Renin–angiotensin system in kidney development: renal tubular dysgenesis

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Autosomal recessive renal tubular dysgenesis (RTD) is a severe disorder of renal tubular development characterized by early onset and persistent fetal anuria leading to oligohydramnios and the Potter sequence. At birth, blood pressure is dramatically low and perinatal death occurs in most cases. Skull ossification defects are frequently associated with RTD. The disease is genetically heterogeneous and linked to mutations in the genes encoding any of the components of the renin–angiotensin system (RAS). An intense stimulation of renin production is noted in the kidneys of patients with mutations in the genes encoding angiotensinogen, angiotensin-converting enzyme, or AT1 receptor, whereas absence or increased renin production is associated with *REN* defects depending on the type of mutation. The severity of the disease underlines the importance of a functional RAS in the maintenance of blood pressure and renal blood flow during fetal life. The absence or poor development of proximal tubules, as well as renal vascular changes, may be attributable to renal hypoperfusion rather than to a morphogenic property of the RAS. The less severe phenotype in mice devoid of RAS may be linked to differences between mice and humans in the time of nephrogenesis and maturation of the RAS. The identification of the disease on the basis of precise clinical and histological analyses and the characterization of the genetic defects allow genetic counseling and early prenatal diagnosis.

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The renin–angiotensin system (RAS) has a major role in the control of extracellular volume and salt metabolism, and in the regulation of blood pressure. Angiotensinogen synthesized by the liver is cleaved in the circulation by renin, an aspartyl protease produced by juxtaglomerular granular cells in the afferent arterioles of the kidney. Angiotensinogen cleavage results in the formation of angiotensin I (ANG I), an inactive decapeptide further converted by angiotensin-1-converting enzyme (ACE) into angiotensin II (ANG II), the active octapeptide. ANG II exerts its effects by binding to two distinct G-protein-coupled receptors AT1 and AT2. AT1 mediates the classical vasopressive action of ANG II whereas AT2 has the opposite effect. Renin is the rate-limiting step in the activation of this catalytic cascade, all other components being produced in excess in normal conditions. Its synthesis and release by juxtaglomerular cells are regulated by signals depending on perfusion pressure in the afferent arteriole, distal tubule sodium concentration at the level of the macula densa, and the sympathetic nervous system. In addition, ANG II exerts a negative feedback control on renin release. Thus blockade of the RAS by ACE inhibitors or ANG II type 1 receptor blockers leading to the absence of production or efficacy of ANG II results in overproduction of renin.

THE RENIN–ANGIOTENSIN SYSTEM DURING FETAL LIFE

All components of the RAS are expressed early, as soon as 5 weeks of gestation, in human embryos.[1,2] Their production is precisely time-regulated suggesting that ANG II could also exert its effects as a growth-promoting agent during kidney development.[3] In humans, as in other mammals whose nephrogenesis is completed before birth, levels of circulating renin and ANG II are higher during fetal life than during postnatal life. Conversely, maximum plasma concentrations of components of the RAS are reached 2–6 weeks after birth in animals, such as rats or mice, completing nephrogenesis after birth.[4] As discussed below, this difference may partly explain the species differences in phenotypes associated with constitutional inactivation of the RAS. Renin does not cross the placenta, and fetal and maternal RAS are independent as shown by measurements of the plasma renin concentration and activity in anephric human and lamb fetuses.[5,6]

Several experiments have shown that RAS is functional during fetal life in mammals. During the second part of gestation, the RAS of the fetal lamb responds to the same stimuli (blood volume depletion, furosemide, hypoxemia, blockade of the RAS, and so on) as the adult.[7,8] In the same way, human fetuses exposed *in utero* to ACE inhibitors or AT1 blockers are severely hypotensive at birth and some of them develop irreversible renal lesions responsible for renal failure and anuria.[9–11] On the other hand, inappropriate activation of the RAS during fetal life may have deleterious consequences. In the twin-to-twin transfusion syndrome resulting from an unbalanced blood supply through placental anastomoses in monochorionic twins, upregulation of renin synthesis in the donor twin, a consequence of chronic hypoperfusion, may negatively affect both fetuses by aggravating hypoperfusion in the donor and increasing blood pressure and volemia in the recipient.[12]

AUTOSOMAL RECESSIVE RENAL TUBULAR DYSGENESIS

In 1983, Allanson *et al.*[13] described the absence or incomplete differentiation of proximal tubules in two stillborn siblings who had developed fetal anuria. They suggested that the tubular lesion could be the marker of a new autosomal recessive syndrome. Since the initial publication, nearly 100 familial or sporadic cases of the disease have been reported using various terms, 'absence of normal-appearing proximal convoluted tubules', 'congenital hypernephronic nephromegaly with tubular dysgenesis', 'isolated congenital renal tubular immaturity', or 'renal tubular dysgenesis'.[14–27] The last denomination has been retained.

Clinical expression

Clinical expression of the disease is quite stereotyped. Oligohydramnios resulting from reduced urine production is the revealing symptom. It is detected as early as 20–22 weeks when the mother is monitored regularly, and persists throughout the gestation period. Normal amniotic fluid volume before 20 gestational weeks has been documented in affected patients, precluding early prenatal sonographic diagnosis. Ultrasound examination shows normal kidneys or mild hyperechogenicity, poor corticomedullary differentiation, or moderate enlargement. Gross kidney malformations are consistently absent. Chronic oligohydramnios leading to fetal compression and decreased intrauterine mobility results in the Potter sequence (facial dysmorphia with large low-set ears, redundant skin, limb-positioning defects, arthrogryposis, and lung hypoplasia). Fetuses may die *in utero*. Most neonates die soon after birth with persistent anuria and respiratory failure. Skull ossification defect with very large fontanels and sutures at birth is a frequent component of the disease. One remarkable feature, in neonates surviving some hours or days on dialysis and respiratory assistance, is the profound life-threatening and refractory hypotension.

Genetic transmission

At this time 76 sporadic or familial cases of renal tubular dysgenesis (RTD) have been reported in 48 families and data concerning 84 additional fetuses/neonates belonging to 56 unrelated families have been reviewed in our laboratory. Male and female patients are similarly affected. Parental consanguinity is present in 32% of families and at least two children are affected in 40% of consanguineous or nonconsanguineous families. Parents are asymptomatic. These data confirm the autosomal recessive inheritance of the disease, initially suggested by Allanson *et al.*[13] No phenotypic difference was observed between apparently sporadic and familial cases of the disease.

Pathological changes

The affected kidneys are usually of normal size but may be enlarged. The corticomedullary architecture is preserved. The morphological hallmark of the disease is the incomplete development of cortical convoluted proximal tubules that have been shown by microdissection to be short and straight.[14] By light microscopy, the number of cortical tubular sections is reduced (Figure 1). Most of them are lined by poorly differentiated cells in which periodic acid Schiff staining and lectin or immunohistological labeling fail to show proximal characteristics: they have no brush border and they are not stained with winged pea lectin or with antibodies to proteins (CD10, CD15, or ACE) normally expressed by proximal tubules[16,17,24] (Figure 1c). Conversely, most tubular sections are labeled with anti-EMA antibodies, normally a marker of distal tubules and collecting ducts (Figure 1f). Some distal tubules at their point of contact with the vascular pole of the glomeruli are enlarged. Lesions are not restricted to the proximal tubule: Henle loops are rare and atrophic and collecting ducts, even if normally labeled with anti-EMA antibodies, are collapsed and surrounded by increased amounts of interstitial mesenchyme (Figure 1d).[14,24] Actually, microdissection studies have shown that all tubular segments from the glomerulus to the collecting duct were markedly hypotrophic in the majority of nephrons.[14,19] Curiously, in one of these studies, dissection of the collecting tree showed an apparent increase in the number of branches.[14] The number of generations of glomeruli estimated according to the 'medullary ray glomerular counting method[28]' is frequently higher than in age-matched controls, but may be lower or normal.[17,24] Glomeruli are normal, slightly retracted, or enlarged as a result of mesangial expansion. They appear to be closely packed because of the reduced number of tubular sections. A constant feature of the disease, clearly demonstrated by α-smooth muscle actin labeling, is the marked thickening and disorganization of the muscular wall of interlobular and preglomerular arteries, also focally present in arcuate arteries (Figure 2).

From the phenotype to the genotype

Renal lesions observed in autosomal recessive RTD are not specific to the genetic disorder. They are very similar to renal ischemic changes produced in rats by partial occlusion of a renal artery and described long ago as 'endocrine kidney'[29] or

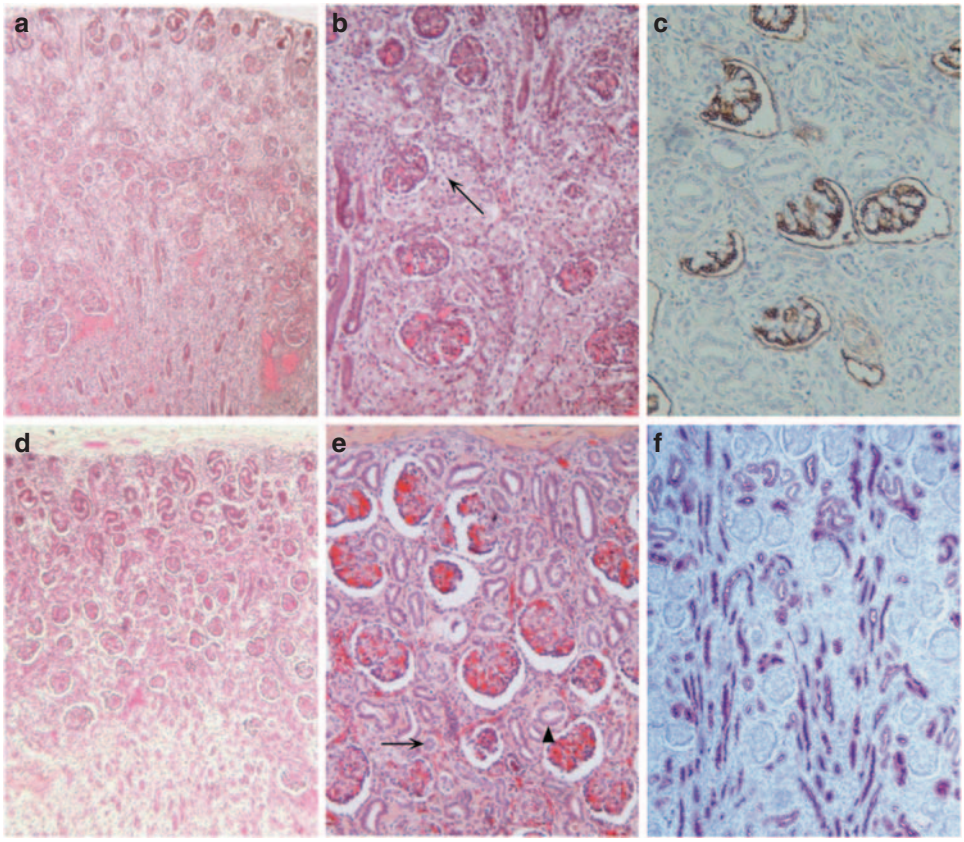

Figure 1 | Renal tubular dysgenesis. Light microscopy. Hematoxylin–eosin (**a**–**b**, **d**–**e**). Compared to age-matched fetal kidney (**a**, **b**), glomeruli in the renal tubular dysgenesis fetus (**d**, **e**) appear closely packed together because of the reduced number of tubular sections. In the medulla, tubules are dedifferentiated or collapsed (**d**). At higher magnification, the reduced number of tubular sections is more evident: some are dedifferentiated (arrows), others, often located near the vascular pole, are hypertrophied (arrowhead) (**e**). Immunolabeling (**c**, **f**). No tubular labeling with anti-CD10 antibody contrasting with normal glomerular epithelial cell staining (**c**). Diffuse tubular labeling with anti-EMA antibody (**f**). Original magnification: (**a**, **d**) ×60; (**b**, **e**) ×270; (**c**) ×300; (**f**) ×180.

to the same changes seen in human kidneys beyond the site of arterial constriction.[30,31] In addition, RTD lesions may be acquired during fetal life in various circumstances, all characterized by a primary failure in renal blood supply. They have been observed in the donor fetus in the twin-to-twin transfusion syndrome, in major cardiac malformations, and in hydrops linked to severe hepatic dysfunction in congenital hemochromatosis.[12] RTD also occurs in those fetuses exposed to ACE inhibitors or to ARBs who develop severe hypotension, fetal anuria and oligohydramnios, and skull ossification defect,[9,10,11] showing the indispensable role of the RAS for human survival in the neonatal period. In all cases of secondary RTD, marked increase in renal renin production is observed (Figure 3b).

Based on the role of the RAS during kidney development and the analysis of secondary RTD, it was suggested that defects in the genes encoding the different components of the RAS could be the causes of autosomal recessive RTD.[18,21,22] To support this hypothesis, we analyzed the renal expression of renin in RTD patients and observed striking abnormalities in all of them. They were of two types: complete absence of the transcript and protein in some cases, or dramatic increase in renin production with massive staining of juxtaglomerular cells, and extensive recruitment of arteriolar smooth muscle cells and mesangial cells in most cases (Figure 3c).[24] No clinical or morphological differences were detected between patients with excess renin production versus loss of renin. Globally, these results reinforce our hypothesis and led us to screen patients for mutations of *RAS* genes and to show they were involved in RTD.[32]

Molecular basis of autosomal recessive RTD

Homozygous or compound heterozygous mutations of the genes encoding angiotensinogen, renin, ACE, or ANG II receptor type 1 were observed in most studied RTD patients.[23,25,27,32] All reported mutations were regarded as pathogenic because they result in the absence of protein, or affect highly conserved residues, segregated with the disease in affected families, and are not present in ethnically matched controls. When parental DNA from familial or apparently sporadic cases is available, mutations at the heterozygous state are present in both parents.

Nonsense or splice site loss-of-function *REN* mutations were, as expected, characterized by the complete absence of renin expression in kidney sections. Conversely, *REN* missense mutations were associated with strong renal

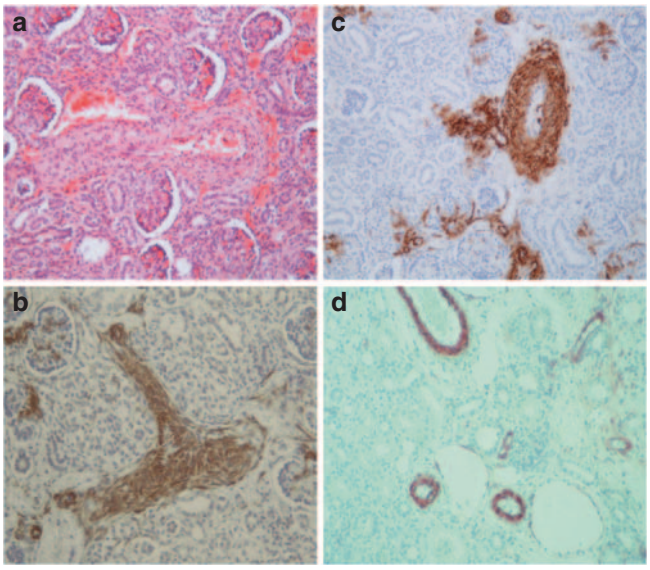

Figure 2 | Vascular changes in RTD. (**a**) Massive thickening of one interlobular artery. (**b**–**d**) Immunolabeling with anti-α-smooth muscle actin antibody (α-SMA). Marked thickening and disorganization of the muscular wall of interlobular and preglomerular arteries are underlined by the α-SMA labeling in renal tubular dysgenesis (RTD) patients (**b**, **c**) compared with an age-paired control (**d**). Original magnification: (**a**) ×270, (**b**) ×270, (**c**) ×300, and (**d**) ×270.

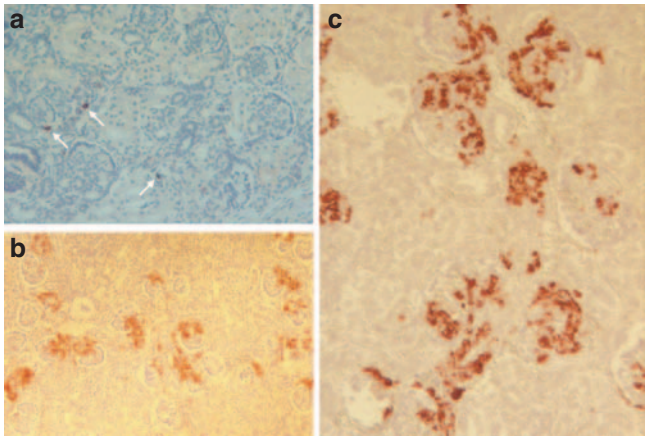

Figure 3 | Immunolabeling with anti-renin antibody. (**a**) In the kidney of a control fetus, only a few cells are renin-positive (arrow). (**b**) In a fetus with secondary renal tubular dysgenesis (RTD; exposure to angiotensin-1-converting enzyme (ACE) inhibitor) there is a strong production of renin: numerous cells are renin-positive in most juxtaglomerular apparatus with recruitment of mesangial and smooth-muscle cells. (**c**) In a fetus with *ACE* mutation, the same strong expression of renin is seen not only in numerous juxtaglomerular cells but also in mesangial cells and arterial smooth-muscle cells at a distance from glomeruli. Magnification (**a**, **c**) ×300; (**b**) ×180.

production of the mutant renin. One of these mutations led to the amino-acid substitution p.D104N in the active catalytic site of the enzyme. Aspartic acid at this site is essential for the release of angiotensin. Its substitution impairs renin function but not production and results in increased renal synthesis of the inactive mutated renin because of the loss of the negative feedback inhibition by ANG II.[32] Another missense *REN* mutation, the p.S135Y, substitution affected a highly conserved serine.[25] It was associated with intracellular renin retention as shown by the high renal renin content contrasting with the very low plasma active renin (<2.5 ng/l; normal range for neonates: 24–850 ng/l). Loss-of-function or missense mutations of the others genes, *ANG*, *ACE*, or *AGTR1*, were always associated with massive increase in renin expression.

The same pattern of mutations was observed in familial and apparently sporadic cases. The clinical phenotype and the pathological changes were remarkably similar regardless of renin production or the gene involved, probably because the final result of all mutations was the absence of ANG II production or function.

In a few anuric fetuses/neonates with RTD lesions, no *RAS* gene mutation was detected. In our experience, the presence of unusual symptoms, such as anasarca or severe intrauterine retardation often linked to extensive areas of ischemic necrosis of the placenta, and/or the absence of changes in renal renin production, suggests that these atypical cases are not due to *RAS* gene mutations. They may be secondary, the cause of which has been missed. Another possibility is that other genes are involved, especially in familial cases. *AGTR2* encoding the AT2 receptor, or *ATP6AP2* encoding the renin/prorenin receptor, can be excluded because of their location on the X chromosome and the presumed consequences of their mutations. The genes encoding the mineralocorticoid receptor or the endothelin family are possible candidates and should be investigated after the classical causes of secondary RTD have been excluded.

Surviving RTD patients with RAS mutations

To date, only four patients have been reported as surviving after days or weeks on peritoneal dialysis and mechanical ventilation. Two had *AGT* mutations, a homozygous missense (p.R375Q) mutation in one, and compound heterozygous nonsense mutations in the other. In them, plasma active renin concentration was very high contrasting with extremely low or null plasma renin activity (estimated by the amount of ANG I produced from angiotensinogen by renin in the patient plasma). Angiotensinogen, ANG I and II levels were all low.[23,25] A gene dose–effect was observed in the brother of the patient having the p.R375Q mutation in the heterozygous state: the plasma level of angiotensinogen was reduced but plasma renin activity was normal due to a compensatory increased production of renin.[25] In the girl patient with the p.R375Q mutation, dialysis could be ceased on day 4 and normalization of blood pressure was obtained after 20 days of vasopressive (adrenaline and dopamine) treatment. At 15 years of age, she is normotensive with moderate chronic renal failure.[25] Two siblings affected with the same disease developed oligo-anhydramnios were anuric and hypotensive at birth, and died at respectively 2 and 4 days of age despite

supportive treatment. In the second patient with AGT mutation peritoneal dialysis was required from day 3 up to day 52 and she has chronic renal failure at 18 months of age. Interestingly, infusions of fresh frozen plasma were effective to correct severe neonatal hypotension (but not renal insufficiency) and had to be maintained up to day 9.[23] At last examination her blood pressure was in the normal range.

One patient had the *REN* missense p.S135Y mutation in the homozygous state associated with very low plasma active renin. She was anuric at birth and recovered diuresis after 5 months on dialysis. Her blood pressure was said to be in the lower range of normal. She had to be transplanted at 4 years of age.[25] In this family, the following gestation was terminated at 30-week gestation because of severe recurrence of the disease.

The last surviving patient had compound heterozygous *ACE* deletions (one in-frame and one truncating). She was anuric at birth, recovered some urination on day 16 but continued to need peritoneal dialysis at 3 years of age. Systemic hypotension present at birth persisted for the first 2 weeks and responded poorly to plasma expanders, catecholamines, and glucocorticoids. Diagnosis of RTD was discussed at 3 years of age on the finding of high plasma renin activity and high active renin concentration contrasting with low ACE concentration. It was confirmed by genetic analysis showing ACE mutations.[27]

No correlation could be established between the clinical course of the disease and the type of mutation. Moreover, as indicated, five affected relatives, with the same *AGT* or *REN* mutations as the surviving patients, died during the perinatal period.[25,27]

From the gene defect, back to the phenotype

The presumed consequence of all mutations observed in RTD is the absence of ANG II production or function, a defect responsible for the severe refractory hypotension observed at birth. The progressive normalization of blood pressure is linked to postnatal development of compensatory mechanisms.

In addition to its vasopressive action, ANG II regulates renal growth during development.[33–36] Thus, a possible explanation for the tubular defect is that it results directly from the loss of the growth-promoting action of ANG II. However this hypothesis cannot be maintained as the same tubular anomalies are observed in fetal conditions associated with stimulation of the RAS (cardiopathies, twin donor in the twin-to-twin transfusion syndrome, renal artery stenosis, and so on.), indicating that other factors are involved. Renal hypoperfusion is probably the common mechanism leading to RTD lesions whatever their cause: blood depletion (twin-to-twin transfusion syndrome), renal artery stenosis, or severe hypotension (autosomal RTD or exposure to RAS blockers). Low blood pressure may also account for the high incidence of ischemic or thrombotic complications seen in RTD patients.[20,21,24,26] Skull ossification defect observed in genetic and secondary RTD is also the consequence of low blood pressure aggravated by oligohydramnios, because skull membranous bones require a high oxygen tension for normal growth.[9,10,13,20,22]

Renal vascular lesions are present in all cases of genetic or secondary RDT. They are characterized by marked thickening of the muscular layer of medium-sized arteries and arterioles, linked to increased number of smooth muscle layers. They are similar to renal vascular changes present in hypertensive nephrosclerosis. Their occurrence in the absence of an effective RAS is surprising because proliferation and hypertrophy of vascular smooth muscle cells may be induced by ANG II through the AT1 receptor. The mechanism may again implicate hypoperfusion as suggested by the occurrence of the same vascular lesions in mice with targeted deletions of the genes encoding components of the RAS.

INACTIVATION OF RAS COMPONENTS IN MICE

The different components of the RAS have been inactivated in mice.[37–47] The resulting phenotype is globally similar whichever gene is invalidated, but is different from the phenotype of RTD patients. Embryonic development is normal in knockout mice but the perinatal mortality rate is high, around 40% in mice lacking both AT_{1A} and AT_{1B} receptors[43] to 60% in *Agt*−/− mice[37] and 80% in *Ren1c*-null mice.[39] Neonates are not anuric but polyuric and their death before weaning results from dehydration. Those that survive spontaneously or by saline infusion show an inability to concentrate urine and an abnormal sensitivity to salt depletion. In all cases they are hypotensive. After weeks or months, according to the gene defect and the genetic background, they develop progressive renal failure.

At the morphological level, kidneys are normal at birth in most mouse models, they do not show nephron reduction or specific changes of proximal tubules. Moderate delay in postnatal glomerular maturation has been observed in *AT1* double nullizygote mice as in *Agt*-null mice, but the glomerular maturity index was normal at 3 weeks.[38,44] Later on, they develop renal abnormalities. They consist in progressive papillary atrophy with tubular atrophy and interstitial fibrosis associated with pelvic dilatation. Cortical atrophy occurs secondarily. As previously indicated, marked thickening of renal arterial walls is present.

Clearly, except for the vascular lesions, the phenotype in mice is different and less severe than the corresponding one in humans:[48] human neonates are anuric with poor or no differentiation of proximal tubules whereas mice are polyuric and develop renal papilla atrophy. Several reasons linked to differences in the timing of nephrogenesis and RAS maturation, or in kidney morphology, may explain the more severe consequences of *RAS* gene disruption in humans. In humans, nephrogenesis is complete at 38 gestational weeks whereas in mice, it continues into the postnatal period for an additional 2 weeks. Hemodynamic changes linked to environmental factors occurring after birth may contribute to normal postnatal proximal tubule maturation. In addition, in humans, *RAS* activity reaches its peak during fetal life at

the time of active nephrogenesis whereas in mice, it is achieved 2–6 weeks after birth, suggesting a less mandatory role in renal development in mice than in humans.[4] Conversely, the long and thin inner medulla of mice might be especially prone to developing ischemic lesions, explaining renal pathology. Another explanation for renal papilla atrophy and calyx dilatation in mice has been proposed by Miyazaki *et al*,[45] based on the similarity with renal lesions in obstructive nephropathy. Angiotensin type 1 receptors are present in pelvic smooth muscles and angiotensin induces the ureteral smooth muscles in organ cultures and the urinary peristaltic machinery during the perinatal period.[49] Lesions that develop in mice defective for the *RAS* genes (but not in anuric RTD patients) should be linked to the defective development of this peristaltic machinery leading to 'functional obstruction' of the urinary tract and its consequences on kidney structures.

Increase in arterial wall thickness is the common feature observed in humans and mice without a functional RAS. The same pattern is present in mineralocorticoid receptor knockout mice that suffer from major renal hypoperfusion,[50] suggesting that vascular changes may result from the stimulation of growth factors provoked by renal hemodynamic anomalies rather than from the absence in ANG II. Actually, enhanced endothelial cell expression of PDGF-B mRNA has been observed in the thickened arteries and arterioles of *Atg−/−* mice.[38] Mechanisms leading to endothelial cell activation are not all known. Hypoxia may play a role by activating the family of HIF transcription factors and by this way, HIF target growth factor genes.[51] On the other hand, hypoperfusion *per se* may be operative: the glycocalyx, covering the endothelial surface in close contact with the flowing blood, has recently been shown to function as a mechanosensor and a mechanotransducer.[52] Syndecans form the largest group of glycocalyx heparan sulfate proteoglycans. They sense and transmit the shear stress to sites where transduction into biochemical signals may occur.[53] Thus they can modulate endothelial cell activity and growth factor production according to hemodynamic conditions. Interestingly, kidney vessels are normal in homozygous mice with ablation of renin-expressing juxtaglomerular cells[54] or inactivation of the *Ren-1^d* gene in 129 mice that possess two renin genes termed *Ren-1^d* and *Ren-2*.[55] This may be explained by the persistent production of renin-2 (from submandibular gland origin in the first model) allowing preservation of normal plasma renin concentration and blood pressure in males and moderate decrease in blood pressure in females with reduced plasma renin concentration.

DOMINANT RENIN MUTATIONS AND TUBULOINTERSTITIAL NEPHROPATHY

Parents and siblings of RTD patients with heterozygous mutation of one or the other *RAS* gene are asymptomatic. However, recently dominant *REN* mutations resulting in either the deletion or the amino-acid exchange of a single leucine residue in the signal sequence of renin have been detected in three unrelated families presenting with early onset anemia, hyperuricemia, and slowly progressive renal failure.[56] Both mutations are predicted by bioinformatics analysis to damage targeting and co-translational translocation of preprorenin into the endoplasmic reticulum, a prediction confirmed by functional studies. It is suggested that expression of the mutant proteins has a dominant toxic effect on renin-expressing cells, reducing their viability, altering the intrarenal RAS, and gradually leading to renal failure.

CONCLUSION

Autosomal recessive RDT is a severe disease of renal tubular development. The diagnosis of this disorder should be considered for fetuses/neonates who present early oligohydramnios with normal or subnormal kidneys at sonography. It is easily confirmed by careful pathological analysis and the exclusion of the possible causes of secondary RDT. The genetic basis of the disease has been elucidated allowing precise genetic counseling and early prenatal diagnosis. The severity of the phenotype linked to RAS mutations shows that there is no redundancy in the systemic RAS and that the alternative enzymatic pathways described *in vitro* are ineffective *in vivo*, at least during human fetal life. Biochemical studies of causative mutations in the different *RAS* genes will help to understand the structure–function relationship of the RAS proteins.

To date, a series of questions may be raised: what is the actual frequency of RTD? What is the hemodynamic condition of asymptomatic heterozygote individuals? Are they protected against hypertension? Are other less severe mutations in the *RAS* genes responsible for milder nephropathies: transient neonatal renal failure, unclassified tubulopathies, or tubulointerstitial nephropathies? The finding of autosomal dominant chronic tubulointerstitial nephropathy linked to *REN* mutations affecting the signal sequence of renin is a first answer to these questions.

DISCLOSURE

The authors declared no competing interests.

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