Renal Tubular Dysgenesis, a Not Uncommon Autosomal Recessive Disorder Leading to Oligohydramnios: Role of the Renin-Angiotensin System


Renal tubular dysgenesis is a clinical disorder that is observed in fetuses and characterized by the absence or poor development of proximal tubules, early onset and persistent oligohydramnios that leads to the Potter sequence, and skull ossification defects. It may be acquired during fetal development or inherited as an autosomal recessive disease. It was shown recently that autosomal recessive renal tubular dysgenesis is genetically heterogeneous and linked to mutations in the genes that encode components of the renin-angiotensin system. This study analyzed the clinical expression of the disease in 29 fetus/neonates from 18 unrelated families and evaluated changes in renal morphology and expression of the renin-angiotensin system. The disease was uniformly severe, with perinatal death in all cases as a result of persistent anuria and hypoxia related to pulmonary hypoplasia. Severe defects in proximal tubules were observed in all fetuses from 18 gestational weeks onward, and lesions also involved other tubular segments. They were associated with thickening of the renal arterial vasculature, from the arcuate to the afferent arteries. Renal renin expression was strikingly increased in 19 of 24 patients studied, from 13 families, whereas no renal renin was detected in four patients from three families. Angiotensinogen and angiotensin-converting enzyme were absent or present in only small amounts in the proximal tubule, in correlation with the severity of tubular abnormalities. No specific changes were detected in angiotensin II receptor expression. The severity and the early onset of the clinical and pathologic expression of the disease underline the major importance of this system in fetal kidney function and development in humans. The identification of the disease on the basis of precise histologic analysis and the research of the genetic defect now allow genetic counseling and early prenatal diagnosis.

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defects, arthrogryposis, and lung hypoplasia. Since the initial description of this condition, at least 63 observations of primary sporadic or familial cases of RTD have been reported (2–22). Fetal anuria persists after birth. Severe and refractory hypotension has been observed in children who survive the immediate neonatal period (14,17,18). Poor calvarial ossification, resulting in wide cranial sutures and large fontanels, is an additional feature of the disease (6–8,13,16–18).

Similar clinical and pathologic features have been observed in several situations, all resulting in renal ischemia (10). These situations include the twin–twin transfusion syndrome that is observed in monochorionic twin pregnancies in which the donor fetus presents RTD (23–25), major cardiac malformations (23,26), severe liver diseases (27–29), and fetal or infantile renal artery stenosis (30; personal observations). RTD with large fontanels also has been described in fetuses that are exposed in utero to angiotensin-converting enzyme (ACE) inhibitors (31,32) or angiotensin II (AngII) receptor antagonists (33,34). These observations of secondary, drug-induced RTD suggested that dysregulation of the renin-angiotensin system (RAS) could be responsible for the hereditary form of the disorder (17). We previously reported abnormal renin accumulation in the preglomerular and interlobular arteries, juxtaglomerular apparatus (JGA), and glomerular mesangial cells in four fetuses/neonates with autosomal recessive RTD; these findings were consistent with a defect in the AngII negative feedback control of renin secretion (9). We recently confirmed the involvement of the RAS by showing that mutations in the genes that encode renin, angiotensinogen, ACE, and AngII receptor type 1 (AT1) are associated with autosomal recessive RTD (35). We report here the results of a clinical and pathologic study of 29 fetuses or neonates who had autosomal recessive RTD and were observed during the past 16 yr.

Materials and Methods
Patients, Tissue, and DNA Specimens
Between 1988 and 2005, autopsy specimens from 44 singleton fetuses or neonates with a prenatal history of severe and persistent oligohydramnios, with normal or nearly normal renal ultrasound scans, were referred to our department for analysis. RTD was diagnosed on the basis of histologic examination. In 15 fetuses, renal involvement was secondary, associated with in utero exposure to ACE inhibitors or AT1 antagonists (nine fetuses), severe neonatal hemispheric liver disease (one fetus), or extensive areas of ischemic necrosis of the placenta (five fetuses). RTD seemed to be primary in the remaining 29 patients, presented here, who belonged to 18 unrelated families. Four of these patients, who belonged to families 4, 10, 11, and 12, were reported briefly previously (9). Families 1 through 9 were studied previously for RAS gene mutations, and homozygous or compound heterozygous mutations were identified in eight of them (families 1 through 7 and 9) (35) (Table 1).

Kidneys were fixed in 10% buffered formalin. Frontal corticomedulary sections were taken through the hilus and embedded in paraffin. Sections (4 μm) were cut and stained with trichrome light green, trichrome safranin, and periodic acid-Schiff (PAS). The number of generations of nephrons was determined as previously reported, according to the “medullary ray counting method” (36,37). Normal kidneys from 21 singleton fetuses (18 to 30 wk of gestation) served as controls. They were obtained from autopsies after the spontaneous abortion of seemingly normal fetuses (five fetuses) or termination of pregnancy because of extrarenal abnormalities (16 fetuses). None of these fetuses presented polyhydramnios or oligohydramnios.

Genomic DNA was isolated from peripheral blood or frozen fetal tissue by standard methods. DNA samples were obtained from 12 affected individuals and 15 unaffected relatives (13 parents and two unaffected siblings) who belonged to 10 families (families 1 through 9 and 14). Linkage analysis and mutation screening were performed as described previously (35). No genetic study could be performed in seven families because of the absence of available blood or frozen tissue and the bad quality of DNA extracted from fixed and paraffin-embedded autopsy specimens and/or because of the absence of parental consent. In family 10, very little DNA was available, allowing only AGTR1 screening.

Antibodies
Anti-renin antibodies, prepared by the group of P. Corvol, were raised against human renin purified from a juxtaglomerular cell tumor. Their specificity was established previously (38). Rabbit polyclonal antibodies against ACE (Y4) (39) and human angiotensinogen (40) also were from P. Corvol. We also used (1) a monoclonal anti–α-smooth muscle actin (α-SMA) antibody (Sigma Immunokemical, St. Louis, MO); (2) a monoclonal anti–epithelial membrane antigen (anti-EMA) antibody, serving as a marker for distal and collecting tubules; (3), a monoclonal anti-CD10 antibody that recognizes the human membrane-associated neutral endopeptidase of podocytes and proximal tubular cells, (4) a monoclonal anti-CD15 antibody that recognizes an early myeloid differentiation antigen and serves as a marker for proximal tubules, and (5) a monoclonal anti-CD34 antibody that labels capillary endothelial cells (Beckman Coulter, Brea, CA). Antibodies were used at the following dilutions: Anti-EMA and anti-CD34, pure (as prediluted by the manufacturer); anti-CD15, 1:100; anti-SMA, 1:500; anti-renin (L24), 1:500; anti-ACE, 1:200; anti-angiotensinogen, 1:100; and anti-CD15, 1:5.

Immunoperoxidase Staining
Formalin-fixed, paraffin-embedded kidneys (and liver and/or lung tissues from six fetuses) were immunostained by the avidin-biotin method, using the Universal Immunostaining Streptavidin-Peroxidase Kit (Beckman Coulter). Deparaffinized 4-μm sections were rehydrated, washed in PBS buffer for 5 min, treated with 3% H2O2 in methanol for 5 min to block endogenous peroxidase activity, and washed in PBS for 2 min. They then were incubated with the protein-blocking agent for 10 min and with the primary antibody for 60 to 120 min. The sections were washed in PBS and incubated for 30 min with the polyclonal secondary biotinylated antibody. Washed sections were incubated for 45 min with streptavidin-peroxidase reagent. They then were incubated for 10 to 20 min with the freshly prepared chromogen solution (H2O2 and the chromogen 3-amino-9-ethylcarbazole). Sections were counterstained with hematoxylin and mounted in aqueous media. Control sections were processed as described above but without the primary antibodies.

Renin content was assessed semiquantitatively on corticomedullary sections, as described previously (25). The number of positive JGA per 100 glomeruli and the number of renin-positive cells per section of JGA were recorded. We also evaluated the presence of renin-positive mesangial, arteriolar, and arterial cells. Normal fetal kidneys served as controls.

Immunofluorescence Staining
Frozen renal and extrarenal tissues were available from one fetus (22 wk gestation; family 7). Immunofluorescence labeling was performed on 3-μm-thick cryostat sections that were fixed in acetone for 10 min
covering the sections with 30°C for 12 min in a microwave oven, postfixed in 4%

and incubated for 20 min with normal swine serum in PBS that contained 0.5% Tween-20 to block nonspecific binding. Sections were incubated for 1 h at room temperature with primary antibodies diluted in this buffer. They were then rinsed three times in PBS and incubated for 30 min with fluorescein-conjugated anti-rabbit antibodies diluted 1:100 in PBS. A mounting medium for immunofluorescence (Fluoprep; BioMérieux, Lyon, France) was used to delay fluorescence quenching.

Table 1. Clinical manifestations of the disease and renin expression in the 29 patients: Results of mutation screening in 11 families

<table>
<thead>
<tr>
<th>Family</th>
<th>Parental Consanguinity</th>
<th>Gender</th>
<th>Oligo-hydrations (wk)</th>
<th>Duration of Gestation (wk)</th>
<th>Age at Death (h/d)</th>
<th>Wide Fontanels</th>
<th>Low BP</th>
<th>Renin Expression</th>
<th>Mutation Screening</th>
<th>Gene</th>
<th>Nucleotide Alteration(s)</th>
<th>Alteration(s) in Coding Sequence</th>
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a++, presence of the feature; --, absence of the feature; het, heterozygous; hom, homozygous; ND, not done; SB, stillbirth; TP, termination of pregnancy.

bNucleotides are numbered with respect to the A of the ATG codon, in accordance with standard nomenclature.

cIn three families, one or two additional fetuses died during the perinatal period.

dConcerns the last nucleotide of the exon.

*In family 10, one female patient recovered partially after several weeks of anuria.

and incubated for 20 min with normal swine serum in PBS that contained 0.5% Tween-20 to block nonspecific binding. Sections were incubated for 1 h at room temperature with primary antibodies diluted in this buffer. They were then rinsed three times in PBS and incubated for 30 min with fluorescein-conjugated anti-rabbit antibodies diluted 1:100 in PBS. A mounting medium for immunofluorescence (Fluoprep; BioMérieux, Lyon, France) was used to delay fluorescence quenching. Labeling was assessed with an Orthoplan microscope equipped for light, fluorescence, and phase-contrast microscopy (Leica Microscopic Systems, Heebrugg, Switzerland).

In Situ Hybridization

Riboprobe Transcription. Recombinant plasmids that encoded the components of the RAS system (renin, angiotensinogen, ACE, AT1, and AT2) were from one of us (J.-M.G.), and the preparation of the riboprobes that were labeled with 35S-UTP (all probes) or digoxigenin (renin probes) has been described elsewhere (25,41).

In Situ Hybridization. In situ hybridization was carried out with 35S-UTP probes, as described previously (41). Deparaffinized 6-μm sections, mounted on silane-treated slides, were boiled in a citric acid buffer (0.01 M [pH 6.0]) for 12 min in a microwave oven, postfixed in 4% paraformaldehyde, and digested for 20 min with proteinase K. Then they were dehydrated and air-dried, and hybridization was performed by covering the sections with 30 μl of diluted riboprobe in hybridization solution (3 to 4 × 106 cpm section) and incubating them overnight in a humid chamber at 50°C. Slides were washed in solutions of various degrees of stringency (from 5× SSC with 50% formamide at 55°C to 0.01× SSC at room temperature) and digested with RNase A (20 μg/ml) to remove the unhybridized single-stranded RNA. Finally, the sections were dehydrated and air-dried. The hybridization signal was estimated macroscopically, by autoradiography of the sections for 1 to 3 d, using a Biomax film (Eastman Kodak, Rochester, NY). Microscopic autoradiographic images then were obtained by dipping the slides in NTB2 liquid emulsion (Kodak). Exposure times varied from 3 to 10 wk. Exposed slides were developed and fixed, then stained with toluidine blue. Slides were examined in bright- and dark-field conditions, using a Leitz microscope.

In situ hybridization with digoxin-labeled renin probes was performed as described previously (25). Renin mRNA levels were assessed semiquantitatively, using the method described for the assessment of renin protein.

Results

Clinical Presentation and Evolution

The 29 fetuses and neonates (16 male, 13 female) belonged to 18 unrelated kindreds of different origins: France, Italy, Slovenia, Syria, Turkey, North Africa, and Pakistan. The parents were first cousins in eight families. In 11 families, two to four children, analyzed in this study or identified by family investigation, were affected by the disease.
All fetuses presented oligohydramnios. It was detected between 16 and 33 wk gestation, mostly around 20 wk, and persisted throughout gestation. On ultrasound examination, the kidneys were normal in size, shape, and echogenicity in four cases. They showed mild hyperechogenic and/or poor corticomedullary differentiation in 10 cases and were enlarged and hyperechogenic in four fetuses. No information about kidney structure was available for the remaining fetuses. There were five stillbirths at 28 to 37 wk gestation, and 10 terminations of pregnancy between 18 and 27 wk gestation because of persistent severe oligohydramnios or recurrence of the disease in five families. Fourteen spontaneous or surgical deliveries, at 30 to 36 wk gestation, resulted in the birth of live infants with dysmorphic features of the Potter sequence and severe respiratory difficulties as a result of pulmonary hypoplasia. Six neonates died within 1 day, from respiratory distress, despite appropriate treatment in intensive care units. Eight infants survived 2 to 36 d, thanks to mechanical ventilation and peritoneal dialysis, but remained completely anuric. BP was abnormally low and not responsive to vasoactive drugs in six infants who survived 3 to 36 d. Surprising, the sister of one patient, previously reported by Kemper et al. (20), recovered diuresis after initial anuria that required peritoneal dialysis. At 13 yr of age, she has chronic renal insufficiency (creatinine clearance 39 ml/min per 1.73 m²). Marked skull hypoplasia with very large fontanel was observed in 10 neonates and four fetuses, examined at 22 to 37 wk gestation (Table 1).

**Morphology and Immunohistochemistry**

**Normal Controls.** In control fetal kidneys, the various stages of nephrogenesis were observed from the outer to the inner cortex. Proximal tubules were labeled with anti-CD10 and anti-CD15 antibodies from the glomerular stage onward, whereas distal tubules and collecting ducts were stained with anti-EMA antibodies. The proximal tubule brush border was strongly PAS positive. Staining for α-SMA was weak in vascular smooth muscle cells and strong in mesangial cells. The cortical and medullary capillary network was identified by staining with anti-CD34 antibodies, which also stained the endothelial cells of larger vessels (data not shown).

**Patients.** At autopsy (15 fetuses and 14 neonates), kidneys appeared grossly normal, with normal lobulation, corticomedullary demarcation, and calyx structure. The kidneys were larger and heavier than normal in half of the patients (+1 to +3 SD), slightly smaller and lighter in one, and normal in the others. The ureters were normal, and the urinary bladders were small and empty. The renal arteries were normal in size and permeable in all cases. Recent renal vein thrombosis was observed in one stillborn fetus of 27 wk gestation, with oligohydramnios since 18 wk gestation, and had occurred in one neonate who died at 36 d of age. Three children developed bowel perforation consistent with ischemic necrosis, leading to bowel resection in one case.

The nephrogenic zone was histologically normal in fetuses, but major cortical and medullary changes were observed (Figure 1, A through C). In the cortex, the most striking abnormality was the small number and irregular size of tubules, most of which were hypoplastic and lined with undifferentiated flat or cuboidal cells (Figure 1, A and B), whereas the distal tubules at their point of contact with the vascular pole of the glomeruli were enlarged in most patients (Figure 1B). In most patients, no proximal tubules could be identified using PAS staining or immunolabeling with anti-CD10 or anti-CD15 antibodies (Figure 1D). In seven patients, such stainings disclosed occasional straight tubular sections, mostly located in the deep cortex (Figure 1F). In contrast, strong apical labeling of all tubules was seen with anti-EMA antibodies (Figure 1, E and G). Focal tubular dilation was observed in three neonates who survived 6, 17, and 35 d, respectively. Glomeruli were closely packed together because of the lack of tubule differentiation, and glomerular tufts were enlarged as a result of mesangial expansion. The number of generations of nephrons was higher than that in age-matched controls in 12 cases, lower in two cases, and normal in the others (Figure 2). Mild interstitial fibrosis was observed. In the medulla, Henle loops were rare and atrophic, collecting ducts were collapsed, and the interstitial mesenchyme was much larger than normal (Figure 1C).

Large interlobar arteries were normal. In contrast, interlobular to afferent arteries showed marked muscular wall thickening and disorganization (Figure 1H), as clearly demonstrated by α-SMA labeling (Figure 1I) compared with labeling in control fetal kidneys (Figure 1M). Similar changes, as a result of both an increase in the number of cell layers and extracellular matrix accumulation, were focally observed within the arcuate arteries (Figure 1I). The cortical and medullary capillary network was normal, as shown by CD34 labeling (Figure 1, K and L). At the glomerular vascular pole, endothelial labeling demonstrated the presence of both afferent and efferent arterioles.

**Expression of the Components of the RAS**

**Normal Fetal Kidneys.** In 18 of 21 normal fetuses, a few cells (1–4) were renin positive in 5 to 10% of the JGA, whereas in the remaining three normal fetuses, renin labeling was observed in 10 to 20% of the JGA (Figure 3A). Mesangial cells displayed no staining for renin. Renin transcripts were detected in a few cells, at the vascular pole of 10 to 30% of the mature glomeruli, and in a few dispersed cells of the pregglomerular arteries (Figure 3B). As previously shown (41,42), anti-ACE antibodies labeled the apical pole of proximal tubular cells in paraffin-embedded tissues (Figure 3C) and also capillary endothelial cells on frozen sections (Figure 3D). Angiotensinogen protein (and RNA) was detected in proximal tubular cells and was abundant in hepatocytes (Figure 3, E and F). On **in situ** hybridization, the AT1 receptor of AngII was detected mostly in mesangial cells (Figures 3, G and H), whereas the AT2 receptor was detected in undifferentiated mesenchyme, immature tubules, and cortical and medullary interstitium (Figure 3, I and J). No labeling was observed in control sections omitting the primary antibody (Figure 3K) or using sense probe (Figure 3L).

**Affected Fetuses and Neonates.** Renin expression was analyzed in 24 patients from 17 families (one to three patients per family), and two different abnormal patterns were seen. In 19 patients from 13 families, very high levels of renin expression...
Figure 1. Renal tubular dysgenesis (RTD). Light microscopy. In the expanded cortex, glomeruli are closely packed together because of the absence of recognizable proximal tubules in an infant who was liveborn at 36 wk gestation (A). Most tubules are small and undifferentiated, but some distal tubular sections, adjacent to the glomerular vascular pole, are hypertrophied in a neonate who was born at 33 wk gestation (B). In the medulla, tubules are collapsed and surrounded by abundant connective tissue (33 wk gestation; C). Immunolabeling. No tubular labeling with an anti-CD10 antibody contrasting with normal podocyte staining (D) and diffuse tubular labeling with anti–epithelial membrane antigen (anti-EMA) antibody (E) in fetuses of 31 and 28 wk gestation, respectively. In one neonate, who was born at 36 wk gestation and died on day 6, we observed dilation of some tubules, focal CD15 labeling of straight parts of proximal tubules (F), and almost diffuse EMA tubular labeling (G). Thickening of the interlobular artery (arrow) in a fetus of 33 wk gestation (H) and of the arcuate arteries in a neonate who was born at 31 wk gestation (I). The arterial wall thickening and disorganization are highlighted by a comparison of α-smooth muscle actin (α-SMA) labeling in patients (J) and control subjects (M). Focally, some peritubular or pericapsular interstitial cells also express α-SMA. Normal cortical and medullary capillary network, in RTD patients, demonstrated by CD34 labeling (K and L). (A through L) RTD fetus/neonate. (M) Control fetal kidney. (A, B, and H) Periodic acid-Schiff staining. (C and I) Trichrome light green. (D through G and J through M) Immunoperoxidase and hematoxylin. Magnifications: ×30 in C and E; ×40 in A and L; ×160 in I, J, and M; ×180 in D, F, and G; ×240 in K; ×270 in B; ×300 in H.
were observed (Figure 4, A through C). Renin-positive cells were found in all JGA in eight patients, with between five and 20 positive cells per JGA section. Renin-positive cells were detected in 60 to 95% of JGA in the other patients. Renin-positive mesangial cells also were present in all 19 patients and were very abundant in 13 patients. Renin expression also was found in the muscular layer of long segments of afferent arterioles and in the interlobular arteries of four patients. In contrast, in four patients from three families, no renin-positive cells were detected (Figure 4D). The results of renin immunolabeling and in situ hybridization were concordant in all patients studied with both techniques. In one stillborn fetus from family 6, kidney tissue was poorly preserved. Renin was detected in only 30% of the JGA and in a few mesangial cells.

No tubular expression of ACE was detected in the kidney tubules of 16 of the 18 patients studied (Figure 4E). In two patients who belonged to families 4 and 14, very focal labeling was seen at the apical pole of small collapsed tubules, showing massive cytoplasmic accumulation of phagolysosomes (Figure 4F). In the frozen specimens of one fetus from family 4, this enzyme was detected clearly in glomerular and peritubular capillary endothelial cells and in lung endothelial cells, contrasting with the absence of tubular labeling (Figure 4, G and H). ACE transcripts also were detected in the renal endothelial cells (Figure 4, I and J) and the lung endothelial cells of the four patients studied (Figures 4, K and L).

Angiotensinogen expression was assessed in 15 patients who belonged to families 2, 3, 4, 5, 6, 9, 10, 11, 12, and 13. Within the kidney, no angiotensinogen was detected in seven patients with no recognizable proximal tubules, but low levels of angiotensinogen mRNA were observed in foci in one of these patients who belonged to family 13 (Figure 4, M and N). A few proximal tubules in the deep cortex produced angiotensinogen (protein and transcripts) in eight patients. In contrast, high levels of angiotensinogen protein and transcripts were observed in the hepatocytes of the four patients who belonged to families 4, 10, 12, and 13 (Figure 4, O and P).

Expression of the AngII receptors AT1 and AT2 was assessed by in situ hybridization in 10 patients. AT1 was normally expressed in the glomerular cells of seven patients, but only a weak signal was obtained in one patient and no signal at all was detected in the other two (Figure 4, Q and R). AT2 expression was normal in all patients studied (Figure 4, S and T).

Correlations between the Renal Expression of RAS Components and the Genotype

No renin expression was detected in four patients from the consanguineous families 1, 2, and 3. Screening for mutations in the renin gene led to the identification of homozygous or compound heterozygous nonsense or splice site REN mutations that are likely to prevent renin production (35). Levels of renin production were much higher than normal in the remaining patients. It was possible to screen patients who belonged to seven families for mutations in genes that encode components of the RAS. Mutations were identified in five of them, affecting the genes that encode renin (family 4) angiotensinogen (family 5), angiotensin-converting enzyme (families 7 and 9), or AngII receptor type 1 (family 6) (35). No mutation was detected in families 8 and 14 (Table 1).

ACE and angiotensinogen were absent or only very weakly expressed in kidney tubules, and this expression profile was correlated with the severity of proximal tubule defect rather than with the genetic defect involved. For example, these proteins were not detected in patients who belonged to families 2, 5, and 9, with no identifiable proximal tubules, and mutations in the REN, AGT, and ACE genes, respectively. Little or no AT1 production was detected in three patients (from families 2, 10, and 11). Screening of AT1 did not lead to the detection of a mutation in the patient from family 10 (E. Clauser, personal communication, 1993), whereas the patient from family 2 had a null mutation in the renin gene.

Discussion

During the past 15 yr, kidneys from 44 singleton fetuses or neonates with RTD were referred to our laboratory. After exclusion of possible alternative causes, RTD was regarded as primary in 29 fetuses from 18 families. The disease displayed autosomal recessive inheritance, as suggested by parental consanguinity in eight families and/or the involvement of several siblings in 11 families. The relevant clinical features, found in all fetuses, were the early occurrence of oligohydramnios, detected at or before 20 wk gestation; the absence of significant renal abnormalities on ultrasound examination; the uniform severity of the disease, with persistent anuria, even in infants who survived for several weeks; and the frequent association with large fontanelles as a result of skull ossification defects. The hallmark of the disease is the absence or paucity of proximal tubules. However, as previously shown by microdissection (2,13), other tubular segments also were affected: Henle loops were rare and atrophic, and collecting ducts were collapsed. We also observed marked thickening and disorganization of arterial and arteriolar walls, as a result of an increase in the number of smooth muscle cells and the abundance of extracellular matrix. These vascular lesions are similar to those in hypertensive nephrosclerosis. Efferent arterioles normally were present, contrasting with previous studies that suggested a
failure of efferent arteriole formation (2). The capillary network seemed to be normal.

Two subgroups of patients were identified on the basis of renal renin expression. No renin production was detected in patients from three unrelated consanguineous families. Screening of the renin gene led to the identification of loss-of-function REN mutations in all three kindreds (35). In contrast, in 19 patients from 13 families, renin levels were much higher than normal, with massive, diffuse staining of the JGA and extensive recruitment of arteriolar smooth muscle and mesangial cells. Patients from seven of these families were tested for mutations in genes that encode components of the RAS. In five of these families, homozygous or compound heterozygous mutations that affect REN, AGT, ACE, or AGTR1 have been detected (35). It is interesting that the REN mutations that were observed in one patient were expected to impair renin function but not renin production. Globally, these mutations are believed to result in AngII’s being absent or ineffective, leading to a loss of negative regulation of renin synthesis. They represent interesting models for analyzing structure–function relationship. Accordingly, their functional consequences are currently being studied in vitro. In addition, the precise phenotype of asymptomatic heterozygotes, especially the BP answer to moderate sodium depletion, is under investigation. An additional question arises from new findings of the role of the RAS in the control and at the different stages of hematopoiesis (43).

No gene defect was detected in two families, one with one affected girl, the other with affected fetuses of both genders. Mutations that affect the noncoding sequences (promotor/enhancer) of the studied genes may have been missed, although other genes possibly are involved in these families. AGTR2 and ATP6AP2, encoding AT2 and the renin receptor, respectively, were excluded because they are located on chromosome X. Genes that encode proteins that belong to the AT1 signaling pathway or the endothelin family are possible candidates. The finding, in mineralocorticoid receptor knockout mice, of a severe phenotype that resembles but is more drastic than the one observed in mice whose RAS genes have been disrupted implicates the MR gene as an additional candidate (44). Studies are in progress for identification of other RTD genes in patients without RAS gene mutations.

One major clinical feature that was observed only in patients

Figure 3. Expression of components of the renin-angiotensin system (RAS) in control fetal kidneys (20 to 30 wk gestation). Renin protein and transcripts are detected in a few cells of the juxtaglomerular apparatus (A and B). Angiotensin-converting enzyme (ACE) protein is seen at the apical pole of proximal tubules in fixed tissue (C), whereas it also is detected in peritubular and glomerular endothelial cells in frozen tissues (D). Angiotensinogen protein is seen in proximal tubules (E) and hepatocytes in the liver (F). AT1 receptor mRNA is detected in cells of the glomerular tuft (G and H). AT2 receptor mRNA is abundant in the cortical mesenchyme, around nephron structures (I and J). (A, C, E, F, and K) Immunoperoxidase. (D) Immunofluorescence. (B) In situ hybridization, using digoxigenin-labeled antisense probes. (G through J) In situ hybridization, using 35S-UTP antisense probes; dark- and bright-field illumination of the same section is shown for each labeling. (K) Immunolabeling of control liver tissue, but omitting the primary antibody. (L) In situ hybridization, using the 35S-UTP sense probe for renin. Magnifications: ×160 in B, E, and L; ×200 in A, C, D, F, and K; ×360 in G through J.
Figure 4. Expression of genes that encode components of the RAS in patients with RTD (21 to 37 wk gestation). Renin: Marked increase in renin protein (A and C) and transcript (B) levels in patients who belonged to families 7, 9, and 10, respectively, contrasting with the complete absence of expression in the patient from family 2 (D). ACE: No ACE expression is seen in fetus from family 11 (E), whereas focal expression is detected at the apical pole of a proximal tubule in one fetus from family 4. Yellow staining is due to the autofluorescence of degradation products (F). On frozen tissue sections of this fetus from family 4, clear ACE expression is seen in kidney and lung endothelial cells (G and H), and ACE transcripts are detected in endothelial cells of a developing glomerulus (arrow; I and J) and of lung capillaries (K and L). Angiotensinogen: Very focal angiotensinogen transcript production is seen in a poorly developed proximal tubule in the patient from family 12 (arrow; M and N). The gene is strongly expressed in hepatocytes in the patient from family 13 (O and P). AT1 and AT2: Normal production of AT1 (Q and R) and AT2 (S and T) transcripts in patients who belonged to families 8 and 13, respectively. (A, C, E and P) Immunoperoxidase. (F through H) Immunofluorescence. (B, D, I through O, and Q through T) In situ hybridization using 35S-UTP antisense probes; dark- and bright-field illumination of the same section is shown for each labeling. Magnification: ×50 in A, B, and E; ×200 in D; ×300 in C, G, H, K, L, Q, and R; ×360 in F, I, J, and M through P.
who had RTD and survived long enough for it to be measured was very low BP, which was refractory to treatment. This symptom persisted until death on day 3, 6, 7, 4, or 36 in five of the patients described here. This feature also was described by other groups in patients who survived 1 d to several weeks (3,6,8,14,17,18), despite treatment with high doses of catecholamine (18). Persistent hypotension during fetal development, leading to chronic low perfusion pressure in the kidney, may account for the defect in proximal tubule development. Similar morphologic lesions—the so-called “endocrine” kidney—have been described in a rat model of chronic renal hypoperfusion (45). Such lesions also are seen in anuric donor twins in twin–twin transfusion syndrome (24,25), in the ipsilateral kidney in renal artery stenosis in children (10,30,46), and in fetuses that are exposed to ACE inhibitors or AT1 antagonists (31–34). As observed by Marcussen (30) in renal artery stenosis, more proximal than distal tubules are destroyed by the ischemic process. Profound hypotension may be responsible for the high incidence of uni- or bilateral renal vein thrombosis, which has been reported in three patients with RTD in previous studies (8,14,19) and was observed in two patients in our series. It also may account for the high incidence of meconium ileus linked to bowel ischemic perforation that was seen in three of our patients and reported in other studies (11,14). It has been suggested that the underdevelopment of cranial bones also may result from low BP–induced hypoxemia of the skull, a membranous bone that requires a high oxygen tension for normal growth (7,14,16).

In mice, complete lack of angiotensinogen, ACE, renin, or AT1 does not reproduce RTD: KO mice are hypertensive but not anuric (47–55). They have normal kidneys at birth or show a modest delay in glomerular maturation (48). They have severely limited capacity for both dilution and concentration (49,50,52,55). Although most die before weaning, they seem to die of dehydration (53) rather than from renal failure. Those that survive several weeks, spontaneously or by saline injection in the 7 d after birth develop marked atrophy of the renal papilla and vascular hypertrophy. Differences in the renal phenotype between humans and mice are not clearly understood. They may be due partly to difference in nephrogenesis timing. In humans, nephrogenesis terminates at 38 wk gestational, whereas in mice, nephrogenesis that begins by 12 to 12.5 d postconception is far from being completed at birth and continues in the postnatal period for 2 additional weeks. Changes in hemodynamic conditions that occur after birth may explain normal proximal tubule differentiation. In addition, the thin and very long inner medulla in the mouse unipapillary kidney may be more susceptible to renal ischemia, a possible explanation for progressive papilla atrophy.

A striking anomaly, thickening and disorganization of the renal arterial and arteriolar walls, is common to both humans with RTD disease and mice with genetic inactivation of the RAS genes. These changes, which occur in the absence of an effective RAS, are surprising because vascular smooth muscle cell proliferation and hypertrophy are known to be induced by AngII via AT1 activation (reviewed in [56]). However, enhanced PDGF-B mRNA expression has been found in the endothelial cells of the thickened renal arteries and arterioles of hypertensive Atg−/− mice, suggesting that the vascular lesions are driven, at least in part, by the same endothelium-dependent mechanism that operates in hypertensive nephrosclerosis (48). Actually, in patients as in RAS-deficient mice, the RAS defect may not necessarily be the direct cause of the renal vascular changes, as similar vascular lesions are observed in secondary RTD in humans (25,34), or in mineralocorticoid receptor knock-out mice (44), all conditions associated with stimulation of the RAS. Low renal BP itself may induce the renal vascular changes.

Conclusion

Autosomal recessive RTD probably is an underrecognized disorder. A diagnosis of RTD should be considered for fetuses who present early oligohydramnios, normal or nearly normal kidneys on ultrasound examination, and skull ossification defects. The diagnosis is confirmed by morphologic analysis of the kidneys and the exclusion, by clinical investigation, of possible causes of secondary RTD. There is a 25% risk for recurrence, and identification of the genetic defects that are responsible now allows precise genetic counseling and early prenatal diagnosis. The clinical and morphologic phenotype was similar in all patients, regardless of renin expression or the gene mutated, showing that there is no redundancy in the systemic RAS and that the enzymatic pathways that are independent of renin and ACE, described in vitro, are ineffective, at least in fetuses. It may be hypothesized that a milder phenotype, such as transient postnatal renal failure or atypical tubulopathy, may be associated with less severe mutations.

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