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Determination of urinary lithogenic parameters in murine models orthologous to Autosomal Dominant Polycystic Kidney Disease

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD), a genetic disease caused by mutations in PKD1 gene, is associated with a high prevalence of nephrolithiasis. The underlying mechanisms may encompass structural abnormalities resulting from cyst growth, urinary metabolic abnormalities or both. An increased frequency of hypocitraturia has been described in ADPKD even in the absence of nephrolithiasis, suggesting that metabolic alterations may be associated with ADPKD per se. We aimed to investigate whether non-cystic *Pkd1*-haploinsufficient (*Pkd1*^{+/-}) and/or nestin-Cre *Pkd1*-targeted cystic (*Pkd1*^{cond/cond};Nestin^{cre}) mouse models develop urinary metabolic abnormalities potentially related to nephrolithiasis in ADPKD. Twenty-four hour urine samples were collected during 3 non-consecutive days from 10–12 and 18–20 week-old animals. At 10–12 weeks of age, urinary oxalate, calcium, magnesium, citrate and uric acid did not differ between test and their respective control groups. At 18–20 weeks, *Pkd1*^{+/-} showed slightly but significantly higher urinary uric acid vs controls while cystic animals did not. Conclusion: The absence of hypocitraturia, hyperoxaluria and hyperuricosuria in the cystic model at both ages and the finding of hyperuricosuria in the 18–20 week-old animals suggest that anatomic cystic distortions per se do not generate the metabolic disturbances described in human ADPKD-related nephrolithiasis, while *Pkd1* haploinsufficiency may contribute to this phenotype in this animal model.

Keywords

ADPKD; Nephrolithiasis; *Pkd1* haploinsufficiency; Renal cysts; Uric acid; Urinary analytes

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Conflict of interest

The authors declare no conflict of interest.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic diseases in humans, with an estimated prevalence of 0.1% in the general population [1]. This disease is characterized by a progressive replacement of renal tubules by cysts, following a gradual process that results in end-stage renal disease in about half of the patients by the 6th decade of life [2]. In addition to the continuous enlargement of cysts that contribute to the loss of renal function, other manifestations also affect the kidneys, including hypertension, urinary tract infection, urinary concentrating deficit, hematuria, acute or chronic flank and abdominal pain and nephrolithiasis [3–6].

Nephrolithiasis reaches 20–36% of the ADPKD patients, an incidence much higher than in the general population [7, 8]. The occurrence of this manifestation is apparently ascribed to structural abnormalities resulting from cyst growth, leading to disruption of the corticomedullary architecture [6, 9–11]. Grampsas et al [9] reported that ADPKD kidneys with stones exhibited a significantly higher number of cysts and a larger predominant cyst size than cystic kidneys without stones. In a stepwise multivariate logistic regression analysis, we have previously shown that a renal volume above 500 mL was a significant predictor of nephrolithiasis in patients with ADPKD and normal renal function, after adjustments for age and hypertension [6]. Taken together, these findings suggested that larger kidneys from patients with ADPKD were more prone to develop stones, probably due to intrarenal anatomic obstruction. On the other hand, we and others have found an increased frequency of hypocitraturia in ADPKD [6, 7, 9], along with other metabolic abnormalities.

ADPKD results from mutations in either *PKD1* gene, which encodes polycystin-1 (PC1), or *PKD2*, whose protein product is polycystin-2 (PC2). Although all ADPKD tubular epithelial cells harbor a *PKD1* or *PKD2* germline mutation in one allele, renal cysts develop in only 1% of the nephrons, since the formation of at least most of them depends on a somatic mutation in the previously normal allele. It has been shown in mice, however, that the inactivation of both *Pkd1* alleles in mature kidneys does not lead to rapid and significant renal cystogenesis [12, 13]. On the other hand, in this case an ischemia/reperfusion renal insult acts as an additional hit, leading to rapid and intense cyst formation [14].

Polycystins are essential for the maintenance of differentiated, polarized, predominantly reabsorptive tubular epithelial phenotypes, as well as for low rates of proliferation and apoptosis. Their functional disruption results in a partially dedifferentiated phenotype, characterized by abnormal intracellular trafficking and protein targeting, excessive fluid secretion, and excessive proliferation and apoptosis [15]. The potential role of *Pkd1* haploinsufficiency on urinary excretion of analytes remains however, essentially unknown. In fact, while the cyst-lining cells present a secretory phenotype, driven by the transepithelial secretion of chloride, the non-cystic tubular cells maintain the reabsorptive phenotype. It is largely unclear, however, whether this reabsorptive pattern harbors defects that could lead to a pro-lithiasis urinary metabolic profile. Grantham et al [16] suggest that cysts prevent the drainage of urine from upstream tributaries, which leads to tubule atrophy and loss of functioning kidney parenchyma by mechanisms similar to those found in ureteral

obstruction. The contribution of cysts to an altered tubular reabsorption, however, also remains unclear.

In human ADPKD the putative renal tubular derangement cannot be distinguished on whether it occurs due to the presence of the germline mutation in the tubular cells or to functional consequences of cyst development following mutations in both copies of the gene. In the current study, we decided to investigate these possible components using genetically modified animal models that could separate each of the scenarios: a *Pkd1*-haploinsufficient non-cystic and a *Pkd1*^{cond/cond};Nestin^{cre} cystic mouse. Both models were submitted to a systematic evaluation of lithogenic parameters.

METHODS

Mouse models

The generation of the cystic model was based on a *Pkd1* floxed allele (*Pkd1*^{cond}) with lox P sites inserted in introns 1 and 4 and a neomycin cassette flanked by FRT sites inserted in intron 1 [17]. By breeding with nestin-Cre transgenic animals, we have produced *Pkd1*^{cond/cond};Nestin^{cre} and *Pkd1*^{cond/cond} animals with the C57BL/6 genetic background. The presence of *Pkd1*^{cond} alleles and the nestin-Cre transgene was determined using two specific PCR reactions [12]. *Pkd1*^{cond/cond};Nestin^{cre} mice develop a renal cystic phenotype that resembles human ADPKD, while their non-cystic *Pkd1*^{cond/cond} littermates were employed as controls.

The *Pkd1*-haploinsufficient model, in turn, was generated on a 129Sv background, based on the construction of a *Pkd1* knockout allele with replacement of part of exon 2 and the entire exon 3 with *lacZ*, followed by the neomycin resistance gene [17]. Genotyping was performed using a three-primer PCR strategy. *Pkd1*^{+/-} mice display virtually no renal cysts by 15 weeks of age, representing a pure *Pkd1*-haploinsufficiency model. Wild-type littermates (*Pkd1*^{+/+}) were utilized as controls. This study was performed in accordance with international standards of animal care and experimentation.

Biochemistry and histological analyses

The animals were housed in metabolic cages and three non-consecutive 24-h urine samples were collected under vaseline in a single recipient, on two distinct periods: 10th, 11th and 12th weeks, representing early-phase ADPKD for *Pkd1*^{cond/cond};Nestin^{cre} animals; and 18th, 19th and 20th weeks, representing a more advanced stage of cystic disease in this model, but still with preserved renal function.

The samples were divided into four aliquots, posteriorly kept at -20°C: one without addition of any preservative, for creatinine determination; two with the addition of 6N HCl (20 mL/L), for determination of oxalate, calcium, magnesium and citrate; and one with added NaHCO₃ (5 g/L), for determination of uric acid as previously described [18]. All urinary determinations were performed in a pool of the three collections obtained from individual mice in the 10th, 11th and 12th weeks and also from the other set of mice in each week as well (18–20 weeks). Blood samples were drawn by retro-orbital bleeding, allowed to clot and centrifuged to obtain serum at the third week of each period. The kidneys were then

removed under lethal dose of ketamine and xylazine, cut longitudinally, fixed with 4% buffered paraformaldehyde and stained using hematoxylin and eosin for histological analysis. Crystal deposition in renal tissue was searched under polarized light microscopy.

Calcium and magnesium were determined in an Olympus AU 400 Fully Automated Analyser (Olympus Corporation, Tokyo, Japan); oxalate was determined by enzymatic method [19] using the Sigma Oxalate Diagnostic Kit (Sigma Chemical Co., St. Louis, MO, USA); and citrate was measured using the citrate lyase methodology [20]. Uric acid was determined by the uricase method [21]. Serum urea was determined according to Crocker's protocol (Celm, Barueri, Brazil) [22], whereas serum and urinary creatinine values were measured using a colorimetric assay (Labtest, Lagoa Santa, Brazil) [23].

Statistical analysis

All parameters were submitted individually to a normality test (*Kolmogorov-Smirnov*) and parametric or non-parametric tests were employed according to the distribution of each variable. All statistical analyses were performed considering a significance level of $p < 0.05$ and a confidence level of 95% was considered appropriate.

RESULTS

The comparison of the haploinsufficient groups with their respective controls disclosed no statistical difference regarding mean body weight of *Pkd1*^{+/-} versus *Pkd1*^{+/+} groups aged 10–12 weeks, 19.8 ± 0.8 g vs 19.4 ± 0.4 g, nor the ones aged 18–20 weeks, 24.7 ± 1.7 g vs 25.2 ± 0.9 g, respectively (data not shown in Tables). No statistical differences were also observed when comparing the body weight of cystic *Pkd1*^{cond/cond}:Nestin^{cre} versus *Pkd1*^{cond/cond} at 10–12 weeks (19.8 ± 2.1 g vs 19.3 ± 0.8 g). Conversely, the mean body weight of cystic animals *Pkd1*^{cond/cond}:Nestin^{cre} aged 18–20 weeks was significantly higher than their controls *Pkd1*^{cond/cond} (30.1 ± 3.5 g vs 25.7 ± 1.5 g, $p = 0.004$).

Table 1 shows the mean serum and the remaining urinary parameters of haploinsufficient mice aged 10–12 weeks and their controls, whereas Table 2 shows the results of cystic groups and their controls. At the end of the study, serum urea was significantly higher in *Pkd1*^{cond/cond}:Nestin^{cre} animals compared with their *Pkd1*^{cond/cond} controls ($p = 0.019$), whereas serum creatinine was not. Urinary oxalate was lower in *Pkd1*^{cond/cond}:Nestin^{cre} vs *Pkd1*^{cond/cond} ($p = 0.01$) and urinary creatinine was slightly higher in *Pkd1*^{cond/cond}:Nestin^{cre} vs *Pkd1*^{cond/cond} ($p = 0.04$). Urinary citrate and other parameters did not significantly differ between the groups. The mean serum and urinary parameters of mice aged 18–20 weeks are shown in Tables 2A and 2B, for the results of haploinsufficient and cystic groups and their controls, respectively. At the end of the study, serum urea was significantly higher in *Pkd1*^{cond/cond}:Nestin^{cre} vs. *Pkd1*^{cond/cond} ($p = 0.04$), but serum creatinine did not change. The *Pkd1*^{+/-} group presented a significantly higher urinary uric acid vs *Pkd1*^{+/+} ($p = 0.03$). In a recently published study [24], we showed that the total kidney weight (TKW)/body weight (BW) ratio was higher in *Pkd1*^{cond/cond}:Nestin^{cre} than *Pkd1*^{cond/cond} mice at 15 weeks of age (1.869 ± 0.289 vs 1.629 ± 0.167 ; $p < 0.05$) and at 24 weeks (2.373 ± 0.649 vs 1.636 ± 0.165 ; $p < 0.05$). Conversely, TKW/BW did not differ between *Pkd1*^{+/-} and *Pkd1*^{+/+} mice at 15 weeks of age (1.964 ± 0.196 vs 1.816 ± 0.217 ; NS). Comparisons between

Pkd1^{cond/cond};Nestin^{cre} or *Pkd1^{cond/cond}* (Table 1B or 2B) with *Pkd1^{+/+}* or *Pkd1^{+/-}* mice (Table 1A or 2A) could not be performed, since the two models were generated on different genetic backgrounds.

Neither crystals nor calculi were found in the renal tissue under polarized light microscopy of both analyzed models. Representative histological images of cystic, *Pkd1*-haploinsufficient and their respective control kidneys are displayed in Figure 1, showing the absence of cysts in *Pkd1^{+/-}* and control mouse kidneys.

DISCUSSION

The causes of a higher prevalence of nephrolithiasis in individuals affected by ADPKD compared to the general population are not yet fully understood. In a retrospective evaluation of ADPKD patients with nephrolithiasis and preserved renal function, Torres et al [7] observed hyperoxaluria in 6/31 (19.4%) and hypocitraturia, the most frequent abnormality, in 10/15 (67%) of the patients, suggesting that metabolic factors might be relevant for stone formation associated with ADPKD. Hyperuricosuria and hypercalciuria were detected in 15% and 11% in a retrospective analysis by the same group [25]. In a prospective evaluation by our group, Nishiura et al [6] detected hyperoxaluria in 18% and hyperuricosuria in 11% of ADPKD cases associated with nephrolithiasis, whereas hypercalciuria was absent. Grampsas et al [9] showed that urinary volumes and urinary magnesium were significantly lower. In addition, 60% of ADPKD patients with associated nephrolithiasis and 49% without it were found to have hypocitraturia [9]. Similarly, hypocitraturia was observed in 15 out of 28 (54%) of ADPKD patients with nephrolithiasis, and in 34/70 (48%) without nephrolithiasis in the series described by Nishiura et al [6]. Whether hypocitraturia is a genetically inherent abnormality in ADPKD patients or is acquired secondarily to disease progression and/or derangements in renal tubular handling, however, could not be determined in these human studies.

The present investigation aimed to evaluate if decreased PC1 levels or distortions in kidney architecture due to the presence of cysts could induce urinary metabolic abnormalities potentially implicated in stone formation. Since in human ADPKD one cannot differentiate the effects of each of these abnormalities upon renal tubular derangements and potential urinary metabolic alterations, in the current study we employed two genetically modified mouse models capable of separating each of the abnormal realities: non-cystic *Pkd1*-haploinsufficient and *Pkd1^{cond/cond};Nestin^{cre}* cystic mice.

The mean values of urinary citrate and magnesium were not lower in haploinsufficient mice (*Pkd1^{+/-}*) nor in cystic animals (*Pkd1^{cond/cond};Nestin^{cre}*) when compared with their respective controls, in both early (10–12 weeks) and more advanced stages (18–20 weeks) of the disease. In Han:SPRD-cy rats, a non-orthologue polycystic kidney disease model, an increased rather than decreased urinary citrate excretion has been observed [26]. The authors speculated that a chloride-dependent secretion from tubular epithelium, favoring intracellular chloride loss, could raise the pH and inhibit the citrate metabolism. Unexpected higher levels of urinary citrate were also detected by Tanner et al [27] in Han:SPRD-cy rats with reduced GFR, a finding that significantly differs from our model. It must be noted, however, that in

our case the animals have preserved renal function. Using orthologue mouse models to ADPKD1, therefore, a strategy that allowed us to distinguish the two main *Pkd1*-deficiency profiles, we were able to show that neither *Pkd1* haploinsufficiency nor the cystic phenotype was consistent *per se* to represent the underlying mechanism for hypocitraturia observed in human ADPKD. Whether an altered content or excretion of citrate and/or other organic anion in rodent polycystic kidney disease may occur, differing from the observed pattern in human ADPKD, could not be addressed by the present study.

The mean urinary oxalate and calcium values did not statistically differ between haploinsufficient *Pkd1*^{+/-} and their controls, both at 10–12 and 18–20 weeks of age. The lower oxaluria detected in the cystic *Pkd1*^{cond/cond}:Nestin^{cre} aged 10–12 weeks vs controls could have been ascribed to a higher mean creatininuria observed in this group, since oxalate has been expressed per urinary creatinine. This higher mean creatininuria could not be ascribed to differences in body weight, which have not been detected between cystic and control groups at 10–12 weeks of age. The reasons for this slightly higher mean urinary creatinine hence remained unexplained. Anyway, it seems that if urinary oxalate had not been biased by a higher creatininuria, it probably would have been similar but not higher than the control group. Accordingly, the data herein disclosed did not reproduce the findings of higher rates of hyperoxaluria in human ADPKD [6, 7, 9]. Nevertheless, the underlying mechanism for human ADPKD hyperoxaluria reported in the literature remains still unclear. The absence of hypercalciuria in our mouse models, in turn, should not be considered a surprise, since this is a finding not often seen in ADPKD patients with nephrolithiasis [6, 9, 25].

Even considering that mice possess uricase [28], which helps to degrade uric acid, mean values of urinary uric acid were slightly but significantly higher in *Pkd1*-haploinsufficient mice than in the wild-type animals only in the 18–20 week, a finding not observed in cystic mice. Why did uric acid increase only in *Pkd1*-haploinsufficient animals and not in fully *Pkd1*-deficient and cystic mice remains unexplained. Although the use of two models with different genetic backgrounds did not allow inter-model comparisons, performance of intra-model comparisons were robust to appropriately evaluate test-control and inter-age analyses in the two abnormal scenarios considered.

Although stone analysis has shown that uric acid is an important constituent of urinary calculi among ADPKD patients with nephrolithiasis [7, 25] and hyperuricosuria has been detected in up to 19% of the cases [6, 7]. Torres et al [7] suggested that low urinary pH may probably represent the main predisposing factor for uric acid calculi in ADPKD. However, a limitation of the present study is that we could not measure pH through a pH electrode, given the difficulties imposed by the very small amounts of urine available before pooling the samples. On the other hand, determination of urinary pH in pooled urine would not provide reliable results, due to eventual evaporation during storage and management of the samples. The reduced ammonium excretion in ADPKD patients, as a possible underlying mechanism for acidosis [29], could not be evaluated in the present study, since ammoniuria was not determined.

Mean serum creatinine did not differ between groups, confirming that the animals had preserved renal function. The slightly elevated serum urea levels observed in cystic mice compared to non-cystic animals, however, suggests an increased tubular urea reabsorption in *Pkd1^{cond/cond};Nestin^{cre}* mice, a finding that may reflect local areas of renal vascular compression secondary to cyst expansion and consequent reduction in perfusion. The mean values of serum urea observed in cystic animals, however, still fall within a normal range [30], therefore not affecting the urinary excretion of the evaluated parameters. Interestingly, potential differences in oxaluria, calciuria, magnesuria, citraturia and uricosuria may exist between *Pkd1^{cond/cond};Nestin^{cre}* and *Pkd1^{+/-}* mice. However, the fact that these models have been generated on distinct genetic backgrounds, did not allow such an analysis in the current study.

We recognize that the absence of documented stone formation in our models is another limitation of the current study. However, most models of nephrolithiasis in rodents, represent in fact nephrocalcinosis [31], with the rat and mouse exceptions being anchored on specific genetic abnormalities [32, 33]. Nevertheless, to the best of our knowledge, this is the first experimental study addressing the potential effects of *Pkd1* haploinsufficiency and of the development of renal cysts secondary to *Pkd1* deficiency on potential lithogenic metabolic factors associated with nephrolithiasis in ADPKD. In fact, the orthologous nature of the current models to human ADPKD brings, relevance to our findings.

In conclusion, the absence of hypocitraturia, hyperoxaluria and hyperuricosuria in the cystic model at both ages suggest that anatomic cystic distortions *per se* may do not generate the metabolic disturbances described in human ADPKD-related nephrolithiasis. Despite of the presence of uricase, the finding of hyperuricosuria in the 18–20 week-old mice suggest that *Pkd1* haploinsufficiency may contribute to this phenotype. However, comparisons to human disease may be limited to the fact that uric acid metabolism of rodents is different from humans. In this experimental model, the potential existence of an excessively acidic urine pH seen in ADPKD patients, could not be properly evaluated due to the inherent technical limitations of the model itself, so that this is a matter that needs to be further explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Wilson PD, Goilav B. Cystic disease of the kidney. *Annu Rev Pathol.* 2007; 2:341–368. [PubMed: 18039103]
2. Peters DJ, Breuning MH. Autosomal dominant polycystic kidney disease: modification of disease progression. *Lancet.* 2001; 358:1439–1444. [PubMed: 11705510]

3. Grantham JJ, Chapman AB, Torres VE. Volume progression in autosomal dominant polycystic kidney disease: the major factor determining clinical outcomes. *Clin J Am Soc Nephrol*. 2006; 1:148–157. [PubMed: 17699202]
4. Bajwa ZH, Sial KA, Malik AB, Steinman TI. Pain patterns in patients with polycystic kidney disease. *Kidney Int*. 2004; 66:1561–1569. [PubMed: 15458452]
5. Eloi SRM, Nishiura JL, Heilberg IP. Translation, cultural adaptation and application of a pain questionnaire for patients with polycystic kidney disease. *J Bras Nefrol*. 2010; 32:386–399. [PubMed: 21541454]
6. Nishiura JL, Neves RFCA, Eloi SRM, et al. Evaluation of nephrolithiasis in autosomal dominant polycystic kidney disease patients. *Clin J Am Soc Nephrol*. 2009; 4:838–844. [PubMed: 19339428]
7. Torres VE, Erickson SB, Smith LH, et al. The association of nephrolithiasis and autosomal dominant polycystic kidney disease. *Am J Kidney Dis*. 1988; 11:318–325. [PubMed: 3354568]
8. Levine E, Grantham JJ. Calcified renal stones and cyst calcifications in autosomal dominant polycystic kidney disease: clinical and CT study in 84 patients. *AJR Am J Roentgenol*. 1992; 159:77–81. [PubMed: 1609726]
9. Grampas SA, Chandhoke PS, Fan J, et al. Anatomic and metabolic risk factors for nephrolithiasis in patients with autosomal dominant polycystic kidney disease. *Am J Kidney Dis*. 2000; 36:53–57. [PubMed: 10873872]
10. Amar AD, Das S, Egan RM. Management of urinary calculous disease in patients with renal cysts: review of 12 years of experience in 18 patients. *J Urol*. 1981; 125:153–156. [PubMed: 7206042]
11. Gambaro G, Fabris A, Puliatta D, Lupo A. Lithiasis in cystic kidney disease and malformations of the urinary tract. *Urol Res*. 2006; 34:102–107. [PubMed: 16416113]
12. Piontek K, Menezes LF, Garcia-Gonzalez MA, et al. A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1. *Nat Med*. 2007; 13:1490–1495. [PubMed: 17965720]
13. Takakura A, Contrino L, Beck AW, Zhou J. Pkd1 inactivation induced in adulthood produces focal cystic disease. *J Am Soc Nephrol*. 2008; 19:2351–2363. [PubMed: 18776127]
14. Takakura A, Contrino L, Zhou X, et al. Renal injury is a third hit promoting rapid development of adult polycystic kidney disease. *Human molecular genetics*. 2009; 18:2523–2531. [PubMed: 19342421]
15. Torres VE, Harris PC. Autosomal dominant polycystic kidney disease: the last 3 years. *Kidney Int*. 2009; 76:149–168. [PubMed: 19455193]
16. Grantham JJ, Mulamalla S, Swenson-Fields KI. Why kidneys fail in autosomal dominant polycystic kidney disease. *Nat Rev Nephrol*. 2011; 7:556–566. [PubMed: 21862990]
17. Piontek KB, Huso DL, Grinberg A, et al. A functional floxed allele of Pkd1 that can be conditionally inactivated in vivo. *J Am Soc Nephrol*. 2004; 15:3035–3043. [PubMed: 15579506]
18. Ferraz RR, Baxmann AC, Ferreira LG, et al. Preservation of urine samples for metabolic evaluation of stone-forming patients. *Urological research*. 2006; 34:329–337. [PubMed: 16896690]
19. Hallson PC, Rose GA. A simplified and rapid enzymatic method for determination of urinary oxalate. *Clin Chim Acta*. 1974; 55:29–39. [PubMed: 4411755]
20. Holt C, Cowley DM, Chalmers AH. Rapid estimation of urinary citrate by use of a centrifugal analyzer. *Clin Chem*. 1985; 31:779–780.
21. Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem*. 1980; 26:227–231. [PubMed: 7353268]
22. Strufaldi, B. *Prática de bioquímica clínica*. Faculdade de Ciências Farmacêuticas da Universidade de São Paulo; São Paulo: 1987.
23. Macfate RP, Cohn C, Eichelberger L, Cooper JA. Symposium on azotemia. *Am J Clin Pathol*. 1954; 24:511–571. [PubMed: 13158285]
24. Fonseca JM, Bastos AP, Amaral AG, et al. Renal cyst growth is the main determinant for hypertension and concentrating deficit in Pkd1-deficient mice. *Kidney Int*. 2014; doi: 10.1038/ki.2013.501

25. Torres VE, Wilson DM, Hattery RR, Segura JW. Renal stone disease in autosomal dominant polycystic kidney disease. *Am J Kidney Dis.* 1993; 22:513–519. [PubMed: 8213789]
26. Ogbron MR, Sareen S, Prychitko J, et al. Altered organic anion and osmolyte content and excretion in rat polycystic kidney disease: an NMR study. *Am J Physiol.* 1997; 272:F63–69. [PubMed: 9039050]
27. Tanner GA, Tanner JA. Dietary citrate treatment of polycystic kidney disease in rats. *Nephron Physiol.* 2003; 93:P14–20. [PubMed: 12411726]
28. Kelly S, Delnomdedieu M, Oliverio M, et al. Diabetes Insipidus in Uricase-Deficient Mice: A Model for Evaluating Therapy with Poly(Ethylene Glycol)-Modified Uricase. *J Am Soc Nephrol.* 12:1001–1009.
29. Torres VE, Keith DS, Offord KP, et al. Renal ammonia in autosomal dominant polycystic kidney disease. *Kidney Int.* 1994; 45:1745–1753. [PubMed: 7933822]
30. Bastos AP, Piontek K, Silva AM, et al. Pkd1 haploinsufficiency increases renal damage and induces microcyst formation following ischemia/reperfusion. *J Am Soc Nephrol.* 2009; 20:2389–2402. [PubMed: 19833899]
31. Khan SR. Nephrocalcinosis in animal models with and without stones. *Urol Res.* 2010; 38:429–438. [PubMed: 20658131]
32. Bushinsky DA, Asplin JR, Grynepas MD, et al. Calcium oxalate stone formation in genetic hypercalciuric stone-forming rats. *Kidney Int.* 2002; 61:975–987. [PubMed: 11849452]
33. Jiang Z, Asplin JR, Evan AP, et al. Calcium oxalate urolithiasis in mice lacking anion transporter Slc26a6. *Nat Genet.* 2006; 38:474–478. [PubMed: 16532010]

Figure 1.

Histological kidney sections at 15 weeks of age: (a) Cystic *Pkd1*^{cond/cond};Nestin^{cre} mouse showing renal cysts (arrows) and (b) noncystic *Pkd1*^{cond/cond} control mouse; (c) *Pkd1*^{+/-} (*Pkd1*-haploinsufficient) mouse displaying no renal cysts and (d) *Pkd1*^{+/+} (wild type) control mouse. No calculi or crystals were observed in both cystic and *Pkd1*-haploinsufficient kidneys. Magnification 400 X.

Table 1Results of Haploinsufficient ($Pkd1^{+/-}$) and Control ($Pkd1^{+/+}$) mice aged 10–12 weeks

	<i>Pkd1^{+/+}</i> (n=11)	<i>Pkd1^{+/-}</i> (n=12)
Serum parameters		
Urea (mg/dL)	53.9 ± 1.3	54.2 ± 2.5
Creatinine (mg/dL)	0.37 ± 0.02	0.35 ± 0.02
Urinary parameters		
Oxalate (mg/mg Creat)	0.16 ± 0.17	0.16 ± 0.10
Calcium (mg/mg Creat)	0.28 ± 0.17	0.41 ± 0.07
Magnesium (mg/mg Creat)	1.10 ± 1.23	1.23 ± 0.26
Citrate (mg/mg Creat)	3.9 ± 1.5	4.2 ± 2.0
Uric acid (mg/mg Creat)	0.39 ± 0.27	0.29 ± 0.15
Creatinine (mg/24h)	35.3 ± 11.7	35.0 ± 8.5

Mean ± SD.

Table 2
Results of Cystic (Pkd1^{cond/cond};Nestin^{cre}) and Control (Pkd1^{cond/cond}) mice aged 10–12 weeks

	Pkd1 ^{cond/cond} (n=18)	Pkd1 ^{cond/cond} ;Nestin ^{cre} (n=17)
Serum parameters		
Urea (mg/dL)	54.0 ± 2.6	57.1 ± 3.1 *
Creatinine (mg/dL)	0.36 ± 0.03	0.36 ± 0.01
Urinary parameters		
Oxalate (mg/mg Creat)	0.16 ± 0.06	0.10 ± 0.04 *
Calcium (mg/mg Creat)	0.15 ± 0.09	0.15 ± 0.07
Magnesium (mg/mg Creat)	0.63 ± 0.17	0.61 ± 0.11
Citrate (mg/mg Creat)	2.8 ± 1.7	3.2 ± 1.3
Uric acid (mg/mg Creat)	0.26 ± 0.06	0.22 ± 0.05
Creatinine (mg/24h)	42.5 ± 4.5	45.4 ± 3.6 *

Mean ± SD.

* p < 0.05 vs. control