*: forward 5′-AAGTCAAGGGCCATCACATC-3′ and reverse 5′-ATGTTTCTGGTCAACAGCCC-3′; *Nphp1*: forward 5′-AGAGAGGAGAGTTAAGCTGTGGCT-3′ and reverse 5′-TGGCATCGAAAAGCTTAAGAAAC-3′; *Nphp2*: forward 5′-ACTTGTTACCCAGCATATGTGGTC-3′ and reverse 5′-AGGAGAAAACATTTGAACCTTGTCTT-3′; *Nphp3*: forward 5′-ACAACTCATTCCCCTAACGTTTTT-3′ and reverse 5′-TGACGGTTAGCTTTGCCCTT-3′; *Nphp4*: forward 5′-TTGGCAATAAGCCAGAATCTCC-3′ and reverse 5′-TGATACAGCCTCAACCGTTTGTC-3′; *Cystin1*: forward 5′-TCTCGGTTTATGTTGCATCCC-3′ and reverse 5′-TGAGGATTCATGGACTGGCA-3′; *Hnf1b*: forward 5′-GTCCAAGCTCACGTCGCTCC-3′ and reverse 5′-CCAGGTCTTTGCAGAGAACTGC-3′; *Ift88*: forward 5′-GCAGGCTTCAACCTCATCCTTA-3′ and reverse 5′-TTTCTCCCGATCTCCAATGG-3′. Gapdh or Rpl13 were used as the normalization controls.

Statistical Analysis

Data were expressed as means ± SEM. Differences between the experimental groups were evaluated using ANOVA, followed when significant ($p < 0.05$) by the Tukey-Kramer test. When only two groups were compared, Mann-Whitney tests were used. The statistical analysis was performed using Graph Prism Software.

Results

Nephron Reduction Induces Severe Tubular Dilations

We have previously shown that nephron reduction (Nx) results in progressive renal lesions in FVB/N genetic background, whereas the C57BL/6 mice undergo compensatory growth alone [13, 17]. In order to investigate if this model might recapitulate some features of ACKD, we characterized the changes of tubular structures during the evolution of the disease. Two months after Nx, we observed that renal lesions were mainly represented by severe tubular dilations (fig. 1A, inset a), which were associated with a marked increase of kidney weight (data not shown). Interstitial fibrosis and glomerulosclerosis were also observed (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000369312). Apical or diffuse accumulation of PAS-positive protein droplets of various sizes was observed in enlarged proximal tubular cells (fig. 1A, inset b). The number and severity of tubular microcystic dilations varied from case to case. They were of several types, either lined by hypertrophic and microvacuolated cells (fig. 1A, inset c), or bordered by a flat dedifferentiated epithelium (fig. 1A, inset d) and/or obstructed by large protein casts (fig. 1A, inset e). Very focally, cortical tubules, dilated or not, were lined by dark basophilic cells (data not shown). Focal tubular necrosis with denudation of the tubular basement membrane and obstruction of the tubular lumen by cellular debris (fig. 1A, inset f) were also observed. A few atrophic or dilated tubules were surrounded by a thick basement membrane (fig. 1A, inset g). Interestingly, cell exfoliation was observed in tubular lumen (fig. 1A, inset h). Using specific markers, we showed that tubular dilations developed in all the segments of the nephron, even though they predominantly arose from proximal tubules (fig. 1B). Quantification confirmed that about 72% of the cysts were from proximal tubules, 22% from Henle's loop, and only 6% arose from collecting ducts (fig. 1B).

Acquired Tubular Dilations Are Associated with Normal Cell Polarity, but Increased Proliferation

We next tried to elucidate the cellular mechanisms involved in the development of ACKD. To this aim, we first focused on events known to induce cyst development in inherited cystic diseases. Immunohistochemical analyses revealed that, at variance with hereditary cystic disease, the apico-basal polarity was preserved after Nx. In fact, we observed that megalin, uromodulin (Tamm Horsfall

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Fig. 1. Tubular dilations after nephron reduction recapitulate many features of ACKD. **A** Morphology of kidneys from 75% nephrectomized (Nx) FVB/N mice visualized by PAS staining, 2 months after surgery. Insets: (**a**) Tubular lesions, (**b**) Apical accumulation of PAS-positive protein droplets in proximal tubular cells (arrow), (**c**) Dilated tubules with a brush border lined by microvacuolated cells (arrows), (**d**) Dilated tubules bordered by a flat and dedifferentiated epithelium (arrow), (**e**) Dilated tubules obstructed by proteinuria (asterisk), (**f**) Dilated tubules obstructed by cellular debris (arrow), (**g**) Dilated tubules surrounded by a thick basement membrane, (**h**) Cell exofoliation in dilated tubules. Magnification: ×200 (**a**) and ×600 (**b–h**). **B** Localization of microcyst dilations in kidneys from 75% nephrectomized (Nx) FVB/N mice, 2 months after surgery, by immunohistochemistry (left panels) using antibodies directed against specific markers of proximal tubules (PT; LTL: Lotus Tetragonolobus Lectin), Henle's loops (HL; TH: Tamm-Horsfall) and collecting ducts (CD; AQP2: Aquaporin 2), and quantification (right panel) of the percentage nephron segments depicting tubular dilations. Magnification: ×200. Pictures are representative samples from 10 Nx mice.
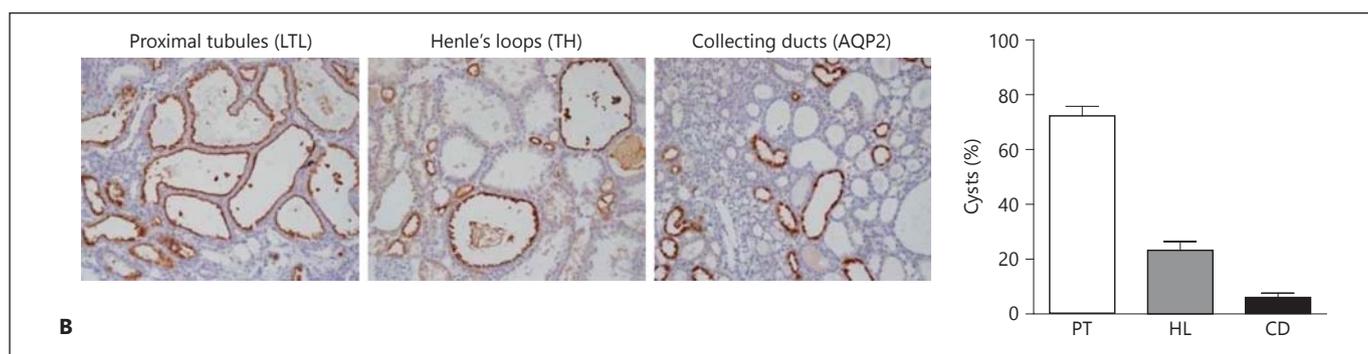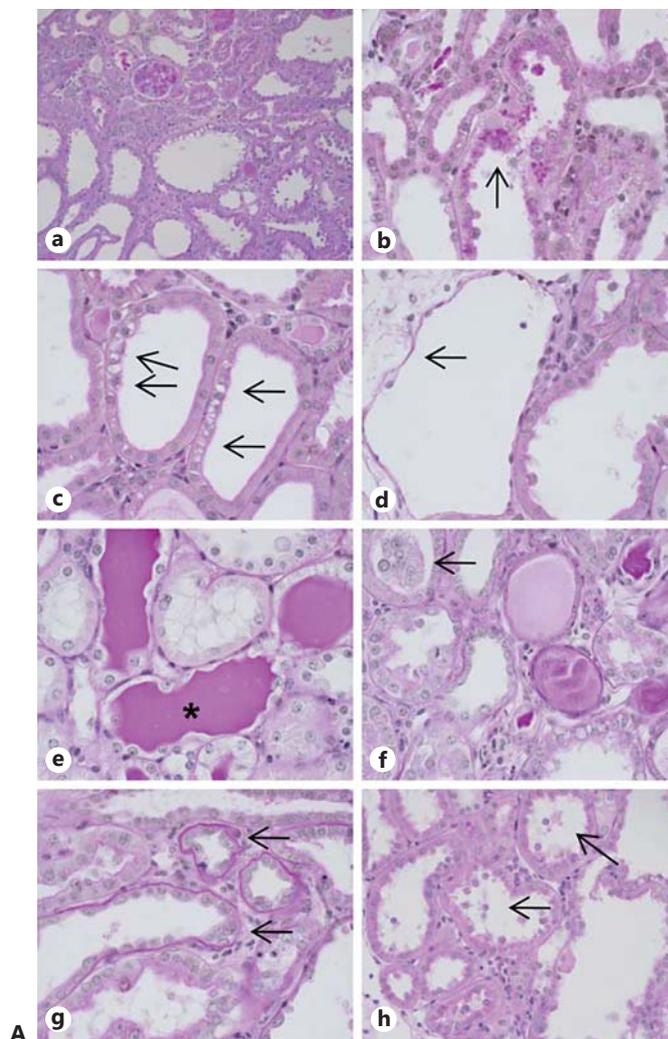
protein), and aquaporin-2 were expressed at the apical membrane of proximal tubules, thick ascending limb of Henle's loop and collecting ducts, respectively (fig. 2a), whereas Na/K ATPase was still expressed at the baso-lateral membrane (fig. 2a). Similarly, acetylated tubulin staining revealed that the primary cilia were still present after Nx (fig. 2b) and that their morphology appeared normal even in the most dilated tubules (fig. 2b). In contrast, we observed that tubular cell proliferation was dramatically increased after Nx (fig. 2c).
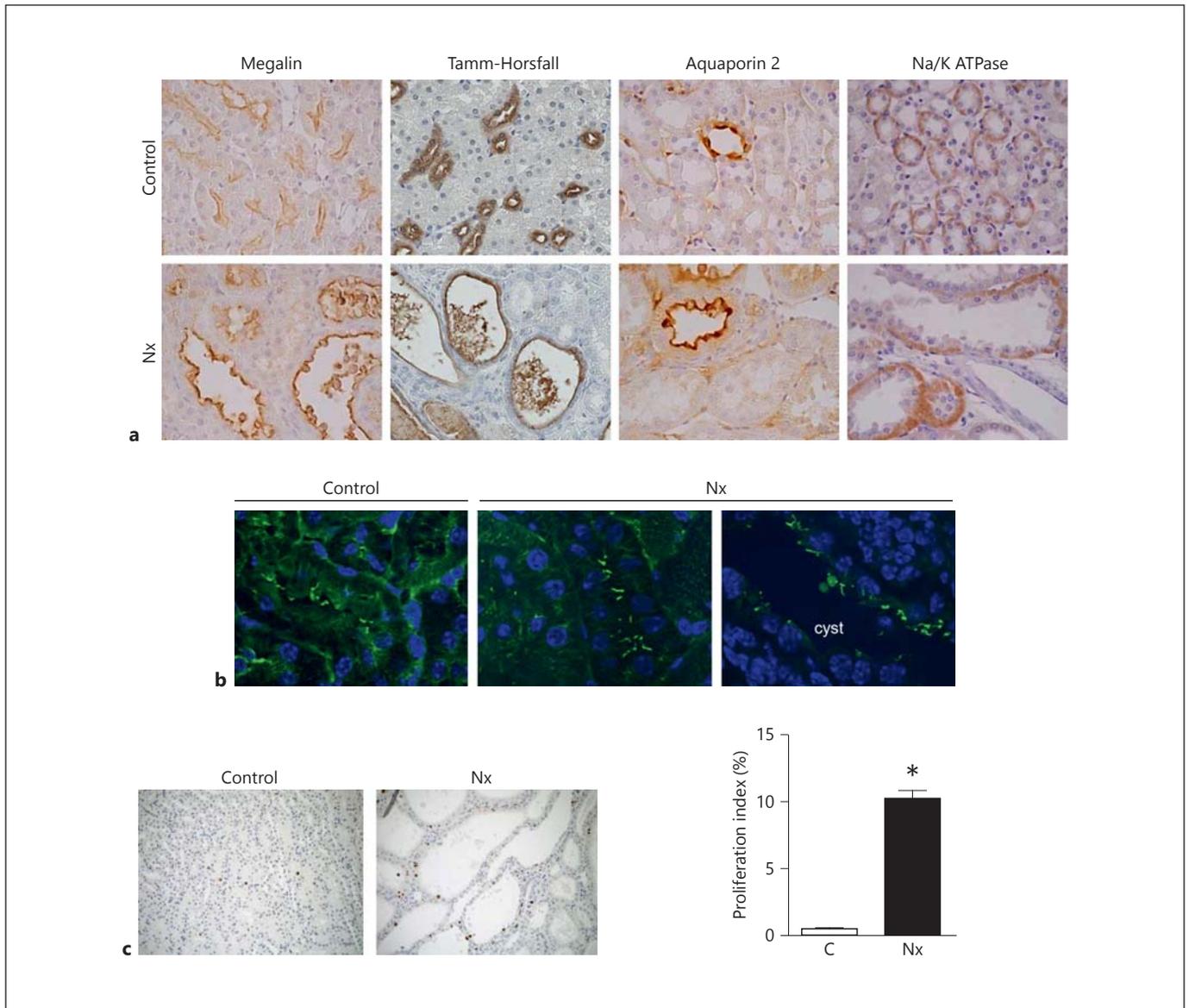
Fig. 2. Cell proliferation, but not dedifferentiation, is associated with tubular dilations after nephron reduction. **a** Megalin, Tamm-Horsfall, Aquaporin 2, and Na/K ATPase expression evaluated by immunohistochemistry in sham-operated (Control) and 75% nephrectomized (Nx) FVB/N mice, 2 months after surgery. Magnification: ×600. **b** Primary cilium visualized by immunofluorescence using anti-acetylated tubulin antibodies, 2 months after nephron reduction. Primary cilia (green) are still present and mor-

phologically normal after nephron reduction, even in the most dilated tubules (cyst). Magnification: ×1,000. **c** Ki-67 staining and quantification of tubular cell proliferation in kidneys from control and Nx FVB/N mice, 2 months after surgery. Magnification: ×200. Data are means ± SEM; n = 4–6 and 6–10 for control and Nx mice, respectively. Mann-Whitney test; control versus Nx mice: * p < 0.05.

Cystic Genes Expression Is Altered in Acquired Cyst Formation

Then, we wondered if the expression of genes involved in hereditary PKD was modified after Nx. Real-time quantitative RT-PCR revealed that the expression of *Pkd1* and

Pkhd1 was significantly increased, while that of *Pkd2* was decreased 2 months after Nx (fig. 3a). We also analyzed the expression of other genes known to be involved in cystic diseases: *Nphp1, 2, 3* and *4*, *Cystin 1*, *Hnf1b*, and *Ift88*. Interestingly, over the seven genes studied, only *Nphp2*
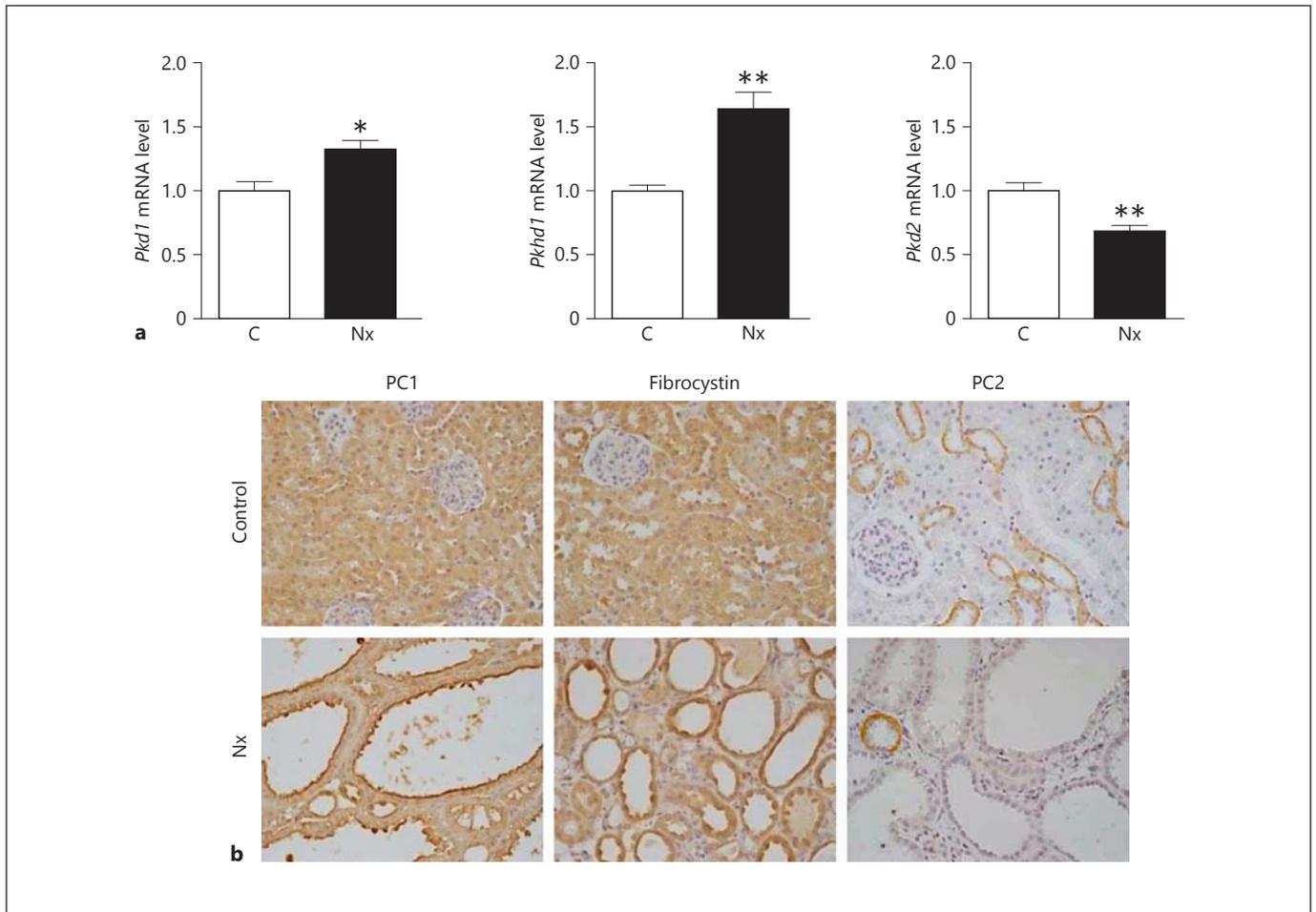
Fig. 3. Cystic disease gene expression is modified after nephron reduction. **a**, **b** Polycystin 1 (PC1), fibrocystin and polycystin 2 (PC2) expression evaluated by (**a**) real-time RT-PCR and (**b**) immunohistochemistry in kidneys from sham-operated (C, Control) and 75% nephrectomized (Nx) FVB/N mice, 2 months after surgery. Pictures are representative samples from at least 6 mice from each group. ×400. Data are means ± SEM; n = 6 and 6–10 for control and Nx mice, respectively. Mann-Whitney test; control versus Nx mice: * p < 0.05, ** p < 0.01.

was slightly decreased after Nx (online suppl. fig. 2), suggesting a specific regulation of cystic genes during ACKD.

Immunohistochemistry revealed that the expression of PC1 and fibrocystin was almost undetectable in sham-operated mice, whereas PC2 was very highly expressed (fig. 3b). Interestingly, the expression pattern was reversed after Nx. In fact, while PC1 and fibrocystin were markedly increased, PC2 was significantly decreased in cells lining the dilated tubules of remnant kidneys 2 months after Nx. Both PC1 and fibrocystin were mainly expressed at the apical membrane of dilated tubules (fig. 3b). In addition, while PC2 expression was turned off in cyst-lining cells, it was still maintained in the few non-dilated tubules of damaged kidneys.

Pkd1 Haploinsufficiency Increases Susceptibility to Renal Damage Following Nephron Reduction

To assess the contribution of cystic genes to ACKD, we examined the impact of Nx in *Pkd1*$^{+/-}$ mice [14]. As *Pkd1*$^{+/-}$ mice were on a mixed genetic background (C57BL/6x129/Sv) that we previously showed to be resistant to Nx [13, 18], we crossed these mice with the FVB/N lesion-prone mice in order to generate a more sensitive strain. In fact, we previously showed that F1 males originated from C57BL/6XFVB/N crosses are sensitive to Nx. In sham-operated mice, we observed no gross differences of renal morphology between *Pkd1*$^{+/+}$ and *Pkd1*$^{+/-}$ mice (data not shown). As expected, after Nx, wild-type mice developed renal lesions, although less severe than
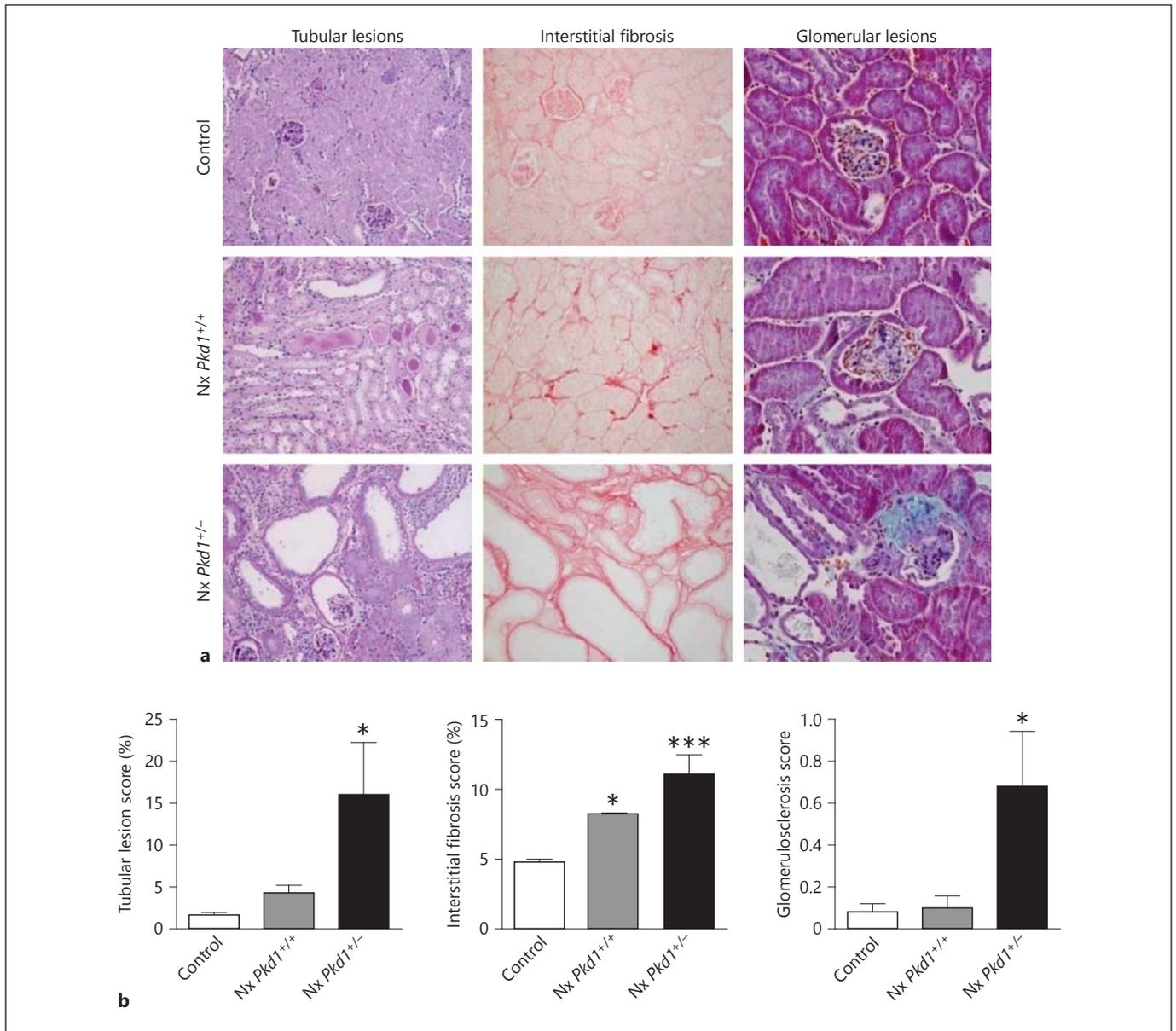
Fig. 4. *Pkd1* haploinsufficiency worsens renal lesions after nephron reduction. **a** Morphology of kidneys from control (upper panels) and 75% nephrectomized (Nx) *Pkd1*$^{+/+}$ (middle panels) and *Pkd1*$^{+/-}$ (lower panels) mice, 4 months after surgery. Magnification: ×200, ×200, ×400 for tubular, interstitial and glomerular lesions, respectively. Because no differences were detected between wild-type and mutant control mice, only one group is shown. **b** Quantification of the kidney lesions in the different experimental groups. Data are means ± SEM; n = 3 and 6 for each control and Nx mice, respectively. ANOVA followed by Tukey-Kramer test; control versus Nx: * $p < 0.05$, *** $p < 0.001$.

those observed in the pure FVB/N genetic background (fig. 4a). The renal lesions were scattered and included tubular dilations and interstitial fibrosis, but no glomerular lesions. Interestingly, the frequency and severity of renal lesions were increased in mice lacking PC1 (fig. 4a). Quantification revealed that *Pkd1*$^{+/-}$ mice had significantly higher scores of tubular, glomerular, and interstitial damages compared to *Pkd1*$^{+/+}$ littermates (fig. 4b). Consistent with these findings, kidney weight was significantly higher in *Pkd1*$^{+/-}$ mice than in *Pkd1*$^{+/+}$ littermates (online suppl. fig. 3). Immunohistochemistry revealed that tubular dilations originated predominantly
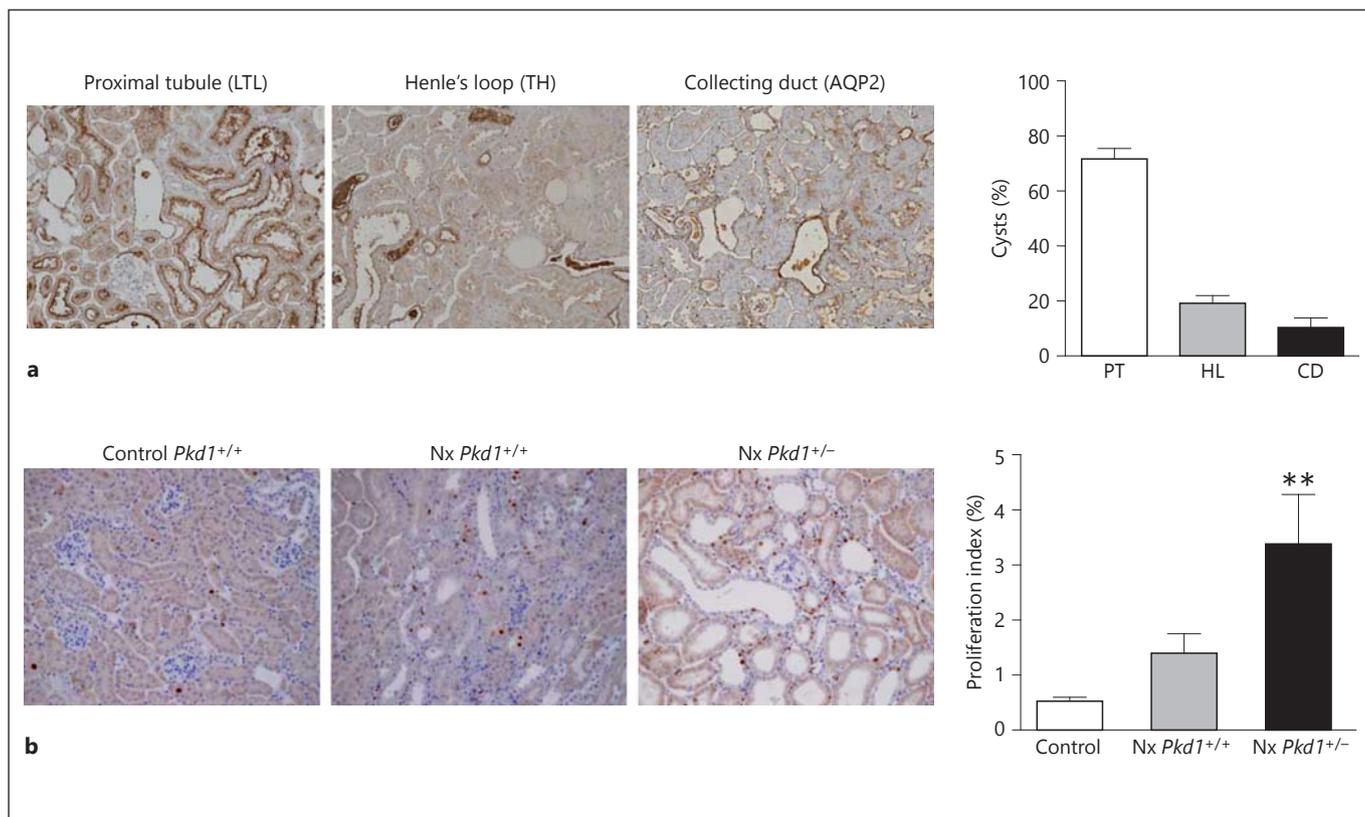
Fig. 5. *Pkd1* haploinsufficiency results in increased tubular proliferation in *Pkd1*$^{+/-}$ mice after nephron reduction. **a** Immunohistochemistry (left panels) using antibodies directed against specific markers of proximal tubules (LTL: Lotus Tetragonolobus Lectin), Henle's loops (TH: Tamm-Horsfall) and collecting ducts (AQP2: Aquaporin 2), and quantification (right panel) of the percentage of nephron segments depicting tubular dilations. Magnification: ×200. **b** Ki-67 staining (left panels) and quantification (right panel) of tubular proliferation in kidneys from control and 75% nephrectomized (Nx) *Pkd1*$^{+/+}$ and *Pkd1*$^{+/-}$ mice, 4 months after surgery. Because no differences were detected between wild-type and mutant control mice, only one group is shown. Magnification: ×200. Data are means ± SEM; n = 3 and 6 for each control and Nx mice, respectively. ANOVA followed by Tukey-Kramer test; control versus Nx mice: ** p < 0.01.

from proximal tubules, even though they were found in all the segments of the nephron (fig. 5a). Interestingly, tubular cell proliferation was much higher after Nx in kidneys of *Pkd1*$^{+/-}$ mice as compared to *Pkd1*$^{+/+}$ littermates (fig. 5b). Of note, *Pkd1* haploinsufficiency did not induce any prompt tubular cell proliferation in control sham-operated mice.

Cystic Genes Expression and Cell Proliferation Are Modified in Human ACKD

Finally, we investigated if these finding were relevant to human ACKD. Immunohistochemistry showed a strong increase of PC1, fibrocystin, and PC2 expression in cystic kidneys from patients with end-stage renal disease as compared to controls (fig. 6a). A careful analysis revealed that these proteins were overexpressed specifi-

cally in cysts, but not in controls. Interestingly, tubular cell proliferation was also markedly increased in cystic human kidneys as compared to control kidneys (fig. 6b).

Discussion

The mechanisms underlying ACKD are still unknown, notably because there is no animal model able to recapitulate this pathology. In this study, we took advantage of an experimental model (subtotal nephrectomy) that we previously showed to induce severe tubular dilations when applied to FVB/N mice to show that the mechanisms involved in inherited PKD might also play a role in ACKD. In particular, we observed that an increase of cell proliferation as well as an imbalance among cystic genes expres-
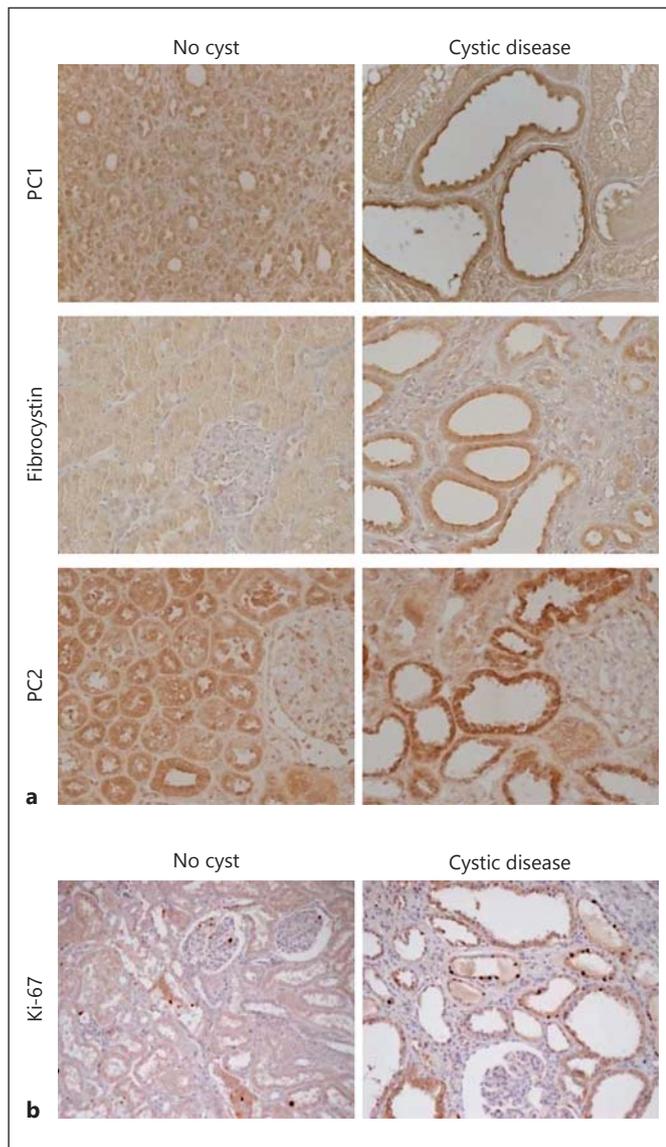
Fig. 6. Cystic disease genes expression and tubular cell proliferation in human ACKD. **a** Polycystin 1 (PC1), fibrocystin and polycystin 2 (PC2) protein expression evaluated by immunohistochemistry in kidneys from controls and ACKD patients. Magnification: ×400. **b** Ki-67 staining of tubular cell proliferation in kidneys from controls and cystic patients. Magnification: ×200.

sion accompanied the development of tubular dilations after nephron reduction. Interestingly, *Pkd1* haploinsufficiency lead to increased tubular dilations and higher proliferation following nephron reduction. These results were relevant to human ACKD, as cystic genes and proliferation were also increased in cystic kidneys. It is worth noting that our experimental model reproduced most of the morphological changes previously reported in human ACKD:

cysts that arose mainly from proximal tubules were surrounded by a flat epithelium and were hyperproliferative [19, 20]. Taken together, these results showed that cystic genes are critically involved in ACKD and uncovered a novel model for elucidating the pathophysiology of ACKD.

An increasing number of evidence supports the idea of a gene-dosage-dependent mechanism for ADPKD where mutations of loss of heterozygosity, haploinsufficiency, or even an overexpression could lead a cystic phenotype [21]. In the present study, we found that the development of tubular dilations following nephron reduction is associated with a particular pattern of cystic gene expression. In fact, if most of the genes were not affected, the expression of PC1 and fibrocystin were selectively increased in cystic-lining cells, whereas that of PC2 was decreased. Interestingly, PC1 expression has been found selectively increased even in cysts of both humans and experimental models of ADPKD [22–24], suggesting that an imbalance or deregulation of PC1 is sufficient to prompt cyst development. Since PC1 and PC2 have been shown to act in the same molecular complex, it is likely that the stoichiometry of the two proteins is essential for their function.

The observation that, despite the dramatic increase of PC1 in dilated tubules, *Pkd1* haploinsufficiency worsened the progression of the disease following nephron reduction is quite interesting. In fact, from results obtained in transgenic mice overexpressing *Pkd1* [22], one could expect that PC1 overexpression participates to lesion development. However, we observed that *Pkd1* inactivation increased both tubular cell proliferation and tubular dilations. Hence, it is tempting to speculate that PC1 overexpression could rather act as a compensatory pathway activated in order to counteract critical events of the cystogenic process, that is, cell proliferation. In favor of this hypothesis, a recent study suggested that PC1 and PC2 may act by inhibiting a cilia-dependent cyst growth-promoting pathway [25]. Disruption of cilia in a context of adult-onset ADPKD reduces cyst growth in complementary models of transgenic mice [25]. In this context, the rate of cell proliferation is higher in cilia devoid of polycystins. Consistent with these results, we have observed that the morphological appearance of cilia appeared normal in our model and that cell proliferation was increased in tubules of $Pkd1^{+/-}$ mice following nephron reduction.

It has been suggested that, in inherited PKD, the translocation of transporters from the basal to the apical membrane may participate to cysts enlargement through an increase of fluid secretion [7, 26]. In the present study, we failed to detect any change of the apico-basal polarity in

cells lining tubular dilations. It is possible that this mechanism is not involved in ACKD. Alternatively, we cannot exclude that at a later stage, fluid secretion might be critically involved in cyst enlargement. Moreover, we cannot argue against the possibility that the location of other transporters known to be involved in the transepithelial secretion of chloride in PKD might be affected [27, 28].

In conclusion, our study revealed that the mechanisms involved in cyst formation in heredity PKD, that is, cell proliferation and cystic gene-dosage, may also play a role in ACKD. In this context, PC1 may act by inhibiting cell proliferation. Together these results indicate that the huge efforts made to develop new therapies for PKD might be beneficial for all those patients who develop cysts in postnatal life, including patients with chronic kidney disease.

References

1 Dunnill MS, Millard PR, Oliver D: Acquired cystic disease of the kidneys: a hazard of long-term intermittent maintenance haemodialysis. J Clin Pathol 1977;30:868–877.

2 Matson MA, Cohen EP: Acquired cystic kidney disease: occurrence, prevalence, and renal cancers. Medicine (Baltimore) 1990;69:217–226.

3 Hughes J, et al: The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. Nat Genet 1995;10:151–160.

4 Qamar S, Vadivelu M, Sandford R: TRP channels and kidney disease: lessons from polycystic kidney disease. Biochem Soc Trans 2007;35:124–128.

5 Nagasawa Y, et al: Identification and characterization of Pkhd1, the mouse orthologue of the human ARPKD gene. J Am Soc Nephrol 2002;13:2246–2258.

6 Torres VE, Harris PC: Strategies targeting cAMP signaling in the treatment of polycystic kidney disease. J Am Soc Nephrol 2014;25:18–32.

7 Stoops EH, Caplan MJ: Trafficking to the apical and basolateral membranes in polarized epithelial cells. J Am Soc Nephrol 2014;25:1375–1386.

8 Verdeguer F, et al: A mitotic transcriptional switch in polycystic kidney disease. Nat Med 2010;16:106–110.

9 Jonassen JA, et al: Deletion of IFT20 in the mouse kidney causes misorientation of the mitotic spindle and cystic kidney disease. J Cell Biol 2008;183:377–384.

10 Patel V, et al: Acute kidney injury and aberrant planar cell polarity induce cyst formation in mice lacking renal cilia. Hum Mol Genet 2008;17:1578–1590.

11 Davenport JR, et al: Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. Curr Biol 2007;17:1586–1594.

12 Lin F, et al: Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. Proc Natl Acad Sci U S A 2003;100:5286–5291.

13 Pillebout E, et al: Proliferation and remodeling of the peritubular microcirculation after nephron reduction: association with the progression of renal lesions. Am J Pathol 2001;159:547–560.

14 Muto S, et al: Pioglitazone improves the phenotype and molecular defects of a targeted Pkd1 mutant. Hum Mol Genet 2002;11:1731–1742.

15 Terzi F, et al: Reduction of renal mass is lethal in mice lacking vimentin. Role of endothelin-nitric oxide imbalance. J Clin Invest 1997;100:1520–1528.

16 Pillebout E, et al: JunD protects against chronic kidney disease by regulating paracrine mitogens. J Clin Invest 2003;112:843–852.

17 Laouari D, et al: TGF-alpha mediates genetic susceptibility to chronic kidney disease. J Am Soc Nephrol 2011;22:327–335.

18 Laouari D, et al: A transcriptional network underlies susceptibility to kidney disease progression. EMBO Mol Med 2012;4:825–839.

19 Vandeursen H, et al: Acquired cystic disease of the kidney analyzed by microdissection. J Urol 1991;146:1168–1172.

20 Costa MZ, Bacchi CE, Franco M: Histogenesis of the acquired cystic kidney disease: an immunohistochemical study. Appl Immunohistochem Mol Morphol 2006;14:348–352.

21 Fedeles SV, Gallagher AR, Somlo S: Polycystin-1: a master regulator of intersecting cystic pathways. Trends Mol Med 2014;20:251–260.

22 Thivierge C, et al: Overexpression of PKD1 causes polycystic kidney disease. Mol Cell Biol 2006;26:1538–1548.

23 Burtey S, et al: Overexpression of PKD2 in the mouse is associated with renal tubulopathy. Nephrol Dial Transplant 2008;23:1157–1165.

24 Kurbegovic A, et al: Pkd1 transgenic mice: adult model of polycystic kidney disease with extrarenal and renal phenotypes. Hum Mol Genet 2010;19:1174–1189.

25 Ma M, et al: Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. Nat Genet 2013;45:1004–1012.

26 Wilson PD, et al: Apical plasma membrane mispolarization of NaK-ATPase in polycystic kidney disease epithelia is associated with aberrant expression of the beta2 isoform. Am J Pathol 2000;156:253–268.

27 Brill SR, et al: Immunolocalization of ion transport proteins in human autosomal dominant polycystic kidney epithelial cells. Proc Natl Acad Sci U S A 1996;93:10206–10211.

28 Terryn S, et al: Fluid transport and cystogenesis in autosomal dominant polycystic kidney disease. Biochim Biophys Acta 2011;1812:1314–1321.