Glomerular and proximal tubule cysts as early manifestations of Pkd1 deletion

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Abstract
Background. The homozygous deletion of Pkd1 in the mouse results in embryonic lethality with renal cysts and hydrops fetalis, but there is no precise data on the segmental origin of cysts and potential changes associated with polyhydramnios.

Methods. We used Pkd1-null mice to investigate cystogenesis and analyze the amniotic fluid composition from embryonic day 12.5 (E12.5) to birth (n = 257 embryos).

Results. Polyhydramnios was consistently observed from E13.5 in Pkd1−/− embryos, in absence of placental abnormalities but with a significantly higher excretion of sodium and glucose from E13.5 through E16.5, and increased cyclic adenosine 3’5-monophosphate (cAMP) levels at E14.5 and glucose from E13.5 through E16.5, and increased maladies but with a significantly higher excretion of sodium and glucose from E13.5 through E16.5, and increased cAMP levels at E14.5 and E15.5. The Pkd1−/− embryos started to die at E13.5, with lethality peaking at E15.5, corresponding to the onset of cystogenesis. The first cysts in Pkd1−/− kidneys emerged at E15.5 in mesenchyme-derived segments at the cortico-medullary junction, with a majority of glomerular cysts and fewer proximal tubule cysts (positive for megalin). The cysts extended to ureteric bud-derived collecting ducts (positive for Dolichos biflorus agglutinin lectin) from E16.5.

Conclusions. These studies indicate that Pkd1 deletion is associated with a massive loss of solutes (from E13.5) and increased cAMP levels (E14.5) associated with polyhydramnios. These abnormalities precede renal cysts (E15.5), first derived from glomeruli and proximal tubules and later from the collecting ducts, reflecting the expression pattern of Pkd1 in maturing epithelial cells.

Keywords: cystogenesis; glomerular and proximal tubule cysts; low-molecular-weight protein; megalin; polyhydramnios

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most prevalent monogenic disorders, leading to end-stage renal disease in approximately half of the affected patients [1]. ADPKD is caused by mutations of either PKD1 or PKD2, the genes that encode polycystin-1 and polycystin-2, respectively. These two proteins, which are located in the primary cilium, interact in vivo to regulate the proliferation and differentiation of renal tubular cells via various signalling pathways [2]. PKD1 and PKD2 are widely expressed throughout different foetal and adult tissues, explaining why ADPKD can affect extra-renal tissues including the liver, the pancreas and the arteries. In ADPKD kidneys, cysts originate from a small number of nephrons and possess functional and molecular characteristics of various nephron segments [3].

During normal human nephrogenesis, PKD1 mRNA is absent from the uninucleated cells and the emerging ureteric bud. From 10 weeks, a strong PKD1 signal appears in the first set of differentiated proximal tubules (PT) from their glomerular origin. From 10 to 24 weeks, the differentiated PT express high levels of PKD1 mRNA. At week 15, a discrete PKD1 expression is also detected in the distal nephron and ureteric bud branches, persisting at a moderate level during foetal life [4]. In mouse embryonic kidneys, Pkd1 is not expressed in the ureteric bud and comma and S-shaped bodies, and weakly expressed in induced mesenchymal mesenchyme from embryonic Day 13.5 (E13.5), to increase intensely in differentiating PT from E15.5 [5]. Several mouse models carrying mutations in Pkd1 have been reported. All Pkd1 knockout (KO) embryos die in utero by developing massive polycystic kidney disease, hydrops fetalis and polyhydramnios [5–10]. Some models are also characterized by vascular fragility [7] and cardiovascular and skeletal development defects [5], suggesting that the type of mutation in Pkd1 may influence the severity of the phenotype and the stage of lethality. Taken together, these studies showed that polycystin-1 does not play a major role in early nephrogenesis, as the latter is normal in Pkd1 mutant embryos [5–7]. Instead, polycystin-1 may participate in epithelial cell differentiation and tubular extension in late nephrogenesis.

While previous studies pointed to the severe renal cystogenesis and extrarenal phenotype of Pkd1 embryos, there
has been no detailed investigation of the time-course and segmental origin of the cysts. Early functional abnormalities in human ADPKD include impaired urinary concentrating capacity [1,2] and urinary excretion of PT markers [11]. However, the factors contributing to polyhydranomias in Pkd1 KO mice, including potential abnormalities in the placenta [12], remain unknown. In this study, we used a mouse model with a targeted deletion of Pkd1, resulting in a Pkd1-null allele [9], to investigate daily survival and cystogenesis in utero, as well as placental morphology and amniotic fluid (AF) volume and composition. Our data show that the loss of Pkd1 is associated with a massive loss of solutes from E13.5 along with increased cyclic adenosine 3'5'-monophosphate (cAMP) levels in the AF. These functional abnormalities precede the renal cysts, which are first detected in mesenchyme-derived glomerulus and PT segments and later in the collecting ducts.

Materials and methods

Pkd1 mice and in utero analyses

Studies were conducted on a Pkd1 mouse model that was obtained by targeting the exon 2 to 5 and part of the exon 6 of Pkd1, built in a null allele [9,13]. The original stock of mice (mixed 129/sv/C57BL/6J background) was later backcrossed (at least six generations) to the C57BL/6J background. Heterozygous Pkd1−/−embryos, aged 10–15 weeks, were crossed to generate homozygous Pkd1−/−embryos. The gestational age was dated by appearance of the vaginal plug on the morning after mating, and designated as Day 0.5 (E0.5). Pregnant mice were sacrificed at mouse age dated by appearance of the vaginal plug on the morning after mating, and designated as Day 0.5 (E0.5). Pregnant mice were sacrificed at

Antibodies and markers

Sheep polyclonal antibodies against megalin (a gift of Dr. P. Verroust, INSERM, Paris, France) and uromodulin (Biodesign Int., Saco, ME); and rabbit polyclonal antibodies against aquaporin-1 (AQP1) (Chemicon-Millipore, Billerica, MA), aquaporin-2 (AQP2) (Sigma, St Louis, MO) and podocin (P35, a gift of Dr. C. Ingelheim, Heidelberg, Germany) in 0.1 mol/L phosphate buffer, pH 7.3, prior to embedding in paraffin as described [13]. Six-micrometre sections were cut and stained with hematoxylin and eosin. Additional sections were incubated for 30 min with 0.3% hydrogen peroxide to block endogenous peroxidase. Following incubation with 10% normal serum for 20 min, sections were incubated for 45 min with the primary antibodies diluted in PBS containing 2% bovine serum albumin (BSA). After washings, sections were successively incubated with biotinylated anti-immunoglobulin (Ig) G antibodies, avidin–biotin peroxidase and aminothiobenzaldehyde (Vectastain Elite, Vector Laboratories). The M.O. kit (Vector Laboratories) was used for mouse-derived antibodies.

Morphometric analyses of the placenta

Volumetric density of four different compartments of the placenta (the chorionic plate with stem villi, the labyrinth, the spongiotrophoblast and the giant cells) was determined by point counting, using a GF Planachromat 12.5× objective on a Nenamed 2 microscope (Jena, Jena, Germany) equipped with GF-PW 10× oculars containing a 100 crosses grid. A random whole histological cross-section was analysed for six placentas from Pkd1−/− and Pkd1−/−embryos at E13.5 by a pathologist unaware of the mouse genotype.

Analyses of AF

The AF samples were prospectively collected from live embryos of pregnant Pkd1−/− females from E12.5 to E18.5. Each embryo was placed inside a pre-weighted chamber before inserting a BD Micro-Fine Insulin needle, 29 G × 12.7 mm, into the amniotic sac for AF aspiration. After careful aspiration, the foetal membrane was ruptured and opened up completely in order to collect all the remaining fluid. The total volume of the AF was measured in pre-weighted sterile tubes (intra-assay error <5%). Aliquots of AF were obtained at the time of aspiration and stored at −20°C. The concentrations of sodium and glucose were measured with a Synchron CX5 PRO analyser (Beckman Coulter, Fullerton, CA). The concentrations of the low-molecular-weight (LMW) protein CC16 (Clara cell protein 16 kD) was determined using a sensitive radioimmunoassay as described [15].

CAMP measurement

For cAMP measurement, AF (30 μl) was mixed with 300 μl of absolute ethanol, vortexed and centrifuged at 3500 g for 20 min at 4°C. The supernatant was collected and lyophilized using a Speed-Vac concentrator. cAMP levels were determined using a cAMP [125I] Biotrak Assay (Amer sham, Buckinghamshire, UK) following the acetylation procedure described in the assay. The lyophilized AF samples and cAMP standards (ranging from 2 to 128 fmol/100 μl) were submitted to acetylation by the addition of a mixture of acetic anhydride triethylamine (1:2; v/v).
A duplicate of 100 μl aliquots from all standards and samples was pipetted into polypropylene tubes, then 100 μl of antiserum (except in tubes for the determination of non-specific), and 100 μl of cAMP [125I] were added into all tubes, prior to being vortexed, and finally incubated for 4 h at 4°C. After the incubation, 500 μl of Amerelex-M secondary antibody reagent was added to each tube. The tubes were vortexed and then incubated for 10 min at room temperature. The antibody-bound fraction was separated by centrifugation at 2500 g for 15 min, and the supernatant liquid was discarded by careful aspiration. The radioactivity was counted in duplicate for 2 min in a gamma counter.

Data analysis
Comparisons between groups were performed using two-tailed unpaired Student’s t-test (GraphPad, San Diego, CA). Significance level was P < 0.05.

Results

Survival rate and polyhydramnios in Pkd1−/− embryos
Embryonic lethality was observed in Pkd1−/− embryos as early as E13.5, with a survival rate that sharply declined at E15.5. Only 25% (3/12) of Pkd1−/− embryos survived at E18.5, and none at birth. By contrast, all wild-type and heterozygous Pkd1 embryos survived to birth (Table 1). The first abnormality found in Pkd1−/− embryos was the polyhydramnios, consistently observed from E13.5 (Figure 1A–C). The time-course analysis revealed a progressive and continuous increase in the total AF volume in Pkd1−/− mice, contrasting with the stability observed between E12.5 and E17.5 in both wild-type and heterozygous mice. The AF volume was significantly higher at all time points from E13.5 to E18.5 in Pkd1−/− vs. both Pkd1+/+ and Pkd1+/− embryos (Figure 1D).

Histological analysis of the Pkd1-mutant placentas
As abnormalities of the placental labyrinth layer have been described in a Pkd1−/− mouse model (K. Piontek et al., unpublished work [12]), we performed a detailed morphometry analysis of the placentas of Pkd1 mice at E13.5, the first stage associated with polyhydramnios. This analysis showed that the volumic density of each placental compartment was similar between Pkd1+/+ and Pkd1+/− embryos.

Table 1. Survival rate of Pkd1-mutant embryos

<table>
<thead>
<tr>
<th>Crossed mice</th>
<th>Embryonic Age</th>
<th>Pkd1−/− n: A (D)</th>
<th>Pkd1+/+ n: A (D)</th>
<th>Pkd1+/− n: A (D)</th>
<th>Total embryos n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>E12.5</td>
<td>3 (0)</td>
<td>3 (0)</td>
<td>12 (0)</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>E13.5</td>
<td>9 (1)</td>
<td>6 (0)</td>
<td>12 (0)</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>E14.5</td>
<td>12 (0)</td>
<td>9 (0)</td>
<td>17 (0)</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>E15.5</td>
<td>10 (4)</td>
<td>11 (0)</td>
<td>19 (0)</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>E16.5</td>
<td>5 (8)</td>
<td>10 (0)</td>
<td>16 (0)</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>E17.5</td>
<td>3 (8)</td>
<td>8 (0)</td>
<td>13 (0)</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>E18.5</td>
<td>3 (9)</td>
<td>9 (0)</td>
<td>15 (0)</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>At birth</td>
<td>0 (4)</td>
<td>6 (0)</td>
<td>13 (0)</td>
<td>23</td>
</tr>
</tbody>
</table>

A, alive; D, dead; n, number.

A, B, C, D, E13.5, E15.5, E16.5, E17.5, E18.5

Fig. 1. Polyhydramnios in Pkd1−/− embryos. (A–C) Pkd1−/− embryos with massive polyhydramnios shown here inside the mother’s uterine membrane at different stages of development in comparison with Pkd1+/+ wild-type embryos: (A) E13.5, (B) E15.5, (C) E16.5. Bar = millimetre scale. (D) Time-course of amniotic fluid volume at each time point according to the Pkd1 genotype (n = 6 to 19 embryos at each time point). The total amniotic fluid volume values were significantly higher in Pkd1−/− vs. Pkd1+/+ and Pkd1+/− from E13.5 to E18.5. *P < 0.0001; #P < 0.0001, Pkd1−/− vs. Pkd1+/−.
embryos, with no detectable abnormalities in the labyrinth and the spongiotrophoblast (Suppl. Fig. 2).

**Hydrops fetalis and vascular fragility in Pkd1−/− embryos**

In addition to polyhydramnios, Pkd1−/− embryos showed a typical phenotype of hydrops fetalis resulting in tissue edema, detectable from E13.5 and throughout gestation (Figure 2A–C). Edema of the back of the body caused a vertical shape, preventing Pkd1−/− embryos from being curved as the wild-type embryos. Moreover, the Pkd1−/− embryos showed areas of focal haemorrhage in different regions of the body, such as the neck and abdomen (Figure 2D, E). The vascular rupture could be observed as early as E13.5 and continuing to E18.5.

**Pattern of cystogenesis in the Pkd1−/− embryonic kidneys**

Histological analysis (Figure 3) showed that the renal cysts in Pkd1−/− embryos were first detected at E15.5 (Figure 3B), consistent with the other Pkd1-mutant mouse models. The first cysts at E15.5 were located in the internal area of the kidney, and a large majority of them were glomerular cysts characterized by the cystic enlargement of the Bowman space and the presence of glomerular tufts (Figure 3F and Figure 4). The glomerulocystic phenotype was only observed for glomeruli located in the deep medulla zone, whereas superficial glomeruli located in the cortex among comma and S-shaped bodies were non-cystic (Figure 4A). At high magnification, the cysts arise from the dilation of the Bowman capsule, with flattened cells and discontinuous cell lineage. The podocytes, typically organized in a crown surrounding the capillaries in the young glomeruli, showed no abnormalities (Figure 4B). Immunostaining for the endothelial marker CD31/PECAM-1 identified the normal glomerular vascularization in these sections (Figure 4C, D).

From E16.5, cystogenesis progressed from the medulla towards the cortical area, still involving glomeruli as well as tubular segments (Figure 3G). By E18.5 the cysts were detected in all areas of the kidney (Figure 3H). Quantification revealed that glomerular cysts accounted for ~65% (128/197) of the total number of cysts at E15.5 and ~45% (110/246) at E16.5 (Table 2). Apart from atrophic lesions of the glomerular tuft, which were observed from E16.5, there was no evidence for fibrosis, inflammatory infiltrate, tubular casts or epithelial hyperplasia in the Pkd1−/− kidneys. Of note, even in mutant embryos, nephrogenesis continued on until birth in the external cortex.

**Segmental origin of the cysts in Pkd1-mutant kidneys**

To further characterize the segmental origin of the cysts, serial sections of Pkd1−/− kidneys were stained with megalin, a multi-ligand receptor that is specifically expressed in PT cells [16], and DBA lectin, a marker of the distal convoluted tubule and the collecting duct [17] (Figure 5). No cyst was observed at E14.5, whereas developing tubular
profiles positive for megalin or DBA lectin were detected (Figure 5A, E). At E15.5, some of the cysts at the cortico-medullary junction were stained with megalin, whereas no cysts were stained with DBA lectin (Figure 5B, F). At E16.5, a fraction of cysts located in medulla and cortico-medullary area were positive for megalin (70/246, 28%), or less frequently, DBA lectin (42/246, 17%) (Figure 5C, G; Table 2). There was no cross-reactivity between megalin and DAB lectin in the same cyst (Figure 5B–H).

Further analyses showed that the staining for megalin, which was strictly apical in wild-type and non-cystic tubule profiles, was less polarized, diffusely increased or
The cell markers megalin and DBA lectin were used to identify PT and collecting duct cells, respectively. Immunohistochemistry on E15.5 kidneys revealed that PT cells expressing megalin were identified at the urinary pole of the Bowman capsule, whereas DBA lectin-positive cells were only seen in puncta within the collecting ducts (asterisk). These findings were consistent with the dynamic activity of the ureteric bud and the expanding mesenchyme during nephrogenesis (Figure 6). The glomerular cysts were unstained, except for some megalin-positive PT cells identified at the urinary pole of the Bowman capsule (Figure 6A). At higher magnification (Figure 6B), the cysts were identified with flattened cells and discontinuous cell lineage (asterisk). The podocytes, typically organized in a crown surrounding the capillaries in the young glomeruli, showed no abnormalities. The first proximal tubules could be seen in some glomerular cysts, without any tubular dilation at E15.5. Immunostaining for CD31 (Figure 6C-D) was used as a marker of glomerular vascularization in cysts identified at E16.5. Bar = 80 μm (A); 20 μm (B, D); 40 μm (C).

Table 2. Segmental origin of the cysts in Pkd1-null embryonic kidneys

<table>
<thead>
<tr>
<th>Age</th>
<th>Kidney sections (n)</th>
<th>Glomerular cysts (n)</th>
<th>Megalin b</th>
<th>DBA lectin b</th>
<th>Undefined cysts (n)</th>
<th>Total cysts (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13.5</td>
<td>8</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E14.5</td>
<td>8</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E15.5</td>
<td>10</td>
<td>128</td>
<td>++</td>
<td>54</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>E16.5</td>
<td>8</td>
<td>110</td>
<td>++</td>
<td>70</td>
<td>42</td>
<td>24</td>
</tr>
</tbody>
</table>

*These sections were obtained from four to five embryos.

bStaining intensity: +, weak positive staining; ++, strong positive staining.

Expression of Pkd1 and polycystin-1 during mouse nephrogenesis

We next investigated the pattern of Pkd1 and polycystin-1 expression in the developing mouse (Figure 7). Using the β-galactosidase reporter gene, Pkd1 expression was not detected in the pronephros or mesonephros prior to the development of the definitive metanephric kidney in the Pkd1del17−21/geo +/− mouse. From E13.5–E15.5, weak Pkd1 expression was seen in the condensed mesenchyme surrounding the ureteric bud tips and weakly in some cells within the uncondensed mesenchyme but not in the ureteric bud tips themselves. Pkd1 expression was also seen in endothelial cells migrating into the S-shaped
body to form the glomerulus. From E15.5, there was marked upregulation of Pkd1 expression within the developing metanephros and in the glomerular parietal epithelium, differentiating PT and collecting ducts (Figure 7A, B). Vascular staining was also detected, whereas early nephron precursors and ureteric bud tips in the peripheral

Fig. 5. Segmentsal origin of cysts in Pkd1−/− embryonic kidneys. Serial sections of Pkd1−/− embryonic kidneys at different stages of development stained with megalin, a marker of the proximal tubule (A–D), and DBA lectin, a marker of the distal tubule and collecting duct (E–H). Non-cystic tubule profiles are stained at E14.5, without cross-reactivity between the two markers (A, E). At E15.5, some cysts are stained with megalin (B), whereas DBA staining is still restricted to non-cystic tubules (F). At E16.5 and E18.5, some cysts are stained with megalin, whereas other cysts are positive for DBA lectin, indicating proximal vs. collecting duct origin respectively (C–D vs. G–H). There was no cross-reactivity between megalin and DAB lectin in the same cyst. Bar = 100 μm.
cortex remained negative. Immunostaining for polycystin-1 (anti-LRR antibodies) detected a specific signal in the glomerular parietal epithelium and in the PT epithelial cells in E15.5 Pkd1+/+ kidneys (Figure 7E–F), whereas no specific staining was observed in the corresponding regions of Pkd1−/− kidneys (Figure 7G). This staining pattern was confirmed (although with a higher background) when using the 7E12 antibody against polycystin-1 (data not shown).

Massive loss of solutes and increased cAMP levels in AF

The AF collected from E13.5 to E16.5 was analyzed in order to calculate the amount of solute excreted in each genotype (Figure 8). The Pkd1−/− embryos were characterized by a significantly higher excretion of sodium and glucose from E13.5 through E16.5 (Figure 8A, B). Time-course analysis of the LMW protein CC16 in the AF of the wild-type embryos revealed a progressive decrease from E13.5 to E16.5, followed by an abrupt rise at E17.5 as a marker of foetal lung growth, as previously described [15]. By contrast, the CC16 excretion progressively increased from E13.5 to E16.5 in the Pkd1−/− embryos, being significantly higher than the wild-type at E15.5 and E16.5 (Figure 8C). Furthermore, there was a progressive increase in the cAMP excreted in the AF of Pkd1-null embryos at E14.5, and even further at E15.5, which was concurrent with cystogenesis (Figure 8D). These data show that deletion of Pkd1 is associated with a substantial loss of solutes, including the LMW protein CC16 before the onset of lung growth, and increased cAMP levels in the AF.

Discussion

In this study, we have analysed the consequences of Pkd1 deletion on the time-course and pattern of cy-
stogenesis, the structure of the placenta and the AF volume and composition in mouse embryos. The $Pkd1$-null embryos start to die at E13.5, with consistent features including hydrops fetalis, renal cysts and vascular fragility, in absence of placental abnormalities. This $Pkd1$-null model is characterized by an early polyhydramnios, with an excessive loss of various solutes, including cAMP, in the AF. These features precede the development of renal cysts, which are first detected in glomeruli and PT, and later in distal nephron segments.

Nephrogenesis in mouse and man is characterized by a repetitive and reciprocal induction between the ureteric bud and the metanephric mesenchyme, resulting in the formation of mature kidneys before birth. The first cysts in $Pkd1^{-/-}$ embryonic kidneys are observed at E15.5, starting in mesenchyme-originated tissues, with the majority of cysts arising from mature glomeruli and a significant proportion from the PT segments as indicated by positive megalin staining. These events are reflected by a rise in embryonic lethality at E15.5. The segmental cystogenesis in our model is consistent with the pattern of $Pkd1$ expression in the mouse as reported by Boulter et al. [5] and further detailed here using lacZ staining on developing kidneys from $Pkd1^{del17-21\betageo-/-}$ mice (Figure 7). In agreement with in situ hybridization data [18], these studies show that $Pkd1$ expression is limited during early nephrogenesis, with weak expression in the mesen-
chyme and no expression in the ureteric bud. From E15.5, Pkd1 expression increased dramatically in induced mesenchymal cells, including maturing PT, and subsequently, more distal nephron segments [5]. The initial and intense expression of Pkd1 in glomerular parietal epithelium and PT is in line with the first cystic lesions observed here and in the Pkd1<sup>del34/del34</sup> mice [6]. Glomerular cysts have also been reported in the Pkd1<sup>L/L</sup> mouse model characterized by a severe phenotype including vascular defect leading to haemorrhagic lesions and lethality by E15.5 [7]. Furthermore, glomerular cysts were detected in the adult kidneys from two models of transgenic mice overexpressing normal PKD1 [19] or Pkd1 [20], suggesting the importance of a precise regulation of polycystin-1 expression for normal glomerular maturation and tubulogenesis.

Hydrops fetalis, a term used to describe foetuses with generalized oedema and cavity effusions, is observed in the Pkd1<sup>−/−</sup> embryos like in the majority of Pkd1 KO mice thus far [5,7–9]. Fluid balance in the foetus integrates placental fluid transfer, capillary filtration, swallowing, lung secretion and urine production [21]. Accordingly, many features observed in the Pkd1 mice may explain an interstitial fluid accumulation, including abnormal vascular permeability, cardiac malformations and impaired renal function. The Pkd1<sup>−/−</sup> embryos investigated here show a significant polyhydramnios, consistently observed from E13.5 and throughout development. By contrast, the AF volume is stable across gestation in both wild-type and heterozygous Pkd1 mice, followed by a sharp decrease at E18.5, similar to the human and mouse situation [21,22]. In addition to our model, polyhydramnios has only been reported in another Pkd1-null mouse [8]. Polyhydramnios may result from salt-losing tubulopathies or increased foetal urine output secondary to diabetes insipidus [23]. The AF fluid analyses demonstrated a massive loss of sodium and glucose in the Pkd1<sup>−/−</sup> embryos starting at E13.5, i.e. 2 days before cystogenesis. There is also an increased excretion of the LMW protein CC16 at E15.5 and E16.5 in the AF of Pkd1-mutant embryos, before
Glomerular and proximal tubule cysts as early manifestations of Pkd1 deletion

The effects of V2R antagonists on cAMP generation and the cystic phenotype in ADPKD are based on the assumption that ADPKD cysts are predominantly of collecting duct origin. However, the deletion of Pkd1 in this mouse model is associated with predominant glomerular cysts at E15.5, followed by the development of (melatin-positive) PT cysts and later by collecting duct cysts. Accordingly, the V2R/AQP2 pathway, which is restricted to the collecting duct and not expressed in the glomerular parietal epithelium or in the PT, is not necessary for cyst development at least in this model. These findings in Pkd1 mice may also yield insights into the segmental origin of cysts in human ADPKD. In the developing human kidney, high PKD1 expression first appears in differentiated PT starting from their glomerular origin and later in the distal nephron and the ureteric bud branches [4]. Glomerular cysts have been reported in patients with ADPKD [33], including in a severe childhood case associated with a PKD1 deletion [34]. Earlier analyses of cyst fluid composition, electric properties and immunoreactivity for segmental markers (including AQP1 and aminopeptidase) have identified a significant number of cysts of PT origin co-existing with collecting duct cysts in end-stage kidneys of ADPKD patients [3,35].

In conclusion, we show that the deletion of polycystin-1 in this mouse model is reflected by polyhydramnios and a massive loss of solutes, including cAMP, in the AF. These changes precede the development of renal cysts, first detected in glomeruli and PT. These features give insights into the role of polycystin-1 in renal development, the mechanisms of cystogenesis and the tubular alterations encountered in ADPKD.

Supplementary data

Supplementary data are available online at http://ndt.oxfordjournals.org

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