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REVIEW ARTICLE

The Life of the Human Kidney Before Birth: Its Secrets Unfold

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Human kidney and lower urinary tract malformations are a major cause of chronic renal failure in young children. Evidence is emerging that at least some of these disorders have a defined genetic basis. This is a rapidly evolving field, with new human mutations and informative animal models described almost monthly. In this review, we provide a background on important animal studies and then address human kidney development, highlighting recent studies where specific genes have been implicated in the pathogenesis of disease.

A potent blend of classical embryology and molecular genetics is illuminating mechanisms of kidney and lower urinary tract development. Most studies use mice in which growth of the adult kidney precursor, the metanephros, is manipulated by up-regulation or down-regulation of molecules expressed during renal development, or nephrogenesis. A recent review (1) documented about 20 varieties of mice with mutations of diverse transcription factors, growth/survival factors, and matrix molecules, all with urinary tract malformations. Impressively, hierarchical networks of "master genes" affecting nephron development are being mapped (1). These experiments also demonstrate that disease phenotypes may depend on yet-to-be-discovered, strain-dependent, modifying genes, as well as mutations of single, key nephrogenesis genes. Furthermore, mutations of homologous genes may summate to disrupt of development (2). The differentiation of more primitive pronephric kidneys of fish (3) and toads (4) is also being explored, revealing remarkable conservation of nephrogenic genes between diverse species.

While it is tempting to speculate that "kidney-specific" master genes exist, so far all mouse genes implicated in urinary tract development are also expressed in diverse other organs: consequently, mutants often have multiorgan malformation syndromes *e.g.* the paired-box 2 (PAX2) transcription factor controls renal and ocular development (5). Other animal experiments indicate that physical obstruction of the fetal urinary tract, teratogenic drugs, exposure to excess glucose from maternal diabetes, as well as deficiencies in maternal protein and vitamin intake, can also perturb normal kidney development (6, 7).

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Apart from furthering our basic knowledge about organogenesis, it is timely to ask whether animal studies help us understand how human kidneys develop, not least because most children with chronic renal failure are born with abnormal urinary tracts (8).

The human metanephros appears at 5 wk after fertilization: it comprises the ureteric bud, an epithelial branch of the mesonephric duct, and renal mesenchyme, a caudal section of intermediate mesoderm. The bud tip branches to generate collecting ducts, while its stalk forms renal pelvis and ureter urothelium. The insertion of the bud into the mesonephric duct becomes incorporated into the cloaca, forming the urinary bladder trigone. Meanwhile, renal mesenchyme transforms into epithelial nephrons, with new glomeruli generated between 8 and 34 wk of gestation (9).

When these complex morphogenetic events go wrong, diverse kidney human malformations occur including agenesis (absent kidney), dysplasia (incomplete differentiation, often with cysts), and hypoplasia (too few nephrons) (10). Moreover, since physical engagement and subsequent interaction between ureteric bud and mesenchyme are essential for differentiation of each partner (1), it is not surprising that kidney malformations are often accompanied by lower urinary tract anomalies including agenesis, duplication, or hydroureter caused by impaired urine flow or vesicoureteric reflux (10, 11).

Alterations of maternal diet perturb nephrogenesis in rats, but there is no hard evidence that this occurs in humans. Although human renal malformations have been documented after exposure to angiotensin converting enzyme inhibitors, excessive glucose and ethanol (10), a history of teratogen exposure is rarely elicited.

More impressive is the common association of human kidney malformations with lower urinary tract obstruction, *e.g.* atretic ureters attached to multicystic dysplastic kidneys, ureteroceles, and urethral valves. In these cases, however, the cause of the obstruction itself remains unexplained, and it could be argued that both the malformed kidney and the obstructive lesion result from a more fundamental problem such as the altered activity of a gene expressed in both upper and lower urinary tract: indeed, genes with such widespread expression patterns exist in mice (12, 13) and humans (14). Finally, in many individuals with dysplastic or hypoplastic kidneys, the urinary tract is patent and hence physical obstruc-

tion cannot be implicated in pathogenesis of the renal malformation.

Data are accumulating that some human urinary tract malformations have genetic bases.

One line of evidence is provided by the observation that such malformations may occur in multiple members of a family, more than would be expected by chance (10). A common example, affecting 1% to 2% of very young children, is vesicoureteric reflux, the retrograde passage of urine into the ureter and, occasionally, the renal parenchyma: in some families this is inherited in a dominant manner with incomplete penetrance (10). Based on a genome-wide analysis in seven Caucasian kindreds with three to seven affected members, Feather *et al.* (15) suggested that the disorder was genetically heterogeneous with some families mapping to chromosome 1p13: the causative gene, however, remains to be defined.

In other human urinary tract malformations, although simple inheritance patterns are not apparent, modifying genetic influences could operate (10). An example is provided by the association of a polymorphism, or common genetic variation, of the angiotensin II type 2 receptor (AT2) gene with a spectrum of disorders including pelviureteric junction obstruction and megaureter (13, 16). The genetic variant most likely affects splicing and mRNA transcript levels of this growth factor receptor implicated in cell survival in developing kidney and lower urinary tract (13). Furthermore, male mice with null mutation of AT2, located on the X chromosome, display a low penetrance of diverse urinary tract malformations (13).

Other clues come from the discovery that specific malformation syndromes affecting the kidney are caused by mutations: in other such multiorgan syndromes, disease loci have been established while genes have yet to be defined. A selection of these disorders are shown in Table 1. Although such syndromes are individually rare, there are a considerable number of them (17, 18), collectively accounting for significant morbidity.

Table 1. *Genetics of human urinary tract malformations*

Apert syndrome (FGFR2* mutation - growth factor receptor): hydronephrosis and duplicated renal pelvis
Bardet Biedl syndrome (loci on 11q13, 16q22, 3p13, 15q21 and 2q31): renal dysplasia and calyceal malformations
Beckwith-Wiedemann syndrome (in a minority of patients, p57KIP2* mutation - cell cycle gene): renal overgrowth, cysts and dysplasia
Branchio-oto-renal syndrome (EYA1* mutation - transcription factor): renal agenesis and dysplasia
Campomelic dysplasia (SOX9 mutation - transcription factor): diverse renal malformations
Carnitine palmitoyltransferase II deficiency (gene for this enzyme is mutated): renal dysplasia
Congenital anomalies of the kidney and urinary tract (CAKUT) syndrome (AT2* polymorphism - growth factor receptor): diverse renal and lower urinary tract malformations
Diabetes and renal malformation syndrome (HNF1 β mutation - transcription factor): renal dysplasia, hypoplasia and glomerular cysts
Di George syndrome (locus on 22q11): renal agenesis, dysplasia and vesicoureteric reflux
Glutaric aciduria type II (glutaryl-CoA dehydrogenase mutation): cystic and dysplastic disease
Fanconi anaemia (FAA family mutation - DNA repair molecule): renal agenesis, ectopic/horseshoe kidney
Kallmann's syndrome (KAL1 mutation - cell signaling molecule): renal agenesis
Meckel syndrome (locus on 17q21-q24): renal cystic dysplasia
Nail-patella syndrome (LMX1B* mutation - transcription factor): glomerulus dysgenesis
Renal-coloboma syndrome (PAX2* mutation - transcription factor): renal hypoplasia and vesicoureteric reflux
Simpson-Golabi-Behmel syndrome (GPC3* mutation - proteoglycan): renal overgrowth, cysts and dysplasia
Smith-Lemli-Opitz syndrome (δ(7)-dehydrocholesterol reductase mutation - cholesterol biosynthesis): renal cysts and dysplasia
WAGR, Denys Drash and Frasier syndromes (WT1* mutation - transcription/splicing factor): lower urinary tract malformations and glomerular sclerosis
Zellweger syndrome (peroxisomal protein mutations): cystic dysplastic kidneys

See text and references 17 and 18 for details; * mutations of these genes are also implicated in mouse malformations.

An early discovery was that deletion of the WT1 gene accounted for genitourinary malformations in the WAGR syndrome, a condition associated with Wilms' tumor and aniridia, the latter caused by deletion of the nearby gene PAX6 (19). WT1 is a transcription factor active in metanephric mesenchyme, and null mutant mice have renal agenesis (1). In the case of PAX2 mutations, mouse and human disease show striking parallels with urinary tract and eye malformations (5): this gene is expressed in mesonephric duct, fetal collecting ducts, and nascent nephrons but is down-regulated at the end of organogenesis (10, 14). I will highlight two more such syndromes in which animal studies act as paradigms for human disease.

The Simpson-Golabi-Behmel syndrome is a human disease in which overgrowth of diverse organs causes malformation (*e.g.* renal cystic dysplasia) and tumor formation (20). It forms part of a spectrum of overgrowth disorders including genetically distinct Beckwith-Weideman and Perlman syndromes (20). Mutations of the glypican-3 (GPC3) gene have been identified in the Simpson-Golabi-Behmel syndrome (21). This codes for a heparan sulfate proteoglycan expressed during human organogenesis (22). The molecule binds to cell surfaces and, *in vitro*, is implicated in cell survival (23). Null mutant mice also develop an overgrowth syndrome with cystic ureteric bud/collecting duct hyperproliferation (24). Superficially similar aberrations of cell turnover have been reported in sporadic cases of human renal cystic dysplasia (14, 25), and although these individuals lack other features of the syndrome, it would be interesting to search for GPC3 mutations in these patients.

The hepatocyte nuclear factor 1β (HNF1β) gene is implicated in endoderm development (26), with human mutations associated with MODY (maturity onset diabetes mellitus of the young): these individuals have a failure of pancreatic insulin secretion (27, 28). The gene is also expressed in developing urinary tract (29) and mutations in the DNA binding and transactivating domains of this transcription factor have been reported in patients with kidney malformations including dysplasia (27), hypoplasia (28), and glomerulocystic disease (30), a form of polycystic kidney in which glomerular cysts predominate. Although embryonic mouse null mutants die before the urinary tract is formed, zebrafish experiments show that intro-

duction of a human mutation perturbs kidney precursor development in a dominant-negative manner with formation of cyst-like structures (4).

Evidence is emerging that some human urinary tract malformations have a genetic basis and key "nephrogenesis genes" are conserved between human, murine, and other animal species. In the near future, it will be possible to envisage that some of these discoveries may form the basis of genetic screening tests for urinary tract malformations: in the much longer term, a better understanding of disease mechanisms could lead to novel drug-based, cellular or genetic therapies to enhance the differentiation of nephrogenic precursor cells. The feasibility of such therapies has at least begun to be explored by the experimental use of metanephric precursor cells that can be genetically altered *ex vivo* and then transplanted into postnatal animals where they differentiate into functioning kidney tissue (31-32).

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