POLYCYSTIN-1 DYSFUNCTION IMPAIRS ELECTROLYTE AND WATER HANDLING IN A RENAL PRE-CYSTIC MOUSE MODEL FOR ADPKD

Eric H.J. Verschuren[1], Sami G. Mohammed[1], Wouter N. Leonhard[2],
Caro Overmars-Bos[1], Kimberly Veraar[2], Joost G.J. Hoenderop[1],
René J.M. Bindels[§,1], Dorien J.M. Peters[§,2], Francisco J. Arjona[#,§,1]

[1] Department of Physiology, Radboud Institute for Molecular Life Sciences,
Radboud university medical center, Nijmegen, The Netherlands

[2] Department of Human Genetics, Leiden University Medical Centre,
Leiden, The Netherlands

Short Title: Renal electrolyte handling in a pre-cystic ADPKD model

[§] These authors have contributed equally to this work
[#] Author for correspondence
Name: Francisco J. Arjona
*Address: Department of Physiology, Ra*dboud Institute for Molecular Life Sciences, Radboud university medical center, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands
Email: Francisco.ArjonaMadueno@radboudumc.nl

ABSTRACT

The *PKD1* gene encodes polycystin-1 (PC1), a mechanosensor triggering intracellular responses upon urinary flow sensing in kidney tubular cells. Mutations in *PKD1* lead to autosomal dominant polycystic kidney disease (ADPKD). The involvement of PC1 in renal electrolyte handling remains unknown since renal electrolyte physiology in ADPKD patients has only been characterized in cystic ADPKD. We thus studied the renal electrolyte handling in inducible kidney-specific *Pkd1* knockout (iKsp-*Pkd1*$^{-/-}$) mice manifesting a pre-cystic phenotype. Serum and urinary electrolyte determinations indicated that iKsp-*Pkd1*$^{-/-}$ mice display reduced serum levels of magnesium (Mg^{2+}), calcium (Ca^{2+}), sodium (Na^{+}) and phosphate (P_i) compared with control (*Pkd1*$^{+/+}$) mice; and renal Mg^{2+}, Ca^{2+} and P_i wasting. In agreement with these electrolyte disturbances, downregulation of key genes for electrolyte reabsorption in the thick ascending limb of Henle's loop (TAL, *Cldn16*, *Kcnj1* and *Slc12a1*), distal convoluted tubule (DCT, *Trpm6* and *Slc12a3*) and connecting tubule (CNT, *Calb1*, *Slc8a1, Atp2b4*) was observed in kidneys of iKsp-*Pkd1*$^{-/-}$ mice compared with controls. Similarly, decreased renal gene expression of markers for TAL (*Umod*) and DCT (*Pvalb*) was observed in iKsp-*Pkd1*$^{-/-}$ mice. Conversely, mRNA expression levels in kidney of genes encoding solute and water transporters in the proximal tubule (*Abcg2* and *Slc34a1*) and collecting duct (*Aqp2*, *Scnn1a* and *Scnn1b*) remained comparable between control and iKsp-*Pkd1*$^{-/-}$ mice, though a water reabsorption defect was observed in iKsp-*Pkd1*$^{-/-}$ mice. In conclusion, our data indicate that PC1 is involved in renal Mg^{2+}, Ca^{2+} and water handling, and its dysfunction resulting in a systemic electrolyte imbalance characterized by low serum electrolyte concentrations.

Keywords: PC1, *Pkd1*, ADPKD, pre-cystic, electrolyte imbalance

60 **INTRODUCTION**

61 The primary function of the kidneys is the removal of waste products from our metabolism. This
62 process accounts for the challenge of filtering an average of 180 liters of blood daily. Upon
63 filtration, the kidney reabsorbs 95% of the electrolytes contained in the filtrate. Consequently, a
64 minor loss of kidney function yields disturbed plasma concentrations due to excessive urinary
65 electrolyte excretion or absorption. This dysregulation of the electrolyte balance results in renal
66 and extrarenal disorders including hypertension, renal stone formation and development of
67 cardiovascular calcifications (8, 14, 21).

68 In the nephron, consecutive epithelial segments, i.e. the proximal tubule (PT), the thick
69 ascending limb of Henle's loop (TAL), the distal convoluted tubule (DCT), the connecting tubule
70 (CNT) and the collecting duct (CD), maintain electrolyte balance through passive and/or active
71 regulation of electrolyte reabsorption. Renal electrolyte handling is accomplished through the
72 interplay of various tight junction proteins and ion channels and transporters expressed alongside
73 the nephron (2, 19, 31, 41, 49). It is largely unknown how the activity of these channels and
74 transporters is regulated. One of the factors that may comprise this regulation is the variable
75 urinary flow in the nephron tubules. After all, renal electrolyte transport needs to be adjusted to
76 the reabsorption demands that are dictated by the variable urinary flow in order to maintain
77 electrolyte balance. In this context, it appears that tubular variable urinary flow is sensed by
78 primary cilia, which are expressed in almost all epithelial cells within the kidney (9). The protein
79 polycystin-1 (PC1), located at the cellular apical plasma membrane and in primary cilia (protruding
80 from the apical surface of renal tubular cells), is suggested to act as a mechanosensory molecule
81 for urinary flow (24, 33, 45, 56).

82 The gene *PKD1* encodes PC1 and is involved in the regulation of various signaling
83 pathways important for the maintenance and differentiation of kidney tubular epithelial cells (5).
84 Mutations in *PKD1* lead to autosomal dominant polycystic kidney disease (ADPKD), which is one
85 of the most common inherited renal diseases accounting for 7 to 10% of all patients on renal

86 replacement therapy (16, 38). ADPKD is characterized by increased cell proliferation, fluid
87 accumulation and altered extracellular matrix synthesis, resulting in cyst formation and eventually
88 in end-stage renal disease (ESRD). In advanced ADPKD, hypertension is common and
89 glomerular filtration rate (GFR) is reduced (6, 48). Electrolyte disturbances in ADPKD are
90 described in literature, but these reports are mostly restricted to cystic ADPKD (4, 11, 13, 34, 39,
91 40, 43, 44, 47, 51, 55, 57, 58). When electrolyte imbalances are detected in cystic ADPKD, it is
92 not possible to discern whether these disturbances are caused by dysfunctional PC1 or by cyst
93 formation or defects in GFR, which dramatically impair renal fluid flow and blood filtration,
94 respectively. In *Pkd1*$^{+/-}$ mice, urinary wasting of Na^+, and reduced urinary Ca^{2+} excretion and
95 serum Na^+ levels have been reported (1). However, *Pkd1*$^{+/-}$ mice are not adequate to disclose
96 PC1 function since one *Pkd1* allele still translates into a functional PC1 protein, while *Pkd1*$^{-/-}$ mice
97 die prematurely. Therefore, use of kidney-specific *Pkd1*$^{-/-}$ mice, which are viable (27) and in a
98 stage preceding cyst formation (pre-cystic), is key to elucidate the involvement of PC1 in renal
99 electrolyte handling. Identification of putative electrolyte disturbances in kidney-specific *Pkd1*$^{-/-}$
100 mice can be of paramount relevance to fully characterize the function of PC1 and thus delineate
101 the physiological consequences of sensing urinary flow along the nephron.

102 The aim of this study was, therefore, to study the function of PC1 in renal electrolyte
103 handling in relation to pre-cystic ADPKD by using an inducible kidney-specific *Pkd1*$^{-/-}$ mouse
104 model.
105
106
107 **METHODS**
108
109 *Animal Procedures*
110 Inducible kidney-specific *Pkd1* knockout mice (iKsp-*Pkd1*$^{lox/lox}$) were used during experimentation.
111 In this mouse model, the *Pkd1*$^{lox/lox}$ allele has Lox-P sites flanking exons 2-11. Tamoxifen was

112 orally administered to iKsp-*Pkd1*$^{lox/lox}$ mice on postnatal days 18, 19 and 20 (PN18) to induce a
113 kidney specific knockout of *Pkd1* (iKsp-*Pkd1*$^{-/-}$) and thus model ADPKD (27, 28). For
114 experimentation, 8 male mice (obtained from 3 litters) received tamoxifen (iKsp-*Pkd1*$^{-/-}$) and 7
115 male mice (obtained from 3 litters) received no treatment (control). Only male mice were used in
116 order to exclude sex as a factor influencing electrolyte handling since estrogen can influence Mg^{2+}
117 absorption rates (8). At PN18 + 22 days and at PN18 + 29 days, mice were placed in metabolic
118 cages for 24hrs to collect urine and faeces. Subsequently, body weight, faeces weight, urinary
119 volume, food and water intake were assessed. Next, mice were anesthetized using isoflurane,
120 and blood was collected *via* eye extraction. Finally, mice were sacrificed by cervical dislocation.
121 Serum was obtained from the blood by centrifugation. Kidneys were extracted and weighed, and
122 different segments of the intestine were collected in liquid nitrogen and stored at -80°C for mRNA
123 and protein isolation. Part of the kidney was fixed in 4% (v/v) formalin before imbedding in paraffin
124 for immunohistochemistry. Urine and faeces were stored at -20°C for assessment of the
125 electrolyte content. The local animal experimental committee of the Leiden University Medical
126 Center and the Commission Biotechnology in Animals of the Dutch Ministry of Agriculture
127 approved the animal procedures performed.

128

129 *Analytical Procedures*

130 Serum, urinary and faecal electrolyte content was measured using inductively coupled plasma
131 mass spectrometry (ICP-MS, ppb, for Mg^{2+}, Ca^{2+}, Na^{+} and K^{+}), a chloride autoanalyzer (ppb, for
132 Cl^{-}), and inductively coupled plasma optic emission spectrometry (ICP-OES, ppm, for total
133 phosphorus (as a measurement of inorganic phosphate, P_i)). Samples were prepared by
134 dissolving 20µl of serum or urine in 50µl nitric acid (HNO_3) and further diluted in 5ml MQ water.
135 Faeces were incubated in 10ml HNO_3 at 50°C for 1hr. Next, total faeces samples were diluted
136 with 10ml MQ water, homogenized by shaking, and 100µl of sample was further diluted with 5ml
137 MQ. Diluted samples were then analyzed for electrolyte content. In addition, blood urea nitrogen

138 (BUN, mg/dl) was analyzed in the serum. Serum glucose (mmol/L) was analyzed using a glucose
139 liquicolor kit (HUMAN GmbH, Germany). Osmolality (mOsm/kg) was assessed in the urine and
140 serum using an osmometer (Osmometer Model 3320, Advanced Instruments Inc, MA, USA).
141 Furthermore, the calculated serum osmolarity was determined using the following formula: 2 x
142 serum[Na^+] + serum[glucose] + [BUN] (52). Non-acetylated cAMP (nmol/24-hrs) was analyzed in
143 the urine using a nonradioactive enzyme immunoassay kit (Cayman Chemical, MI, USA). The
144 weight of both kidneys (2KW) was compared to the total body weight (BW) in order to determine
145 the 2KW/BW ratios (%) for each mouse.
146
147 *Histology & Cystic Index*
148 Formalin fixed kidneys were embedded in paraffin and sections (4μm) were prepared. Sections
149 were stained with periodic-acid Schiff (PAS) and hematoxylin and eosin (HE) using standard
150 procedures. PAS and HE stainings were analyzed in order to examine features such as tubular
151 dilation and/or cyst formation. The cystic index of kidneys from control and iKsp-*Pkd1*$^{-/-}$ mice was
152 defined as the percent of lumen area over the total image area and assessed from total scans of
153 hematoxylin and eosin-stained kidney sections (Figure 1). The stained lumen content of larger
154 dilations and/or potential small cysts was removed from the images using Photoshop CC 2017
155 (Adobe Systems, CA, USA). Cystic index, using the ratio of total renal area plus lumen and total
156 renal area minus lumen was determined by ImageJ software (National Institute of Health, MA,
157 USA).
158
159 *Immunohistochemistry*
160 Specific nephron segments were distinguished by immunofluorescence using segment specific
161 primary antibodies, namely rat anti-breast cancer resistance protein (BCRP) for the PT (1:250 in
162 Tris-NaCl-blocking buffer (TNB), Kamiya Biomedical Company, WA, USA), sheep anti-Tamm-
163 Horsfall protein (THF) for the TAL (1:200 in TNB, Biotrend, Germany), rabbit anti-NCC for the

164 DCT (1:200 in TNB, Millipore, MA, USA), guinea pig anti-TRPV5 for the CNT (1:2000 in TNB) (20)
165 and rabbit anti-Aquaporin-2 (AQP2) for the CD (1:100 in TNB, Millipore, MA, USA). Sections were
166 deparaffinized in xylene and subjected to heat-mediated antigen retrieval in citrate buffer (pH 6.0,
167 Sigma-Aldrich, MI, USA) for 15min. Sections were incubated in 0.1% (v/v) PBS-Triton for 15min
168 for permeabilization. Sections with staining for BCRP, THF, NCC and AQP2 were blocked for
169 30min in TNB and incubated with primary antibodies overnight. Next, sections were washed with
170 Tris-NaCl (TN-Tween) buffer and incubated with secondary antibodies for 1hr in dark at room
171 temperature: goat anti-rat Cy5 (1:100 in TNB, for BCRP, Jackson ImmunoResearch, PA, USA),
172 goat anti-sheep Alexa594 (1:300 in TNB, for THF, Molecular Probes, OR, USA) and goat anti-
173 rabbit Alexa594 (1:300 in TNB, for NCC and AQP2, Molecular Probes, OR, USA). Finally, sections
174 were washed with TN buffer and mounted (DAPI Fluoromount-G, SouthernBiotech, AL, USA).
175 For anti-TRPV5, after permeabilization, sections were blocked with 0.3% (v/v) H_2O_2 for 30min for
176 endogenous peroxidase activity. Next, sections were blocked with a few droplets of endogenous
177 Avidin and Biotin (Vector Laboratories, CA, USA) for 15min each. Subsequently, sections were
178 blocked using TNB for 30min and incubated with primary antibody overnight. Next, sections were
179 washed with TN-Tween buffer and incubated with secondary antibody for 1hr in dark at room
180 temperature: goat anti-guinea pig Biotin SP (1:2000 in TNB, Jackson ImmunoResearch, PA, USA).
181 Subsequently, sections were incubated in strep-HRP (1:100 in TNB, PerkinElmer, MA, USA) for
182 30min followed by fluorescein tyramide (1:50 in amplification diluent, PerkinElmer, MA, USA) for
183 7min. Finally, sections were mounted (DAPI Fluoromount-G, SouthernBiotech, AL, USA) and
184 analyzed with a fluorescence microscope (Axio Imager 2, Zeiss, Germany).
185
186 *Quantitative Real-Time PCR*
187 Tissue RNA was extracted using TriZol/chloroform extraction (Invitrogen, CA, USA). After DNase
188 treatment (Promega, WI, USA), cDNA was synthesized using Molony-Murine Leukemia Virus-
189 Reverse Transcriptase (Invitrogen, CA, USA) as previously described (18). The cDNA was mixed

190 with Power SYBR green PCR master mix (Applied Biosystems, CA, USA) and with primers
191 (400nM) for the gene of interest as previously described (3). The expression of the following genes
192 was assessed via RTqPCR (7min at 95°C, 40 cycles of 15 sec at 95°C and 1 min at 60°C) in the
193 kidney; *Abcg2, Atp2b4, Aqp2, Calb1, Cldn16, Cldn19, Cnnm2, Kcnj1, Kim-1, Prom1, Prom2,*
194 *Pvalb, Scnn1a, Scnn1b, Slc8a1, Slc12a1, Slc12a3, Slc34a1, Slc41a3, Trpm6, Trpm7, Trpv5* and
195 *Umod* (Table 1). In the intestine, the expression of the following genes was assessed: *Atp2b4,*
196 *Cnnm4, Trpm6* and *Trpv6*. As a reference gene, *Gapdh* was used, and negative controls (samples
197 where the reverse transcriptase was omitted during cDNA synthesis, and non-template samples)
198 were taken along with each gene. The relative gene expression was analyzed using the Livak
199 method ($2^{-\Delta\Delta Ct}$).
200
201 *Statistical Analyses*
202 Differences between groups were assessed using an unpaired Student's *t*-test. All data were
203 expressed as mean ± SEM. Statistical significance was accepted at $P < 0.05$. Statistical analyses
204 were performed using GraphPad Prism 6 (GraphPad, San Diego, CA, USA).
205
206
207 **RESULTS**
208
209 **Pre-cystic kidneys of iKsp-*Pkd1*$^{-/-}$ mice do not manifest tubular dilation in TAL, DCT and**
210 **CNT**
211 Normal renal histology was observed in the kidneys of mice treated without tamoxifen (controls)
212 by Periodic acid-Schiff (PAS) staining, whereas tamoxifen-treated mice (kidney specific *Pkd1*$^{-/-}$
213 (iKsp-*Pkd1*$^{-/-}$) mice) displayed mild dilated tubules in the cortex, outer and inner medulla at PN18
214 + 29 days (Figure 1, 2A). In detail, after immunofluorescent staining for specific nephron segments,
215 only mild tubular dilation, restricted to the PT and CD, was observed. Importantly, no tubular

216 dilation was observed in TAL, DCT and CNT (Figure 2B). Remarkably, at this pre-cystic stage,
217 *Kim-1* (Kidney injury molecule-1) mRNA expression was significantly increased ($P < 0.05$),
218 whereas the Blood Urea Nitrogen (BUN) levels were not altered between control and iKsp-*Pkd1*$^{-}$
219 $^{/-}$ mice (Figure 2C-D). Furthermore, a significantly increased 2KW/BW ratio (1.3 ± 0.1% and 1.6 ±
220 0.1% for control versus iKsp-*Pkd1*$^{-/-}$ mice, respectively, $P < 0.05$) and cystic index (1.8 ± 0.2%
221 and 3.6 ± 0.4% for control versus iKsp-*Pkd1*$^{-/-}$ mice, respectively, $P < 0.05$) was observed (Figure
222 2E-F), indicative of enlargement of the kidneys due to the mild tubular dilations seen in the PT
223 and CD.
224

225 **Pre-cystic iKsp-*Pkd1*$^{-/-}$ mice display disturbances in renal electrolyte and water handling**
226 Serum and 24-hrs urine were collected to characterize the renal electrolyte and water handling in
227 iKsp-*Pkd1*$^{-/-}$ mice with pre-cystic kidneys, and in control mice. In detail, at PN18 + 22 days, urinary
228 wasting of Ca^{2+} and Mg^{2+} was observed ($P < 0.05$) (Table 2); however, this effect was not
229 observed at PN18 + 29 days (Table 2). Conversely, analysis at PN18 + 29 days showed that iKsp-
230 *Pkd1*$^{-/-}$ mice exhibited lower serum Ca^{2+}, Mg^{2+}, Na^{+} and P_i levels ($P < 0.05$) and a renal P_i leakage
231 ($P < 0.05$) (Table 2). A non-statistically significant increase in urinary volume was observed in
232 iKsp-*Pkd1*$^{-/-}$ mice as compared to controls ($P = 0.23$ and $P = 0.08$ for PN18 + 22 days and PN18
233 + 29 days, respectively). No changes in urine osmolality and cAMP levels at PN18 + 22 days
234 were observed. However, at PN18 + 29 days, urine osmolality was significantly lower ($P < 0.05$)
235 in iKsp-*Pkd1*$^{-/-}$ *versus* control mice. At this time point, urinary cAMP was significantly higher ($P <$
236 0.05) in iKsp-*Pkd1*$^{-/-}$ mice as compared to controls (Table 2), indicating an activation of the
237 arginine vasopressin (AVP)-cAMP-AQP2 axis. Significant changes in serum glucose were not
238 observed between iKsp-*Pkd1*$^{-/-}$ and control mice at PN18 + 29 days. Serum osmolality was similar
239 between iKsp-*Pkd1*$^{-/-}$ and control mice at PN18 + 29 days. The calculated serum osmolarity was
240 significantly lower in iKsp-*Pkd1*$^{-/-}$ mice as compared to controls. Furthermore, control and iKsp-
241 *Pkd1*$^{-/-}$ mice had a comparable food and water intake (Table 2).

242

243 **Decreased expression of key genes for electrolyte reabsorption in TAL, DCT and CNT**

244 To assess whether the electrolyte imbalances in iKsp-*Pkd1*$^{-/-}$ mice resulted from aberrant gene
245 expression, the mRNA expression of key genes relevant for electrolyte handling in the kidney
246 were examined. At PN18 + 29 days, downregulation of the mRNA levels in whole kidney of several
247 key genes for electrolyte reabsorption in TAL, DCT and CNT was observed in iKsp-*Pkd1*$^{-/-}$ mice
248 compared to control mice. In TAL, the expression of *Cldn16* (Claudin16), *Kcnj1* (ROMK) and
249 *Slc12a1* (NKCC2) was decreased ($P < 0.05$) (Figure 3B). In DCT, reduced expression of *Trpm6*
250 (TRPM6) and *Slc12a3* (NCC) was observed ($P < 0.05$) (Figure 3C). The expression of *Calb1*
251 (Calbindin1), *Slc8a1* (NCX1) and *Atp2b4* (PMCA4) was downregulated in the CNT ($P < 0.05$)
252 (Figure 3D). Genes encoding channels and transporters in the PT (*Abcg2* and *Slc34a1*) and CD
253 (*Aqp2*, *Scnn1a* and *Scnn1b*) were not affected (Figure 3A, 3E). Gene expression of *Trpm7*
254 (TRPM7), a gene ubiquitously expressed along the nephron, was similar in iKsp-*Pkd1*$^{-/-}$ and
255 control mice (Figure 3F).

256

257 **Decreased gene expression of renal segment markers in pre-cystic iKsp-*Pkd1*$^{-/-}$ mice**

258 The expression of *Umod* (Uromodulin), a marker of the TAL (46), and *Pvalb* (Parvalbumin), a
259 marker of the DCT (36), was downregulated in iKsp-*Pkd1*$^{-/-}$ mice compared to control mice ($P <$
260 0.05) (Figure 4A). Furthermore, decreased expression of *Prom2* (Prominin-2), a marker of TAL,
261 DCT, CNT and CD was also observed iKsp-*Pkd1*$^{-/-}$ mice compared to control mice ($P < 0.05$),
262 whereas *Prom1* (Prominin-1) expression, a marker of the PT (23), was similarly expressed in the
263 kidneys of control and iKsp-*Pkd1*$^{-/-}$ mice (Figure 4B).

264

265

266 **Compensation of the renal electrolyte disturbances in the intestine**

267 In order to disclose extra-renal mechanisms compensating for the electrolyte imbalances elicited
268 by knocking out *Pkd1* in the mouse kidney, we assessed the mRNA expression of genes relevant
269 for electrolyte handling in the intestine. Interestingly, *Trpv6* (TRPV6) expression was increased in
270 the duodenum ($P < 0.05$) of iKsp-*Pkd1*$^{-/-}$ mice as compared to controls (Figure 5A), whereas in
271 colon, *Trpm6* expression was decreased ($P < 0.05$). In duodenum and caecum, no changes in
272 *Trpm6* expression were observed. Furthermore, in colon and caecum, no changes in gene
273 expression were observed between iKsp-*Pkd1*$^{-/-}$ and control mice for *Cnnm4*, *Trpv6* and *Atp2b4*
274 (Figure 5B, 5C).
275
276
277 **DISCUSSION**

278 This study is the first characterization of the renal electrolyte and water handling in a model of
279 ADPKD during the renal pre-cystic phase. We show that the knockout of PC1 in the mouse kidney
280 leads to decreased serum Mg^{2+}, Ca^{2+}, Na^{+} and P_i levels; and urinary wasting of Mg^{2+} and Ca^{2+}
281 during the pre-cystic stage, illustrating the role of PC1 in renal Mg^{2+} and Ca^{2+} handling. In addition,
282 our data support the involvement of PC1 in the regulation of water reabsorption in the kidney. The
283 Mg^{2+} and Ca^{2+} imbalances elicited by dysfunctional PC1 were likely caused by a decrease in the
284 expression of key genes for the reabsorption of $Mg^{2+}$and Ca^{2+} in TAL, DCT and CNT of the
285 nephron.

286 By characterizing the renal electrolyte and water handling, and its influence on serum
287 electrolyte levels, in the renal pre-cystic stage of iKsp-*Pkd1*$^{-/-}$ mice, information about the early
288 stages of development of ADPKD is provided. Most studies using models for ADPKD have only
289 investigated renal cystic stages, and thus, later stages to the pre-cystic phase. The mice used in
290 our study clearly show a renal pre-cystic phenotype. This is supported by the low 2KW/BW ratios,
291 the low cystic index, and the absence of cysts in the PAS-stained kidney sections of *Pkd1*$^{-/-}$ mice.
292 We only observed a mild tubular dilation restricted to the PT and CD (cystic index: 3.6 ± 0.4%).

293 Models with a cystic phenotype generally display a cystic index of 20 to 60%, depending on the
294 model (10, 17, 28, 35).

295 iKsp-*Pkd1*$^{-/-}$ mice showed renal Mg^{2+} and Ca^{2+} wasting at PN18 + 22 days, pointing to a
296 role of PC1 in the reabsorption of Mg^{2+} and Ca^{2+} in the kidney. This Mg^{2+} and Ca^{2+} leak in the
297 kidney of iKsp-*Pkd1*$^{-/-}$ mice was manifested as reduced serum Mg^{2+} and Ca^{2+} levels as compared
298 with control mice at a later time point of PN18 + 29 days. Changes in urinary electrolyte excretion
299 precede changes in serum electrolyte concentrations (12). Thus, the renal Mg^{2+} and Ca^{2+} leak
300 detected in iKsp-*Pkd1*$^{-/-}$ mice compared to control mice at PN18 + 22 days illustrates evolving
301 systemic (serum) Mg^{2+} and Ca^{2+} disturbances, which become apparent at PN18 + 29 days. The
302 comparable Mg^{2+} and Ca^{2+} excretion between control and iKsp-*Pkd1*$^{-/-}$ mice at PN18 + 29 days
303 illustrate further the inability of the kidneys at this time point to restore the serum electrolyte
304 balance by increasing Mg^{2+} and Ca^{2+} reabsorption. These data are consistent with adult *Slc41a3*$^{-}$
305 $^{/-}$ and *Trpm6*$^{+/-}$ mice of 8-12 weeks, that display lower serum Mg^{2+} concentrations and a
306 comparable urinary Mg^{2+} excretion compared with control (*Slc41a3*$^{+/+}$ and *Trpm6*$^{+/+}$, respectively)
307 mice (7, 54).

308 In addition to renal Mg^{2+} and, Ca^{2+} wasting, urinary P_i excretion was increased in iKsp-
309 *Pkd1*$^{-/-}$ mice compared to control mice at PN18 + 29 days. This finding relates PC1 function to the
310 control of renal P_i excretion in addition to regulating renal Mg^{2+} and Ca^{2+} handling.

311 In agreement with the decreased Na^{+} levels in serum found in our iKsp-*Pkd1*$^{-/-}$ mice
312 compared with control mice, haploinsufficient *Pkd1* mice that do not develop cysts, had lower
313 serum Na^{+} levels than *Pkd1*$^{+/+}$ mice (1). A decreased serum Na^{+} concentration relates to an
314 excess of water in the blood (32) or a renal salt wasting resulting in hypovolaemia (30). However,
315 control and iKsp-*Pkd1*$^{-/-}$ mice had a similar serum osmolality (PN18 + 29 days), though the
316 calculated serum osmolarity was lower in iKsp-*Pkd1*$^{-/-}$ mice. Control and iKsp-*Pkd1*$^{-/-}$ mice
317 displayed a comparable water intake and urine output, not indicating water overload or

318 hypovolaemia, respectively. Thus, the origin of the lower levels of Na^+ in the serum of iKsp-*Pkd1*$^{-/-}$

319 mice compared with controls remains elusive.

320 In contrast with serum osmolality, urine osmolality was significantly decreased at PN18 +

321 29 days in iKsp-*Pkd1*$^{-/-}$ mice as compared to controls. Taking into account the increase in urine

322 production between iKsp-*Pkd1*$^{-/-}$ *versus* control mice (though not statistically significant) (Table 2),

323 these data clearly indicate an inability of the kidneys of iKsp-*Pkd1*$^{-/-}$ mice to concentrate ions in

324 urine. This is supported by increased urinary cAMP levels in iKsp-*Pkd1*$^{-/-}$ mice, which indicates a

325 compensatory response to the decreased water reabsorption by activation of the AVP-cAMP-

326 AQP2 axis (42).

327 Importantly, BUN, a common marker for kidney function, remained unchanged in *Pkd1*$^{-/-}$

328 mice, indicating that the disturbances in Mg^{2+}, Ca^{2+} and Na^+ balance observed are not caused by

329 defects in glomerular filtration. However, an increase in the expression of *Kim-1* in the pre-cystic

330 kidneys of iKsp-*Pkd1*$^{-/-}$ mice was observed as compared with control mice. These findings point

331 to mild tubular injury as a result of *Pkd1* gene disruption. *Kim-1* encodes a membrane protein,

332 which is up-regulated in proliferating and dedifferentiated tubular cells after renal ischemia (25).

333 *Kim-1* is postulated to be a potential biomarker for ADPKD progression (15, 39). Our data further

334 support this notion, especially when considering ADPKD in a pre-cystic stage.

335 The underlying cause of the impaired renal Mg^{2+} and Ca^{2+} handling observed in iKsp-

336 *Pkd1*$^{-/-}$ mice is likely the decreased renal gene expression of *Cldn16*, *Kcnj1* and *Slc12a1*, key

337 genes for paracellular Mg^{2+} and Ca^{2+} transport in the TAL; of *Trpm6*, *Slc12a3* and *Cnnm2*, relevant

338 genes for transcellular Mg^{2+} reabsorption in the DCT; and *Calb1*, *Slc8a1* and *Atp2b4*, genes

339 coding the players that facilitate transcellular Ca^{2+} reabsorption in the CNT. Some of these genes,

340 i.e. *Cldn16*, *Slc12a1* and *Slc12a3*, encode proteins that are also involved in Na^+ reabsorption and

341 thus might evoke aberrant renal Na^+ transport in iKsp-*Pkd1*$^{-/-}$ mice. Therefore, renal PC1

342 dysfunction seems to predominantly affect the TAL, DCT and CNT of the nephron, eliciting

343 aberrant gene expression of regulators of Mg^{2+}, Ca^{2+} and Na^+ transport in these segments. In

344 contrast with serum Na^{+} levels, the concentration of Ca^{2+} and Mg^{2+} in serum is influenced by renal
345 Mg^{2+} and Ca^{2+} transport (8). Thus, the decreased expression of genes relevant for Mg^{2+} and Ca^{2+}
346 in the TAL, DCT and CNT, can explain the lower serum Mg^{2+} and Ca^{2+} concentrations observed
347 in iKsp-*Pkd1*$^{-/-}$ mice compared to controls. In addition, a compensatory mechanism for the renal
348 Ca^{2+} leak was detected in the duodenum of iKsp-*Pkd1*$^{-/-}$ mice as an increased mRNA expression
349 of *Trpv6* was observed in this segment of the intestine in comparison with control mice. The same
350 phenomenon was observed in wild-type mice on a low Ca^{2+} diet in a previous study (53).
351 Conspicuously, in correlation with the decreased expression of key genes for electrolyte
352 reabsorption in the kidney, a lower gene expression of TAL (*Umod*) and DCT (*Pvalb*) segment
353 markers was observed in iKsp-*Pkd1*$^{-/-}$ compared to control mice, pointing to a potential remodeling
354 of TAL and DCT segments evoked by renal PC1 dysfunction. This finding is supported by the
355 decreased expression of *Prom2*, a marker for TAL, DCT, CNT and CD, whereas the expression
356 of *Prom1*, a marker for PT, was not decreased in iKsp-*Pkd1*$^{-/-}$ mice when compared to control
357 mice. While remodeling events, eventually leading to cyst formation, are clearly intertwined with
358 ADPKD (5, 38), this study is the first to show that remodeling due to PC1 dysfunction in a pre-
359 cystic context results in broad electrolyte imbalances. The association of the electrolyte
360 imbalances in iKsp-*Pkd1*$^{-/-}$ mice with remodeling events in the kidney is congruent with the de-
361 differentiation and persistent cell proliferation already reported for altered PC1 expression in
362 kidneys (22, 29).
363 In conclusion, we have demonstrated that dysfunction of PC1 impairs renal Mg^{2+}, Ca^{2+}
364 and water reabsorption in pre-cystic kidneys leading to serum Mg^{2+} and Ca^{2+} levels. These
365 electrolyte disturbances preceding cyst formation observed in our model provide novel insights
366 into PC1 function (Table 3). More research is required to disclose whether the electrolyte
367 disturbances shown in this study might serve as early biomarkers of disease progression in
368 ADPKD and/or might aid the development of treatment options in this early stage of the disease.
369

ACKNOWLEDGEMENTS

The authors thank the Leiden University Medical Centre animal facility for breeding/maintaining the mice and Janne Plugge for helpful support.

GRANTS

This work was supported by a grant from the Dutch Kidney Foundation (15OP03) to D.J.M. Peters and R.J.M. Bindels.

DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

E.V., R.B., D.P., and F.A. conceived and designed the research reported here; E.V., S.M., W.L., C.B., and K.V. performed the experiments; E.V., S.M., W.L., C.B., R.B., D.P., and F.A. analyzed the data; E.V., S.M., W.L., J.H., R.B., D.P., and F.A. interpreted the results of experiments; E.V., and S.M. prepared figures; E.V. drafted the manuscript; E.V., S.M., R.B., D.P., and F.A. edited and revised the manuscript.

REFERENCES

395 1. **Ahrabi AK, Terryn S, Valenti G, Caron N, Serradeil-Le Gal C, Raufaste D, Nielsen S,**
396 **Horie S, Verbayatz JM, Devuyst O.** PKD1 haploinsufficiency causes a syndrome of
397 inappropriate antidiuresis in mice. *J Am Soc Nephrol* 18: 1740-1753, 2007.
398 2. **Ares GR, Caceres PS, Ortiz PA.** Molecular regulation of NKCC2 in the thick ascending limb.
399 *Am J Physiol Renal Physiol* 301: 1143-1159, 2011.
400 3. **Arjona FJ, de Baaij JH, Schlingmann KP, Lameris AL, van Wijk E, Flik G, Regele S,**
401 **Korenke GC, Neophytou B, Rust S, Reintjes N, Konrad M, Bindels RJ, Hoenderop JG.**
402 CNNM2 mutations cause impaired brain development and seizures in patients with
403 hypomagnesemia. *PLoS Genet* 10:e1004267, 2014.
404 4. **Bastos AP, Piontek K, Silva AM, Martini D, Menezes LF, Fonseca JM, Fonseca II,**
405 **Germino CG, Onuchic LF.** Pkd1 haploinsufficiency increases renal damage and induces
406 microcyst formation following ischemia/reperfusion. *J Am Soc Nephrol* 20: 2389-2402, 2009.
407 5. **Chapin HC, Caplan MJ.** The cell biology of polycystic kidney disease. *J Cell Biol* 191: 701-
408 710, 2010.
409 6. **Chapman AB, Stepniakowski K, Rahbari-Oskoui F.** Hypertension in autosomal dominant
410 polycystic kidney disease. *Adv Chronic Kidney Dis* 17: 153-163, 2010.
411 7. **De Baaij JH, Arjona FJ, Van den Brand M, Lavrijsen M, Lameris AL, Bindels RJ,**
412 **Hoenderop JG.** Identification of SLC41A3 as a novel player in magnesium homeostasis. *Sci*
413 *Rep* 6:28565, 2016.
414 8. **De Baaij JH, Hoenderop JG, Bindels RJ.** Magnesium in man: implications for health and
415 disease. *Physiol Rev* 95: 1-46, 2015.
416 9. **Deane JA, Ricardo SD.** Emerging roles for renal primary cilia in epithelial repair. *Int Rev Cell*
417 *Mol Biol* 293: 169-193, 2012.
418 10. **Fedeles SV, Tian X, Gallagher AR, Mitobe M, Nishio S, Lee SH, Cai Y, Geng L, Crews**
419 **CM, Somlo S.** A genetic interaction network of five genes for human polycystic kidney and

420 liver diseases defines polycystin-1 as the central determinant of cyst formation. *Nat Genet* 43:
421 639-647, 2011.
422 11. **Ferraz RRN, Fonseca JM, Germino GG, Onuchic LF, Heilberg IP.** Determination of urinary
423 lithogenic parameters in murine models orthologous to autosomal dominant polycystic kidney
424 disease. *Urolithiasis* 42: 301-307, 2014.
425 12. **Fleming CR, George L, Stoner GL, Tarrosa VB, Moyer TP.** The importance of urinary
426 magnesium values in patients with gut failure. *Mayo Clin Proc* 71: 21-24, 1996.
427 13. **Fonseca JM, Bastos AP, Amaral AG, Sousa MF, Souza LE, Malheiros DM, Piontek K,**
428 **Irigoven MC, Watnick TJ, Onuchic LF.** Renal cyst growth is the main determinant for
429 hypertension and concentrating deficit in Pkd1-deficient mice. *Kidney Int* 85: 1137-1150,
430 2014.
431 14. **Fujita T.** Mechanism of salt-sensitive hypertension: focus on adrenal and sympathetic
432 nervous systems. *J Am Soc Nephrol* 25: 1148-1155, 2014.
433 15. **Gauer S, Urbschat A, Gretz N, Hoffmann SC, Kränzlin B, Geiger H, Obermüller N.** Kidney
434 Injury Molecule-1 is Specifically Expressed in Cystically-Transformed Proximal Tubules of the
435 PKD/Mhm(cy/+) Rat Model of Polycystic Kidney Disease. *Int J Mol Sci* 17: 802, 2016.
436 16. **Happé H, Peters DJ.** Translational research in ADPKD: lessons from animal models. *Nat Rev*
437 *Nephrol* 10: 587-601, 2014.
438 17. **Happé H, Van der Wal AM, Salvatori DC, Leonhard WN, Breuning MH, de Heer E, Peters**
439 **DJ.** Cyst expansion and regression in a mouse model of polycystic kidney disease. *Kidney Int*
440 83: 1099-1108, 2013.
441 18. **Hoenderop JG, Hartog A, Stuiver M, Doucet A, Willems PH, Bindels RJ.** Localization of
442 the epithelial Ca(2+) channel in rabbit kidney and intestine. *J Am Soc Nephrol* 11: 1171-1178,
443 2000.
444 19. **Hoenderop JG, van Leeuwen JP, van der Eerden BC, Kersten FF, van der Kemp AW,**
445 **Mérillat AM, Waarsing JH, Rossier BC, Vallon V, Hummler E, Bindels RJ.** Renal Ca^{2+}

446 wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. *J Clin Invest*
447 112: 1906-1914, 2003.
448 20. **Hoenderop JGJ, Voets T, Hoefs S, Weidema F, Prenen J, Nilius B, Bindels RJM.** Homo-
449 and heterotetrameric architecture of the epithelial Ca^{2+} channels TRPV5 and TRPV6. *EMBO*
450 *J* 22: 776-785, 2003.
451 21. **Hoorn EJ, Walsh SB, McCormick JA, Zietse R, Unwin RJ, Ellison DH.** Pathogenesis of
452 calcineurin inhibitor-induced hypertension. *J Nephrol* 25: 269-275, 2012.
453 22. **Hopp K, Ward CJ, Hommerding CJ, Nasr SH, Tuan H, Gainullin VG, Rossetti S, Torres**
454 **VE, Harris PC.** Functional polycystin-1 dosage governs autosomal dominant polycystic
455 kidney disease severity. *J Clin Invest* 122: 4257-4273, 2012.
456 23. **Jászai J, Farkas LM, Fargeas CA, Janich P, Haase M, Huttner WB, Corbeil D.** Prominin-
457 2 is a novel marker of distal tubules and collecting ducts of the human and murine kidney.
458 *Histochem Cell Biol* 133: 527-539, 2010.
459 24. **Kathem SH, Mohieldin AM, Nauli SM.** The roles of primary cilia in polycystic kidney disease.
460 *AIMS Mol Sci* 1: 27-46, 2014.
461 25. **Kuehn EW, Park KM, Somlo S, Bonventre JV.** Kidney injury molecule-1 expression in
462 murine polycystic kidney disease. *Am J Physiol Renal Physiol* 283: 1326-1336, 2002.
463 26. **Kwon TH, Frøkiær J, Nielsen S.** Regulation of aquaporin-2 in the kidney: A molecular
464 mechanism of body-water homeostasis. *Kidney Res Clin Pract* 32: 96-102, 2013.
465 27. **Lantinga-van Leeuwen IS, Leonhard WN, van der Wal A, Breuning MH, de Heer E, Peters**
466 **DJ.** Kidney-specific inactivation of the Pkd1 gene induces rapid cyst formation in developing
467 kidneys and a slow onset of disease in adult mice. *Hum Mol Genet* 16: 3188-3196, 2007.
468 28. **Leonhard WN, Happe H, Peters DJ.** Variable cyst development in autosomal dominant
469 polycystic kidney disease: the biologic context. *J Am Soc Nephrol* 27: 3530-3538, 2016.

470 29. **Leonhard WN, Zandbergen M, Veraar K, Van den Berg S, Van der Weerd L, Breuning M,**
471 **de Heer E, Peters DJM.** Scattered Deletion of PKD1 in Kidneys Causes a Cystic Snowball
472 Effect and Recapitulates Polycystic Kidney Disease. *J Am Soc Nephrol* 26: 1322-1333, 2015.
473 30. **Maesaka JK, Imbriano LJ, Ali NM, Ilamathi E.** Is it cerebral or renal salt wasting? *Kidney Int*
474 76: 934-938, 2009.
475 31. **Mount DB.** Thick ascending limb of the loop of Henle. *Clin J Am Soc Nephrol* 9: 1974-1986,
476 2014.
477 32. **Mullins LJ, Bailey MA, Mullins JJ.** Hypertension, kidney, and transgenics: a fresh
478 perspective. *Physiol Rev* 86: 709-746, 2006.
479 33. **Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM,**
480 **Quinn SJ, Ingber DE, Zhou J.** Polycystins 1 and 2 mediate mechanosensation in the primary
481 cilium of kidney cells. *Nat Genet* 33: 129-137, 2003.
482 34. **Nishiura JL, Neves RF, Eloi SR, Cintra SM, Ajzen SA, Heilberg IP.** Evaluation of
483 nephrolithiasis in autosomal dominant polycystic kidney disease patients. *Clin J Am Soc*
484 *Nephrol* 4: 838-844, 2009.
485 35. **Novalic Z, Van der Wal AM, Leonhard WN, Koehl G, Breuning MH, Geissler EK, De Heer**
486 **E, Peters DJM.** Dose-Dependent Effects of Sirolimus on mTOR Signaling and Polycystic
487 Kidney Disease. *J Am Soc Nephrol* 23: 842-853, 2012.
488 36. **Olinger E, Schwaller B, Loffing J, Gailly P, Devuyst O.** Parvalbumin: calcium and
489 magnesium buffering in the distal nephron. *Nephrol Dial Transplant* 27: 3988-3994, 2012.
490 37. **Olteanu D, Yoder BK, Liu W, Croyle MJ, Welty EA, Rosborough K, Wyss JM, Bell PD,**
491 **Guay-Woodford LM, Bevensee MO, Satlin LM, Schwiebert EM.** Heightened epithelial Na+
492 channel-mediated Na+ absorption in a murine polycystic kidney disease model epithelium
493 lacking apical monocilia. *Am J Physiol Cell Physiol* 290: 952-963, 2006.
494 38. **Ong AC, Devuyst O, Knebelmann B, Walz G.** Autosomal dominant polycystic kidney
495 disease: the changing face of clinical management. *Lancet* 385: 1993-2002, 2015.

496 39. **Parikh CR, Dahl NK, Chapman A, Bost JE, Edelstein CL, Comer DM, Zeltner R, Tian X,**
497 **Grantham JJ, Somlo S.** Evaluation of urine biomarkers of kidney injury in Polycystic Kidney
498 Disease. *Kidney Int* 81: 784-790, 2012.
499 40. **Pavik I, Jaeger P, Kistler AD, Poster D, Krauer F, Cavelti-Weder C, Rentsch KM,**
500 **Wüthrich RP, Serra AL.** Patients with autosomal dominant polycystic kidney disease have
501 elevated fibroblast growth factor 23 levels and a renal leak of phosphate. *Kidney Int* 79: 234-
502 240, 2011.
503 41. **Pearce D, Soundararajan R, Trimpert C, Kashlan OB, Deen PM, Kohan DE.** Collecting
504 duct principal cell transport processes and their regulation. *Clin J Am Soc Nephrol* 10: 135-
505 146, 2015.
506 42. **Pedersen EB, Thomsen IM, Lauridsen TG.** Abnormal function of the vasopressin-cyclic-
507 AMP-aquaporin2 axis during urine concentrating and diluting in patients with reduced renal
508 function. A case control study. *BMC Nephrol* 11: 26, 2010.
509 43. **Pietrzak-Nowacka M, Safranow K, Bober J, Olszewska M, Birkenfeld B, Nowosiad M,**
510 **Ciechanowski K.** Calcium-phosphate metabolism parameters and erythrocyte Ca2+
511 concentration in autosomal dominant polycystic kidney disease patients with normal renal
512 function. *Arch Med Sci* 9: 837-842, 2013.
513 44. **Pietrzak-Nowacka M, Safranow K, Palacz J, Golembiewska E, Marchelek-Myśliwiec M,**
514 **Ciechanowski K.** Association of kidney and cyst dimensions with anthropometric and
515 biochemical parameters in patients with ADPKD. *Ren Fail* 37: 798-803, 2015.
516 45. **Praetorius HA.** The primary cilium as sensor of fluid flow: new building blocks to the model.
517 A review in the theme: cell signaling: proteins, pathways and mechanisms. *Am J Physiol Cell*
518 *Physiol* 308: 198-208, 2014.
519 46. **Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O.** The rediscovery of uromodulin
520 (Tamm-Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease. *Kidney*
521 *Int* 80: 338-347, 2011.

522 47. **Rohatgi R, Greenberg A, Burrow CR, Wilson PD, Satlin LM.** Na transport in autosomal
523 recessive polycystic kidney disease (ARPKD) cyst lining epithelial cells. *J Am Soc Nephrol*
524 14: 827-836, 2003.
525 48. **Schrier RW, Abebe KZ, Perrone RD, Torres VE, Braun WE, Steiman TI, Winkhofer FT,**
526 **Brosnahan G, Czarnecki PG, Hogan MC, Miskulin DC, Rahbari-Oskoui FF, Grantham**
527 **JJ, Harris PC, Flessner MF, Bae KT, Moore CG, Chapman AB; HALT-PKD Trial**
528 **Investigators.** Blood pressure in early autosomal dominant polycystic kidney disease. *N Engl*
529 *J Med* 371: 2255-2266, 2014.
530 49. **Subramanya AR, Ellison DH.** Distal convoluted tubule. *Clin J Am Soc Nephrol* 9: 2147-2163,
531 2014.
532 50. **Terryn S, Ho A, Beauwens R, Devuyst O.** Fluid transport and cystogenesis in autosomal
533 dominant polycystic kidney disease. *Biochim Biophys Acta* 1812: 1314-1321, 2011.
534 51. **Veeramuthumari P, Isabel W.** Clinical study on autosomal dominant polycystic kidney
535 disease among south Indians. *Int J Clin Med* 4: 200-204, 2013.
536 52. **Worthley LI, Guerin M, Pain RW.** For calculating osmolality, the simplest formula is the best.
537 *Anaesth Intensive Care* 15: 199-202, 1987.
538 53. **Woudenberg-Vrenken TE, Lameris AL, Weißgerber P, Olausson J, Flockerzi V, Bindels**
539 **RJ, Freichel M, Hoenderop JG.** Functional TRPV6 channels are crucial for transepithelial
540 Ca2+ absorption. *Am J Physiol Gastrointest Liver Physiol* 303: 879-885, 2012.
541 54. **Woudenberg-Vrenken TE, Sukinta A, Van der Kemp AW, Bindels RJ, Hoenderop JG.**
542 Transient receptor potential melastatin 6 knockout mice are lethal whereas heterozygous
543 deletion results in mild hypomagnesemia. *Nephron Physiol* 117: 11-9, 2011.
544 55. **Yamaguchi T, Hempson SJ, Reif GA, Hedge AM, Wallace DP.** Calcium restored a normal
545 proliferation phenotype in human polycystic kidney disease epithelial cells. *J Am Soc Nephrol*
546 17: 178-187, 2006.

547 56. **Yoder BK, Hou X, Guay-Woodford LM.** The polycystic kidney disease proteins, polycystin-
548 1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol* 13: 2508-
549 2516.
550 57. **Zatti A, Chauvet V, Rajendran V, Kimura T, Pagel P, Caplan MJ.** The C-Terminal Tail of
551 the Polycystin-1 Protein Interacts with the NA,K-ATPase α-Subunit. *Mol Biol Cell* 16: 5087-
552 5093, 2005.
553 58. **Zheleznova NN, Wildson PD, Staruschenko A.** Epidermal growth factor-mediated
554 proliferation and sodium transport in normal and PKD epithelial cells. *Biochim Biophys Acta*
555 1812: 1301-1313, 2011.
556

557

558 **FIGURE LEGENDS**
559

560 **Figure 1. Illustrative examples of the images used for the calculation of the cystic index in**
561 **iKsp-*Pkd1*$^{-/-}$ and control kidneys.** The cystic index of kidneys from control and iKsp-*Pkd1*$^{-/-}$ mice
562 were assessed from total scans of hematoxylin and eosin-stained kidneys sections (A, D). The
563 area of the total kidney minus the stained lumen area was calculated (C, F) and subtracted from
564 the total renal plus lumen area (B, E).
565

566 **Figure 2. Kidneys of iKsp-*Pkd1*$^{-/-}$ mice display a pre-cystic phenotype at PN18 + 29 days.**
567 (A, B and C) iKsp-*Pkd1*$^{lox/lox}$ mice untreated (control) or treated (kidney specific *Pkd1*$^{-/-}$) with
568 tamoxifen on post natal days 18, 19 and 20 (PN18) and sacrificed 29 days later (PN18 + 29 days).
569 (A) Periodic acid-Schiff (PAS) staining indicating normal renal histology in mice without tamoxifen
570 treatment (control) and mild tubular dilation 29 days after tamoxifen treatment (*Pkd1*$^{-/-}$). Mild
571 tubular dilation is observed in the cortex, outer and inner medulla. (B) Mild tubular dilation was

572 observed predominantly in the PT (anti-BCRP, green) and CD (anti-AQP2, red). No significant
573 tubular dilation was observed in the TAL (anti-THF, red), DCT (anti-NCC, red) and CNT (anti-
574 TRPV5, green). (C) Increased *Kim-1* mRNA expression observed in pre-cystic kidneys of *Pkd1*$^{-/-}$
575 mice. (D) Blood Urea Nitrogen (BUN) levels displayed as mg/dL, no significant differences were
576 observed. (E) Ratio of the kidney weight to body weight expressed as a percentage (2KW/BW %)
577 and (F) the calculated cystic index showing the percentage of dilated/cystic area, values are
578 presented as means ± SEM (n = 7-8), $*P < 0.05$ is considered statistically significant.

579

580 **Figure 3. Renal expression of transporters relevant for electrolyte reabsorption.** (A-F) iKsp-
581 *Pkd1*$^{lox/lox}$ mice were either untreated (control, white bars) or treated (kidney specific *Pkd1*$^{-/-}$, black
582 bars) with tamoxifen on post natal days 18, 19 and 20 (PN18) and sacrificed 29 days later (PN18
583 + 29 days). (A) Relative mRNA expression of genes enriched in the PT. The genes assessed
584 were *Abcg2* (encoding BCRP) and *Slc34a1* (encoding NaPi-2a). (B) Relative mRNA expression
585 of genes enriched in the TAL. Genes measured were *Cldn19*, *Cldn16*, *Kcnj1* (encoding ROMK)
586 and *Slc12a1* (encoding NKCC2). (C) Relative mRNA expression of genes enriched in the DCT.
587 Genes measured were *Trpm6*, *Slc12a3* (encoding NCC), *Cnnm2* and *Slc41a3*. (D) Relative
588 mRNA expression of genes enriched in the CNT. Genes measured were *Trpv5*, *Calb1* (encoding
589 calbinin-D_{28K}), *Slc8a1* (encoding NCX1) and *Atp2b4* (encoding PMCA4A). (E) Relative expression
590 to controls of genes enriched in the CD. Genes measured were *Aqp2*, *Scnn1a* (encoding ENaCα)
591 and *Scnn1b* (encoding ENaCβ). (F) Relative mRNA expression of *Trpm7* (ubiquitous expressed
592 along the nephron). (A-F) mRNA levels were assessed by RTqPCR and normalized against the
593 reference gene *Gapdh*. Gene expression data were calculated using the Livak method ($2^{-\Delta\Delta Ct}$),
594 and they represent the mean fold difference (mean ± SEM, n = 7-8) from the calibrator group
595 (control mice). $*P < 0.05$ is considered statistically significant.

596

597 **Figure 4. Decreased gene expression of markers for TAL and DCT.** (A-B) iKsp-*Pkd1*$^{lox/lox}$ mice
598 were either untreated (control, white bars) or treated (kidney specific *Pkd1*$^{-/-}$, black bars) with
599 tamoxifen on post natal day 18, 19 and 20 (PN18) and sacrificed 29 days later (PN18 + 29 days).
600 (A) Relative mRNA expression of genes encoding specific renal segment markers, namely *Umod*
601 (encoding Uromodulin) for the TAL and *Pvalb* (encoding Parvalbumin) for the DCT. (B) Relative
602 mRNA expression of genes encoding for a specific marker of the PT, namely *Prom1* (encoding
603 Prominin-1) and *Prom2* (encoding Prominin-2), a marker for distal tubules. mRNA expression
604 levels were assessed by RTqPCR and normalized against the reference gene *Gapdh*. Gene
605 expression data were calculated using the Livak method ($2^{-\Delta\Delta Ct}$), and they represent the mean
606 fold difference (mean ± SEM, n = 7-8) from the calibrator group (control mice). *$P < 0.05$ is
607 considered statistically significant.

608

609 **Figure 5. Intestinal expression of transporters relevant for electrolyte reabsorption.** (A-C)
610 iKsp-*Pkd1*$^{lox/lox}$ mice were either untreated (control, white bars) or treated (kidney specific *Pkd1*$^{-/-}$,
611 black bars) with tamoxifen on post natal day 18, 19 and 20 (PN18) and sacrificed 29 days later
612 (PN18 + 29 days). Relative mRNA expression of key genes for Ca^{2+} and Mg^{2+} absorption in the
613 duodenum (A), caecum (B) and colon (C). Genes assessed were *Trpm6*, *Cnnm4*, *Trpv6* and
614 *Atp2b4* (encoding PMCA4A). mRNA levels were assessed by RTqPCR and normalized against
615 the reference gene *Gapdh*. Gene expression data were calculated using the Livak method ($2^{-\Delta\Delta Ct}$),
616 and they represent the mean fold difference (mean ± SEM, n = 7-8) from the calibrator group
617 (control mice). *$P < 0.05$ is considered statistically significant.