Proximal Tubular Cysts in Fetal Human Autosomal Recessive Polycystic Kidney Disease

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Abstract. Standard texts describe human autosomal recessive polycystic kidney disease (ARPKD) as a cystic kidney disease in which renal cysts are localized to collecting tubules. Murine models of ARPKD consistently demonstrate an early phase of proximal tubular (PT) cystic involvement, which disappears shortly after birth. This is followed by a phase of collecting tubular (CT) cyst formation and progressive enlargement leading to compromise of renal function and death. Because the description of cystic lesions in human ARPKD has been largely based on postnatal specimens, PT cyst formation was hypothesized to be a characteristic feature of fetal human, as well as murine, ARPKD. This study examines nephron segment-specific cyst localization histochemically by lectin binding in 11 human ARPKD specimens obtained at different fetal and postnatal ages. PT cysts were found in human fetal specimens from gestational age 14 wk to 26 wk. The percentage of cysts involving PT segments ranged from 2 to 41%. The cystic index of PT cysts ranged from 2 to 5. In all specimens in which PT cysts were found, both the percentage of CT cysts and their cystic index were equal to or greater than the percentage of PT cysts and the associated PT cystic index. PT cysts were absent in all kidney specimens older than 34 wk gestational age. It is concluded that human ARPKD, like murine ARPKD, has a transient phase of PT cyst formation during early fetal development.

Autosomal recessive polycystic kidney disease (ARPKD) is one of a number of human genetic and acquired diseases in which renal cysts are a central pathologic feature. Although standard texts (1,2) describe human ARPKD as a cystic kidney disease in which lesions are localized to collecting tubules, murine models of ARPKD consistently demonstrate an early phase of proximal tubular (PT) cystic involvement, which disappears shortly after birth. This is followed by a phase of collecting tubular (CT) cyst formation and progressive enlargement leading to compromise of renal function and death. Because description of cystic lesions in human ARPKD has been largely based on postnatal specimens, PT cyst formation was hypothesized to be a characteristic feature of fetal human, as well as murine, ARPKD. We therefore examined nephron segment-specific cyst localization in human ARPKD specimens obtained at different fetal and postnatal ages. The results demonstrate that human ARPKD, like murine ARPKD, has a transient phase of PT cyst formation during fetal development.

Materials and Methods

Specimens: ARPKD Diagnosis

We examined 11 kidney specimens of human ARPKD from nine families. ARPKD was diagnosed in all cases by consensus histopathologic, clinical, radiographic, and genetic criteria (6). Fresh ARPKD specimens 2 (gestational age 17 wk [GA17]), 4 (GA20), 8 (GA24), and 11 (postnatal age 12 wk [PA12]) were fixed immediately after harvesting in 4% paraformaldehyde, embedded in Immuno-bed (Poly-science, Warrington, PA) plastic resin, and sectioned at 4 μm. The remaining human ARPKD specimens were received as formalin-fixed, paraffin-embedded blocks, which were then sectioned at 6 μm. Normal kidney specimens were received as a formalin-fixed, paraffin-embedded blocks and used as a control.

Immunohistochemistry

Cyst localization was examined using our previously described segment-specific lectin binding method with biotin-labeled Lotus Tetragonolobus (LTA; Sigma Chemical Co., St. Louis, MO) as a marker for proximal tubules (visualized with Fast Red) and biotin-labeled Arachis Hypogaea (PNA; Sigma Chemical Co.) as a marker for collecting tubules (visualized with diaminobenzidine) (3,4,7–9).

Lectin Profile Analysis of Cyst Formation and Cystic Index

After immunohistologic preparation, LTA and PNA lectin profile analyses were performed on the kidney sections of human ARPKD.
Published data and morphometric analyses in our laboratory of normal fetal lectin-identified PT and CT diameters established a mean PT diameter of $0.010 \pm 0.004$ mm (range, 0.004 to 0.020) at GA14, which increased only slightly to $0.013 \pm 0.006$ mm (range, 0.006 to 0.025) at PA12 (10). PT luminal diameter had to exceed the mean diameter by twofold and the highest normal value by 1.5-fold to be considered cystic. The degree of tubular cyst formation was quantified by utilization of a cystic index. The index has been derived from basic light microscopic morphometric methods (11) and has been standardized to quantify cyst formation in vivo and in vitro (3,4,7–9). After routine histologic preparation, 10 to 12 evenly spaced 4-μm sections were graded for cyst formation in LTA-positive tubular segments (PT) or in PNA-positive tubular segments (CT), on the following scale (9): 0, no cysts observed; 1, single or multiple cysts >0.03 and ≤0.05

Figure 1. Proximal tubular (PT) cysts in human autosomal recessive polycystic kidney disease (ARPKD). Sections were stained with *Lotus Tetragonobolus* (LTA) (red), a PT marker, and *Arachis Hypogaea* (PNA) (brown), a collecting tubule (CT) marker. Representative photos from control specimen, GA17 (A); specimen 2, GA17 (B); specimen 4, GA20 (C); specimen 8, GA24 (D); and specimen 11, PA12 (E). Cysts of proximal tube origin (asterisks) are present amid morphologically normal proximal tubules, collecting tubules, and CT cysts (B and C). Cysts of proximal tube origin (asterisks) are present amid an increasing number of CT cysts (D). All cysts are collecting tubules in origin (E). Magnification: ×400 in A; ×500 in B through D; ×250 in E.
mm; 2, multiple cysts >0.05 and ≤0.10 mm; 3, multiple cysts >0.10 and ≤0.15 mm; 4, multiple cysts >0.15 and ≤0.20 mm; 5, multiple cysts >0.20 mm.

The numbers of LTA-positive, PNA-positive, and total cysts were counted in six separate fields having more than 40 glomeruli, with low-power magnification (×32).

Results

PT cysts were found in human fetal specimens from GA14 to GA26. PT cysts were absent in specimen 10 (GA34) and specimen 11 (PA12). Representative photos from specimens 2 (GA17), 4 (GA20), 8 (GA24), and 11 (PA12) are shown in Figure 1. The percentage of cysts involving PT and CT segments ranged from 0 to 41% and from 52 to 96%, respectively. During successive fetal stages, there was a gradual shift in the site of cystic nephron involvement, from PT to CT, as defined by lectin binding. The cystic index of PT cysts ranged from 0 to 5. In all specimens in which PT cysts were found, the percentage of CT cysts and their cystic index was equal to or greater than the percentage of PT cysts and the associated PT cystic index (Table 1).

Discussion

This study demonstrates that human ARPKD, like murine ARPKD, has a transient phase of PT cyst formation during fetal development. These findings suggest that ARPKD is a developmental kidney disease in which the shift in the site of tubular lesions may involve developmental regulation of cystic (and/or modifier) gene expression. Identification of these regulatory elements may provide new insights into segment-specific normal and abnormal kidney differentiation and growth.

Acknowledgments

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Table 1. Lectin profile of cyst formation and cystic index in human ARPKD

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Age (wk)</th>
<th>LTA+ (% of cysts)</th>
<th>PT CI Size Range (mm)</th>
<th>PNA+ (% of cysts)</th>
<th>CT CI Size Range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GA14</td>
<td>41</td>
<td>PT 5 (0.04 to 0.22)</td>
<td>52</td>
<td>CT 5 (0.04 to 0.25)</td>
</tr>
<tr>
<td>2</td>
<td>GA17</td>
<td>34</td>
<td>PT 3 (0.05 to 0.15)</td>
<td>64</td>
<td>CT 4 (0.10 to 0.20)</td>
</tr>
<tr>
<td>3</td>
<td>GA17</td>
<td>39</td>
<td>PT 5 (0.04 to 0.50)</td>
<td>57</td>
<td>CT 5 (0.04 to 0.25)</td>
</tr>
<tr>
<td>4</td>
<td>GA20</td>
<td>15</td>
<td>PT 4 (0.05 to 0.20)</td>
<td>82</td>
<td>CT 5 (0.10 to 0.35)</td>
</tr>
<tr>
<td>5</td>
<td>GA22</td>
<td>21</td>
<td>PT 4 (0.04 to 0.20)</td>
<td>77</td>
<td>CT 5 (0.05 to 0.45)</td>
</tr>
<tr>
<td>6</td>
<td>GA22</td>
<td>23</td>
<td>PT 4 (0.03 to 0.16)</td>
<td>77</td>
<td>CT 5 (0.05 to 0.32)</td>
</tr>
<tr>
<td>7</td>
<td>GA23</td>
<td>15</td>
<td>PT 3 (0.03 to 0.15)</td>
<td>79</td>
<td>CT 5 (0.05 to 0.40)</td>
</tr>
<tr>
<td>8</td>
<td>GA24</td>
<td>9</td>
<td>PT 4 (0.03 to 0.16)</td>
<td>88</td>
<td>CT 5 (0.05 to 0.30)</td>
</tr>
<tr>
<td>9</td>
<td>GA26</td>
<td>2</td>
<td>PT 2 (0.03 to 0.07)</td>
<td>91</td>
<td>CT 5 (0.05 to 0.27)</td>
</tr>
<tr>
<td>10</td>
<td>GA34</td>
<td>0</td>
<td>(No cysts)</td>
<td>96</td>
<td>CT 5 (0.10 to 0.50)</td>
</tr>
<tr>
<td>11</td>
<td>PA12</td>
<td>0</td>
<td>(No cysts)</td>
<td>96</td>
<td>CT 5 (0.20 to 0.60)</td>
</tr>
</tbody>
</table>

a ARPKD, autosomal recessive polycystic kidney disease; GA, gestational age; PA, postnatal age; PT, proximal tubular; CT, collecting tubular; CI, cystic index; LTA+, *Lotus Tetragonobolus*-positive; PNA+, *Arachis Hypogaea*-positive.
References

Access to UpToDate on-line is available for additional clinical information at http://www.lww.com/JASN.