I. Normal Development of Human Kidney and Lower Urinary Tract

A. Introduction

Normal development of the human kidney and lower urinary tract is a highly complex process that not uncommonly goes wrong. Furthermore, congenital malformations of these structures, involving absent, immature, or poorly-grown organs, account for most young children with long-term kidney failure who require dialysis and transplantation. Evidence is emerging that some of these individuals have mutations of genes, such as transcription factors, expressed in prenatal development of the kidney and ureter. In other patients, physical obstruction of the lower tracts causes urinary flow impairment, an event that triggers diverse aberrations of kidney development, and teratogens can occasionally be identified. It is also possible that modifications of maternal diet may affect kidney development, although this hypothesis is currently more persuasive for animal models than for human disease. Understanding the mechanisms of pathogenesis of these diseases is important for genetic counseling, avoidance of possible teratogens, and planning possible therapeutic interventions, including surgical decompression of obstructed, fetal urinary tracts.
B. The Pronephros

The human pronephros appears at the 10 somite stage on day 22 of gestation (i.e., 22 days after fertilization), which is morphologically equivalent to embryonic day 9 in mice. Initially, it consists of a small group of nephrotomes with segmental condensations, grooves, and vesicles between the second and sixth somites. Nephrotomes are nonfunctional, probably representing vestiges of the pronephric kidney of lower vertebrates (Chapter 3). This stage is essential for subsequent kidney development, however, because the pronephric duct develops from the intermediate mesoderm lateral to the notochord, elongates caudally to reach the cloacal wall on day 26, and is then renamed the mesonephric, or Wolffian duct, as mesonephric tubules develop. The nephrotomes and pronephric part of the duct involute and cannot be identified by 24 or 25 days gestation.

C. The Mesonephros

In humans, the elongated sausage-like mesonephros appears around 24 days after fertilization and consists of the mesonephric duct and adjacent mesonephric tubules (Chapter 1). The duct is initially a solid rod of cells, which then canalizes in a caudocranial direction after fusion with the cloaca. Mesonephric tubules develop medial to the duct by a “mesenchymal-to-epithelial” transformation from the intermediate mesoderm. This phenotypic transformation is subsequently reiterated during nephron formation in the metanephros. In humans, a total of around 40 pairs of mesonephric tubules are produced (several per somite), but the cranial tubules regress as caudal ones are forming so that there are never more than 30 pairs at any time. Each tubule consists of a medial cup-shaped sac encasing a knot of capillaries, which appears analogous to the Bowman’s capsule and glomerulus of the mature kidney, and a lateral portion in continuity with the mesonephric duct. Other segments of the tubule resemble mature proximal and distal tubules histologically but there is no loop of Henle. The human mesonephros is reported to produce small quantities of urine between 6 and 10 weeks of gestation, which drains via the mesonephric (Wolffian) duct; the murine organ is much more rudimentary and does not contain well-differentiated glomeruli. Mesonephric structures involute during the third month of human gestation, although caudal mesonephric tubules contribute to the efferent ducts of the
epididymis and the mesonephric duct contributes to the duct of the epididymis, the seminal vesicle, and ejaculatory duct.

D. The Metanephros

Human metanephric development begins at day 28 after fertilization when the ureteric bud sprouts from the distal part of the mesonephric duct. The tip of the bud then penetrates a specific area of sacral intermediate mesenchyme, termed the metanephric blastema around day 32. Next the mesenchyme condenses around the tip of the growing ureteric bud. The ureteric bud branches sequentially to form the ureter, renal pelvis, calyces, and collecting tubules, while the mesenchyme undergoes an epithelial conversion to form the nephrons from the glomerulus to the distal tubule. The ureteric bud also gives rise to the urothelium of the renal pelvis and the ureter, and the junction of the bud and the mesonephric duct becomes incorporated into the cloaca, forming the urinary bladder trigone (see later).

As the epithelial ureteric bud grows into the metanephric blastema, it becomes invested with condensed mesenchyme, and interactions between the two cell types are associated with proliferation and branching of the bud and its derivatives. The branch tips are highly proliferative, as are the surrounding nephrogenic mesenchymal cells (Winyard et al., 1996a and b). Branching continues at the tips of the...
bud in the outer (nephrogenic) cortex until completion of nephrogenesis around 34 weeks of human gestation. The final result is an arborized (tree-like) system of collecting ducts, which is connected to nephrons that develop concurrently from the mesenchymal condensates adjacent to the ampullary tips (see later). Mature collecting ducts drain first into minor calyces, which then empty into the major calyces of the renal pelvis and ureter. Formation of these structures requires "remodeling" of many of the early branches of the ureteric bud, a process that has been recognized since Kampmeier (1926) described "vestigial" and "provisional" zones in the human medulla. The exact number of generations of branches that are remodeled is unknown, although Potter (1972) estimated that the first three to five generations form the pelvis and the next three to five give rise to the minor calyces and papillae. Nephrons that were initially attached to these early branches are thought to either transfer to a later branch or degenerate during development.

Each nephron develops from the mesenchyme that condenses around the ampullary tips of the ureteric bud. The condensed mesenchyme undergoes phenotypic transformation into epithelial renal vesicles, which then elongate to form a comma shape before folding back on themselves to form S-shaped bodies. The distal portion of the S shape elongates and differentiates into the proximal convoluted tubule, the descending and ascending limbs of the loop of Henle, and the distal convoluted tubule, which fuses with the adjacent branch of the ureteric bud to form a continuous functional unit. The proximal S shape differentiates into the glomerular epithelium, and capillaries develop in the glomerular crevice. During this "sculpting" process, a degree of programmed cell death occurs, with apoptosis prominent in the nephrogenic zone, proximate to primitive nephrons (Winyard et al., 1996a). The first vascularized metanephric glomeruli are formed around 8 to 9 weeks of human gestation, and new nephron formation ceases around 34 weeks of gestation. The number of nephrons, as assessed by counting glomeruli, varies widely in different species, probably reflecting the number of branches of the ureteric bud required to both induce these nephrons and form the collecting ducts to drain urine from them. It has been calculated that 9 to 10 rounds of branching occur in mice, which generates 10–20,000 nephrons per kidney, and a further 10 branching generations occur in human to give rise to approximately a million nephrons in each kidney (Ekblom et al., 1994). In fact, studies suggest that about two-thirds of nephrons are generated in the last third of human gestation and that the normal range of nephrons found in healthy human kidneys is rather large, around 0.5–1.0 million (Hinchliffe et al., 1991, 1992, 1993). During the second half of human gestation, fetal urine is the main component of amniotic fluid and is thought to be important for fetal lung growth.

E. The Ureter

As reciprocal inductive interactions begin to occur between the ureteric bud and the metanephric mesenchyme, the urinary bladder starts to form. The cloaca separates into the urogenital sinus (primordial urinary bladder) and rectum and, by 28 days of human gestation, the mesonephric duct drains into the urogenital sinus. At this time, the epithelium of the sinus and mesonephric duct become fused and the ureteric bud arises as a diverticulum from the posteroMedial aspect of the mesonephric duct where the duct enters the primordial bladder. Between 28 and 35 days of gestation the entire length of the ureter is patent and it has been assumed, because the cloaca is imperforate at that time, that mesonephric urine might lead to increased intraluminal pressure, thus maintaining ureteral patency. Between 37 and 47 days gestation, a membrane temporarily occludes the junction between the ureter and the bladder (Alcaraz et al., 1991). In the same period, the ureter becomes occluded throughout its entire length. Recanalization has been reported to begin in the middle third of the ureter and is temporally related to longitudinal growth of the ureter (Ruano-Gil et al., 1975). The cause of the apparent obstruction and recanalization is not understood. At 8 weeks of gestation the ureter is a non muscularized but patent tube. Between 32 and 56 days, the metanephros "ascends" relative to spinal landmarks from a position at the level of the upper sacral segments to the level of the upper lumbar vertebrae. This is explained by caudal growth of the spine and ureteric elongation. The developing kidney also rotates medially on its polar axis so that the renal pelvis faces the spine and the hilum is directed anteromedially. Between 10 and 14 weeks of gestation, the epithelium of the ureter changes from a simple monolayer to a more mature pseudo-multilayered arrangement, and by the end of this period the ureter begins its submucosal course as it enters the urinary bladder. Production of metanephric urine at 8–9 weeks of gestation, coinciding with the first layer of vascularized glomeruli, precedes muscularization of the upper ureter and may stimulate ureteric myogenesis, which starts at 12 weeks (Matsuno et al., 1984; Escala et al., 1989). The important anatomical landmark, the ureteropelvic junction, is apparent at 18 weeks.

F. The Urinary Bladder

On day 28 of gestation, the urorectal septum (Tournoux’s fold) extends caudally through the cloaca toward the cloacal membranes. In addition, two tissue folds (Rathke’s plicae) advance from the lateral aspect meeting by 44 days of gestation. This separation of the cloaca into the primitive urogenital sinus and rectum is the first step in the development of the urinary bladder. The portion of the urogenital sinus cranial to the mesonephric ducts is called the vesico-urethral canal, and the part caudal to the mesonephric ducts
is called the urogenital sinus. By 33 days of gestation, the mesonephric duct below the ureteric bud dilates, and this "common excretory duct" is absorbed into the urogenital sinus as the precursor of the trigone. In this manner, the origin of the ureteric bud "enters" the bladder directly by day 37 to become the ureteric orifice; thereafter, the ureteric orifices migrate in a cranial and lateral direction. Continued growth of the epithelium of the absorbed common excretory ducts separates the ureteral orifices laterally and establishes the framework of the primitive trigone. The mesonephric duct above the ureteric bud becomes the vas deferens in the male, but this portion of the duct involutes in the female. The urogenital membrane ruptures on day 48 of gestation, hence providing a connection between the emerging bladder and the outside of the body. The human allantois, another potential outflow tract on the anterior of the developing bladder, appears at 21 days of gestation; the allantois will involute by 12 weeks of gestation, persisting as a remnant, the median umbilical ligament. After 10 weeks of gestation, the urinary bladder appears as a cylindrical tube lined by connective tissue. By 13 weeks of gestation the bladder mesenchyme has developed into circular and longitudinal smooth muscle fibers and, by 16 to 20 weeks, discrete inner and outer longitudinal layers and a middle circular muscle layer exist.

II. Varied Phenotypes of Human Kidney and Lower Urinary Tract Maldevelopment

As might be expected from such an anatomically and functionally complex entity as the kidney and lower urinary tract, there are several disease manifestations which arise when normal development goes wrong. The detailed histology of these malformations has been well described (Risdon and Woolf, 1998b). Some of the main types of structural disorders are discussed briefly.

A. Renal Agenesis

The most basic structural anomaly is "agenesis" when the organ is absent. In theory, in order to prove such a diagnosis, one would have to provide evidence that the metanephros had never formed. Clearly, this is impossible in humans unless a fetus is available for direct anatomical inspection just as the metanephros should be forming; this would constitute a very rare event. In the "imperfect world" of clinical medicine, renal agenesis is generally diagnosed radiologically, e.g., at the routine midgestation fetal ultrasonography or, postnatally, during investigation of renal tract disease. Less commonly, unilateral renal agenesis is noted incidentally at autopsy. Renal agenesis is usually accompanied by an absence of the ureter on the same side. Unilateral renal agenesis is considered a rather common congenital anomaly with an incidence of 1:1000. Being born with a solitary functioning kidney is not generally associated with significant morbidity, although there is some evidence that the incidence of hypertension (high blood pressure) and renal functional impairment is greater than expected (Woolf, 1998; Duke et al., 1998). Bilateral renal agenesis is an order of magnitude less common than unilateral disease and would inevitably lead to neonatal death without medical intervention. Because urine is the main constituent of amniotic fluid in the second half of gestation, a reduced amount of amniotic fluid (oligohydramnios) will accompany bilateral renal agenesis. It is notable that fetal renal excretory function is not required for prenatal life, as waste products are effectively dialyzed across the placenta into the maternal circulation; soon after birth, however, affected individuals would die from uremia unless treated by dialysis. Renal agenesis can occur as an isolated anomaly or be part of a multiorgan syndrome, some of which have defined genetic bases; a good example is Kallmann’s syndrome, as discussed later.

B. Renal Dysplasia

This term refers to a kidney that has begun to form, but which fails to undergo normal cellular differentiation. Dysplastic kidneys can be considerably larger than normal, when they take the form of the multicystic dysplastic kidney; here, the organ is distended by cysts and generally attached to an "atretic" ureter that contains at least one segment with no lumen. With the increasing use of fetal and infant ultrasonography, multicystic kidneys have often been noted to involute prenatally or in the first year of life; in fact, some cases of human "renal agenesis" diagnosed by radiography may represent multicystic kidneys, which have "disappeared" beyond the lower limit of detection (Mesrobian et al., 1993). Hence, multicystic dysplastic kidneys can be likened to "supernovae," which have spectacular phases of growth and regression. However, dysplastic organs can be much smaller than normal; these varieties are sometimes associated with abnormal lower urinary tracts, e.g., obstructive lesions including ureteroceles (a pouch at the lower end of the ureter, which often has an ectopic opening into the urinary bladder or urethra) and posterior urethral valves (occluding leaflets in the male urethra). The definition of renal dysplasia is technically a histological one, with poorly branched tubules surrounded by stromal/mesenchymal-type cells; often, islands of cartilage can be seen in the latter compartment (Daikha-Dahmane et al., 1997; Risdon and Woolf, 1998b). The appearances hold whatever the gross size of the malformed organ. As with renal agenesis, renal dysplasia can occur either as an isolated anomaly or be part of a multiorgan syndrome; an example is the renal cysts and diabetes syndrome, as discussed later.
C. Renal Hypoplasia

This is another histological term that refers to a kidney with significantly fewer nephrons than normal. Hypoplastic kidneys are small but, on biopsy, do not contain undifferentiated tissues, as does the dysplastic kidney. Although there are too few nephrons in hypoplastic kidneys, the glomeruli and tubules are sometimes enlarged greatly, a condition called oligomeganephronia (Salomon et al., 2001). Renal hypoplasia can occur as part of a multorgan congenital syndrome; e.g., it occurs in the renal coloboma syndrome, discussed in detail in Chapter 23.

D. Polycystic Kidney Disease

This term describes a large group of human diseases in which the major early steps of renal development are normal but an aberration of terminal epithelial differentiation follows so that the kidney becomes filled with fluid-filled cysts. One common variety is called autosomal-dominant polycystic kidney disease (ADPKD), which is discussed in detail in Chapter 24. Most cases are associated with mutations of PKD1, a gene coding for a large membrane-spanning protein called polycystin 1; in this condition, cysts can arise from all parts of the nephron. Another well-recognized variety is called autosomal-recessive polycystic kidney disease (ARPKD); here cystic dilatation of the ureteric bud-derived collecting ducts is the major feature. The gene for ARPKD has been defined; it is called poly- cystic kidney disease; here, cystic dilatation of the glomerular Bowman’s space is the key feature. It has been discovered that some cases of glomerulocystic kidney disease are associated with mutations of a transcription factor called heptocyte nuclear factor 1 (HNF1), as discussed later.

E. Other Disorders of Kidney Differentiation

Other kidney diseases can be considered disorders of renal differentiation, although they do not lead to gross structural congenital malformations. Among these diseases, which are addressed in detail later in this volume, are congenital nephrotic syndromes, diseases in which the differentiation of podocytes in the glomerular tuft are abnormal and lead to massive leakage of protein into the urinary space (Chapters 22 and 27); congenital diseases in which tubule physiology is abnormal (Chapter 26); and childhood and adult renal neoplasms, such as Wilms’ tumor and clear cell carcinomas (Chapters 22 and 25) in which there is a major deregulation of growth, sometimes involving genes active in normal nephrogenesis.

F. Malformations of the Lower Urinary Tract

In many cases, major renal malformations are accompanied by aberrant development of the lower urinary tract. Because development of the ureteric bud and renal mesenchyme depends on mutual interactions (Grobstein, 1967), it is not surprising that a primary defect of either component will affect its partner’s development in vivo. This provides one explanation for the clinical observation that an abnormal position of the insertion of the ureter into the bladder is associated with congenital renal parenchymal defects (Schwartz et al., 1981; Vermillion and Heale, 1973). In such cases, the altered ureteric anatomy may reflect a delayed or premature branching of the ureteric bud from the mesonephric duct. During early organogenesis, this abnormal trajectory could result in the bud failing to fully engage the segment of intermediate mesoderm destined to become nephrogenic mesenchyme. The end result would be defective or absent nephron formation together with a failure of mesenchyme-driven ureteric bud growth and branching morphogenesis. In other cases, renal dysplasia can occur with physical obstruction of the lower urinary tract at the level of the ureter or urethra; hence, it can also be postulated that the renal anomaly occurs secondary to the impairment of urine flow, which is discussed in detail later in this chapter. Another, not mutually exclusive, hypothesis for the coexistence of upper and lower urinary tract anomalies is that the same genes are expressed in both tissues and that mutations would therefore perturb development of the kidney and the lower urinary tract.

A very common malformation of the lower urinary tract is called primary vesicoureteric reflux, which is thought to affect about 1% of all young children (Feather et al., 1996). In this condition, there is a retrograde passage of urine from the bladder into the ureter, renal pelvis, and sometimes into the renal parenchyma itself. This is associated with a leaky valve mechanism at the junction of the ureter and urinary bladder. Another type of anomaly, also with an incidence of about 1%, are double or “duplex” ureters and kidneys (Whitten and Wilcox, 2001) in which the upper part of the kidney drains into a ureter with an ectopic, lower insertion into either the urinary bladder or even the urethra; the lower half of a duplex kidney drains into a separate ureter, which crosses the lower ureter, to drain into an ectopic, lateral insertion into the urinary bladder. Up to 40% of young children with renal failure requiring long-term dialysis and renal transplantation have dysplastic kidneys associated with lower urinary tract obstruction; in these cases, posterior urethral valves, a disorder unique to males, is the commonest specific diagnosis (Woolf and Thiruchelvam, 2001). Obstructive lesions can also occur at the junction of the renal pelvis and the top of the ureter (Josephson et al., 1993), or at the junction of the ureter and the urinary
bladder, the latter category including obstructive megaureters (Liu et al., 1994) and ureterocele (Austin et al., 1998).

G. Clinical Impact of Human Kidney and Lower Urinary Tract Malformations

Routine midgestation human fetal ultrasonographic scanning is used increasingly to detect organ malformations. Hence, more renal tract malformations are being diagnosed before birth (Anderson et al., 1997; Hiraoka et al., 1997; Yeung et al., 1997; Jaswon et al., 1999), accounting for up to 30% of all anomalies diagnosed prenatally (Noia et al., 1996). Many have little clinical significance, but for conditions such as bilateral renal agenesis or dysplasia, early diagnosis allows consideration of termination or active therapeutic intervention such as surgical decompression of obstructed fetal urinary tracts (e.g., by forming an artificial conduit between the fetal bladder and the amniotic cavity) (Freedman et al., 1999). It is well established that renal malformations are the major cause of chronic renal failure in children (Ehrich et al., 1992; Drozdzi et al., 1998; Lewis, 1999; Woolf and Thiruchelvam, 2001). With advances in technology, babies with minimal renal function can be dialyzed from birth and toddlers can receive kidney transplants from the age of 1 year. These strategies, together with general improvements in the care of children with chronic renal failure, will ultimately mean an increase in long term survival into the adult period for these individuals. However, in the severest cases, accompanying lung hypoplasia can be life-threatening in the neonatal period even if dialysis is technically feasible.

III. Causes of Maldevelopment of Human Kidney and Lower Urinary Tract

In general terms, we can divide the possible causes of human kidney and lower urinary tract malformations into two categories: (1) mutations, and possibly polymorphisms, of genes expressed during developmental and (2) environmental influences on development, which can be subdivided into (a) changes that originate outside the fetus, such as alterations of maternal diet, and (b) changes within the fetus, which disrupt normal development, e.g., impairment of normal fetal urinary flow due to physical obstruction of the urinary tract. We will now explore these themes in more detail.

A. Mutations

1. Studies of Mutant Mice: Lessons for Human Disease

Kuure et al. (2000) (also see Chapter 20) listed known renal tract developmental defects associated with mutations of about 20 genes encoding diverse molecules, several of which are thought to modulate cell survival/apoptosis, proliferation, differentiation, and morphogenesis. It is certain that the eventual number of key genes involved in kidney and urinary tract is higher than this. In common with human disease, the resulting malformations include absent, poorly differentiated and small kidneys, respective phenotypes which represent defects in the regulation of formation of the metanephros, tubule differentiation, and nephron numbers. With regard to human disease, several general conclusions from mouse genetic experiments provide potentially important paradigms for understanding the genetics of human kidney and lower urinary tract malformations.

1. Many of the genes implicated in mouse kidney and lower urinary tract development are also expressed in other differentiating organs. Consequently, mouse mutants often have multiorgan malformation syndromes. For instance, the PAX2 transcription factor is expressed not only in the embryonic kidney, but also in the eye; hence, mice with PAX2 mutations have both renal and ocular malformations (Favor et al., 1996). The same disease spectrum applies to humans with the renal-coloboma syndrome; these individuals also have PAX2 mutations (Sanyanusin et al., 1995; Salomon et al., 2001; Chapter 23). In fact, there are a considerable number of human malformation syndromes that involve both the kidney and lower urinary tract, as well as the central nervous system, the heart, the limbs, and other organs. Some of these conditions, several of which are inherited and hence must have a defined genetic basis, are listed in Table 21.2. For a complete listing of such syndromes, the reader is referred to the constantly updated McKusick’s Online Mendelian Inheritance in Man (http://www4.ncbi.nlm.nih.gov/Omim/).

2. Mutant mice also demonstrate that the same kidney and urinary tract malformation can result from the mutation of different genes. For example, null mutations of either of the two transcription factor genes, WT1 or PAX2, cause renal agenesis (Kreidberg et al., 1993; Torres et al., 1995), and the same phenotype is generated in mice with a genetically deleted glial cell line-derived neurotrophic factor signaling system (Schuchardt et al., 1994; Sanchez et al., 1996). Hence, one would predict in human disease that similar disease phenotypes might result from mutations of different genes. As an example of this phenomenon, unilateral renal agenesis has been described with mutations of both KAL-1, which codes for a cell–cell signaling molecule, and HNF1β, a transcription factor gene (see later for details).

3. Mouse experiments also demonstrate that kidney and lower urinary tract malformation phenotypes can vary between different mouse strains (Threadgill et al., 1995), an observation that might be explained by polymorphisms, or variants, of genes that act during development. In many
Table 21.2 Genetics of Some Human Renal Tract Malformations and Inherited Cystic Diseases

<table>
<thead>
<tr>
<th>Malformation</th>
<th>Genetics</th>
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<tbody>
<tr>
<td>Apert syndrome (FGFR2a mutation — growth factor receptor)</td>
<td>hydropteresis and duplicated renal pelvis with premature fusion of cranial sutures and digital anomalies (Cohen and Kreiborg, 1993; Wilkie et al., 1996)</td>
</tr>
<tr>
<td>Autosomal-dominant polycystic kidney disease (PKD1 and PKD2 mutations — membrane proteins, the latter may be a cation channel)</td>
<td>cysts arise from all nephron segments, with liver cysts and cerebral aneurysms (Hughes et al., 1995; Gonzalez-Perret et al., 2001; Chapter 24)</td>
</tr>
<tr>
<td>Autosomal-recessive polycystic kidney disease (PKHD1 — possible cell surface receptor)</td>
<td>kidney collecting duct cysts and hepatic fibrosis (Ward et al., 2002)</td>
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<tr>
<td>Bardet–Biedl syndrome (several locigenes involved — functions unknown)</td>
<td>renal dysplasia and calseque malformations with retinopathy, digit anomalies, obesity, diabetes mellitus, and male hypogonadism (Harnett et al., 1988; Katsanis et al., 2001)</td>
</tr>
<tr>
<td>Beckwith–Wiedemann syndrome (in a minority of patients, p57KIP2 mutation — cell cycle gene)</td>
<td>widespread somatic overgrowth with large kidneys, cysts, and dysplasia (Lam et al., 1999; Choyke et al., 1999)</td>
</tr>
<tr>
<td>Branchio-oto-renal syndrome (EYA1 mutation — possible transcription factor)</td>
<td>renal agenesis and dysplasia with deafness and branchial arch defects such as neck fistulae (Konig et al., 1994; Abdelhak et al., 1997)</td>
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<tr>
<td>Campomelic dysplasia (SOX9 mutation — transcription factor)</td>
<td>diverse renal and skeletal malformations (Houston et al., 1983; Wagner et al., 1994)</td>
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<tr>
<td>CHARGE association (genetic basis unknown)</td>
<td>coloboma, heart malformation, choanal atresia, retardation, genital and ear anomalies; diverse urinary tract malformations can occur (Regan et al., 1999)</td>
</tr>
<tr>
<td>Congenital anomalies of the kidney and urinary tract (CAKUT) syndrome (AT2 polymorphism — growth factor receptor)</td>
<td>diverse renal and lower urinary tract malformations (Nishimura et al., 1999)</td>
</tr>
<tr>
<td>Denys Drash syndrome (WT1 mutation — transcription/splicing factor)</td>
<td>mesangial cell sclerosis and calyeal defects (Jadresic et al., 1990; Coppes et al., 1993; Chapter 22)</td>
</tr>
<tr>
<td>Di George syndrome (microdeletion at 22q11 — probably several genes involved)</td>
<td>renal agenesis, dysplasia, vesicooureteric reflux, with heart and branchial arch defects (Budarf et al., 1995; Czarnecki et al., 1998; Stewart et al., 1999)</td>
</tr>
<tr>
<td>Glutaric aciduria type II (glutaril-CoA dehydrogenase mutation): cystic and dysplastic disease (Wilson et al., 1989)</td>
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<tr>
<td>Hypoparathyroidism, sensorineural deafness, and renal anomalies (HDR) syndrome (GATA3 mutation — transcription factor)</td>
<td>renal agenesis, dysplasia, and vesicooureteric reflux (van Esch and Bilous, 2001)</td>
</tr>
<tr>
<td>Fanconi anemia (six mutatant genes reported — involved DNA repair): renal agenesis, ectopic/horseshoe kidney, anemia and limb malformations (Lo et al., 1996; Yamashita and Nakahata, 2001)</td>
<td></td>
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<tr>
<td>Kallmann’s syndrome (KAL1 mutation — cell signaling molecule): renal agenesis (Duke et al., 1998; Hardelin, 2001; see this chapter)</td>
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<tr>
<td>Meckel syndrome (loci at 1q1 and 17q — genes unknown): cystic renal dysplasia, central nervous system, and malformations (Salonen, 1984; Paavola et al., 1995; Roume et al., 1998)</td>
<td></td>
</tr>
<tr>
<td>Nail-patella syndrome (LMX1B mutation — transcription factor): malformation of the glomerulus and renal agenesis (Gubler and Levy, 1993; Haga et al., 1997; Dreyer et al., 1998)</td>
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<tr>
<td>Oral facial digital syndrome type 1 (OFD1 mutation — function unknown): glomerular cysts with facial and digital anomalies (Feather et al., 1997; Ferrante et al., 2001)</td>
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<tr>
<td>Renal-coloboma syndrome (PAX2 mutation — transcription factor): renal hypoplasia and vesicooureteric reflux (Sanyanusin et al., 1995; Salomon et al., 2001; Chapter 23)</td>
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</tr>
<tr>
<td>Renal cysts and diabetes syndrome (HNF1B mutation — transcription factor): renal dysplasia, cysts, and hypoplasia (Bingham et al., 2000, 2001; Kolatsi-Joannou et al., 2001; see this chapter)</td>
<td></td>
</tr>
<tr>
<td>Simpson–Golabi–Behmel syndrome (GPC3 mutation — proteoglycan): renal overgrowth, cysts, and dysplasia (Hughes-Benze et al., 1994; Pillia et al., 1996; Gonzalez et al., 1998)</td>
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<tr>
<td>Smith–Lemli–Opitz syndrome (d(7)-dehydrocholesterol reductase mutation — cholesterol biosynthesis): renal cysts and dysplasia (Akl et al., 1977; Wallace et al., 1994)</td>
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<tr>
<td>Townes–Brocks syndrome (SALL1 mutation — transcription factor): renal dysplasia and lower urinary tract malformations (Salerno et al., 2000)</td>
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<tr>
<td>Urofacial (Ochoa) syndrome (locus on 10q — gene unknown): congenital obstructive bladder and kidney malformation with abnormal facial expression (Wang et al., 1997)</td>
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<tr>
<td>Urogenital adysplasia syndrome (some cases associated with HNF1β mutation): renal dysplasia and uterine anomalies (Bingham et al., 2002)</td>
<td></td>
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<tr>
<td>VACTERL association (basis unknown apart from one report of mitochondrial gene mutation): vertebral, cardiac, tracheoesophageal, renal, radial, and other limb anomalies (Dumain et al., 1996)</td>
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<tr>
<td>von Hippel Lindau disease (VHL mutation — tumor suppressor gene): renal and pancreatic cysts, renal tumors (Chatha et al., 2001; Chapter 25)</td>
<td></td>
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<tr>
<td>WAGR syndrome (WT1 and PAX6 contiguous gene defect — transcription factors): Wilms’ tumor, aniridia and genital and renal malformations (Pritchard-Jones, 1999; Chapter 22)</td>
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<tr>
<td>Zellweger syndrome (peroxisomal protein mutation): cystic dysplastic kidneys (Powers and Moser, 1998; Shimozawa et al., 1992)</td>
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* Mutations of this gene also implicated in mouse kidney and/or lower urinary tract malformations.
in normal controls. The variant polymorphism affects RNA splicing and mRNA transcript levels (Nishimura et al., 1999). Furthermore, male mice with null mutation of AT2 display a low penetrance of diverse renal tract malformations (Nishimura et al., 1999).

4. In mice, mutations of two homologous genes (e.g., homeobox or retinoic acid receptor) may summate to generate a major disruption of kidney differentiation (Mendelsohn et al., 1994; Davis et al., 1995; Patterson et al., 2001). Furthermore, metanephrin organ culture studies have shown that different growth factors can have complex, interacting effects on development (Plisov et al., 2001). There are several human examples of kidney anomalies in which more than one gene is mutated. For example, in Bardet Biedl syndrome, a condition in which kidney malformations are associated with retinopathy, digit anomalies, obesity, diabetes mellitus, and male hypogonadism, more than one gene can be mutated in an affected individual (Katsanis et al., 2001). In addition, clinically severe polycystic kidney disease has been noted in individuals who have a mutation of both PKD1 and PKD2 genes (Pei et al., 2001) or who have contiguous gene defects that delete PKD1 and TSC2 (Kleymenova et al., 2001; Martigoni et al., 2002).

2. X-Linked Kallmann’s Syndrome: A Genetically Defined Example of Human Renal Agenesis

The X-linked form of this disease is caused by mutations of KAL-1 (Franco et al., 1991; Legouis et al., 1991; Hardelin, 2001). In affected males, anosmia (absent sense of smell) and hypogonadotrophic hypogonadism (testicular failure secondary to defective release of stimulating hormones from the hypothalamus) occur because of defective fetal elongation of olfactory neuron axons and migration of gonadotrophin-releasing hormone synthesizing neurons from the nasal placode into the forebrain; furthermore, the olfactory bulb fails to grow and is hypoplastic (Schwanzel-Fukuda et al., 1989). About one-third of patients with the syndrome have a solitary functioning kidney, with presumed unilateral renal agenesis (Kirk et al., 1994; Duke et al., 1998). Other anomalies occur less commonly, including duplex systems, hydronephrosis, and vesicoureteric reflux. Patients with urinary tract agenesis can also lack the vas deferens, a structure derived from the mesonephric duct, which also gives rise to the ureteric bud and its derivatives. Deeb et al. (2001) reported two children with the syndrome and unilateral multicystic dysplastic kidney: they suggested that the apparent “agenesis” phenotype seen in older individuals with the syndrome might be the result of spontaneous involution of multicystic organs. In vitro studies demonstrate an adhesive role for the protein coded by KAL-1 (Soussi-Yanicos et al., 1998). In vivo KAL-1 is expressed in the embryonic human central nervous and excretory systems (Duke et al., 1995; Hardelin et al., 1999). The protein immunolocalizes to the epithelial interstitial matrix and basement membranes of the mesonephric collecting tubules and mesonephric duct and to the first generations of metanephrin collecting duct branches of the ureteric bud. It is possible that failure of growth of either the ureteric bud or its first branches, perhaps due to alterations in cell adhesion, would lead to a failure of metanephric formation and hence renal agenesis. Certainly, other adhesion molecules have been considered critical for normal nephrogenesis in murine models (Klein et al., 1988; Noakes et al., 1995; Kreidberg et al., 1996; Muller et al., 1997; Bullock et al., 2001).

3. Renal Cysts and Diabetes (RCAD) Syndrome: A Genetically Defined Cause of Renal Dysplasia

HNF1β mutations cause the recently described RCAD syndrome. Human mutations of this transcription factor gene were initially associated with MODY (maturity onset diabetes mellitus of the young): affected individuals have a failure of pancreatic insulin secretion. Diverse mutations, both inherited and occurring de novo, in the DNA-binding and transactivating domains of this transcription factor have been reported in patients with kidney malformations including absent kidney (Bingham et al., 2002), renal dysplasia (Bingham et al., 2000, 2002), renal hypoplasia with oligomeganephronia (Lindner et al., 1999), and the glomerulocystic type of polycystic kidney disease (Bingham et al., 2001), a form of polycystic kidney in which glomerular cysts predominate. Embryonic mouse null mutants die before the urinary tract is formed, but in Xenopus, the introduction of one of the human mutations perturbs kidney precursor development in a dominant-negative manner with the formation of cyst-like structures (Wild et al., 2000). Furthermore, experimental disruption of the homologous zebrafish gene (Sun and Hopkins, 2001) apparently implicates it in the determination of the border between the pronephric glomerulus and the tubule; normal gene expression seems necessary for the maintenance of PAX2 and the downregulation of WT1 in the tubule. HNF1β is expressed in murine embryonic kidney and lower urinary tract (Coffinier et al., 1999; Barbacci et al., 1999). Kolatsi-Joannou et al. (2001) reported that the gene is widely expressed in human embryos: e.g., in normal human metanephiroi the highest levels of transcripts localized to fetal medullary and cortical collecting ducts, and low levels of expression in nephrogenic cortex mesenchyme, primitive nephron tubules, and immature glomeruli. It has been postulated that a primary defect in development of the ureteric bud/collecting duct could lead to either a very severe disruption of development (dysplasia) or to a more mild phenotype, namely the generation of glomerular cysts, perhaps secondary to the impairment of fetal urine flow from the proximal to distal part of the developing tubule (Woollf et al., 2002). The gene is also expressed in the human embryonic
pancreas, liver, gut, and lung (Kolatsi-Joannot et al., 2001). Most likely, this is an example of a gene involved in epithelial differentiation and branching in several organ systems.

4. Genetics of Human Kidney and Lower Urinary Tract Malformations Not Associated with Multiorgan Syndromes

Kindreds have been reported with multiple individuals affected by kidney and/or lower tract malformations that are not associated with multiorgan syndromes. These disease phenotypes include families with renal agenesis and/or renal dysplasia (Cain et al., 1974; Roodhoft et al., 1984; McPherson et al., 1987; Murugasu et al., 1991; Arfeen et al., 1993), multicystic dysplastic kidney (Moazin et al., 1997; Murakami et al., 1992), oligomeganephronic renal hypoplasia (Moerman et al., 1984), pelviureteric junction obstruction (Izquierdo et al., 1992), duplex kidneys and ureters (Atwell, 1985), and posterior urethral valves (Farrar and Pryor, 1976). A good example is primary vesicoureteric reflux — it is called “primary” because there is no outflow tract obstruction. It is associated with a “nephropathy” that represents a mixture of congenital dysplasia and/or hypoplasia (Risdon et al., 1993) and the renal disease accounts for about 10% of both children and adults who require treatment with renal dialysis and kidney transplantation (Lewis, 1999; Smellie et al., 2001). In some families, primary vesicoureteric reflux is clearly inherited in a dominant manner, with evidence in some families for a locus on chromosome 1p13 (Feather et al., 1996; 2000); at present the causative gene is undefined.

B. Effects of Maternal Diet and Diverse Teratogens on Urinary Tract Development

On occasion, human kidney or lower urinary tract malformation have been reported in association with teratogens. These include angiotensin converting enzyme inhibitors, drugs used to treat high blood pressure (Pryde et al., 1993; Barr et al., 1994; Sedman et al., 1995), cocaine (Battin et al., 1995), corticosteroids (Hulton and Kaplan, 1995), ethanol (Moore et al., 1997; Taylor et al., 1994), gentamycin, an antibiotic (Hulton and Kaplan, 1995), glucose (Novak and Robinson, 1994; Lynch et al., 1997; Woolf, 2000), nonsteroidal anti-inflammatory drugs (Voyer et al., 1994), and vitamin A and its derivatives (Von Lennep et al., 1985; Rothman et al., 1995). In several cases, however, evidence for association is anecdotal (e.g., for gentamycin and nonsteroidal anti-inflammatory drugs) and may not be based on hard epidemiological data. Clearly, the effect of individual agents can be very complicated. For example, animal experiments indicate that there may be an optimal intake of vitamin A and its retinoid derivatives for the development of several organs with both too much and too little inhibiting normal differentiation; retinoids may affect organogenesis by altering patterning (e.g., by perturbing homeobox gene expression) or by altering cell-cell signaling by growth factors (e.g., the GDNF/RET axis) (Padmanabhan, 1998; Moreau et al., 1998; Mendelsohn et al., 1999; Pitera et al., 2001).

More intriguingly, relatively subtle changes in maternal diet, in terms of protein intake, may have an effect on metanephric development. Imposition of a severe dietary protein restriction (5–6% versus 18% control diet) during pregnancy reduced the final numbers of glomeruli generated in rat kidneys (Levy and Jackson, 1993; Merlet-Benichou et al., 1994). Studies have also shown that milder maternal protein restriction is also associated with a final nephron deficit and high systemic blood pressure in young adult animals (Langley-Evans et al., 1996, 1999; Welham et al., 2002). How such an effect might be mediated, and when it might occur during nephrogenesis, has, until recently, been unknown. Kwong et al. (2000) demonstrated that maternal low-protein diets reduced cell numbers in the inner cell mass of preimplantation embryos. Using the study of Kwong et al. (2000) as a paradigm, Welham et al. (2002) postulated that low-protein maternal diets might affect cell turnover in early metanephrinogenesis (Fig. 21.3). Rats were supplied with one of three isocaloric diets from day 0 of pregnancy: control (18% protein) or low protein (9 or 6%) diets. At 2 weeks postnatally, when nephrogenesis has finished in this species, controls had a mean of $17 \times 10^5$ glomeruli/kidney, whereas offspring exposed to either 9 or 6% protein diets had significantly fewer nephrons. At embryonic day 13, when the rat metanephros has just formed, metanephiroi in all dietary groups contained the same number of cells (about $2 \times 10^6$). In all diets, apoptosis was noted in condensing mesenchyme (nephron/glomeruli precursors) and loose mesenchyme (interstitial precursors), yet it was increased significantly in the mesenchyme in developing kidneys exposed to low-protein diets. At embryonic day 15, when rat mesenchyme begins forming primitive nephrons but glomeruli are still absent, the average control metanephros had $2 \times 10^6$ cells, whereas time-matched embryos exposed to either 6 or 9% protein had significantly fewer. Other studies emphasise that the deregulation of apoptosis affects metanephric growth. Caspase inhibitors reduce renal mesenchymal cell death and perturb epithelial morphogenesis in mouse metanephric organ culture (Araki et al., 1999), and null mutation of AT2, which decreased cell death around the lower urinary tract, causes a variety of kidney and ureter malformations (Nishimura et al., 1999). In addition, an excess of metanephric cell death can be triggered genetically, e.g., in PAX2+/− and BCL2−/- mutant mice (Sorenson et al., 1995; Porteus et al., 2000), or by exposure to specific cytokines, such as tumor necrosis factor α (Cale et al., 1998); all these maneuvers lead to hypoplastic organs with too few nephrons.
Hence, in rats, maternal low protein diets reduce the final numbers of glomeruli in association with the enhanced deletion of mesenchymal cells at the start of kidney development. In humans, the equivalent developmental time frame would be 5 to 7 weeks gestation (Risdon and Woolf, 1998a), which might represent a critical window when kidney morphogenesis might be affected by dietary influences. Of note, Hinchliffe et al. (1993) reported nephron deficits, as assessed by counting glomeruli, in infants with intrauterine growth retardation, and epidemiological studies have found that individuals born to mothers with poor diets are prone to hypertension (Barker 1998); furthermore, others have speculated that congenital “nephron deficits” predispose individuals to hypertension later in life (Brenner et al., 1988).

C. Fetal Urinary Flow Impairment

The presence of cartilage and smooth muscle cells, as well as epithelial/mesenchymal intermediate forms, in human dysplastic kidneys indicates major aberrations of cell differentiation. In addition, the abnormal growth and involution seen in some of these organs are likely to be related to the balance between proliferation and death at different stages of evolution of the disease. Studies have been made regarding gene expression and cell turnover in human dysplastic kidneys, some of which are attached to obstructed lower urinary tracts (Bussieres et al., 1995; Winyard et al., 1996a, b; Granata et al., 1997; Matsell et al., 1997; Kolatsi-Joannou et al., 1997; Winyard et al., 1997; Calé et al., 2000; Poucell-Hatton et al., 2000; Yang et al., 2001; MacRae et al., 2000; Woolf and Winyard, 2000) (Fig. 21.4). These studies show that cystic epithelia express PAX2 and BCL2, both of which enhance cell survival, and these cells tend to be hyperproliferative, as assessed by expression of the proliferating cell nuclear antigen. Indeed, genetically engineered mice with forced overexpression of PAX2 (Dressler et al., 1993) acquire renal cysts, suggesting a role for this gene in epithelial overgrowth. In contrast, stromal cells show a high level of apoptosis, as assessed by in situ end-labeling and the presence of pyknotic nuclei; although some of these stromal cells express WT1, suggesting they might have been induced to form nephrons, BCL2 expression is low. Some stromal cells proximate to dysplastic tubules show a shift to a smooth muscle lineage, as assessed by expression of αSMA (Yang et al., 2000). Yang et al. (2000) postulated that an upregulation of transforming growth factor-β (TGF-β), found in human dysplastic organs, might have a biological role in the disease; they found that this cytokine induced an epithelial to smooth muscle shift in cultured dysplastic tubule cells and also demonstrated in vivo that rare dysplastic epithelial cells expressed αSMA, whereas rare stromal cells expressed cytokeratin, an epithelial marker.

Experimental urinary tract obstruction in fetal sheep generates a histological appearance that resembles human renal dysplasia, with malformed tubules, a loss of the normal nephrogenic zone, transformation of precursor cells towards a smooth muscle phenotype, and the generation of cysts (Peters et al., 1992; Attar et al., 1998; Yang et al., 2000).
expression is predominantly in the dysplastic epithelium. Bar: 30 μm.

(A) Very low levels of WT1 protein are detected in condensing mesenchyme (arrowheads), but expression increases in nephron precursors, reaching maximal intensity in glomerular (g) podocytes. (B) Conversely, BCL2 levels are highest in the mesenchymal condensates and decrease as the nephron matures. Note that neither WT1 nor BCL2 is expressed in the mesenchyme but is prominent in cystic epithelia. Further-

Figure 21.4 Comparison of WT1 and BCL2 expression in normal human kidney development and in dysplastic kidneys. Sections of the midgestation metanephric outer (nephrogenic) cortex (A and B) and postnatal dysplastic kidneys (C and D) immunostained for WT1 (A and C) or BCL2 (B and D) counterstained with methyl green (A–C) or hema-

developing opossum kidney (Steinhardt et al., 1995). Furthermore, after experimental ureteric obstruction of the neonatal rat kidney (in rats, only about 10% of glomeruli have formed at birth), administration of either insulin-like growth factor or epidermal growth factor reduces damage (Chevalier et al., 1998, 2000). Animal models have also been used to answer the question whether decompression of the fetal obstructed urinary tract can rescue normal development. In utero decompression performed before the end of the nephrogenic period can partially prevent renal dysplasia and the loss of glomeruli in fetal sheep (Glick et al., 1984; Edouga et al., 2001). In the neonatal rat model alluded to earlier, decompression attenuated but did not reverse renal injury resulting from 5 days of ureteric obstruction (Chevalier et al., 1999). Probably, in the human scenario, effective therapeutic intervention, e.g., by surgical shunting to allow flow of urine from the obstructed urinary tract into the amniotic cavity, would need to be performed early in the genesis of this disease, before severe changes have occurred.

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heterogeneous with a locus on chromosome 1.


