Abstract. c-ErbB2 (also referred to as Neu or HER2), a transmembrane glycoprotein with intrinsic tyrosine kinase activity, is structurally related to epidermal growth factor receptor (EGFR) and forms active heterodimers with EGFR as well as other members of the EGFR family. c-ErbB2 is reported to mediate differentiation and proliferation in epithelial cells and is expressed in a tissue-specific and developmental stage-specific manner. Given the role of EGFR in cystic renal epithelial hyperplasia and the immature phenotype of cystic renal epithelial cells, the segment-specific expression pattern of c-ErbB2 in human autosomal recessive polycystic kidney disease (ARPKD) was examined in nine ARPKD kidney specimens ranging from gestational age 17 wk through postnatal age 4 wk. c-ErbB2 staining of human ARPKD samples showed increased expression with increasing gestational age compared with normal human fetal and postnatal kidneys. This increased c-ErbB2 expression was primarily localized to the apical surfaces of cystic collecting tubule cells, similar to the pattern of EGFR expression, and paralleled collecting tubular cyst formation and growth.

Autosomal recessive polycystic kidney disease (ARPKD) is invariably characterized by the formation and enlargement of renal collecting tubular cysts as well as hepatic biliary ectasia and fibrosis (1,2). Extensive morphologic analysis and mathematical modeling of cyst formation and growth indicate that epithelial hyperplasia is a necessary element in the formation and growth of cystic lesions in polycystic kidney disease (PKD) (1). Evidence from a number of laboratories has established a role for the epidermal growth factor (EGF)-transforming growth factor-α (TGF-α)-epidermal growth factor receptor (EGFR) axis in promoting epithelial hyperplasia and collecting tubular cyst enlargement in PKD (3). These studies demonstrate that EGFR is overexpressed and mislocated to the apical surface of cystic tubular epithelium in human ARPKD, in human autosomal-dominant PKD (ADPKD), and in murine models of ARPKD (cpk, bpk, and orpk) and ADPKD (4–8). Furthermore, in murine ARPKD, inhibition of EGFR tyrosine kinase through genetic or pharmacologic manipulation drastically reduces cyst formation and growth (5,9).

The c-erbB2 oncogene (also referred to as neu or HER2) encodes a 185-kD transmembrane glycoprotein with intrinsic tyrosine kinase activity. The c-ErbB2 protein is structurally related to EGFR and is able to form active heterodimers with EGFR (10,11). c-ErbB2 is commonly expressed in fetal epithelial cells of the human kidney and to a lesser degree in normal human adult kidneys (12,13). Amplification of c-ErbB2 has been demonstrated in human breast, ovarian, and pancreatic carcinoma, and some studies have suggested that c-ErbB2 amplification may have significant prognostic implications (14–19). These findings suggest that c-ErbB2 plays a role in both normal proliferation and neoplastic transformation of epithelial cells. Given these data, as well as the established role of EGFR in cystic renal epithelial hyperplasia, we sought to examine the segment-specific expression pattern of c-ErbB2 in fetal and infantile human ARPKD.

The results demonstrate segment-specific and cyst formation/growth-related c-ErbB2 expression in human ARPKD. These data suggest a potential role for c-ErbB2 in proximal tubular cyst formation and, in concert with EGFR, in progressive collecting tubular cyst formation and enlargement in ARPKD.

Materials and Methods
Specimens: ARPKD Diagnosis
We examined nine human renal ARPKD specimens ranging from gestational age 17 wk (GA17) to postnatal age 4 wk (PA4) from seven families (1 GA17, 2 GA22, 1 GA23, 1 GA26, 1 GA29, 1 GA33, 1 GA34, and 1 PA4). ARPKD was diagnosed in all cases by consensus clinical, radiographic, and genetic criteria (20). Five normal kidney specimens aged GA12, GA13, GA14, GA15, PA4, and PA16 were received as formalin-fixed, paraffin-embedded blocks and used as controls.

Immunohistochemistry
Sections were incubated for 1 h at room temperature with anti-c-ErbB2 antibody (A485, affinity-purified rabbit polyclonal, 1:100; DAKO, Carpinteria, CA), and serial sections were incubated for 1 h at room temperature with anti-EGFR (mouse monoclonal clone 29.1, 1:200; Sigma Chemical Co., St. Louis, MO) and lectins. Cyst localization was examined using our previously described segment-specific lectin binding method with biotin-labeled Lotus tetragonolobus.
(Sigma) as a marker for proximal tubules (PT; visualized with Fast Red) and biotin-labeled *Arachis Hypogaea* (Sigma) as a marker for collecting tubules (CT; visualized with DAB) (4,5,7,9,21–24). Staining was scored semiquantitatively with the use of computer video image analysis as follows: 0, absent; 1 to 3, weak; 4 to 7, moderate; and 8 to 10, strong.

**Results**

**c-ErbB2 and EGFR Expression in Normal Human Fetal and Postnatal Kidneys**

In normal kidneys GA12, GA13, GA14, GA15, PA4, and PA16, PT demonstrated weak cytoplasmic expression of c-ErbB2 and CT demonstrated moderate basolateral as well as apical expression of c-ErbB2 (Table 1, Figure 1, A and C). In normal kidneys, c-ErbB2 expression was greater in CT than in PT, and expression in all segments decreased with increasing gestational age (Figure 1, A and C). EGFR expression in normal fetal kidney specimens demonstrated apical as well as basolateral EGFR expression in CT (Figure 1B). However, by PA4, EGFR expression was restricted to the basolateral membrane domain in all tubular segments (Figure 1D). The postnatal expression pattern of c-ErbB2 remained apical as well as basolateral in CT and primarily cytoplasmic in PT (Figure 1C). The intensity, however, was markedly decreased when compared with fetal specimens, suggesting a temporal decrease in c-ErbB2 expression.

**Proximal Tubule: c-ErbB2 and EGFR Expression in Human ARPKD**

At GA17, both noncystic and cystic PT demonstrated cytoplasmic expression of c-ErbB2 (Figure 2B, Table 1). However, expression of c-ErbB2 was greater in cystic compared with noncystic PT (Figure 2B). c-ErbB2 expression in cystic PT decreased with advancing gestational age, paralleling decreasing proximal tubular cyst numbers and decreasing PT cyst size (Table 1, Figure 3) (22). The difference in c-ErbB2 expression level between cystic PT and noncystic PT decreased with advancing gestational age. All ARPKD and normal specimens demonstrated cytoplasmic and basolateral EGFR staining in PT (Figure 2, C and F).

**Collecting Tubule: c-ErbB2 and EGFR Expression in Human ARPKD**

At GA17, both noncystic and cystic CT demonstrated basolateral and apical expression of c-ErbB2 in equal intensity (Figure 2B, Table 1). In contrast to decreasing CT expression with increasing age in normal specimens, c-ErbB2 expression in cystic CT showed little decline with advancing gestational age, particularly on the apical cell surface of cystic CT (Figure 2, B insert and E insert). This increased c-ErbB2 expression in cystic CT paralleled collecting tubular cyst formation and growth, as well as increased apical EGFR expression in cystic CT (Table 1, Figures 2, E and F, and 3). c-ErbB2 expression in noncystic CT was less intense than in cystic CT and decreased with advancing gestational age similar to that seen in normal fetal specimens (Figure 2, B and E, Table 1). At GA33, expression of c-ErbB2 in noncystic CT was weak and the difference of c-ErbB2 expression between cystic and noncystic CT was striking. At PA4, the expression level of c-ErbB2 in cystic CT began to decline slightly, whereas expression level of apical EGFR remained high in cystic CT (Table 1, Figure 2, C and F).

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<th>Table 1. Segment-specific developmental expression of c-ErbB2 and EGFR*</th>
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* EGFR, epidermal growth factor receptor; ARPKD, autosomal recessive polycystic kidney disease; PT, proximal tubule; CT, collecting tubule; BL, basolateral; AP, apical.
All ARPKD specimens demonstrated apical as well as basolateral expression of EGFR in cystic CT (Figure 2F and insert). This apical expression of EGFR in CT was limited to cystic tubules except in kidneys at GA13, GA17, and GA22 that had apical EGFR expression in noncystic CT (Figure 1B, Table 1).

**Discussion**

Human ARPKD has been classically described as a cystic kidney disease in which lesions are localized to CT (1). However, studies of the ontogeny of cyst formation in murine models of ARPKD have demonstrated two distinct phases in cystic nephron involvement (4,7,23–25). Recently, we established that human ARPKD, like murine ARPKD, has a transient phase of proximal tubular cyst formation during fetal development (22). Given the role of c-ErbB2 and EGFR in mediating proliferation and differentiation in renal epithelium (12,13,21,26,27), we examined segment-specific c-ErbB2 and EGFR expression in normal and ARPKD human fetal kidneys.

In agreement with previous studies, all five normal kidney specimens in the current study demonstrated weaker c-ErbB2 expression in PT compared with CT (12). However, in human fetal ARPKD kidneys at GA17, c-ErbB2 expression in cystic CT was higher, at any given age, than c-ErbB2 expression in normal PT (A). c-ErbB2 expression in normal CT declines with increasing gestational age and remains apical and basolateral (C). EGFR expression in normal fetal kidney specimens demonstrated apical as well as basolateral EGFR expression in CT at GA13 (B). However, by PA4, EGFR expression was restricted to the basolateral membrane domain in all tubular segments (D). In normal kidneys, c-ErbB2 expression was greater in CT than in PT, and expression in all segments decreased with increasing gestational age, suggesting a temporal decrease in c-ErbB2 expression. Magnifications: ×800 in A and B; ×2000 in C and D.

![Image of immunoperoxidase staining of c-ErbB2 and EGFR in human fetal kidneys](image-url)
Figure 2. Lectin staining of GA17 (A) and PA4 (D) human autosomal recessive polycystic kidney disease (ARPKD) kidney (red \(\text{Lotus Tetragonolobus} = \text{PT}\), brown \(\text{Arachis Hypogaea} = \text{CT}\)). Immunoperoxidase staining of c-ErbB2 (B) and EGFR (C) in GA17 human ARPKD kidney and c-ErbB2 (E) and EGFR (F) in PA4 human ARPKD kidney. Inserts show high-power c-ErbB2 staining of CT cysts in GA17 (B) and PA4 (E) ARPKD kidney and EGFR staining of CT cysts in GA17 (C) and PA4 (F) ARPKD kidney. Photographs show that in human fetal ARPKD kidneys at GA17, c-ErbB2 expression in cystic PT was equivalent to that in cystic CT and was more intense than that in noncystic PT (B). At GA17, both noncystic and cystic CT demonstrated basolateral and apical expression of c-ErbB2 (B) in equal intensity. c-ErbB2 expression in cystic CT showed little decline with advancing gestational age, particularly on the apical cell surface of cystic CT (insert, B and E). In cystic CT, EGFR expression is strong throughout gestation and remains apically and basolaterally expressed (F and insert) in contrast to normal CT.
PT was equivalent to that in cystic CT and was more intense than that in noncystic PT. The expression level in cystic PT after GA17 decreased with advancing gestational age, paralleling decreases in PT cyst formation and enlargement (22). These findings suggest that c-ErbB2 may be involved in the developmentally regulated pathophysiology of PT cyst formation in ARPKD. The contribution of cystic PT to the total population of kidney cysts decreases during disease progression. This is most likely due to restriction of proximal tubular cystogenesis to a specific developmental stage in the context of ongoing nephrogenesis and progressive formation of collecting tubular cysts (25). The shift in nephron involvement during renal organogenesis suggests that there may be a developmentally regulated pattern of cystic gene expression or tubular responsiveness to cyst-promoting processes. Regulation of PT c-ErbB2 expression may be one factor involved in this process.

In cystic CT from human fetal ARPKD kidney specimens, c-ErbB2 expression remained strong with advancing gestational age, particularly on the apical cell surface. This increased c-ErbB2 expression in CT paralleled collecting tubular cyst formation and growth as well as apical EGFR expression in cystic CT lesions. In contrast, c-ErbB2 expression in noncystic CT decreased with advancing gestational age. However, at PA4, expression level of c-ErbB2 in cystic CT began to decrease, whereas expression level of apical EGFR remained high.

These findings suggest that c-ErbB2 may also be involved, in a developmentally regulated manner, in the pathophysiology of early to mid-CT cyst formation. Herrera (28) reported c-ErbB2 amplification in ADPKD (3 of 3 specimens) but not ARPKD specimens (0 of 3 specimens). Ages of ARPKD specimens were not described in that report, and differences between those and the current findings may relate to stage or severity of the cystic lesions and/or ages of the specimens.

EGFR is overexpressed and mislocated to the apical surface of cystic tubular epithelium in human ARPKD and ADPKD and in the murine models of ARPKD (cpk, bpk, and orpk) and ADPKD (3–8). In this study, we report apical EGFR expression in noncystic CT in specimens at GA17 and GA22 and in four normal human fetal kidney specimens aged between GA12 and GA15 as well as in cystic CT in all ARPKD kidney specimens regardless of age. Apical EGFR expression in noncystic CT is not seen in human ARPKD older than GA22. As previously reported, apical EGFR expression may be a normal pattern in CT of developing human kidneys (3,6). These findings confirm that cystic renal CT demonstrates features of an immature epithelial phenotype (29,30).

In summary, developmental expression patterns of c-ErbB2 suggest a potential role for this glycoprotein in proximal tubular cyst formation and enlargement and in concert with EGFR, in progressive collecting tubular cyst formation and enlargement in ARPKD. These findings suggest that c-ErbB2, like EGFR, may be a potential target for cyst reduction therapy in ARPKD.

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References