Development of the Kidney and Lower Urinary Tract

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1.1 Introduction

Congenital abnormalities of the kidney and urinary tract (CAKUT) are the cause of 30–50 % of end-stage renal disease in young children [307]. CAKUT are represented by a heterogeneous group of renal, ureter, and bladder malformations across a wide range of clinical severity (Table 1.1). The incidence of renal and urinary tract anomalies in humans is 0.3–1.6 per 1,000 live born and stillborn infants [359]. Renal malformations account for 20–30 % of all solid-organ birth defects detected by antenatal sonography during pregnancy [273]. Thirty percent of cases occur in association with extrarenal malformations [359] and may be found as part of over 100 congenital syndromes (Table 1.2) [173].

This chapter approaches CAKUT from an embryological perspective with emphasis on morphologic, cellular, and molecular events in normal urinary tract development. The science of human embryology relates to form and process of tissue development and integrates molecular, cellular, and structural factors within a dynamic spatiotemporal framework. A clear understanding of human embryology provides a foundation for understanding structure-function relationships within a given tissue or organ. It also renders insight into the pathological basis of congenital malformation resulting from perturbations in normal organ development and leads to the recognition of associated malformations within the same organ system (e.g., genitourinary system) when developmental mechanisms are shared between tissues (e.g., kidney and ureter). Consequently, the principles of urinary tract embryology described in this chapter are fundamental to the diagnosis and clinical management of CAKUT in fetuses and newborns and crucial to understanding the long-term impact of CAKUT on overall health.

Developmental events in kidney, ureter, and bladder morphogenesis are highly conserved across vertebrate species [75]. The use of model organisms such as mice, zebrafish, and frogs has been invaluable for defining gene expression patterns in the embryonic urinary tract system and for providing a spatiotemporal framework upon which to study gene function. Understanding relationships between gene expression and function has been greatly facilitated by the creation of a molecular atlas of gene expression for the developing urinary tract, which can be accessed online through the GenitoUrinary Development Molecular Anatomy Project (GUDMAP; http://www.gudmap.org/index.html) [117]. Gene function has been largely elucidated through the anal-

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Table 1.1 Examples of renal, ureter, and bladder malformations

Renal malformations
Renal agenesis
Renal hypoplasia
Renal dysplasia
Renal duplication
Horseshoe kidney
Renal ectopia (e.g., pelvic kidney)
Cross-fused ectopia
Cystic kidney diseases
Polycystic kidney disease (autosomal dominant, autosomal recessive)
Multicystic dysplastic kidney
Medullary cystic kidney
Nephronophthisis
Ureteral malformations
Ureteral agenesis
Ureteral duplication
Ureteropelvic junction obstruction
Ureteral stricture (distal to the ureteropelvic junction)
Hydroureter (nonobstructive)
Ectopic ureter
Ureterocele
Bladder malformations
Bladder exstrophy
Bladder diverticulum
Vesicoureteral reflux

Table 1.2 Human congenital malformation syndromes associated with CAKUT

Syndromes with cystic dysplasia	
Apert	Meckel-Gruber
Bardet-Biedl	Meckel syndrome, type 7
Branchio-oto-renal	Melnick-Needles
Campomelic dysplasia	Pallister-Hall
Cornelia de Lange	Patau (trisomy 13)
Down (trisomy 21)	Senior-Loken
Edwards (trisomy 18)	Tuberous sclerosis
Jeune asphyxiating thoracic dystrophy	von Hippel-Lindau
	Zellweger
Syndromes with polycystic kidneys	
Congenital rubella	Peutz-Jeghers
Ehlers-Danlos	Pyloric stenosis
Kaufman-McKusick	Roberts
Noonan	Short rib-polydactyly, types II, III, and IV
	Zellweger
Syndromes with horseshoe kidney	
Abruzzo-Erickson	Pallister-Hall
Bowen-Conradi	Pyloric Stenosis
Cerebro-oculo-facio-skeletal (Pena-Shokeir)	Roberts
Facio-cardio-renal (Eastman-Bixler)	Trisomy 18 (Edwards)
Focal Dermal Hypoplasia (Goltz-Gorlin)	Trisomy 21 (Down)
Oral-cranial-digital (Juberg-Hayward)	Turner
Syndromes with unilateral renal agenesis	
Acro-renal-mandibular	Ivemark
Adrenogenital (21-OH-ase deficiency)	Klippel-Feil
Alagille	Lacrimo-auriculo-dento-digital
Cardiofacial	Larsen
Cat-Eye	Lenz microphthalmia
Cerebro-oculo-facio-skeletal (Pena-Shokeir)	Mayer-Rokitansky
Chondroectodermal dysplasia (Ellis-van Creveld)	Miller-Dieker, lissencephaly
Coffin-Siris	Oculo-auriculo-vertebral dysplasia (Goldenhar)
Congenital rubella	Olfactogenital dysplasia (Russell-Silver)
Fetal alcohol	Spondylocostal dysostosis

ysis of embryonic mouse mutant phenotypes generated either by targeted mutagenesis, which disrupts gene function universally, or by conditional mutagenesis, which renders in loss of gene function in a cell-specific or time-dependent manner [159]. This chapter will make reference to genetic studies in mice to gain insight into morphogenetic, molecular, and cellular mechanisms which underlie normal development of the human kidney, ureter, and bladder. By convention, text references to human genes will be noted in capitalized italics whereas mouse genes are in sentence case italics [262].

This chapter is subdivided into broad categories representing stereotypic processes in urinary tract formation. These include descriptions of the

Table 1.2 (continued)

Syndromes with renal and/or ureteral duplications	
Achondrogenesis	Congenital Rubella
Acrocephalosyndactyly (Saethre-Chotzen)	Denys-Drash
Acro-renal (Dieker-Opitz)	Fetal Alcohol
Adrenogenital (21-OH-ase deficiency)	Klippel-Feil
Bowen-Conradi	Noonan
Branchio-oto-renal	Oculo-auriculo-vertebral dysplasia (Goldenhar)
Braun-Bayer	Rubinstein-Taybi
Cerebro-oculo-facio-skeletal (Pena-Shokeir)	Trisomy 21
	Turner
Syndromes with hydroureter or hydronephrosis	
Apert	Johanson-Blizzard
Campomelic Dysplasia	Kaufman-McKusick
Chondroectodermal dysplasia (Ellis-van Creveld)	Larsen
Coffin-Siris	Menkes
Cornelia de Lange	Noonan
DiGeorge	Pyloric stenosis
Fetal Alcohol	Spondylocostal dysostosis
	Wolfram
Syndromes with renal ectopia	
Cardiofacial	Marfan
Cerebro-cost-mandibular	Mayer-Rokitansky
Craniosynostosis-radial aplasia (Baller-Gerold)	Oculo-auriculo-vertebral dysplasia (Goldenhar)
Denys-Drash	Pallister-Hall
Klippel-Feil	Seckel
	Spondylocostal dysostosis
Syndromes with renal hypoplasia	
Branchio-oto-renal	Ivemark
Campomelic Dysplasia	Poland
Cornelia de Lange	Pyloric Stenosis
Fetal Alcohol	Seckel
	Townes-Brock
Syndromes with bladder exstrophy	
Syndactyly, type IV (Haas)	

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early embryonic origins of the kidneys, ureters, and bladder, as well as descriptions of later events involved in renal collecting duct and nephron morphogenesis, ureter formation, and bladder development. Within each subdivision, attention is given to genetic or molecular control mechanisms essential for executing key developmental programs. Specific references to urinary tract morphogenesis in embryonic mice are made when orthologous events in humans have not been fully characterized. Consequently, the information in this chapter serves as a framework for understanding the breadth and complexity of anatomical and functional defects of the urinary system that present clinically in the fetus and newborn infant.

1.2 Embryonic Origins of the Urinary System

1.2.1 Overview of the Early Urinary Tract Embryology

The mammalian urinary system has embryologic cellular origins in the mesodermal and endodermal germ layers of the post-gastrulation embryo [76]. Mesodermal derivatives comprise all epithelial cell types of the mature nephron, renal pelvis, and ureter, as well as non-epithelial cell types including glomerular endothelial and mesangial cells, renal parenchymal interstitial cells (also known as stromal cells), ureteral and bladder smooth muscle cells, and adipocytes and connective tissue-producing fibrocytes of the renal capsule and ureter and bladder adventitia. Endodermal tissue, on the other hand, gives rise to the luminal epithelial cells of the bladder and urethra. Kidney and ureter development requires the initial formation of a mesoderm-derived embryonic structure known as the nephric duct (also known as the Wolffian duct or mesonephric duct). Conversely, bladder development is preceded by formation of the urogenital sinus. The following sections (Sects. 1.2.2 and 1.2.3) describe formation of the nephric duct and urogenital sinus, respectively. Table 1.3 compares the chronology of human and mouse urinary tract development.

Table 1.3 Embryonic time table for nephrogenesis: human versus mouse

	Human	Mouse
Pronephros		
First appearance	22 days	9 days
Regresses by	25 days	10 days
Mesonephros		
First appearance	24 days	10 days
Regresses by	16 weeks	14 days
Metanephros appears	28–32 days	11 days
Collecting tubules and nephrons	44 days	13 days
Glomeruli	8–9 weeks	14 days
Nephrogenesis ceases	34–36 weeks	4–7 days after birth
Length of gestation	40 weeks	19 days

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1.2.2 Nephric Duct Morphogenesis

Mesoderm-derived ancestors of the kidneys and ureters originate from within a narrow strip of tissue termed intermediate mesoderm (IM) which is located bilaterally on each side of the embryonic midline. IM is evident in human fetuses by week 4 of gestation based on the appearance of the nephric duct [139]. In mice, IM is detected by embryonic day 8.5 (E8.5) based on tissue-specific patterns of gene expression [76, 336]. IM extends bilaterally in an anteroposterior, or rostral-caudal, direction from the level of the twelfth somite at the embryonic midsection to the cloaca, which is a midline embryonic structure located in the embryonic hind region [139]. During week 4 of human fetal gestation (E9.0 in mice), IM is induced along its anteroposterior axis to form a paired set of single-cell-layered epithelial tubes which are the nephric ducts. Fully formed nephric ducts extend the full length of IM and deviate at its posterior end towards the midline to insert into the cloaca (see Sect. 1.2.3).

While the nephric duct is formed, surrounding intermediate mesoderm (referred to as mesenchyme) is induced to undergo epithelial transformation. This inductive process results in the sequential generation of three morphologically unique nephrogenic primordia – the pronephros, mesonephros, and metanephros – which connect to the anterior, mid-, and posterior sections of the nephric duct, respectively (Fig. 1.1) [75]. The pronephros is a primitive paired organ characterized by a single glomerular and tubular filtrative unit (Fig. 1.1a). In lower-order animals such as amphibians and fish, the pronephros functions as a transient embryonic excretory organ. Conversely, in higher-order mammals including humans, pronephric structures are transiently represented by nonfunctioning rudimentary tubules which degenerate by apoptosis [86, 286]. The mesonephros is a more sophisticated filtrative system characterized by several well-developed glomerular- and tubular-like structures that connect directly to the nephric duct at its midsection (Fig. 1.1b). In adult fish and amphibians, the mesonephros replaces the pronephros as the definitive filtrative organ [75]. In humans, mesonephric development begins late in the fourth week of gestation and results in the transient production of fetal urine [118, 242]. By week 5, most mesonephric tissue undergoes degeneration while the remaining tissues in males contribute to the formation of the reproductive system, including the efferent ductules of the testis, vas deferens, epididymis, and seminal vesicle. In female fetuses, the mesonephros regresses although vestigial structures may persist and are represented clinically as Gartner's duct cysts, epoophoron, and paroophoron [226]. As mesonephric degeneration takes place, IM surrounding the posterior nephric duct (termed metanephric mesenchyme) is induced to form the metanephros, which represents the nascent mammalian kidney (Fig. 1.1c). Induction of metanephric mesenchyme (MM) is dependent on outgrowth of an epithelial diverticulum from the nephric duct termed the ureteric bud (UB) which occurs at approximately week 5 of human fetal gestation (E10.5 in mice). Invasion of MM by the UB initiates a series of reciprocal inductive interactions that triggers formation of the adult mammalian kidney and ureters. Detailed descriptions of kidney and ureter morphogenesis are provided in Sects. 1.3 and 1.4.

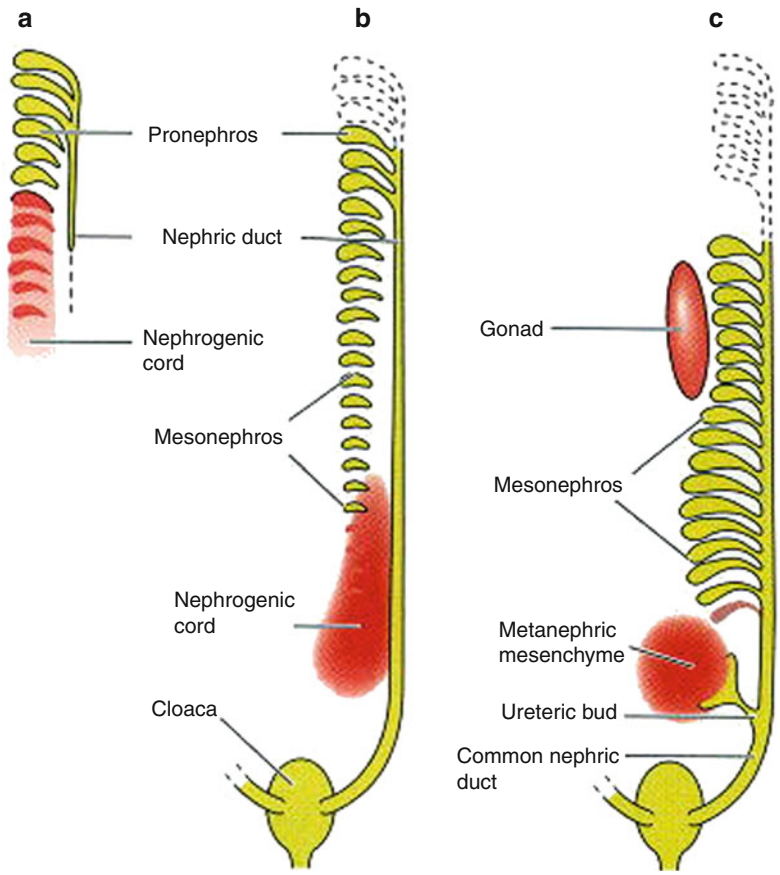

Fig. 1.1 Formation of nephrogenic primordia. (**a**) The nephrogenic cord and pronephros. (**b**) The mesonephros. (**c**) The metanephros

1.2.2.1 Molecular Pathways Involved in Nephric Duct Morphogenesis

Genetic studies in mice point to critical roles played by four transcription factor genes – *Lhx1*, *Pax2*, *Pax8*, and *Osr1* – in specifying IM for kidney and ureter development. *Lhx1* (Lim homeobox protein 1) encodes a member of the Lim family of homeodomain proteins which are essential to forming anterior embryonic structures [311]. *Pax2* and *Pax8* are paired box domain DNA-binding proteins which function as master regulators of tissue development in several organ systems, including kidney [26]. *Lhx1*, *Pax2*, and *Pax8* mRNAs are among the earliest gene transcripts detected in IM [26]. All three genes ultimately show mRNA expression in nephric duct cells, and their functions are essential to normal nephric duct formation. Mice lacking *Lhx1* function fail to form nephric ducts [336], whereas mice homozygous for a null *Pax2* mutation form posteriorly truncated nephric ducts [34, 335]. Combined inactivation of *Pax2* and *Pax8* results in complete absence of the nephric duct [26], suggesting that Pax family members have overlapping functions in anterior regions of IM. Combined *Pax2/8* function may be important for demarcating IM from lateral plate and paraxial mesoderm since *Pax2* mRNA are exclusively detected at the boundaries of these mesodermal compartments at stages prior to nephric duct formation [336]. Expression of *Pax2* and *Pax8* in this region appears to be under the positive control of bone morphogenetic protein 4 (BMP4; encoded by the *Bmp4* gene) which is secreted by cells in adjacent lateral plate mesoderm and in overlying ectoderm [142, 143, 236]. Negative control over these inductive interactions appears to be

provided by other as-yet undefined factors secreted by nearby somites [190].

Osr1 encodes an odd-skipped related zinc-finger DNA-binding protein and is required to specify IM for kidney development [141]. *Osr1* is expressed in IM surrounding the nephric duct along its entire length and is excluded from nephric duct cells. Mice lacking *Osr1* function form nephric ducts but lack kidneys, which suggest that *Osr1* plays an important role in specifying posterior IM for kidney development.

Hox genes encode a large family of homeodomain proteins and are organized into related gene subgroups or clusters sharing functions that coordinate regional expression of other genes involved in axial patterning of a wide range of embryonic tissues [71]. Two mouse hox gene clusters – *Hox4* and *Hox11* – have been implicated in establishment of anterior and posterior IM cell identity, respectively, along the early embryonic AP axis. A role for *Hox4* genes in promoting anterior IM cell fate is suggested by the mRNA expression pattern for *Hoxb4* which is detected in early mesoderm at the anterior boundary of prospective IM [9]. The notion that *Hox4* genes establish an anterior code for IM is supported by studies in cultured chick embryos which revealed an anterior shift in expression of IM-specific markers *Lhx1* and *Pax2* within chick mesoderm when the anterior limits of *Hoxb4* expression were experimentally manipulated [263]. Conversely, evidence in mice suggests that *Hox11* cluster genes (*Hoxa11*, *Hoxc11*, and *Hoxd11*) control posterior IM cell fate and promote differentiation of cells within this region along a metanephric cell lineage. In a study which involved the use of tissue-specific promoter sequences in transgenic mice to expand mesodermal expression of *Hoxd11* anteriorly into a region of IM normally fated for mesonephros development, ectopically activating *Hoxd11* in this region resulted in transformation of mesonephric tubules into a more metanephric phenotype [212]. This observation suggested that *Hox11* cluster genes are necessary for instructing IM cells to differentiate along a metanephric cell fate instead of a mesonephric cell fate. Hox11 genes also appear to be required for enabling posterior IM cells to respond appropriately to inductive cues which initiate kidney and ureter development. This is revealed in compound mutant mice when either combinations of two or all three paralogous *Hox11* genes are mutated, which results in severe kidney hypoplasia or complete agenesis of kidneys and ureters, respectively [356]. In vitro, proteins encoded by *Hox11* genes form a DNA-binding complex with proteins encoded by *Pax2* and the Eyes absent 1 (*Eya1*) proteins and directly activate the expression of key metanephric regulators, glial-derived neurotrophic factor (*Gdnf*) and sine oculis homeobox 2 (*Six2*) (see Sect. 1.3.3.1) [105]. Consequently, the complete absence of kidneys and ureters in *Hox11* triple mutant mice is likely due to a failure of *Hox11* genes to appropriately activate the expression of other genes critical for initiating urinary tract development.

1.2.3 Urogenital Sinus Morphogenesis

The urogenital sinus is an embryonic structure which originates as a sub-compartment of the cloaca. The cloaca is an endoderm-derived transient hollow structure located midline in the embryonic hind region. It connects bilaterally with the posterior ends of the paired nephric ducts (Fig. 1.2). In humans, the cloaca is formed by the third week of fetal gestation from confluence of the allantois and hindgut [267]. The allantois precedes the umbilicus as the conduit for embryonic gas and solute exchange with the placenta, while the hindgut ultimately forms distal colonic structures including the rectum and the upper part of the anal canal. Between weeks 6 and 7, the cloaca is subdivided into dorsal and ventral chambers by a fold of mesodermal tissue which projects into the cloacal cavity and creates a transverse ridge known as the cloacal septum (also known as the urorectal septum; Fig. 1.2a). The dorsal chamber generates the anorectal canal which communicates with the hindgut and ultimately develops into the rectum and anus. The ventral chamber forms the urogenital sinus which is connected at its anterior end to the allantois via

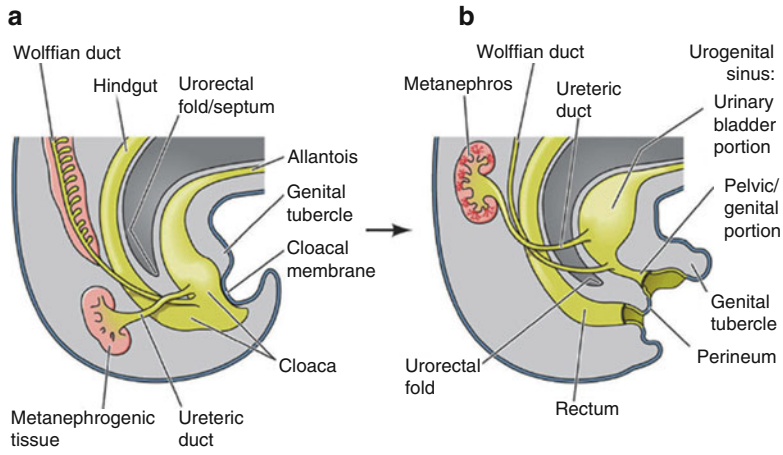

Fig. 1.2 Development of the urogenital sinus. (**a**) The cloaca and its anatomical location relative to other early embryonic urogenital tissues. (**b**) The primitive bladder forms from the anterior portion of the urogenital sinus. The posterior portion contributes to the formation of the urethra and external genital organs (Reproduced from Tasian et al. [331], Copyright 2010, with permission from Elsevier)

the vesico-allantoic canal or urachus (Fig. 1.2b). By week 12, the vesico-allantoic canal closes completely and remains as the median umbilical ligament in the fully developed fetus. Failure of this obliteration may result in urachal remnants which may present in the newborn infant as a vesico-umbilical fistula, vesico-urachal diverticulum, urachal sinus, and urachal cyst [317]. Once cloacal septation is completed by week 7, the urogenital sinus is further subdivided into upper and lower zones of endodermal cell differentiation [171]. Endodermal cells lining the upper zone of the urogenital sinus differentiate into bladder epithelium, while lower-zone urogenital sinus cells give rise to urethral epithelial cells. Morphogenetic and molecular processes involved in bladder development will be discussed in Sect. 1.5.

Failure to initiate or complete cloacal septation during fetal development has severe clinical consequences and is thought to underlie the pathogenesis of a wide range of urogenital and anorectal human malformations [170, 249]. Morphogenetic and molecular mechanisms underlying cloacal septation are poorly understood. Several processes have been implicated, including regional changes in mesenchymal cell proliferation along the cloacal periphery [306] which may be responsible for generating the tissue folds associated with septation [135, 229, 341]. Genetic studies in mice with gene disruptions affecting Sonic Hedgehog signaling have shown that this pathway is essential to the process of partitioning the cloaca into urogenital and anorectal sub-compartments [56, 116, 252]. Activating Sonic Hedgehog signaling seems to be necessary for stimulating cloacal mesenchyme cell proliferation as removing *Shh* function prior to cloacal septation associated with reduced levels of mesenchymal cell proliferation along the anterior and lateral cloacal margins, which resulted in anorectal and genitourinary defects [267, 306].

1.3 Embryology of the Kidney

1.3.1 Overview of Kidney Morphogenesis

The mammalian kidney develops as a result of reciprocal inductive interactions between the ureteric bud, which is an epithelial outgrowth of the caudal nephric duct, and the metanephric mesenchyme, which is represented by a region of undifferentiated mesodermal cells surrounding the caudal nephric duct. Cellular descendants of the ureteric bud contribute to formation of the renal collecting system, which includes both cortical and medullary collecting ducts, the epithelial lining of the renal calyces and pelvis, and also the urothelial lining of the ureter. Conversely, metanephric mesenchymal cells engage in a complex morphogenetic program which involves mesenchymal-to-epithelial transformation and cellular differentiation and results in formation of

all nephron segments, including the glomerulus, proximal and distal convoluted tubules, and loops of Henle. In the sections that follow, morphogenetic stages which result in formation of the collecting system and nephron will be described. In addition, the functions of genes and molecules known to control normal renal branching morphogenesis and nephron development will be discussed.

1.3.2 Ureteric Bud Outgrowth

Outgrowth of the ureteric bud (UB) from the nephric duct heralds the onset of kidney development. The UB forms in response to inductive signals provided by metanephric mesenchyme (MM). These signals induce the UB to elongate and extend towards MM. In humans, UB outgrowth occurs during week 5 of fetal gestation and in mice, at embryonic day 10.5 (E10.5) [261, 298]. Defects in ureteric bud induction are likely to underlie CAKUT phenotypes such as unilateral or bilateral renal aplasia which result from complete failure of UB outgrowth or in duplex kidney which occurs as a result of supernumerary UB formation [256].

The cellular basis of UB outgrowth was recently investigated in murine kidney organ culture using time-lapse imaging to follow the fate of nephric duct cells labeled with a fluorescent marker [60]. The first sign of UB formation is the appearance of an epithelial swelling on the dorsal surface of the posterior nephric duct. This morphologic event is associated with local increases in nephric duct epithelial cell proliferation and restructuring of the nephric duct luminal surface from a simple, single-layered epithelium to a pseudostratified epithelium. The significance of epithelial pseudostratification in this region prior to UB outgrowth is unknown. Similar restructuring of epithelial cell domains has been shown to occur in other developing organ systems where epithelial budding takes place, including liver, thyroid, and mammary gland [24, 93]. It is thought that converting the future budding site from a simple to pseudostratified epithelium might render a higher density of cells for rapid expansion within this region in order to form the UB, although this notion has not been confirmed experimentally [68].

In addition to local changes in cell number, changes in epithelial cell shape and movement are noted to precede regional swelling in the posterior nephric duct. Evidence in mice suggests that cellular rearrangements are essential for enriching the epithelial domain where budding will take place with cells that are capable of responding to inductive signals from surrounding metanephric mesenchyme [60].

1.3.3 Molecular Control of Ureteric Bud Outgrowth

Induction of UB outgrowth depends on molecular interactions between secreted peptides produced by MM cells with receptors on the surface of UB cells. These inductive interactions are under the control of transcriptional networks acting within MM or UB cells to coordinate gene expression and regulate the capacity of UB cells to respond to inductive cues within a precise spatiotemporal framework. This section summarizes the roles played by selected genes and molecular pathways in UB outgrowth as revealed through mutational analyses in mice and, where applicable, in humans.

1.3.3.1 Positive Control of UB Outgrowth

Glial-derived neurotrophic factor (GDNF) and two members of the fibroblast growth factor (FGF) family of signaling peptides, FGF7 and FGF10, are secreted growth factors which play important roles in UB outgrowth. In mouse kidney organ culture, recombinant GDNF, FGF7, and FGF10 proteins are each shown to be potent in vitro inducers of ureteric bud outgrowth [197, 268, 288]. At stages which precede UB outgrowth, endogenous GDNF and FGF10 are secreted by MM cells which surround the posterior nephric duct [197, 288]. These peptides exert their actions by binding and activating cell surface receptor tyrosine kinases (RTKs) expressed on the surface of posterior nephric duct and

nascent UB cells. GDNF binds an RTK encoded by the Ret proto-oncogene (*Ret*) in complex with the GDNF family receptor alpha 1 (*Gfrα1*) co-receptor [38, 82]. Fibroblast growth factor receptor 2 (*Fgfr2*) is the candidate RTK receptor for FGF10 given its expression within the nephric duct and UB [343, 379].

RTK activation initiates downstream signaling through a number of pathways including Ras-ERK/MAPK, phospholipase C gamma (PLCγ)/Ca++, and phosphoinositidyl-3-kinase (PI3K) [179]. Ret and Fgfr2 receptor activation results in the expression of *Etv4* and *Etv5* (Fig. 1.3), which are genes that encode two members of the E-26 (ETS) family of transcription factors. ETS proteins are involved in a wide variety of functions including the regulation of cellular differentiation, cell cycle control, cell migration, cell proliferation, apoptosis, and angiogenesis [237]. During kidney development, *Etv4* and *Etv5* function as genetically downstream of *Gdnf* and *Ret*. Their activities are necessary for regulating expression of other genes essential to UB outgrowth [179].

Complete loss of *Gdnf* or *Ret* function in vivo in mice leads to UB outgrowth failure and results in renal and ureter agenesis in 70–80 % of affected littermates [38, 253, 293, 303, 304]. Renal agenesis phenotypes were also generated in mice with homozygous null mutations in genes that encode transcription factors *Sall1*, *Eya1*, *Pax2*, and *Hox11* homeodomain proteins which are required for normal *Gdnf* expression [33, 34, 356, 366, 367] (Fig. 1.4, left panel). Table 1.4 provides a list of gene mutations which cause renal agenesis in mice. Conversely, mutations in genes that normally limit the domain of GDNF expression, such as *Slit2*, *Robo2*, and *Foxc2*, result in the formation of multiple UBs, leading to duplex ureters and kidneys [107, 166] (Fig. 1.4, right panel).

Additional control over UB outgrowth is provided by mechanisms which regulate *Ret* expression in nephric duct cell and nascent UB cells. During normal kidney development, *Ret* expression in the embryonic mouse nephric duct is positively regulated by *Pax2* and *Pax8*. In vitro, Pax2 and Pax8 proteins activate *Ret* transcription by binding directly to the *Ret* gene promoter [65].

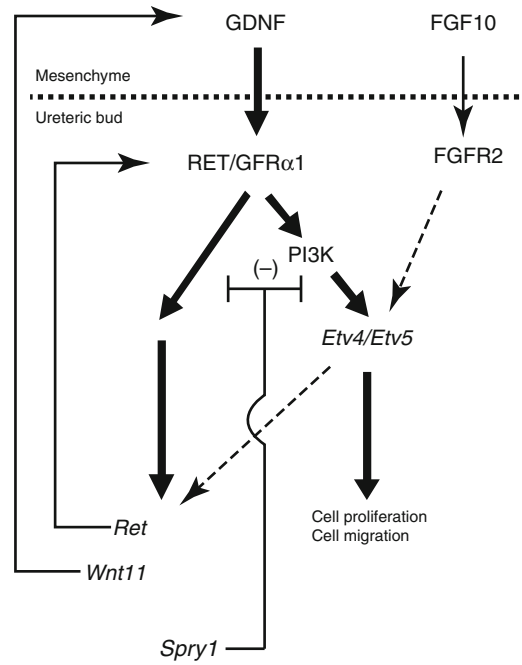

Fig. 1.3 Model of the role for *Etv4* and *Etv5* in a gene network controlling ureteric bud branching morphogenesis. GDNF secreted by metanephric mesenchyme signals to the ureteric bud through a RET/GFR**α**1 receptor complex to activate the expression of a number of genes, including *Etv4* and *Etv5*, which promote ureteric bud growth and branching. Positive effects on branching may be mediated through cell proliferation and cell migration. Expression of *Etv4* and Etv5 requires PI3K activity. A weaker contribution to the regulation of *Etv4/5* expression may be provided by FGF10, acting via FGFR2. Positive regulation of this network is provided by *Wnt11*, which lies downstream of GDNF-RET signaling. Negative regulation is provided by *Spry1* which antagonizes RET signaling post-ligand activation. *Bold arrows* depict strong effects. *Dashed arrows* denote weak or possible effects (Reproduced from Costantini [68], with permission)

The UB fails to form in mice with homozygous null *Pax2* mutations and in mice with compound homozygous null mutations in *Pax2* and *Pax8* due to severe defects in nephric duct formation [26]. However, genetic studies in heterozygous *Pax* mutants suggest that *Ret* expression in the nephric duct is highly sensitive to the level of *Pax2* gene activity. This was revealed in mice heterozygous for a null *Pax2* mutation, which demonstrate renal hypoplasia and lower levels of *Ret* expression compared with mice heterozygous for a null *Ret* mutation, which have normal

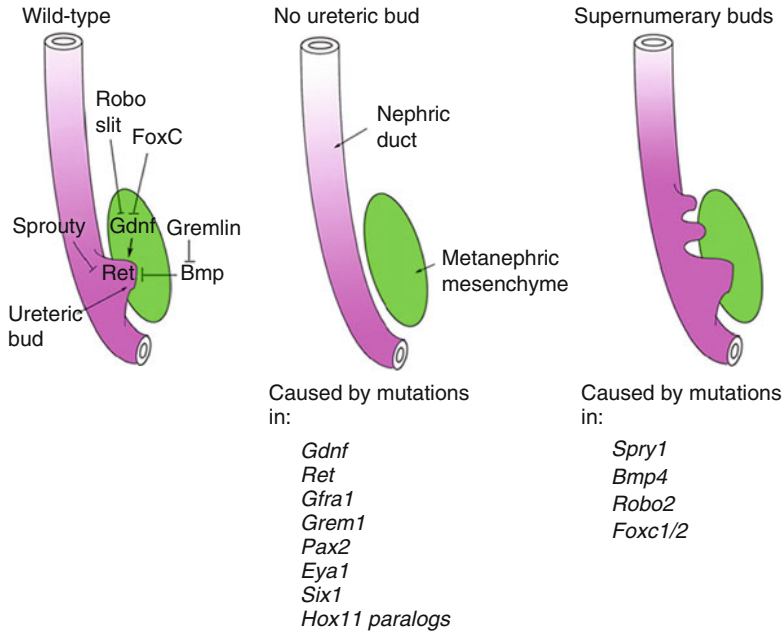

Fig. 1.4 Signals that promote or suppress ureteric bud outgrowth. *Left panel* highlights a subset of molecular interactions that control normal ureteric bud outgrowth by negatively regulating the stimulatory actions of Gdnf and Ret. *Pink area* represents the nephric duct. Green area denotes metanephric mesenchyme. *Middle* and *right panels* illustrate the effect on ureteric bud outgrowth in mice following genetic disruption. Below the images in the *middle* and *right panels* are listed a subset of gene mutations which result in failure to induce a ureteric bud (*middle panel*) or supernumerary budding (*right panel*). See Sect. 1.3.3.1–2 for details (Reproduced with modifications from Dressler [76], with permission)

kidneys [65]. A more severe phenotype was generated in compound mutant mice heterozygous for null mutations in both *Pax2* and *Ret*, which displayed a high frequency of unilateral or bilateral renal agenesis [65]. Collectively, these data suggest that one important function of *Pax2* in nephric duct morphogenesis is to establish the expression domain of *Ret*.

Gata-binding protein 3 (*Gata3*) encodes a zinc finger transcription factor with a role in maintaining *Ret* expression during nephric duct morphogenesis and UB outgrowth. Genetic studies in mice suggest that *Gata3* functions within a regulatory pathway that lies downstream of beta-catenin (encoded by *Ctnnb1*), a cytoplasmic protein with DNA-binding properties which functions as a transcription factor in the presence of Wnt-mediated signals [108]. At stages that precede UB outgrowth, *Gata3* is necessary to maintain *Ret* expression in the caudal nephric duct, which is essential for nephric duct extension towards the cloaca. Nephric ducts of mice lacking *Gata3* function are deficient for *Ret* and show severe nephric duct cell proliferation and differentiation defects [108, 109]. Some nephric duct cells located rostral to the presumptive budding zone maintain *Ret* expression, which leads to ectopic UB formation. This observation suggests that one role for *Gata3* may be to coordinate the recruitment of cells with high levels of Ret to the presumptive budding zone.

The mechanism by which GDNF-Ret signaling causes UB outgrowth is not entirely known. The effects of Ret receptor activation on bud formation may be in response to chemotactic effects of GDNF on nascent UB cells. This is revealed in two reports showing chemoattractant properties for GDNF in vitro on cultured kidney cells [328, 329]. Another study involving chimeric mice made up of a mosaic of cells with varying levels of *Ret* activity suggested that *Ret* is necessary to recruit highly sensitized nephric duct cells to the

Table 1.4 Mouse models of renal agenesis

Gene	Type of protein	Defects in humans	References
Involved in nephric duct formation			
Pax2 and Pax8	Transcription factor	Renal coloboma syndrome	[26, 295, 335]
Gata3	Transcription factor	HDR syndrome	[109, 342]
Lhx1	Transcription factor	–	[157, 247, 336]
Involved in ureter budding			
GDNF-Ret/GFRα1 pathway components			
Gdnf	Secreted molecule, neurotrophin family	Hirschsprung disease	[8, 210, 253, 293]
Ret	Receptor tyrosine kinase	Hirschsprung disease	[83, 303]
GFRα1	GPI-linked neurotrophin receptor	Hirschsprung disease	[38, 88]
Required for GDNF expression			
Eya1	Transcription factor	Branchio-oto-renal	[1, 366]
Hox11 paralogs	Transcription factor	–	[356]
Sall1	Transcription factor	Townes-Brocks syndrome	[160, 232]
Six2	Transcription factor	–	[367]
Pax2	Transcription factor	Renal coloboma syndrome	[34]
Osr1	Transcription factor	–	[141]
Required for Ret expression			
Gata3	Transcription factor	HDR syndrome	[108, 109, 342]
Ctnnb1 (β-catenin)	Transcriptional co-activator, Wnt pathway	–	[188]
Emx2	Transcription factor	–	[204]
Involved in metanephric mesenchyme induction			
WT1	Transcription factor	Denys-Drash	[165]

Reproduced with modifications from Uetani and Bouchard [340], with permission
HDR hypoparathyroidism-deafness-renal anomalies syndrome

site of ureteric budding so that they can readily respond to GDNF [60] (Fig. 1.5).

The role of FGF proteins on UB induction is less clear. In vitro, recombinant FGF7 and FGF10 proteins were capable of inducing UB outgrowth in isolated nephric duct organ cultures [185]. The stimulatory effects of FGF proteins on UB outgrowth were augmented by co-incubating cultures with an inhibitor of TGF-β signaling, suggesting that the nephric duct is normally desensitized to FGFs by local inhibitory feedback. Since *Fgf7* mRNA are not expressed in MM at stages preceding UB outgrowth, *Fgf10* is the more likely candidate for activating FGFR-mediated inductive responses in vivo. Targeted deletion of *Fgf10* results, however, in mice which form UBs but display later defects in UB branching [60]. A similar phenotype was generated in mice with inactivating mutations in *Fgfr2* conditionally limited to the UB cell lineage, which are able to form a UB but show defects in UB branching that lead to renal hypoplasia [316, 379], suggesting that other mechanisms (viz., GDNF-RET signaling) are able to compensate for loss of FGF signaling in these mutants. A recent study showed that complete penetrance of UB agenesis was generated in $Fgf10^{-/-}$ mutants by introducing a single null allele for GDNF [60]. Taken together, these studies strongly suggest that GDNF is the dominant inducer of UB outgrowth whereas FGF10 has a weaker role, possibly due to local variances in gene expression or inhibitory feedback on RTK signaling [60].

1.3.3.2 Negative Control of UB Outgrowth

Experimentally, the nephric duct is competent to respond to ectopic GDNF via RET and initiate ureteric bud formation and branching at multiple

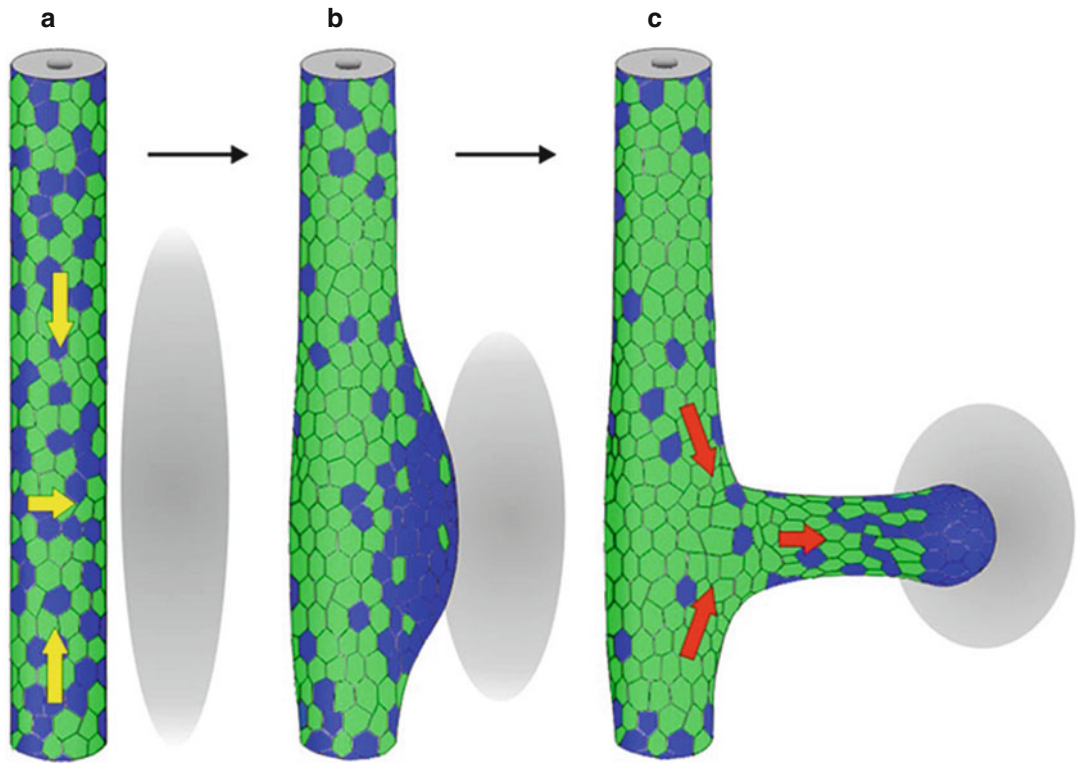

Fig. 1.5 Model for the rearrangement of nephric duct cells at different stages of ureteric bud formation. (**a**) Pre-ureteric bud induction. (**b**) Onset of ureteric bud induction. (**c**) Ureteric bud outgrowth and early elongation. *Blue* and *green areas* represent nephric duct cells with high and low relative levels of RET activity, respectively. *Shaded regions* denote nephrogenic mesenchyme. *Yellow arrows* in (**a**) illustrate the convergence of cells with high levels of RET activity along the long axis of the posterior nephric duct. *Red arrows* in (**c**) demonstrate the recruitment of cells with high levels of Ret signaling to the ureteric bud tip (Reproduced from Chi et al. [60], Copyright 2009, with permission from Elsevier)

sites along its anteroposterior axis [250, 288]. This propensity for random budding may underlie the cause of renal malformations that result when either more than one UB is formed (e.g., duplex kidney) or when induction occurs in the wrong position along the nephric duct's anteroposterior axis (e.g., VU reflux). Consequently, a number of inhibitory mechanisms are used to control the stimulatory response of nephric duct cells to inductive signaling and ensure that UB outgrowth is limited to a single site.

Sprouty proteins provide inhibitory control over signaling pathways activated by GDNF and FGF. Sprouty proteins are intracellular peptides which are known to antagonize RTK signaling downstream of ligand-receptor interactions [84]. In the developing kidney, Sprouty1 (*Spry1*) expression is induced downstream of GDNF-RET signaling in nephric duct and nascent UB cells. Sprouty2 (*Spry2*) and Sprouty4 (*Spry4*) are additional family members expressed in UB cells following outgrowth [377], which suggests that Sprouty proteins also exert negative feedback inhibition at later stages of UB morphogenesis.

At the UB outgrowth stage, *Spry1* plays a role in repressing the nephric duct's response to GDNF and FGF10 and controlling their stimulatory effects on the nephric duct [68]. This inhibitory role was revealed genetically in a series of two reports involving complex analyses of mouse mutants with single or combined loss-of-function mutations in *Spry1*, *Gdnf*, and *Fgf10*. Loss of *Spry1* function in mice resulted in renal malformations including multiple ureters, multiplex

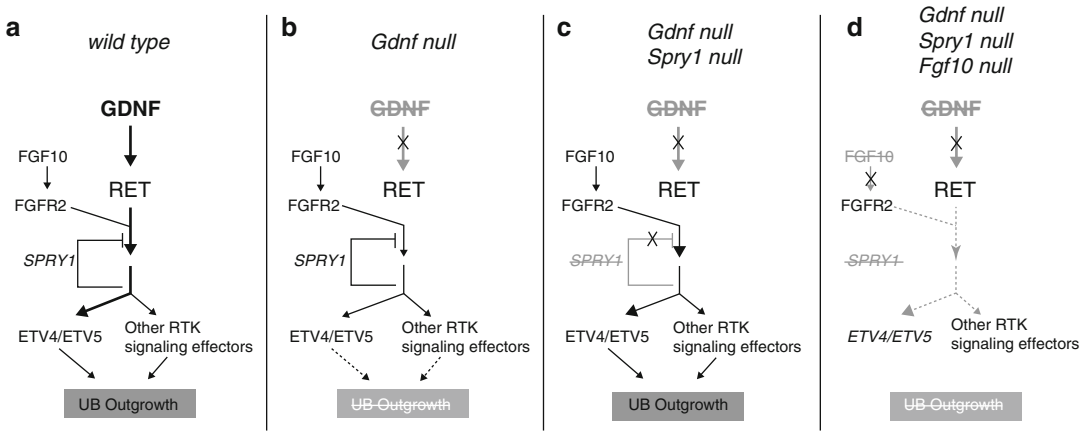

Fig. 1.6 Summary of results from genetic studies in mice supporting a model for *Gdnf* and *Fgf10* cooperating in the induction of ureteric bud outgrowth under the inhibitory control of *Spry1*. (**a**) Wild-type scenario. GDNF acts as the principal inducer (depicted by *bold arrows*) by activating RET, resulting in the upregulation of genes which stimulate ureteric bud outgrowth. FGF10 provides a substantially weaker effect via FGFR2. *Spry1* exerts inhibitory control by antagonizing RTK signaling. (**b**) When *Gdnf* is genetically inactivated, FGF10 is incapable of overcoming the inhibitory effects of *Spry1* on FGFR2 signaling, resulting in failed ureteric bud outgrowth. (**c**) The stimulatory effects of FGF10 on ureteric bud outgrowth are unmasked in mice lacking *Gdnf* by additionally removing *Spry1*, thereby rescuing ureteric bud outgrowth failure in *Gdnf* null mice. (**d**) Confirmation that FGF10 is providing the inductive cue in *Gdnf*/*Spry1* double null mice is provided by additionally removing Fgf10 function, which prevents formation of a ureteric bud (Reproduced with modifications from Michos et al. [197])

kidneys, and hydroureter [14, 15]. These features were associated with ectopic ureteric bud induction, increased expression of *Gdnf* in the metanephric mesenchyme, and expanded expression of GDNF-RET target genes (viz., *Wnt11*). The presence of multiple ureters and the occurrence of renal and ureteral duplications in *Spry1* $^{-/-}$ mice represented a loss of negative control over the stimulatory actions of GDNF. Reducing the *Gdnf* gene dosage in *Spry1* $^{-/-}$ mutant mice by 50 % was sufficient to restore control over UB outgrowth and eliminate the formation of supernumerary buds [14], indicating that inhibitory molecular mechanisms which control GDNF-RET signaling activity are critical to the formation of a normal, single ureteric bud. In another study, the effect of inactivating *Spry1* in mice with complete loss of *Gdnf* function (*Gdnf* $^{-/-}$ mice) was examined (Fig. 1.6). Removing *Spry1* function in mice lacking *Gdnf* was sufficient to rescue renal agenesis and result in generation of a single ureteric bud (Fig. 1.6), likely because the nephric duct now became sensitized to the inductive effects of *Fgf10* [197]. Additionally removing *Fgf10* in *Spry1* $^{-/-}$, *Gdnf* $^{-/-}$ compound mutant mice resulted in renal agenesis [197], which served as evidence that *Fgf10* was capable of fulfilling the role of inducer in the absence of Gdnf only when *Spry1*-mediated inhibition was lifted. A number of studies suggest that angiotensin II signaling may be involved in regulating the levels of *Spry1* mRNA expression in the developing UB, although the local response to angiotensin II in UB cells appears to be dependent on which angiotensin receptor, AT1R or AT2R, is activated [372, 373]. These data support the existence of negative feedback mechanism involving *Spry1* and activation of angiotensin signaling which controls UB outgrowth by regulating which nephric duct cells will respond to GDNF- and FGF10-mediated signals.

Bone morphogenetic protein-4 (*Bmp4*) also negatively regulates UB outgrowth and is locally suppressed by Gremlin 1 (*Grem1*), allowing budding to occur in the correct position. *Bmp4* belongs to a family of genes that encode signaling peptides secreted by MM and UB cells in the embryonic mouse kidney and shown to regulate various stages of kidney development [39]. Kidney organ culture studies have revealed

inhibitory roles for BMP4 and other BMP family members including BMP2 and BMP7 in ureteric bud branching morphogenesis [42, 255, 259, 274]. At stages prior to UB induction, *Bmp4* mRNA are expressed broadly in mesenchymal cells surrounding the nephric duct [29, 80, 199]. The expression domain for *Bmp4* partially overlaps with the expression of *Grem1*, which encodes an extracellular antagonist that sequesters secreted BMPs and reduces local BMP signaling activity [199]. The role of *Bmp4* as a negative regulator of UB outgrowth is suggested by the appearance of ureteral duplication and severe hydronephrosis in mutant mice heterozygous for a null *Bmp4* allele [206]. In contrast, *Grem1-deficient* mouse embryos display renal agenesis, presumably due to unopposed inhibitory actions of BMP4 on the nephric duct [199]. At the molecular level, *Grem1* is necessary to maintain and propagate the expression of *Wnt11* in the UB tips, which promotes *Gdnf* expression in MM via a positive feedback mechanism. This infers that BMP4 functions within an inhibitory feedback mechanism that suppresses UB induction by antagonizing the local effect of GDNF-RET signaling on the nephric duct [206].

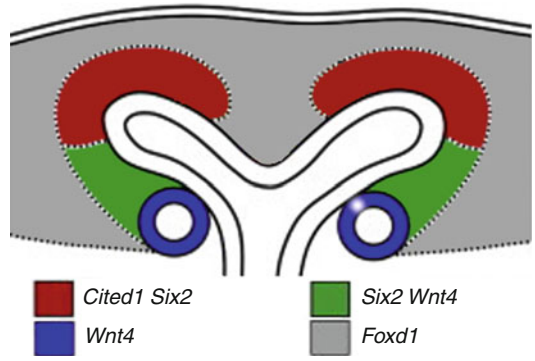

Fig. 1.7 *Six2*, *Cited1*, *Wnt4*, and *Foxd1* gene expression domains in condensed mesenchyme. Depicted is a graphic representation of condensed mesenchyme at the tips of two ureteric bud branches, signifying that metanephric mesenchyme induction has occurred. Cells with high levels of *Six2* and *Cited1* expression (*red area*) represent a subpopulation of condensed mesenchyme cells that are self-renewing and undifferentiated. These cells give rise to a second cell subpopulation within condensed mesenchyme (*green area*) which feature upregulation of *Wnt4* and downregulation of *Cited1*. These cells represent progenitor cells that are fated to undergo cell differentiation and generate all specialized epithelial cells of the mature nephron. Downregulation of *Six2* in cells which express *Wnt4* (*blue area*) signifies the transition from a mesenchymal to an epithelial cell phenotype. Surrounding mesenchymal condensates and epithelial progenitors are stromal cells which require *Foxd1* expression and are necessary for normal nephron development (Reproduced with modifications from Mugford et al. [214], Copyright 2009, with permission from Elsevier)

1.3.4 Induction of Metanephric Mesenchyme

Prior to UB induction (E9.5 in mice), MM is characterized by a population of undifferentiated, non-polarized cells which are dispersed loosely along the ventral caudal nephric duct and marked by the expression of *Osr1* [76]. At this stage of development, *Osr1*-positive (*Osr1* $^{+}$) cells in mice represent a multipotent cell population which are the precursors for epithelial, stromal, and vascular tissue elements in the mature kidney [27]. With advancing gestational age, *Osr1* $^{+}$ cells become progressively restricted in their fate as progenitors of nephron epithelial cells [213] and are transformed into a tightly associated, polarized cell population that forms primitive tubules. Further differentiation of epithelial cell types within these primitive tubules occurs in a spatially organized proximal-distal pattern, resulting in formation of the glomerular and tubular segments of the mature nephron.

Invasion of MM by the UB marks a critical stage in the divergence of mesenchymal cell fate. Upon contact with the UB, the MM condenses to form a discrete zone 4–5 cells thick around the ampulla of the advancing UB [12, 155]. The appearance of condensed mesenchyme (CM) around the tip of the UB signifies that MM induction has occurred. Based on patterns of gene expression, CM is subdivided into two cell populations. One population is comprised of undifferentiated rapidly proliferating cells which are marked by upregulated expression of transcriptional regulators *Six2* and Cbp/p300-interacting transactivator 1 (*Cited1*) (Fig. 1.7) and are thought to represent a pool of self-renewing multipotent epithelial progenitors [28, 158]. The

second group is a subpopulation of the first and represents cells which have embarked on the pathway towards epithelial differentiation along an epithelial lineage and are marked by the upregulated expression of the secreted peptide wingless-type MMTV integration site family, member 4 (*Wnt4*) and downregulated expression of *Cited1* [214] (Fig. 1.7). A third population of MM cells is marked by the expression of the forkhead box D1 transcription factor *Foxd1* (Fig. 1.7), which surrounds cap condensates and is thought to give rise to interstitial or stromal cells [122, 214, 308]. Stromal cells secrete extracellular matrix and growth factors and are thought to provide a supportive framework around the developing nephrons and collecting system. Genetic fate mapping studies in mice suggest a common origin for nephron epithelial and stromal lineages which diverges just before UB induction [28, 158, 213]. However, conflicting reports on stromal lineage have shown that some stromal cells originate in paraxial mesoderm [110] or migrate into the developing kidney as neural crest precursors once UB has invaded MM [287, 297].

1.3.5 Control Mechanisms Involved in Mesenchymal Induction

Metanephric mesenchyme condensation occurs in response to a Wnt signal, likely Wnt9b, secreted by the UB. The kidneys of mouse embryos lacking *Wnt9b* are rudimentary and devoid of nephrons [48]. The renal defect is characterized by developmental arrest at the stage where the UB invades metanephric mesenchyme and branches to form a T-shaped structure. Consequently, loss of *Wnt9b* prevents mesenchymal condensation from occurring and blocks initiation of tubulogenesis.

Wnt9b induces the expression of *Wnt4* in cap mesenchyme, which initiates the program that converts mesenchyme to epithelial cells [48]. This interaction appears to be mediated by canonical signaling pathways involving beta-catenin. In one study, the inductive functions of *Wnt9b* were prevented by removing beta-catenin in condensed mesenchyme cells of embryonic mice by conditional mutagenesis [244]. In the same study, activating beta-catenin through overexpression of a constitutively active, stabilized form in MM functionally rescued the inductive defects in *Wnt9b* and *Wnt4* mutants and was capable of initiating the tubulogenic program for epithelial cell differentiation. These data strongly implicate canonical signaling pathways in carrying out the initial functions of *Wnt9b* protein during MM induction and nephron epithelial cell differentiation. However, there appears to be a need to attenuate canonical Wnt signaling once induction has taken place since normal epithelial tubules do not form in embryonic mouse kidneys when activated beta-catenin continues to be overproduced in MM [244]. Consequently, the tubulogenic response to Wnt signaling following MM induction is likely to involve noncanonical signaling mechanisms.

Six2, *Sall1*, and *Wt1* are three transcription factor-encoding genes which are implicated in maintaining a sufficient number of self-renewing nephron progenitor cells to generate all the nephrons that ultimately comprise the mature kidney. *Six2* is a homeodomain transcriptional regulator [158, 308], *Sall1* encodes for a mammalian spalt-like homeotic gene [160, 232], and *Wt1* encodes a zinc finger transcription factor with both a DNA- and RNA-binding properties [348]. Dominant mutations in *SALL1* are the cause of Townes-Brock syndrome (OMIM: 107480), which features renal hypoplasia, dysplasia, or vesicoureteral reflux in addition to anal and limb anomalies [160, 292]. Denys-Drash syndrome (OMIM: 194080) is an autosomal dominant disorder caused by inactivating mutations for *WT1* and characterized by gonadal dysgenesis, diffuse mesangial sclerosis, and increased risk of Wilms tumor [67, 177].

During embryonic mouse kidney development, *Six2*, *Sall1*, and *Wt1* are expressed at low levels in uninduced mesenchyme and upregulated in condensed mesenchyme [158, 160, 232, 308, 370]. *Six2* plays a major role in maintaining the multipotency of nephron progenitors, while *Sall1* and *Wt1* appear to have a more important role in progenitor cell survival. The kidneys of

mice homozygous for *Six2* loss-of-function mutations are small and malformed and exhibit ectopic nephron formation along the tips and stalks of ureteric bud branches [308]. The nephrogenic defect in *Six2* mutant mice is attributed to premature epithelial cell differentiation, presumably in response to uncontrolled *Wnt9b* activity, which exhausts the progenitor pool and results in fewer nephrons. In contrast, in the absence of *Sall1* and *Wt1*, MM undergoes apoptosis resulting in unilateral or bilateral renal agenesis in two-thirds of *Sall1* knockout mice [232] and bilateral renal agenesis in 100 % of mice homozygous for inactivating *Wt1* mutations [165].

The functional properties of *Sall1* in kidney development were assessed in an in vitro colony-forming assay. When cultured in the presence of an inducing signal (viz., Wnt4), isolated MM from wild-type mice formed colonies which were reconstituted in organ culture into kidney-like tissues with glomerular and tubular components [239]. Molecular studies on these cultured rudiments revealed expression of epithelial cell markers for glomeruli, proximal and distal tubules, and loops of Henle. In contrast, although MM cells from *Sall1*-deficient mice were able to form colonies and induce the expression of tubulogenic genes, colony size was significantly smaller, suggesting that the dominant role of *Sall1* is to promote progenitor cell survival. Additional support for a survival role was provided by the analysis of MM induction in organ culture, which showed that isolated mesenchyme from *Sall1* mutant mice retained their competence to respond to an inducer but resulted in the formation of smaller tubules [232]. Other genes involved in promoting mesenchymal survival include bone morphogenetic protein 7 (*Bmp7*) [79, 104], *Pax2* [333], and *Fgfr2* [260].

Wt1 is required for MM survival and appears to be necessary for induced mesenchyme to condense around the UB following induction. Evidence in favor of these roles is suggested by the analysis of MM induction when *Wt1* function is removed either by genetic deletion in vivo [165] or by morpholino knockdown in embryonic kidney organ culture [120]. When cultured ex vivo, isolated mesenchyme from *Wt1*-deficient mice failed to aggregate in response to inducing signals from wild-type UB and subsequently underwent apoptosis [73]. A similar result was achieved by co-culturing wild-type kidney explants in the presence of WT1 morpholinos, which modify gene function by preventing translation. Instead of forming a tightly packed cap around the UB, MM was loosely arranged around UB tips in morpholino-treated explants. It was suggested that this finding may be due to defects in cytoskeletal rearrangements or in the formation of focal adhesion complexes on the basis of transcriptional profiling of *Wt1* targets in normal embryonic mouse kidney tissue [120]. Additional targets of *Wt1* which were revealed by this analysis included several MM survival genes with promoters that bind WT1 protein, including *Pax2*, *Sall1*, *Bmp7*, and *Fgfr2* [120]. Thus, one function of *Wt1* may be to regulate cellular events such as cell survival and cell-cell adhesion in a concerted manner which promotes nephron cell fate.

1.3.6 Development of the Collecting System

1.3.6.1 Overview of Collecting Duct Morphogenesis

The collecting duct system refers to the cortical and medullary collecting ducts, the renal calyces, and the renal pelvis of the mature kidney [261, 298]. Development of the collecting duct system involves an embryonic process termed renal branching morphogenesis, which refers to growth and branching of the UB [130]. This process is dependent on reciprocal inductive interactions between MM and UB cells within the embryonic kidney. As a developmental process, branching morphogenesis is essential to the formation of several tissues including kidney, lung, mammary tissue, exocrine pancreas, and salivary glands (reviewed in Hu and Rosenblum [130]). In kidney development, renal branching morphogenesis may be considered as a sequence of related events, which include (1) outgrowth of the ureteric bud, (2) iterative branching of the ureteric bud and derivation of its daughter collecting ducts, (3) patterning of the cortical and medullary

collecting duct system, and (4) formation of the pelvicalyceal system.

In human kidney development, renal branching morphogenesis commences between the fifth and sixth week of fetal gestation [261]. In mice, this process is initiated at E11.5 when the ureteric bud invades the metanephric mesenchyme and forms a T-shaped, branched structure [298]. This T-shaped structure subsequently undergoes further iterative branching to generate approximately 8 branch generations in the embryonic mouse kidney [51, 298] and 15 generations of branching in the human fetal kidney [261]. In humans, the first 9 generations of ureteric bud branching are completed by approximately the 15th week of fetal gestation [261]. Throughout this time, new nephrons are induced at the newly formed tips of ureteric bud branches through reciprocal inductive interactions with surrounding metanephric mesenchyme. By the 20th–22nd week, ureteric bud branching is completed, and the remainder of collecting duct development occurs by extension of peripheral (or cortical) segments and remodeling of central (or medullary) segments [261]. During these final stages of UB morphogenesis, new nephrons continue to form predominantly through the induction of approximately four to seven nephrons around the tips of terminal collecting duct branches which have completed their branching program while retaining the capacity to induce formation of multiple new nephrons [261, 298].

1.3.6.2 Cellular Events Involved in UB Branching Morphogenesis

Throughout normal kidney development, the branching UB recapitulates a sequence of morphogenetic events. This sequence is characterized by (1) expansion of the advancing ureteric bud branch at its leading tip (called the ampulla); (2) remodeling of the ampulla, leading to the formation of new UB branches; and (3) growth and elongation of the newly formed branch segment (referred to as the branch "stalk" or "trunk").

Insight into cellular events that take place in UB morphogenesis has been provided by studies of embryonic mouse kidney development in organ culture using time-lapse imaging to follow the fate of UB cells labeled with a visible marker (e.g., enhanced green fluorescent protein; EGFP) in the developing UB as it grows and branches [174, 310, 351]. From these studies, it is evident that cells at the UB tips undergo a burst of cell proliferation which causes regional expansion two to three times the diameter of the parental trunk, leading to ampulla formation [310, 351] (Fig. 1.8a–c). Once the ampulla forms, there is significant cell movement within the ampulla resulting in asymmetric redistribution of highly proliferating cells to the very tips of the UB branch and cells with lower rates of proliferation closer to the branch trunk [310]. Simultaneously, branch formation occurs by an as-yet uncharacterized morphogenetic process of remodeling which causes the ampulla to bifurcate (Fig. 1.8d–f). Studies show that rapidly dividing cells remain at the leading edge of the newly formed branch as they advance forward, whereas cells with lower rates of proliferation remain behind and are incorporated into the trunk [310] (Fig. 1.8g). Based on gene expression patterns, tip cells are viewed as progenitor cells for the formation of new branches throughout kidney development since these cells tend to express genes associated with growth and cell proliferation [49, 179, 300]. For the most part, trunk cells are represented by gene expression patterns associated with the acquisition of specialized functions such as acid-base homeostasis and water balance [22]. Some trunk cells, however, retain the potential to generate new branches, as suggested by patterns of lateral branching (described below).

The majority of UB bifurcations are symmetrical wherein the ampulla flattens and extends in two opposite directions. Asymmetrical patterns of branching have been demonstrated in embryonic mouse kidney organ culture (e.g., trifid branching, which occurs when three daughter branches arising from one ampulla, and lateral branching, which results from de novo branch formation arising from a UB truncal segment) [351]. Morphometric analyses of individual branch growth parameters have revealed a conserved hierarchical pattern of diminishing final length for successive UB branch generations such that sixth-generation branches are shorter

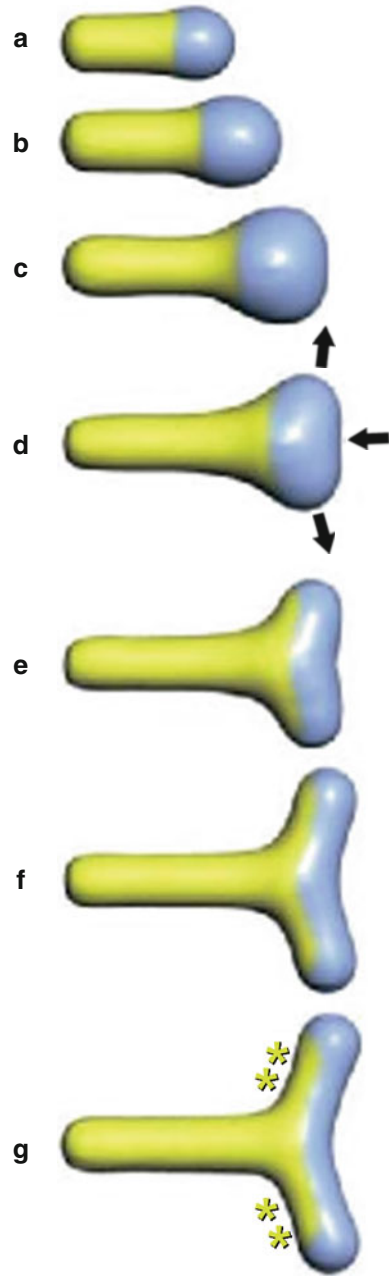

Fig. 1.8 A model for the fate of tip and trunk cells during ureteric bud elongation and branching. (**a**) Shown is a ureteric bud branch segment. The *yellow area* denotes cells residing within the branch trunk. The *blue area* represents cells within the ureteric bud branch tip. At subsequent stages of growth and branching (**b**)–(**g**), *yellow* and *blue regions* denote cells which are derived from the original primary branch trunk and tip segments. (**b**) and (**c**) The trunk elongates through an internal mechanism. Tip cells (*blue*) proliferate under the influence of GDNF/Ret signaling to form the enlarged ampulla. (**d**) The ampulla and the adjacent trunk epithelium are remodeled by an as-yet uncharacterized mechanism which leads to branch bifurcation (**e**). (**f**) and (**g**) Elongation of the newly formed branch generates a new, secondary branch segment with its own trunk and tip zones. Cells which originated as primary branch trunk cells (*yellow*) occupy the proximal sides (adjacent to the *asterisks*) of the two new secondary branch trunks. Cells from the primary branch ampulla (*blue*) form the two new tips and the distal epithelium of the secondary branch trunks (Reproduced from Shakya et al. [310], Copyright 205, with permission from Elsevier)

than fifth-generation branches, etc. [351]. However, in kidney organ culture, asymmetric branching has been described such that branches forming in the posterior region of kidney explants elongate at a slower growth rate compared with branches formed in anterior regions [42]. Presumably, mechanisms that promote asymmetrical branch growth may be important to achieving the final nonspherical shape of the fully formed, mature kidney [51, 351].

1.3.6.3 Molecular Mechanisms that Stimulatory Renal Branching Morphogenesis

GDNF-RET signaling is shown in vivo and in vitro to be a major stimulus for subsequent ureteric bud branching [250, 309]. The ability of *Gdnf* to stimulate UB branching in vivo is revealed by the demonstration of multiple branched ureteric buds in transgenic mice which express *Gdnf* ectopically along the full length of the nephric duct [309]. In response to RET receptor activation, UB tip cells secrete Wnt11 (Fig. 1.9), which acts on MM cells to maintain *Gdnf* expression and thereby promote branching through positive feedback [186]. Signaling responses to GDNF are negatively regulated by Sprouty genes as demonstrated in organ culture experiments with embryonic kidney explants from *Spry1* mutant mice, which show increased sensitivity to GNDF [14]. In addition, loss of *Spry1* function was also associated with increased MM expression of *Wnt11* and *Gdnf* which resulted in an increased number and diameter of UB branches [60, 152, 250], illustrating that negative feedback mechanisms are an important determinant of normal UB branch patterning.

The functional role of GDNF-RET signaling in branching morphogenesis is not well understood. Recombinant GDNF has been shown to be a potent stimulant of UB cell proliferation in organ culture studies [250], leading to the assumption that *Gdnf* stimulates UB branching by promoting UB cell proliferation within the ampulla. It is unlikely, however, that stimulating cell proliferation alone is sufficient to induce

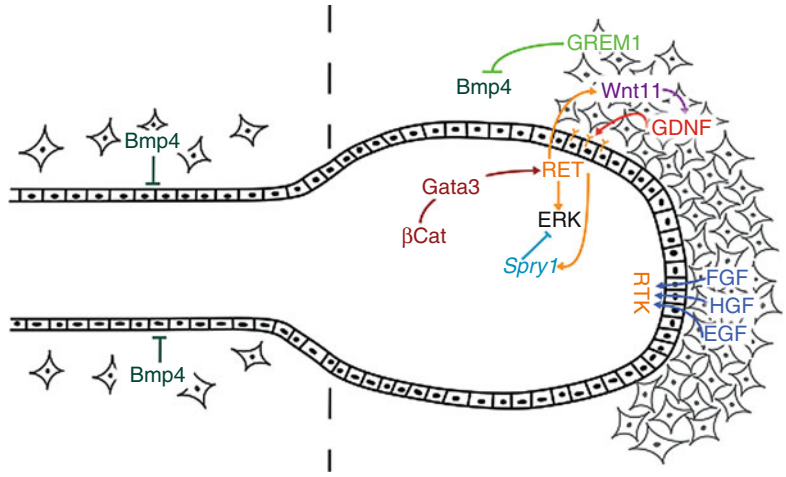

Fig. 1.9 Molecular interactions between stimulatory and inhibitory signals in control of ureteric bud branching morphogenesis. Shown is a sketch of a ureteric bud branch tip surrounded by loose mesenchyme around its truncal segment (*left*) and condensed mesenchyme at the leading edge of the ampulla (*right*). GDNF-RET signaling activity is localized to the branch tip. Wnt11, a molecular target of GDNF-RET, is secreted by branch tip cells and acts on surrounding metanephric mesenchyme to maintain *Gdnf* expression. RET activation in tip cells also upregulates expression of *Spry1*, which feeds back negatively on RET to modulate GDNF-RET signaling activity within the ampulla. Other RTK signaling pathways such as FGF, EGF, and HGF provide additional stimulatory control over branching. BMP4, expressed in mesenchymal cells surrounding the branch trunk exert an inhibitory effect on branching. Gremlin, expressed in mesenchyme around the branch tip, antagonizes BMP4 and facilitates ureteric bud branching by negating local inhibitory effects of BMP4 (Reproduced from Michos [196], Copyright 2009, with permission from Elsevier)

branching since mitotic cells are found in all regions of the ampulla [195]. It is possible that activating RET is necessary to induce cell movements in the ampulla that promote branching [310], much in the way that RET is essential for cellular rearrangements that drive UB outgrowth [60]. The availability of high-resolution imaging systems combined with transgenic technologies that enable tracking the future destination of UB cells will likely provide the means to better understand the mechanistic basis of GDNF-RET signaling on branching.

In vitro studies show that recombinant GDNF alone is not sufficient to induce robust branching in isolated ureteric bud culture [269, 288], suggesting that parallel signaling mechanisms are also involved in controlling ureteric bud branching. Several in vivo and in vitro reports suggest that FGF signaling has direct effects on the UB branching. Following UB outgrowth, several FGFs and their RTK receptor counterparts are expressed in the developing mouse kidney, including ligand-encoding genes *Fgf2*, *Fgf7*, *Fgf8*, and *Fgf10*, as well as receptor genes *Fgfr1* and *Fgfr2*, isoform IIIb (*Fgfr2IIIb*) [43, 268, 270]. All members of the FGF family of ligands are capable of exerting effects on UB growth and cell proliferation as demonstrated in organ culture experiments when isolated UB are exposed to recombinant FGF proteins [268]. These in vitro observations are consistent with in vivo reports describing decreased ureteric bud branching, decreased cell proliferation, and increased ureteric bud apoptosis in Fgf7 and Fgf10 knockout mice and in mouse mutants following conditional deletion of the *Fgfr2* in the ureteric bud lineage [260].

Different FGF family members exert unique spatial effects on ureteric bud cell proliferation in vitro. For example, FGF10 preferentially stimulates cell proliferation at the ureteric bud tips, whereas FGF7 induces cell proliferation in a nonselective manner throughout the developing collecting duct system [268]. These data suggest that multiple FGF family members may function in complex morphogenetic programs that control three-dimensional growth of the developing collecting duct system. FGF7 is also shown to induce the expression of the Sprouty gene, *Spry2*, in developing collecting ducts in vitro [59]. Consequently, FGF7 may participate in a negative feedback loop that controls UB branching by regulating *Gdnf* and *Wnt11* expression.

Two additional RTK ligands hepatocyte growth factor (HGF) and epidermal growth factor (EGF) represent two additional RTK ligands which are expressed in the developing kidney and have been shown to act synergistically in promoting UB branching during mouse kidney development [138, 290, 363]. HGF and EGF proteins bind their respective RTK, MET and EGFR, and activate downstream signaling through PI3K, ERK, and a number of other RTK-mediated pathways [149]. HGF has been shown to promote UB branching in kidney organ culture [45, 209], yet inactivating *Met* function exclusively in the UB lineage using a conditional knockout approach resulted in only a modest decrease in UBB and a 30 % reduction in nephron number [138]. Since *Egfr* is upregulated in Met-deficient UB cells and recombinant EGF can partially rescue UB branching in these Met mutant kidneys, it has been suggested that HGF/MET and EGF/EGFR signaling play complementary roles in UB morphogenesis [138]. Complementary functions for *Met* and *Egfr* were revealed in vivo by combining UB-specific deletion of *Met* with a hypomorphic *Egfr* mutation, which resulted in embryos with small kidneys and severely reduced UB branching [138]. The interaction between HGF/MET and EGF/EGFR serves as an example for molecular cross talk between other RTK signaling mechanisms, including pathways activated by FGF-FGFR interactions, that may act cooperatively with GDNF/RET to control UB branching morphogenesis (Fig. 1.9).

1.3.6.4 Inhibitory Pathways Involved in Control of Renal Branching Morphogenesis

Negative regulators of UB branching include activin A and TGFβ1 [37, 64, 259, 279, 291], semaphorins 3a and 4D (Sema3a and Sema4D) [163, 338], and several BMP family members (reviewed in Cain et al. [39]) based on evidence of their abilities to inhibit branch formation in

UB organ or cell culture models or in mouse genetic mutants which cause downstream signaling to be blocked or attenuated in UB cells.

Bone morphogenetic proteins represent a large group of signaling peptides which share a conserved signaling mechanism. BMP signaling involves ligand binding to receptor complexes that include serine threonine kinase receptors called activin-like kinases (ALKs) and downstream signaling through receptor-activated SMAD proteins, which become phosphorylated upon ligand-receptor binding and translocate to the nucleus to control transcription of several target genes involved in cell adhesion and differentiation [39]. Modulation of this pathway may be provided by interactions between BMP-ALK receptor complexes with extracellular molecules which may either augment signaling (e.g., glypican-3 heparan sulfate proteoglycans; *Gpc3*) [44, 121, 140] or attenuate signaling (e.g., gremlin; *Grem1*) [199] depending on context.

BMP ligands *Bmp-2*, *-4*, *-5*, *-6*, and *-7* and Alk receptors Alk-3 and -6 are expressed in the developing kidney in distinct but partially overlapping domains [72, 80, 103, 345]. Genetic studies suggest that *Alk3* is the dominant signaling receptor in the UB since inactivating *Alk3* specifically in the UB cell lineage by conditional mutagenesis results in medullary hypoplasia and cortical cysts [119] while the embryonic kidneys of *Alk6-deficient* mice are normal [371]. Kidney organ culture studies have revealed inhibitory roles for BMP-2, -4, and -7 in ureteric bud branching morphogenesis ex vivo [42, 255, 259, 274]. The kidneys of *Bmp7*-deficient mouse embryos are small which is associated with decreased UB branching and nephron formation [79, 104]. The renal phenotype in this mutant is attributed, however, to a cell survival defect within the mesenchymal compartment causing the loss of mesenchyme-derived factors that promote UB branching rather than a direct effect on UB cells.

Embryonic mice that carry germline null mutations in *Bmp2* or *Bmp4* die before kidney development begins, thus precluding an analysis of their individual roles in nephrogenesis [81, 360, 376]. The presence of one *Bmp2* null allele does not seem to affect UB branching since the kidneys of *Bmp2*$^{+/-}$ heterozygotes appear normal [121]. However, an inhibitory function for *Bmp2* was revealed by additionally removing the enhancing function of glypican-3 (*Gpc3*) in *Bmp2* heterozygous mutant mice, which resulted in an abnormal increase in UB branching and cell proliferation [121].

Understanding the role of *Bmp4* in UB branching through in vivo analysis of *Bmp4* heterozygotes is made complex because *Bmp4* heterozygous null mice display a broad spectrum of kidney and ureter malformations [206]. An inhibitory function for *Bmp4* in regulating UB branching is suggested by studies in kidney explants from wild-type mice, in which recombinant BMP4 protein suppressed kidney growth, UB branching, and nephron formation in a dose-dependent manner [37, 42, 274]. Modulating the level of BMP4 activity in vivo is shown to alter the three-dimensional architecture of UB branching as revealed when antagonists of BMP signaling (viz., *Cer1* (cerberus homolog 1) and *Grem1*) are dysregulated in embryonic mice [58, 228]. BMP antagonism appears to function by coordinating the inhibitory activities of BMP4 (and possibly BMP2) with the stimulatory effects of GDNF and WNT11, thus providing a mechanism for spatial patterning of UB branching [58, 199].

Recent studies have shown that Sonic Hedgehog signaling is required to pattern UB branching during kidney development. In the embryonic kidney, *Shh* is initially expressed exclusively in the UB and later is restricted in its expression to the ureter epithelial and medullary collecting ducts [374]. An early requirement for *Shh* in UB induction was revealed by the analysis of mice with germline null *Shh* mutation [374]. These mice exhibited severe developmental abnormalities which included bilateral renal aplasia or the presence of a single dysplastic kidney. Dysplastic kidneys from these mutants showed decreased levels of *Pax2* and *Sall1*, implying that Sonic Hedgehog signaling is necessary to regulate genes that promote UB induction. In addition, removing *Shh* activity resulted in downregulation of the levels of Gli proteins which normally activate transcription (i.e., Gli activator) in the face of Gli3 proteins which

normally repress transcription (i.e., Gli3 repressor). Eliminating *Gli3* function in *Shh* mutant mice rescued kidney development and restored *Pax2* and *Sall1* levels. This analysis suggests that one early role of *Shh* in kidney development is to counterbalance the inhibitory influence of Gli3 repressor activity at the time of UB induction. This notion of balancing Gli activator and repressor functions is later important in establishing cortical and medullary domains within the collecting duct system as shown in mice when Sonic Hedgehog signaling is inappropriately activated in the developing renal cortex. Ectopic HH signaling was induced in the renal cortex of mice with targeted inactivation of *Ptc1* in the UB cell lineage, which resulted in decreased UB branching and caused renal hypoplasia [41]. The branching defect in *Ptc1* conditional mutants was associated with decreased levels of *Gdnf, Ret*, and *Wnt11* expressions, which were restored in *Ptc1* mutant mice by additionally overexpressing a constitutively active form of Gli3 repressor protein in these mutants [41]. These data suggest that a gradient of HH signaling activity exists within the developing kidney which is characterized by low levels of Gli activator function and high levels of Gli3 repressor in the cortex. This pattern of Hedgehog activity appears to be necessary for setting up gene expression patterns in the renal cortex which promote UB branching and repress branching in the medulla.

1.3.6.5 Development of the Renal Medulla and Pelvicalyceal System

Between weeks 22 and 34 of human fetal gestation [261], or at E15.5 in embryonic mice [298], the renal cortex and medulla of the developing kidney become morphologically distinct. The cortex contains all glomerular, proximal, and distal tubular tissue elements as well as adjoining segments of the descending and ascending loops of Henle and cortical collecting ducts. It is organized as a relatively compact, outer rim of tissue and represents about 70 % of total kidney volume at birth [51]. The renal medulla lies deep to the cortex. It is comprised of longitudinal arrays of collecting ducts and loops of Henle which are interspersed with peritubular capillaries that surround both tubular tissues.

During embryonic kidney development, distinct morphologic differences emerge between collecting ducts located in the medulla compared to those located in the renal cortex during this stage of kidney development. Medullary collecting ducts are organized into elongated, relatively unbranched linear arrays which converge centrally to form the papilla. In contrast, collecting ducts located in the renal cortex actively induce metanephric mesenchyme until nephrogenesis is complete. The most central segments of the collecting duct system that are formed from the first five generations of ureteric bud branching undergo remodeling by increased ductile growth and dilatation to form the pelvis and calyces (reviewed in Al-Awqati and Goldberg [4]). The renal pelvis is lined by UB-derived epithelium and is surrounded by smooth muscle at its confluence with the proximal ureter. The calyces, in turn, represent extensions of the renal pelvis which surround the papilla.

The developing renal cortex and medulla exhibit distinct axes of growth. The renal cortex grows along a circumferential axis, resulting in a tenfold increase in volume while preserving compact organization of cortical tissue around the developing kidney [51]. In this manner, developing glomeruli and tubules maintain their relative position in the renal cortex with respect to the external surface of the kidney or renal capsule. Preserving this spatial relationship between developing nephrons and the renal capsule appears to be crucial as revealed by defective nephron development in mice that fail to form a renal capsule [169].

In contrast to the circumferential pattern of growth exhibited by the developing renal cortex, the developing renal medulla expands 4.5-fold in thickness along a longitudinal axis perpendicular to the axis of cortical growth [51]. This pattern of growth is attributed to elongation of outer medullary collecting ducts [51]. Elongation of medullary collecting ducts is governed by oriented cell division (OCD), a process which involves aligning cell mitotic spindles with the long axis of a structure [98]. OCD leads to duct elongation

without a change in lumen diameter. Conversely, cystic dilatation of ducts occurs when OCD is disrupted [98, 284].

Signaling mechanisms implicated in control of OCD include planar cell polarity and Wnt signaling. Fat4 is a single-pass transmembrane protein and component of the planar cell polarity signaling pathway which is required for OCD as demonstrated in *Fat4*-deficient mice. Targeted disruption of *Fat4* in embryonic mice caused randomization of OCD in developing collecting ducts, which resulted in the formation of dilated collecting ducts and cystic kidneys [284]. The Wnt7 signaling molecule, encoded by *Wnt7b*, is expressed in UB trunk cells and is necessary for CD elongation which takes place during formation of the medulla and papilla [375]. When *Wnt7b* function is lost in UB-derived cells by conditional inactivation, CDs that form the medulla and papilla become grossly dilated [375]. *Wnt9b*, which is expressed in UB tip cells and which plays an important role in MM induction, is later expressed in medullary CD cells where it acts to control OCD through an autocrine signaling mechanism [150]. The role of *Wnt9b* in this process is reflected by the occurrence of cysts and disruption of OCD in collecting ducts of mice when *Wnt9b* is deleted from UB-derived cells [150].

Development of the medulla coincides with the appearance of stromal cells between the 7th and 8th generations of ureteric bud branches [51]. It is suggested that stromal cells provide stimulatory cues which promote growth of medullary collecting ducts [51]. Additional support for this hypothesis is provided by analyses of mutant mice lacking stromal transcription factors Pod1 (also known as TCF21) [69, 272] or Foxd1 [11, 69, 122, 272], which demonstrate defects in medullary collecting duct patterning.

Mutations in a number of genes that encode molecular components of the renin-angiotensin system, which is best known for its role in controlling renal hemodynamics, cause abnormalities in the development of the renal calyces and pelvis. Mice homozygous for a null mutation in the angiotensinogen gene (*Agt*) demonstrate progressive widening of the calyx and atrophy of the papillae and underlying medulla [230]. Identical defects occur in homozygous mutants for the angiotensin receptor-1 (*Agtr1*) gene [208]. The underlying defect in these mutants appears to be decreased cell proliferation of the smooth muscle cell layer lining the renal pelvis, resulting in decreased thickness of this layer in the renal pelvis and proximal ureter. Mutational inactivation of *Agtr2* (encoding angiotensin receptor-2) results in a range of anomalies including vesicoureteral reflux, duplex kidney, renal ectopia, ureteropelvic or ureterovesical junction stenoses, renal dysplasia or hypoplasia, multicystic dysplastic kidney, or renal agenesis [231]. *Agtr2* null mice demonstrate a decreased rate of apoptosis of the cells around the ureter, suggesting that *Agtr2* also plays a role in morphogenetic remodeling of the ureter.

1.3.6.6 Terminal Differentiation of Collecting Ducts

Ureteric bud epithelial cell differentiation results in the formation of two functionally distinct mature cell types – principal cells and intercalated cells. Principal cells (PC) are characterized by the expression of aquaporin-2 water channels (encoded *Aqp2*) and are necessary for water homeostasis [22]. Intercalated cells (IC) are required for acid-base balance and excrete either hydrogen ions (alpha-intercalated cells) or bicarbonate (beta-intercalated cells) [347]. In the mature kidney, PCs and ICs are relatively distributed throughout the entire collecting duct system in a manner such that the ratio of PC to IC increases towards the papilla [22]. In the developing kidney, immature PCs and ICs initially express functional markers for both mature cell types [22]. Terminal differentiation results in PCs and ICs acquiring gene expression patterns that reflect their cell type-specific functions.

A number of signaling mechanisms that have been implicated in controlling the relative ratio of PCs to ICs during collecting duct development include the forkhead transcription factor gene, *Foxi1*, Notch signaling, and Wnt signaling. *Foxi1* is required for IC cells to form as *Foxi1* null mice displayed embryonic kidneys with collecting ducts lacking in IC cells without abnormal

formation of PC cells [22]. A mechanism involving activation of Notch receptor signaling appears to favor the formation of PCs over ICs as the ratio of PC/IC is reduced in mice when Notch signaling is perturbed [147, 193]. Wnt signals play a role in patterning cell types within the collecting duct system by maintaining UB cells in an immature state. This was shown in mice by deleting beta-catenin (*Ctnnb1*) in the UB lineage, which resulted in premature cell differentiation as revealed by the ectopic expression of mature CD cell markers (e.g., ZO1 and aquaporin-2; *Aqp2*) in UB tip cells [32, 137, 188]. In addition, *Wnt7b*, which is expressed in branch trunks, appears to promote the formation of PC cells by regulating collecting duct elongation and OCD [375].

1.3.7 Nephron Development

1.3.7.1 Overview of Nephron Development

In humans, the first functioning nephrons are formed by week 9 and excrete urine by week 12. By 34–36 weeks gestation, fetal nephrogenesis is completed, following which no new nephrons are formed [126, 261]. Total nephron number varies tenfold in humans with normal kidney function and is reported to range from 200,000 to 2,000,000 [129, 131]. Environmental and genetic factors can impact on total nephron number [127, 211], and low nephron number at birth is associated with the development of hypertension and renal disease in adulthood [30, 31, 183]. Infants with low birth weight as a result of intrauterine growth retardation and premature birth are particularly susceptible to adult renal disease since low birth weight correlates strongly with reduced nephron endowment (Table 1.5) [74, 131, 278].

Nephron formation is initiated when a subpopulation of condensed mesenchyme cells is induced to enter a program of epithelial cell differentiation. This process of differentiation is termed mesenchymal-epithelial transformation (MET) and ultimately generates all epithelial cell types comprising the mature nephron, including the visceral and parietal epithelium of the glomerulus, the proximal convoluted tubule, the ascending and descending limbs of the loops of Henle, and the distal convoluted tubule [261, 298]. MET results in the conversion of loosely associated, non-polarized mesenchymal cells into tightly associated, polarized epithelial cells, which form primitive tubules. Tubule formation, or tubulogenesis, is characterized by the appearance of morphologically distinct epithelial structures (viz., pre-tubular aggregates, renal vesicles, comma-shaped bodies, and S-shaped bodies) which are generated throughout kidney development according to stereotypic sequence (Fig. 1.10). Pre-tubular aggregates are solid cell clusters which represent the progenitor cell subpopulation of condensed mesenchyme that is induced to differentiate along a nephron-specific epithelial cell lineage. Renal vesicles are hollow structures deriving from pre-tubular aggregates which are comprised of immature epithelial cells that begin to show patterns of nephron segment-specific gene expression. Comma-shaped bodies are transitional structures which convert renal vesicles into S-shaped bodies. S-shaped bodies are tubular structures which may be considered as primitive nephrons based on segment-specific differences in cellular morphology and gene expression. The following sections describe morphogenetic events which occur at each step of nephron development based on observations in human fetuses and mouse embryos [261, 298].

Table 1.5 Susceptibility factors for hypertension and renal disease in humans associated with low nephron number [181]

Susceptibility factor	References
Low birth weight	[125, 187, 278]
Preterm birth	[125, 278]
Short stature	[129, 238, 315]
Reduced kidney mass or volume	[234, 301, 321, 378]
Maternal gestational diabetes	[7, 225]

1.3.7.2 Early Stages of Nephron Development

Pre-tubular aggregates form as a localized oval-shaped cluster of cells separated from condensed mesenchyme situated near the base of the UB ampulla. Appearance of the pre-tubular

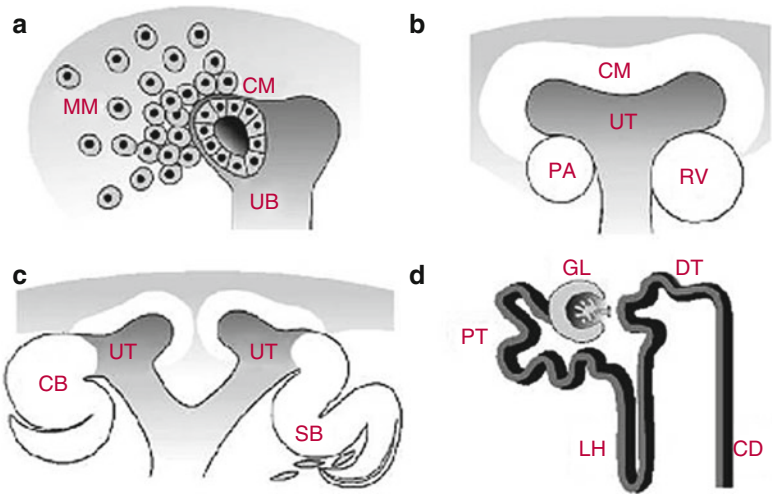

Fig. 1.10 Overview of nephron morphogenesis. (**a**) Metanephric mesenchyme (MM) responds to signals from the ureteric bud (UB) branch tip by condensing around its leading edge. Condensed mesenchyme (CM) contains self-renewing nephron progenitor cells which contribute to the generation of all glomerular and tubular epithelial cells of the mature nephron. (**b**) A subpopulation of CM proximal to ureteric bud branch form a pre-tubular aggregate (PTA). PTA cells subsequently undergo epithelial transformation and form a hollow structure, the renal vesicle (RV), which connects with its adjacent ureteric bud branch tip (UT). (**c**) Transitional structures are generated by the formation of a distal and proximal cleft within the RV, resulting in formation of a comma-shaped body (CB) and S-shaped body (SB). The distal cleft is invaded by endothelial cell precursors which form the glomerular capillary network. (**d**) The mature nephron is characterized by morphologically and functionally distinct epithelial segments: the glomerulus (GL), proximal convoluted tubule (PT), loop of Henle (LH), and distal convoluted tubule (DT). The nephron is connected at its distal end to the ureteric bud-derived collecting duct (CD) (Reproduced with modifications from Little et al. [176], Copyright 2010, with permission from Elsevier)

aggregate coincides with MET as mesenchymal cells within this cluster acquire a polarized, epithelial cell phenotype, including the formation of adherens junctions and a partial basement membrane [12, 101]. Simultaneous with epithelialization, an internal cavity forms within the pre-tubular aggregate, at which point the structure is called a renal vesicle. The initial renal vesicle is a spherical hollow ball which elongates and widens at the end which is distal to the UB ampulla. The renal vesicle subsequently abuts against the ampulla and forms a connection between these two structures, permitting the UB lumen to communicate with the internal cavity of the renal vesicle [12]. Fusion with the ampulla is a relatively late event in renal vesicle morphogenesis and requires restructuring of the UB cell basement membrane [101]. A completely patent lumen linking the primordial nephron to the UB is evident by the late comma-/early S-shaped body stage [101].

The establishment of glomerular and tubular domains (referred to as nephron segmentation) is initiated by the sequential formation of two clefts in the renal vesicle [261]. Creation of a lower cleft that termed the vascular cleft heralds formation of the comma-shaped body. The comma-shaped body is a transient structure that rapidly undergoes morphogenetic conversion into an S-shaped body following the appearance of an upper cleft. Segmentation is clearly distinguished in S-shaped bodies by the organization of cells into three distinct sub-compartments or limbs. The middle and upper limbs give rise to the proximal and distal tubular segments of the mature nephron, respectively, while visceral and parietal epithelia of the mature glomerulus derive from the lower limb [261, 298]. Prospective cells of the loop of Henle are thought to be first positioned at the junctional region of the middle and upper limbs of the S-shaped body [223].

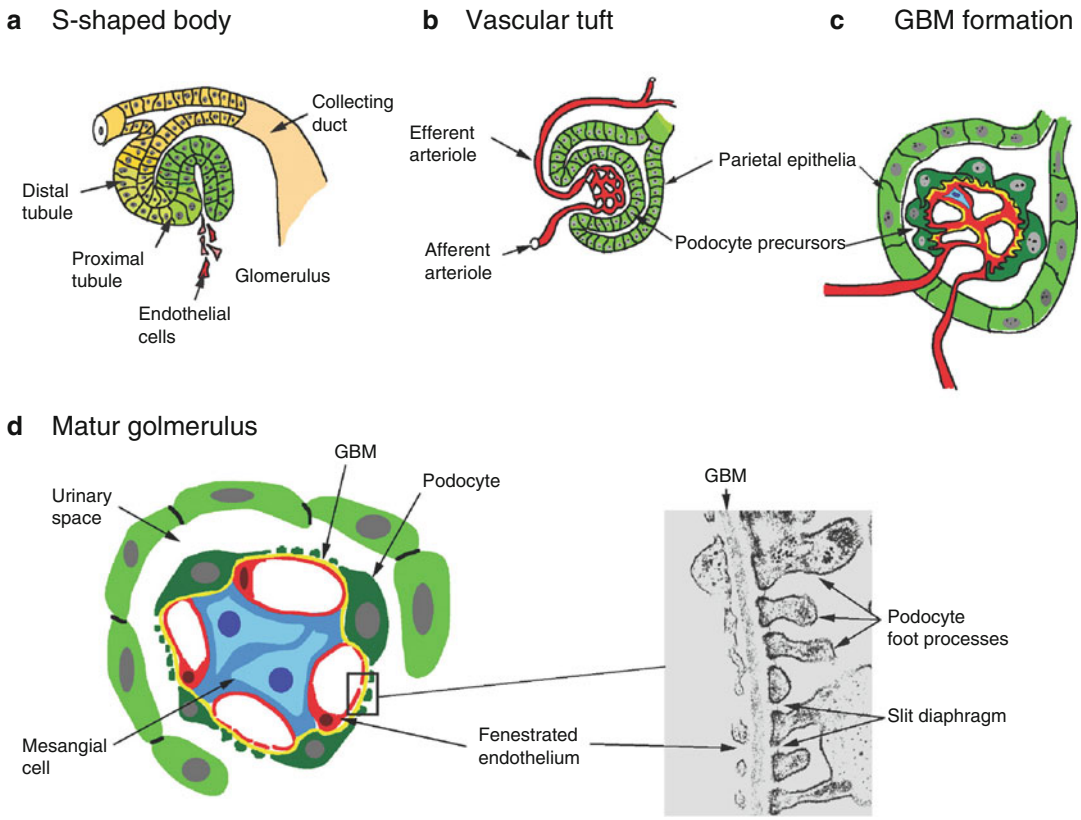

Fig. 1.11 Glomerular development. (**a**) Endothelial cells are recruited into the cup-shaped glomerular precursor region of the S-shaped body forming a primitive vascular tuft. (**b**) Podocyte precursors contact invading endothelial cells and begin to differentiate. In turn, endothelial cells form a primitive capillary plexus (capillary loop stage). (**c**) An interposed glomerular basement membrane forms between podocytes and endothelial cells. Parietal epithelial cells encapsulate the developing glomerulus. (**d**, *left panel*) Elaboration of podocyte primary and secondary cellular processes accompanies podocyte differentiation and formation of the glomerular filtration barrier. (**d**, *right panel*) A high-magnification electron micrograph showing the fenestrated endothelium, podocyte foot processes and slit diaphragms located between the interdigitating foot processes (Reproduced from Piscione and Waters [257], with permission)

As the vascular cleft broadens and deepens, the lower limb of the S-shaped body forms a cup-shaped unit (Fig. 1.11a). Epithelial cells lining the inner wall of this cup will ultimately generate visceral glomerular epithelial cells, or podocytes [164, 261]. Cells lining the outer wall of the cup form parietal glomerular epithelium, or Bowman's capsule. The vascular cleft also serves as an entry portal for the migration of endothelial and mesangial progenitor cells which subsequently differentiate and organize into the glomerular vascular tuft [89, 277]. Recruitment of endothelial and mesangial precursors into the vascular cleft coincides with the formation of a primitive vascular plexus and the deformation of the S-shaped body lower limb into a cup-like structure [261] (Fig. 1.11b). This denotes the capillary loop stage of glomerulogenesis.

The fetal origin of endothelial and mesangial cells is unknown. Endothelial and mesangial cells have been thought to share a similar origin based on experimental evidence involving autologous transplantation of embryonic kidney rudiments into adult renal cortex. These studies suggest that glomerular endothelial and mesangial precursors originate from a unique subpopulation of induced metanephric mesenchyme that does not differentiate along epithelial or stromal

lineages [134, 275, 277]. This theory is supported by another study showing a conserved mechanism for both endothelial and mesangial precursor cell recruitment into the developing glomerulus [89]. However, conflicting evidence comes from experiments involving rodent fetal kidneys engrafted onto avian chorioallantoic membrane which support the potential role of angiogenesis in glomerular capillary tuft [296].

Cells residing along the inner surface of the lower S-shaped body limb represent nascent podocytes (Fig. 1.11a). At this stage, immature podocytes are proliferative and exhibit a columnar shape, apical cell attachments, and a single-layer basement membrane [164]. At the capillary loop stage, podocytes lose their mitotic capability [219] and begin to demonstrate a complex cellular architecture (Fig. 1.11d, left panel), including the formation of actin-based cytoplasmic extensions, or foot processes, and the formation of specialized intercellular junctions, termed slit diaphragms [100, 246] (Fig. 1.11d, right panel).

1.3.7.3 Nephron Maturation

Maturation of glomerular and tubular segments of the developing nephron is characterized by morphological changes reflecting growth. The most striking changes occur in the proximal convoluted tubule and loop of Henle, which show increased tortuosity with maturation and elongation, respectively [261]. At a cellular level, proximal tubular epithelial cells transition from a columnar to a cuboidal cell phenotype and develop microvilli on their apical surfaces [92]. Proximal tubule growth is reflected by a gradual increase in tubular diameter and length and is dependent on oriented cell division [324]. At birth, the human kidney exhibits variation in proximal tubule length from the outer to inner cortex, which becomes uniform by approximately 1 month of postnatal life [97]. From a functional perspective, proximal tubule length correlates strongly with sodium reabsorption such that immature proximal tubules have a limited capacity to reabsorb filtered sodium [322].

The descending and ascending limbs of the primitive loop of Henle are first recognizable as a U-shaped structure in the periphery of the developing renal cortex [221, 223]. Maturation of the primitive loop involves elongation of both ascending and descending limbs through the corticomedullary boundary. Longitudinal growth of the medulla contributes to lengthening of the loops of Henle such that all but a small percentage of the loops of Henle extend below the corticomedullary junction in full-term newborn infants [261]. As the kidney increases in size postnatally, the loops of Henle further elongate and reach the inner two-thirds of the renal medulla in the mature kidney.

Continued maturation of the loop of Henle is accompanied by specialization of descending and ascending limb epithelial cells as they acquire unique transport functions [224]. Regional specification of cell function is essential to the kidney's urine-concentrating mechanism as differential transport of urine water and solutes along its descending and ascending limbs, respectively, contributes to generation of an interstitial medullary tonicity gradient. Maintenance of this interstitial tonicity gradient is functionally coupled to urine concentration by rendering a favorable gradient within the medulla for water reabsorption from collecting ducts. Consequently, longer loops are more capable of generating steeper medullary tonicity gradients, hence favoring an increased urine-concentrating capacity. The clinical significance of this relationship is best illustrated in extremely premature newborn infants who have small kidneys that feature short loops of Henle owing to the reduced distance between the renal capsule and the renal papilla. Consequently, the urine-concentrating capacity of the premature kidney is limited by generation of a shallow medullary tonicity gradient.

Glomerulogenesis is completed in human fetuses by 36 weeks gestation in accordance with cessation of nephrogenesis. At birth, superficial glomeruli are chronologically the last to be formed and are significantly smaller than juxtamedullary glomeruli, which are the earliest formed glomeruli [97]. Subsequent growth and maturation involves hypertrophy, with glomeruli reaching adult size by 3 ½ years of age [97]. Glomerular hypertrophy likely plays an important

role in glomerular filtration rate (GFR) maturation in term infants who have completed kidney development and enter postnatal life with their full complement of glomeruli [282]. Other factors affecting glomerular function in the newborn term and preterm infant are renal blood flow, which increases at birth [156, 344], and glomerular filtration barrier surface area, which expands progressively from birth to adulthood [97].

The glomerular filtration barrier is a physiologic module comprised of glomerular capillary endothelial cell fenestrations, slit diaphragms which are modified cell-cell junctions between adjacent podocyte foot processes, and the glomerular basement membrane which lies interposed between podocytes and glomerular capillary endothelium (reviewed in Kreidberg [164]; Pavenstadt et al. [246]). The capillary loop stage of glomerulogenesis marks a critical time point in glomerular maturation since it is at this stage that endothelial fenestrations form and podocytes transition from an immature to mature cellular phenotype [89, 164].

1.3.7.4 Genes Implicated in Mesenchymal-to-Epithelial Transformation and Nephron Development

Throughout embryonic kidney development, undifferentiated nephron epithelial progenitor cells are marked by high levels of expression for *Cited1* and *Six2* in condensed mesenchyme surrounding the UB tips. Mesenchymal-to-epithelial transformation (MET) coincides with an initial decrease in the expression of *Cited1* and a subsequent progressive decline in *Six2* expression within a subpopulation of cells which will eventually form pre-tubular aggregates. It is not known what regulates these changes in *Cited1* and *Six2* expression, although a decline in *Cited1* expression within condensed mesenchyme cells seems to be necessary for epithelial transformation to occur [214].

In response to Wnt9b, cells with declining *Six2* activity show upregulated expression of a number of genes necessary for the formation of epithelial tubes, including *Fgf8*, *Wnt4*, and *Lhx1* [244] (Fig. 1.12). *Fgf8* and *Wnt4* are essential for initiating the epithelial cell differentiation as demonstrated by the epithelialization defects in embryonic kidneys of *Fgf8* and *Wnt4* mutants, which show kidney development arrest at the PTA stage [106, 251, 323]. *Lhx1* is not essential for the initial stages of epithelialization since PTA is evident in kidneys of *Lhx1* embryonic mutant mice before kidney development is halted at the RV stage [157]. Molecular studies have revealed that *Fgf8* is expressed earlier than *Wnt4* and is required for the expression of *Wnt4* and *Lhx1* in induced MM [106, 251]. Once MET is initiated, Wnt4 maintains its own expression and likely functions in a cell-autonomous fashion [151]. The initial functions of Wnt proteins during MET are thought to be dependent on canonical signaling mechanisms involving beta-catenin. Activation of this pathway is likely transient since sustained beta-catenin activity within *Cited1*-negative, *Six2*-positive, *Wnt4*-positive cells is shown to prevent epithelial tubule formation in

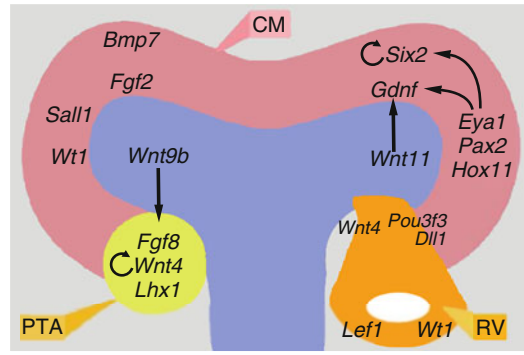

Fig. 1.12 Genes involved in regulating mesenchymal-to-epithelial transformation and establishment of proximal and distal nephron cell fates during early nephron development. Shown is a schematic representation of a branched ureteric bud (*blue area*) with condensed mesenchyme (CM; *pink area*) surrounding the *branch tips*. A pre-tubular aggregate (PTA; *yellow area*) and renal vesicle (RV; *orange area*) are also represented. The diagram illustrates expression domains of genes involved in several stages of early nephron morphogenesis: mesenchyme induction (*Gdnf*, *Eya1*, *Pax2*, *Hox11* paralogs, *Wnt9b*), nephron progenitor cell self-renewal and/or survival (*Six2*, *Sall1*, *Bmp7*, *Wt1*, and *Fgf2*), epithelial transformation (*Fgf8*, *Wnt4*, *Lhx1*), and early nephron segmentation (*Wnt4*, *Lef1*) and the formation of proximal and distal nephron segments (*Wt1*, *Dll1*, *Pou3f3*) (Reproduced with modifications from Little et al. [176], Copyright 2010, with permission from Elsevier)

transgenic mice [244]. Several reports suggest that epithelial cell differentiation and tubulogenesis depend on noncanonical Wnt/planar cell polarity (PCP) signaling pathways [36, 330]. This transition in Wnt signaling may involve *Dkk1*, an inhibitor of canonical Wnt signaling, which is upregulated in Wnt4-expressing cells as they transition from pre-tubular aggregates to renal vesicles [101].

Nephron segmentation refers to the process which establishes gene expression domains that promote the formation of glomerular and tubular epithelial cells along the proximal-distal axis of the developing nephron. As early as the renal vesicle stage, proximal-distal expression domains are established as illustrated by the complementary gene expression of patterns of *Wnt4* and *Lef1* (encoding lymphoid enhancer-binding protein 1) (Fig. 1.12), which is a downstream target of Wnt signals [214]. Expression studies show that *Wnt4* is localized to distal RV cells which are closest to the UB, while proximal RV cells which are furthest from the UB express *Lef1*. Other genes which define the "distal domain" include the POU domain homeobox gene *Pou3f3* (aka *Brn1*), which is required for the formation of loops of Henle [221], and Delta-like 1 (*Dll1*) and Jagged1 (*Jag1*), which are membrane-associated proteins that activate Notch receptor signaling and are implicated in setting up the proximal-distal axis during early nephron development [55]. Conversely, proximal domain markers include *Wt1*, which is necessary for normal podocyte development [112], and *Tmem100*, which encodes a transmembrane protein whose function in kidney development is unknown [101].

Establishing and/or maintaining segment-specific patterns of epithelial cell differentiation during early nephron development is dependent on the functions of *Lhx1* and genes encoding members of the Notch signaling pathway. *Lhx1* appears to control the decision between adopting a glomerular and a tubular epithelial cell fate. This role was revealed in a chimeric study involving the analysis of embryos comprised of a mosaic of wild-type cells and cells deficient in *Lhx1* activity [157]. This study showed that *Lhx1* function was dispensable for the formation of glomerular epithelial cells as only wild-type cells (i.e., cells with full *Lhx1* activity) were detected within proximal and distal tubular segments of the mature nephron, whereas glomerular epithelial cells (i.e., podocytes and cells of Bowman's capsule) were comprised of a mosaic of wild-type cells and Lhx1-deficient cells [157]. Analyses of temporal patterns of gene expression within the developing kidney reveal that the onset of *Lhx1* expression in cells of pre-tubular aggregates follows the induction of *Fgf8* and *Wnt4* and is subsequently expressed in all cells of renal vesicles as these structures progress along their morphogenetic sequence [157]. *Lhx1* promotes the expression of *Pou3f3* and *Dll1*, which have explicit roles in segment-specific epithelial cell differentiation. *Pou3f3* is essential for formation of loops of Henle as well as terminal differentiation of distal tubule epithelial cells [221]. In contrast, *Dll1* is implicated in the formation of proximal tubules as embryonic mice with a hypomorphic mutation for *Dll1* showed a reduction in proximal tubules [55].

Proteins encoded by *Dll1* (Delta-like 1) and *Jag1* (Jagged1) belong to a family of membrane-bound ligands which bind and activate transmembrane Notch receptors on the surface of neighboring cells [312, 313]. Notch receptor activation regulates progenitor cell proliferation and differentiation in many organ systems (reviewed in Chiba [62]). The defect in proximal tubule formation exhibited by *Dll1* hypomorphic mutants is consistent with several reports showing that Notch signaling is essential for the formation of glomerular and proximal tubule segments of the nephron [55, 191]. Among genes which encode Notch signaling pathway molecules in mammals, *Notch1* and *Notch2* receptor genes and ligands *Jag1* and *Dll1* are expressed in RVs and S-shaped bodies in non-overlapping expression domains [54, 258]. Genetic studies show that *Notch2* is the dominant receptor since conditional deletion of *Notch2* in MM results in the formation of rudimentary nephrons comprised of cells expressing distal tubule markers and lacking podocytes and proximal tubule cells [55]. In contrast, a requirement for *Notch1* in proximal nephron formation was only revealed in *Notch1* conditional mutants

when the gene dosage of *Notch2* is halved [324]. At later stages of tubular development, *Notch1* and *Notch2* cooperatively regulate OCD in developing proximal tubules, which is necessary to prevent cystic dilatation and malignant transformation to renal cell carcinoma-like tumors [324]. A role for Notch signaling in glomerular development was suggested by the analysis of a mouse model for Alagille syndrome caused by a hypomorphic mutation in *Notch2* [191, 192]. In humans, Alagille syndrome is characterized by a multisystem disorder featuring heart, eye, liver, skeletal, and renal defects with variable penetrance [5, 87]. The kidneys of mice with two hypomorphic *Notch2* alleles or one hypomorphic *Notch2* allele combined with one *Jag1* loss-of-function allele were hypoplastic and featured glomeruli arrested in their development at the capillary loop stage [191, 192]. The primary glomerulogenesis defect in these mice was attributed to failure to form the vascular tuft, although it was not clear from the analysis whether this resulted from a defect in the initial formation or differentiation of podocytes or, alternatively, abnormal migration of endothelial and mesangial cells into the vascular cleft. Thus, while Notch signaling appears to be critical for the formation of glomeruli and proximal tubules, additional studies are still required to determine what role, if any, Notch signaling plays in governing cell fate decisions which affect podocyte cell differentiation.

Mechanisms that control the differentiation of nephron epithelial cell progenitors into podocytes are poorly understood although evidence in nonmammalian urogenital systems, such as zebrafish and Xenopus, have implicated two transcription factor genes, *Foxc2* and *Wt1*, in this process [235, 358]. In the developing mouse kidney, *Foxc2* expression is first detected along the inner surface of the vascular cleft in comma-shaped bodies, which is the position occupied by podocyte progenitors during the morphological transition from comma-shaped body to S-shaped body [326]. Subsequently, *Wt1* expression is upregulated in the same cells at the S-shaped body stage [248]. The role of *Wt1* in podocyte differentiation is not evident through the analysis of *Wt1* knockout mice since development is arrested at MM induction [165]. However, a role for *WT1* in podocyte differentiation is suggested by the occurrence of diffuse mesangial sclerosis in humans with dominant mutations in *WT1* [67, 144, 177, 245]. Diffuse mesangial sclerosis is a congenital form of proteinuric glomerulopathy which is characterized by defects in podocyte differentiation [369] and is featured along with gonadal defects in humans with Denys-Drash (OMIM: 194080) and Frasier syndromes (OMIM: 136080), which are caused by dominant WT1 mutations [10, 67, 154]. The identical glomerular phenotype in mice is demonstrated in mice with targeted *Wt1* mutations genetically similar to the *WT1* mutation in humans with Denys-Drash syndrome [99, 112, 114, 245], which serves as additional evidence that *Wt1* has an important role in podocyte differentiation. Several studies have shown that *Wt1* acts as a transcriptional activator by binding to the promoters of a number of genes expressed in developing and mature podocytes including *Vegf*, *Nphs1* (encoding nephrin), and *Podxl* (podocalyxin) [111, 115, 243, 349]. Recent studies in zebrafish and Xenopus have suggested that functions of *Wt1*, *Foxc2*, and Notch signaling converge to regulate the expression of podocyte-specific genes [235, 358]. Further identification of genes whose promoters bind WT1 will be essential to our understanding of WT1 function in glomerular development [120].

Podocyte terminal differentiation relies on the function of a number of transcription factors, including those encoded by transcription factor 21 (*Tcf21*; also known as *Pod1*), LIM homeobox transcription factor 1 beta (*Lmx1b*), and the Kreisler leucine zipper homolog *Mafb*. In mice with loss-of-function mutations in *Tcf21*, *Lmx1b*, and *Mafb*, glomerulogenesis is disrupted, and podocytes show altered expression of structural genes (e.g., nephrin, collagen type IV) which become evident at the capillary loop stage (in the case of *Tcf21* mutant mice) or later (in the case of *Lmx1b* and *Mafb* mutants) [201, 272, 285]. In humans, *LMX1b* mutations are identified in patients with Nail-Patella syndrome, which is associated with focal segmental glomerulosclerosis [78].

Recruitment of endothelial and mesangial precursors and subsequent formation and assembly of the glomerular capillary tuft require vascular endothelial growth factor (VEGF) and platelet-derived growth factor-B (*Pdgfb*). VEGF is secreted by podocyte precursors of early S-shaped bodies and continues to be secreted by mature podocytes in postnatal life [153]. During early glomerulogenesis, VEGF promotes recruitment of angioblasts into the vascular cleft [337]. This process is tightly regulated as severe glomerular defects result in mice when the gene dosage of *Vegf* is genetically manipulated [89, 90].

Pdgfb is expressed by endothelial cells and binds to its receptor, PDGF receptor-beta (*Pdgfrb*) [175], on the surface of mesangial cell precursors to promote inward migration into the vascular cleft of S-shaped bodies. The function of this axis is required for proliferation and assembly of glomerular capillaries and mesangium as revealed by the absence of glomerular capillary tufts in mice deficient of either *Pdgfb* or *Pdgfrb* [168, 320].

During the S-shaped stage, podocyte progenitors express a primitive glomerular basement membrane which is composed predominantly of laminin-1 and α-1 and α-2 subchains of type IV collagen [202]. During glomerular development, composition of the glomerular basement membrane undergoes transition as laminin-1 is replaced by laminin-11, and α-1 and α-2 type IV collagen chains are replaced by α-3, α-4, and α-5 subchains [202]. As demonstrated in several mouse models, failure of these changes result in severe structural and functional defects [200, 203, 233].

1.3.7.5 Cessation of Nephrogenesis

At the morphologic level, the final stages of nephrogenesis are marked by a progressive absence of ureteric bud ampullae and a decline in the density of *Six2*-positive, *Cited1*-positive nephron epithelial progenitor cells within cap condensates [158]. The mechanism that triggers this change in progenitor cell self-renewal and differentiation is unknown. A recent report described three-dimensional modeling of the nephrogenic zone in postnatal mice and revealed that a rapid wave of mesenchymal cell differentiation occurs during the final stages of nephrogenesis with the induction of multiple nephrons around a single UB tip [283]. Thus, it is possible that mass induction exhausts the self-renewing progenitor cell pool, perhaps because the signal for self-renewal is no longer present in sufficient quantity [123]. In line with this view, nephrogenesis might be prolonged if progenitor cell self-renewal and/or survival was restored by providing the necessary signal or stimulus [123]. A study involving uninephrectomized fetal sheep showed that removing one kidney in utero accelerated the rate nephron induction in the remaining kidney [74], indicating that the developing kidney in some species may compensate for loss of nephron mass in utero. It is tempting to speculate, then, that nephrogenesis could be "rescued" in utero in humans by intervening prior to 34-week gestation in human fetuses with renal hypoplasia or in fetuses exposed to maternal factors (e.g., maternal malnutrition) that affect final nephron endowment [167, 187, 362, 380]. The evolution of functional genomics and high-throughput gene expression technologies has rapidly advanced our understanding of genes and pathways involved in starting and stopping nephrogenesis [35, 52, 305] in humans [124, 264]. Validating these studies through functional analyses should provide a clearer understanding of the mechanisms involved in establishing final nephron number.

1.4 Ureter Development

The mature ureter is a muscle-walled tube which acts as a conduit for urine flow from the kidney to the bladder. Its luminal surface is covered by a water-impermeable, stratified epithelium which is termed urothelium. Smooth muscle cells encompass the mature ureter along its proximal-distal length (Fig. 1.13) and propagate urine flow by peristalsis. In humans, ureteral malformations are represented by a heterogeneous group of anatomical defects, including ureteral agenesis, duplication, ectopia, and strictures, as well as ureteral dilatation (or hydroureter) which may be

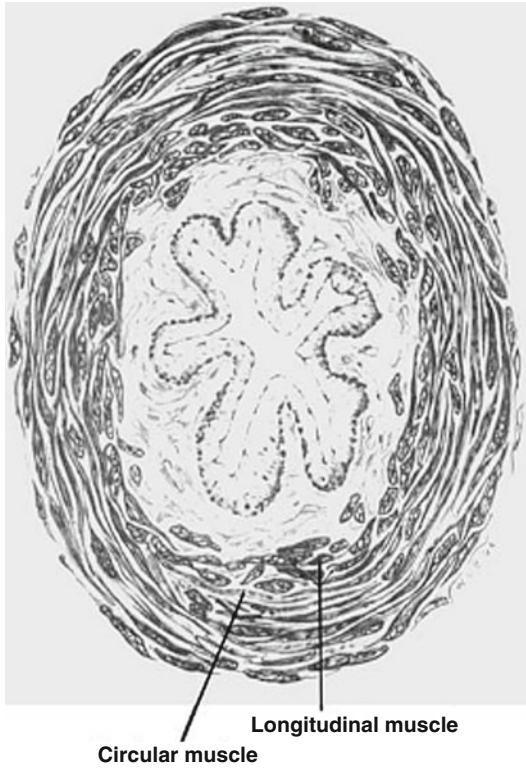

Fig. 1.13 Photomicrograph of a mature, human ureter in cross section at the mid-length. Longitudinal and spiral or circular smooth muscle layers are denoted (Reproduced from Woodburne [361], with permission)

associated either with urinary tract obstruction above or below the bladder or secondary to an intrinsic defect in smooth muscle peristalsis [205]. Traditional views on normal ureter development have stemmed largely from pathological descriptions of human fetal ureter defects from autopsy specimens [184]. Conventional concepts on ureter development have been challenged by the results of recent studies which have rendered new insights on normal and abnormal ureter morphogenesis [17, 61, 339]. Likewise, phenotypic analyses in mouse genetic mutants with ureteral malformations have been highly informative in clarifying developmental disruptions which underlie these anomalies. The following section reviews current concepts of ureter development in humans and draws inferences from studies in mice. This information serves as a foundation for understanding the pathophysiological basis of human congenital urinary tract obstruction syndromes.

1.4.1 Normal Ureter Morphogenesis

Normal ureter development follows a complex sequence of events initiated by UB outgrowth and elongation and succeeded by a process of maturation which involves smooth muscle and urothelial cell differentiation, distal ureter remodeling, and formation of a connection with the developing bladder (Fig. 1.14). Following UB outgrowth, the truncal segment of UB elongates and initiates a series of reciprocal interactions with surrounding mesenchyme that induces periureteral mesenchyme to differentiate into ureteral smooth muscle and UB cells to transform into urothelium. Initially, the ureter is separated from the primitive bladder (represented at this stage by the upper urogenital sinus) by the common nephric duct, which intervenes between the ureteric bud and the urogenital sinus. The common nephric duct subsequently degenerates, and the ureter separates from the nephric duct to form a connection to the bladder. A more detailed description of this process is provided in Sect. 1.5.2.

The initial stages of ureter development are common to both sexes. In humans, this occurs at approximately weeks 11–12 of gestation [189, 325] and E15.5 in mice [16]. Once the common nephric duct degenerates and the ureter separates to connect with the bladder, the disconnected nephric duct migrates posteriorly and unites with tissues derived from the lower urogenital sinus, which adopt different fates in males and females. In males, the paired nephric ducts enter the presumptive prostatic urethra and contribute to the bilateral formation of seminal vesicles and ejaculatory ducts. In females, the anterior nephric duct degenerates in response to a lack of androgen production. Posterior segments become incorporated into the Mullerian duct-derived lower third of the vagina and may persist in mature females as vestigial structures [23, 77, 276].

The newly formed ureter is represented by a single-cell-lined epithelial tube surrounded by a loose network of undifferentiated mesenchymal cells. Studies on first-trimester fetal autopsy specimens have revealed that the immature ureter is patent between gestation weeks 5 and 7 and then is periodically obstructed by epithelial cells

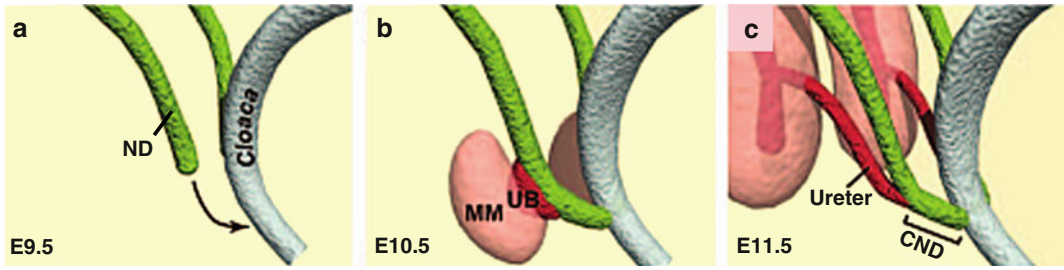

Fig. 1.14 Early stages of distal ureter morphogenesis. (**a**) Ureter formation requires that the nephric duct (ND) extend posteriorly to reach and fuse with the cloaca/urogenital sinus. (**b**) The ureteric bud (UB) develops as an outgrowth of the posterior nephric duct in response to signals emanating from the metanephric mesenchyme (MM). (**c**) The UB invades the MM and branches to form the collecting duct system. The section of ureteric bud located between the MM and ND differentiates into the ureter. The nephric duct segment located between the ureter and cloaca/urogenital sinus represents the common nephric duct. This segment is eliminated by apoptosis during distal ureter maturation (Reproduced with modifications from Uetani and Bouchard [340], with permission)

and proceeds through repetitive cycles of recanalization during the elongation phase of development [6, 281]. By gestation week 12, this process of recanalization is complete, and the normal ureter remains as a hollow tube for the rest of its development. The purpose of this cycle of obstruction and recanalization in human fetal ureter development is not known; however, these processes appear to be unique to ureter morphogenesis since they are not documented in other ureteric bud-derived structures, such as renal collecting ducts and the pelvicalyceal system [240, 241]. One suggestion is that this process may be required to transform the luminal of the nascent ureter from a single layer of cells into a stratified urothelium [182]. Between weeks 12 and 15, the immature ureter epithelium transforms from a monolayer to a pseudostratified epithelium which is characterized by the expression of apical proteins called uroplakins (in mice, encoded by *UpkII* or *UpkIIIa*) [162]. Uroplakins confer water impermeability to the urothelial surface. In humans, mutations in *UPKIIIA* have been associated with ureteral malformations including vesicoureteral reflux and multicystic dysplastic kidney (OMIM: 611559), the latter which is associated with a ureter that lacks a patent lumen [146, 302].

Between 5 and 8 weeks gestation, the fetal kidney ascends to its stereotypic position from the sacral region to the upper lumbar segments. Ureteral smooth muscle formation is evident by approximately week 12 of gestation in the human fetus [189, 325] (E15.5 in mice) [50, 374]. Initial events involve the aggregation and proliferation of periureteral mesenchymal cells which form condensations around the nascent ureters [374]. Condensates are subsequently induced to undergo smooth muscle cell differentiation and are characterized by an increase in smooth muscle actin, smooth muscle myosin heavy chain, and SM22, which is an early marker of smooth muscle cell differentiation [50, 374]. Smooth muscle cell differentiation is initiated in the region surrounding the proximal ureter and renal pelvis and continues in a proximal to distal manner until the entire ureter is covered by smooth muscle by week 22 [189, 325]. Differentiated smooth muscle cells are assembled into fibers which are arranged in spiral bundles around the developing ureter. By week 17, a second inner layer of smooth muscle is generated and assembled into longitudinal bundles. Longitudinal smooth muscle fibers are restricted to the region surrounding the distal two-thirds of the ureter and extend into the tunnel formed by the ureter as it passes through the dorsal bladder wall [218, 346].

Urine flow from the kidney to the bladder is an active process and is mediated by the peristaltic activity of ureter smooth muscle. Peristaltic activity is initiated within the region of the ureteropelvic junction (UPJ) and propagates distally along the length of the ureter in a coordinated fashion [66, 368]. During development, ureter

peristalsis is evident by E15.5 in mice as soon as the process of smooth muscle cell differentiation has begun [70]. Onset of peristaltic activity coincides with the presence of two pacemaker cell populations – Cajal-like cells and HCN3 cells. Cajal-like cells show similarity to interstitial cells of Cajal (ICCs), which are pacemaker cells that control smooth muscle peristalsis in the intestinal tract [294]. ICCs and Cajal-like cells are marked by the cell surface expression of the receptor tyrosine kinase, c-kit [70, 334]. Cajal-like cells are found within the lamina propria and between smooth muscle fibers of the embryonic mouse renal pelvis and proximal ureter regions [40, 70, 133]. HCN3 cells are characterized by the activity of hyperpolarization-activated cation channels. These cells play a pivotal role in regulating cell excitability in cardiac and enteric nervous tissues [96, 365]. HCN3 cells are specifically localized to the UPJ, and their function in this region is thought to initiate unidirectional propagation of smooth muscle peristalsis from proximal to distal [133]. Based on immunohistochemistry, abnormal patterns of pacemaker cell distribution or number have been described in resected ureteral tissues from children with congenital UPJ obstruction, suggesting that the cause of this disorder may involve intrinsic defects in pacemaker cell differentiation, survival, or migration [161, 319]. A recent report described the occurrence of severe nonobstructive hydronephrosis and hydroureter accompanied by complete absence of c-kit-positive cells in the renal pelvis and proximal ureter in a mouse model of Pallister-Hall syndrome (OMIM: 146510) [40]. Pallister-Hall syndrome is a multisystem congenital disorder in humans with urinary tract malformations in approximately 60 % of cases [113, 136]. PHS has been mapped to the GLI3 locus [148], which encodes a transcription factor in the Sonic Hedgehog signaling pathway that has activator or repressor functions depending on it cleavage state [132]. In PHS, nonsense and frameshift mutations result in constitutive GLI3 repressor activity, which abrogates Sonic Hedgehog signaling [148]. When Sonic Hedgehog signaling was genetically inactivated in a tissue-specific manner by deleting smoothened (*Smo*), reduced numbers of c-kit positive and HCN3 cells were associated with nonobstructive hydronephrosis and ureter dyskinesia [40]. Thus, Sonic Hedgehog signaling appears to play a critical role in normal ureter development and may cause of congenital urinary tract obstruction when activating this signaling pathway is disrupted.

1.4.2 Molecular Control of Ureter Development

Ureter smooth muscle formation and urothelial cell maturation is coordinated by reciprocal inductive interactions between mesenchymal and epithelial compartments of the developing ureter. Early in ureter development, immature urothelial cells derived from the UB stalk secrete Sonic Hedgehog, which acts on peri-ureteral condensed mesenchyme through its cell surface receptor Patched1 to stimulate cell proliferation and induce expression of *Bmp4* [374]. Inactivating *Shh* in the mouse urinary tract delays ureteral smooth muscle cell differentiation [374]. Proximal ureter segments of *Shh* null mice show reduced amounts of smooth muscle whereas distal segments lack smooth muscle completely. *Bmp4* expression is completely lost in *Shh* mutant ureters, suggesting that one role of *Shh* is to maintain *Bmp4* expression in the ureteral mesenchyme [3].

Bmp4 is uniformly expressed in ureter mesenchyme at early developmental stages and becomes localized to areas of active smooth muscle cell differentiation [374]. In vitro, BMP4 protein promotes the differentiation of peri-ureteral mesenchyme into smooth muscle [29, 207, 274, 350]. In vivo, decreased BMP4 signaling either by gene deletion in peri-ureteral mesenchyme cells or by direct antagonism in organ culture with proteins that sequester BMP4 protein (e.g., noggin, gremlin) resulted in a gradual reduction of ureter smooth muscle [350]. These data serve as strong evidence that *Bmp4* plays a regulatory role in the formation of ureteral smooth muscle.

The ability of ureter mesenchyme to condense and respond to epithelial-derived cues during mouse ureter development depends on the gene function of *Tbx18*. *Tbx18* encodes a transcription factor belonging to a family which share a highly conserved DNA-binding region known as the T-box and act as activators or repressors depending on molecular context [220]. *Tbx18* is exclusively activated in distal ureter mesenchyme prior to condensation and before *Bmp4* expression is induced in this compartment [2]. When *Tbx18* function is lost due to gene disruption in mice, ureteral mesenchyme fails to condense, and mesenchymal *Ptc1* expression is significantly downregulated, rendering mutant mesenchyme unresponsive to *Shh* signals [2]. Moreover, urothelial cell expression of *Shh* is present but detected at lower than normal levels in *Shh* mutants, suggesting that the response of peri-ureteral mesenchymal cells to secreted *Shh* is dose dependent. Consequently, *Bmp4* activation is blocked in mutant mesenchyme, and $Tbx18^{-/-}$ mutant mice display severely dilated and truncated ureters that lack smooth muscle. *Tbx18* may also control local molecular cross talk between mesenchymal and epithelial cell populations that supports urothelial cell differentiation. This is revealed in $Tbx18^{-/-}$ mutants by showing that ureter epithelial cells fail to proliferate and differentiate into a functional urothelium, leading to the generation of a flat, single-layered urothelium devoid of uroplakin [2]. These data support the concept that growth and differentiation programs of ureter smooth muscle and urothelium are tightly coupled during ureter development [3].

Smooth muscle formation proceeds in a stereotypic manner from proximal to distal during normal ureter development. Sonic Hedgehog signaling is believed to promote this myogenic sequence by controlling mesenchymal cell proliferation along the proximal-distal axis of the developing ureter. The proliferative functions of *Shh* on peri-ureteral mesenchyme are first observed at the proximal end of the ureter, which initiates the myogenic program in that region before more distal regions. In the absence of *Shh*, smooth muscle formation is significantly delayed, such that the fewer mesenchymal cells form condensations and transform into smooth muscle at the proximal end of the ureter and smooth muscle formation is absent at the distal end of the ureter [374]. The effects of *Shh* on smooth muscle development may be dependent on *Bmp4* since *Bmp4* mRNA expression is lost in *Shh* mutants. Evidence from mouse embryonic organ culture studies suggests that *Bmp4* is sufficient to promote mesenchymal cell differentiation into a ureter-specific smooth muscle phenotype. This was demonstrated in experiments using agarose beads soaked in recombinant BMP4 which were placed ectopically near UB-derived renal collecting ducts. These studies showed that BMP4 was sufficient to induce ureter smooth muscle differentiation around collecting ducts, which secondarily caused renal collecting duct cells to express urothelial cell markers [29, 198].

Other genes implicated in proximal-distal ureter smooth muscle patterning include teashirt zinc finger homeobox 3 (*Tshz3*), which belongs to a family of transcription factors with roles in anterior-posterior patterning of embryonic tissues [91, 94]. *Tshz3* function is dispensable in early ureter myogenesis for mesenchymal condensation and proliferation events but is required later in proximal smooth muscle differentiation programs [50]. During normal development, *Tshz3* is expressed in the mesenchyme along the entire length of the ureter and is shown to be upregulated by recombinant BMP4 in organ culture ex vivo [50]. Mice homozygous for a null *Tshz3* mutation exhibit nonobstructive hydroureter and akinetic ureters which lack evidence of smooth muscle cell differentiation within the proximal regions of the ureter yet display normal ureter smooth muscle cell differentiation distally. The defect in *Tshz3* null mice is thought to lie downstream of *Shh* and *Bmp4* since *Ptc1* and *Bmp4* expressions are unaffected in condensed proximal mesenchyme and exogenous BMP4 is incapable of rescuing proximal ureter smooth muscle differentiation in *Tshz3* mutant ureter organ culture.

1.5 Development of the Bladder

1.5.1 Normal Bladder Morphogenesis

The mature urinary bladder is comprised of an inner epithelial cell layer (i.e., bladder urothelium) and an outer smooth muscle layer (anatomically referred to as detrusor muscle). Bladder urothelial cells are endoderm-derived and originate from the luminal epithelial cells from the upper zone of the definitive urogenital sinus. In contrast, bladder smooth muscle cells are mesodermal derivatives arising from surrounding splanchnic mesenchyme.

Mesenchyme encompassing the primordial bladder is spatially organized into three morphologically distinct cell layers: (1) an outer adventitial layer, (2) an inner subepithelial layer (which lies next to the basal surface of immature urothelium), and (3) an interposing subadventitial layer [46]. Cells within all three layers have been shown experimentally to be capable of differentiating into smooth muscle cells [13]. However, only mesenchymal cells within the adventitial and sub-adventitial layers undergo smooth muscle cell differentiation during normal bladder development. In contrast, cells residing within the subepithelial layer remain undifferentiated throughout development.

In the human fetus, bladder smooth muscle cell differentiation is variably reported to occur between 7- and 12-week gestation (E13.5 in mice) [102, 227, 314]. At 10 weeks, the primitive bladder has a distinct outer muscle layer consisting of a continuous sheet of closely packed cells [102]. By 11 weeks, muscle fibers of the bladder wall form a meshwork of interlacing smooth muscle bundles. With the exception of the bladder neck, bladder musculature in the human fetus consists of large smooth muscle bundles arranged as an intermingling network in the absence of distinct layers by week 12 of gestation [102]. In the bladder neck, smooth muscle is replaced by a layer of undifferentiated mesenchyme which is in continuity with similar cells surrounding the urethral wall. Between 12 and 14 weeks, a collar of circularly oriented smooth muscle forms around the bladder neck. At 18 weeks, a relatively thin layer of smooth muscle is distinguished in the region of the trigone and extends into the bladder neck to merge with urethral smooth muscle.

1.5.2 Molecular Mechanisms Involved in Bladder Development

Bladder smooth muscle cell differentiation occurs in response to inductive signals emanating from urothelial cells [13]. Expression studies implicate Sonic Hedgehog as a strong candidate for this role since *Shh* is expressed in developing bladder urothelium, and genes which are activated by Sonic Hedgehog signaling (e.g., *Ptc1*, *Gli1*, *Bmp4*) are expressed in adjacent mesenchyme [314]. In organ culture experiments, exogenous Sonic Hedgehog was shown to be sufficient to induce mesenchyme cell proliferation and smooth muscle cell differentiation in bladder mesenchymal explants which had been separated from urothelium [47]. These data are consistent with genetic studies in mice which showed dysregulated mesenchymal cell proliferation and decreased bladder smooth muscle cell differentiation in mice by eliminating the function of *Gli1* and *Gli2*, which are downstream effectors of activated Sonic Hedgehog signaling [56]. The proliferative effects of *Shh* are dose dependent as increasing concentrations of exogenous Sonic Hedgehog increased bladder size and mesenchymal cell number ex vivo [47]. These data are consistent with a model in which mesenchymal cells closest to bladder urothelium are induced to proliferate in response to high concentrations of Shh and thus maintain a pool of undifferentiated smooth muscle cell progenitors. However, the effect of *Shh* on smooth muscle cell differentiation may be a secondary phenomenon since blocking Sonic Hedgehog signaling in bladder explants had no effect on the expression of smooth muscle cell terminal differentiation markers once smooth muscle formation had already been initiated [314]. These data suggest that while *Shh* may be involved in

initiating smooth muscle cell differentiation, other pathways are likely involved in maturation of bladder smooth muscle.

Based on expression studies, molecular cross talk between Sonic Hedgehog and BMP4 signaling may be important for radial patterning of smooth muscle during bladder development. Expression analyses in developing mouse bladder are consistent with Bmp4 being a target of Sonic Hedgehog signaling as high levels of *Bmp4* mRNA are detected in subepithelial mesenchyme of embryonic mouse bladder and follow the onset of *Gli1* and *Gli2* expression in this compartment by one day [178]. Conversely, lower levels of *Bmp4* mRNA coincide with reduced *Gli1* and *Gli2* mRNA levels in peripheral mesenchyme. It has been suggested that *Bmp4* opposes the mitogenic effects of Sonic Hedgehog signaling based on an analysis of embryonic bladders from *Gli2* knockout mice. Tissue analysis of these mutants showed ectopic smooth muscle formation in regions of the subepithelial mesenchyme layer in which *Bmp4* mRNA expression was lost [56]. Other factors, including TGF-beta and serum response factor (SRF), may be involved in regulating smooth muscle cell differentiation as their expressions coincide with upregulation of several smooth muscle cell differentiation markers, such as *SMAA*, *SMMHC*, smooth muscle protein 22-α (*SM22α*), and *calponin* [172].

1.5.3 Formation of the Ureterovesical Junction

The route taken by the ureter through the smooth muscle layer of the bladder trigone is critical to forming a competent anti-reflux mechanism (Fig. 1.15). It is designed in such a way that retrograde flow of urine to the ureters and kidney is prevented by effectively compressing the intramural ureter against the smooth muscle wall during bladder contractions. A non-refluxing ureterovesical junction (UVJ) necessitates the normal formation of a tunnel through which the ureter passes through the bladder wall, an appropriate angle of entry of the ureter into the bladder, a well-formed trigone, and a correctly positioned ureteral orifice within the trigone. The clinical condition in humans resulting from an incompetent anti-reflux mechanism is vesicoureteral reflux (VUR), which is one of the most common urinary tract disorders in young children with a reported incidence as high as 1 % of live births (reviewed in Murawski et al. [217]).

The traditional view of UVJ morphogenesis was largely based on pathological studies of autopsy specimens from fetuses with lower urinary tract anomalies [327, 355, 357]. The model arising from these studies described UVJ formation as resulting from fusion of the two common nephric ducts with the posterior wall of the developing bladder, thus creating the transmural tunnel

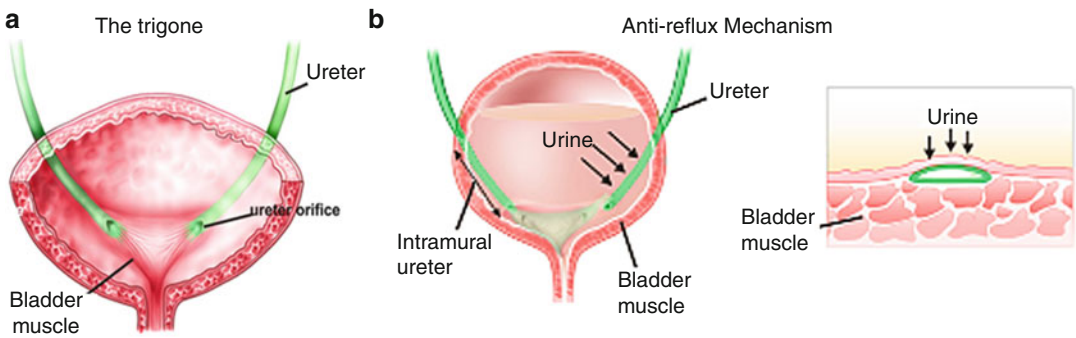

Fig. 1.15 Diagrammatic representation of the trigone and bladder anti-reflux mechanism. (**a**) The trigone is a triangular-shaped region located at the bladder base. It is bordered anterolaterally by bilateral ureteral orifices and posteriorly by the urethral orifice. (**b**) Schematic of the trigone and its connections with the ureters showing the intramural ureter segment that is normally compressed to prevent back-flow of urine to the ureters and kidneys. (**b**, *inset*) Schematic showing compression of the intramural ureter (Reproduced with modifications from Viana et al. [346], with permission)

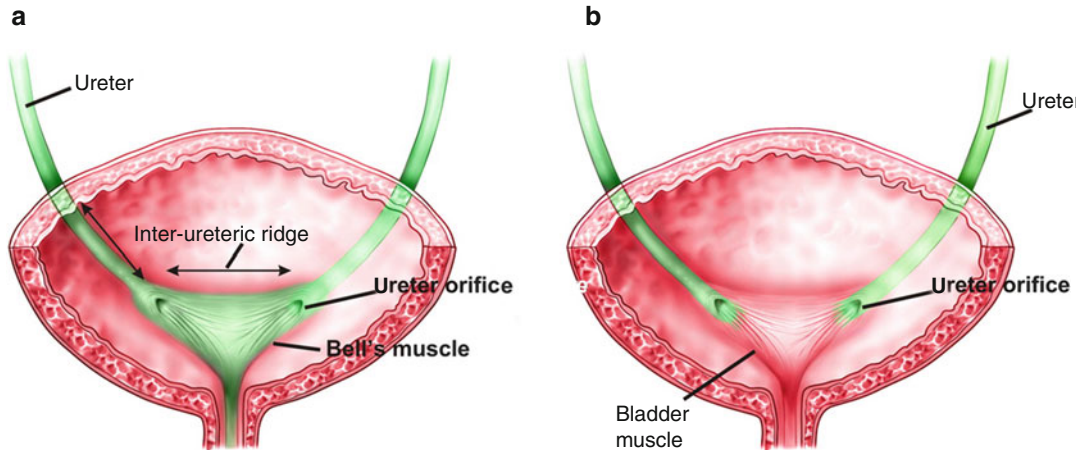

Fig. 1.16 Traditional and revised models of ureterovesical junction (UVJ) formation. Shown are schematic representations of the bladder, ureter, and trigone. Embryonic origins are illustrated by *green* (mesoderm-derived) and *red* (endoderm-derived) areas. (**a**) Traditional model of UVJ formation. The trigone forms in large part from ureteral fibers that fan out across the basal surface of the bladder generating the interureteric ridge and Bell's muscle. In this instance, the trigone is considered to form independently of the bladder. (**b**) Revised model of UVJ formation. The trigone and smooth muscle surrounding the intramural ureter are derived from bladder muscle. Ureteral fibers which extend into the bladder wall contribute a small amount to the intramural ureter tunnel (Reproduced with modifications from Viana et al. [346], with permission)

and the trigone [184, 357]. Based on this view, the outer muscle and inner epithelial layers of the bladder trigone were thought to share a similar mesodermal origin with the muscle and epithelial layers of the ureter (Fig. 1.16a). The traditional model of UVJ formation has been recently challenged by recent studies on distal ureter maturation in wild-type mice at multiple stages of embryonic development [16, 17, 339, 346]. Accordingly, a revised model of UVJ formation has emerged in which the trigone shares an endodermal origin with smooth muscle and epithelial layers of the developing bladder (Fig. 1.16b).

The first critical event in UVJ formation is degeneration of the common nephric duct. Elimination of the common nephric duct occurs as a result of cell apoptosis which occurs progressively once ureteric bud outgrowth takes place [17, 339] (Fig. 1.17a). As a consequence of common nephric duct degeneration, the nascent ureter approximates with the dorsal surface of the urogenital sinus (i.e., the primitive bladder) as ureter extends posteriorly towards the bladder and as the bladder grows and expands anteriorly towards the ureter [339]. At this stage, the distal end of the nascent ureter is observed to rotate 180° at the point where it connects to the nephric duct (Fig. 1.17b), which causes the distal ureter to make contact tangentially with the dorsal surface of the primitive bladder wall (Fig. 1.17c). The mechanism of ureter rotation around the nephric duct at this stage is unknown, although the rotation process appears to be facilitated by expansive growth of the primitive bladder [17]. The section of distal ureter which lies in contact with the bladder subsequently undergoes a second round of apoptosis. With that distal segment eliminated, the ureter becomes detached from the nephric duct, thus generating an open-ended tube which is connected to a single kidney proximally. The distal end of the newly formed ureter proper is subsequently observed to migrate towards the base of the bladder where it is properly oriented to penetrate the muscular exterior surface of the future trigone (Fig. 1.17d). Concomitantly, the nephric duct, which becomes detached from the newly formed ureter, remains in the region to connect with the urethra.

It is widely held that the location of ureteric budding along the long axis of the nephric duct determines the final position at which the ureter enters the bladder and results in vesicoureteral

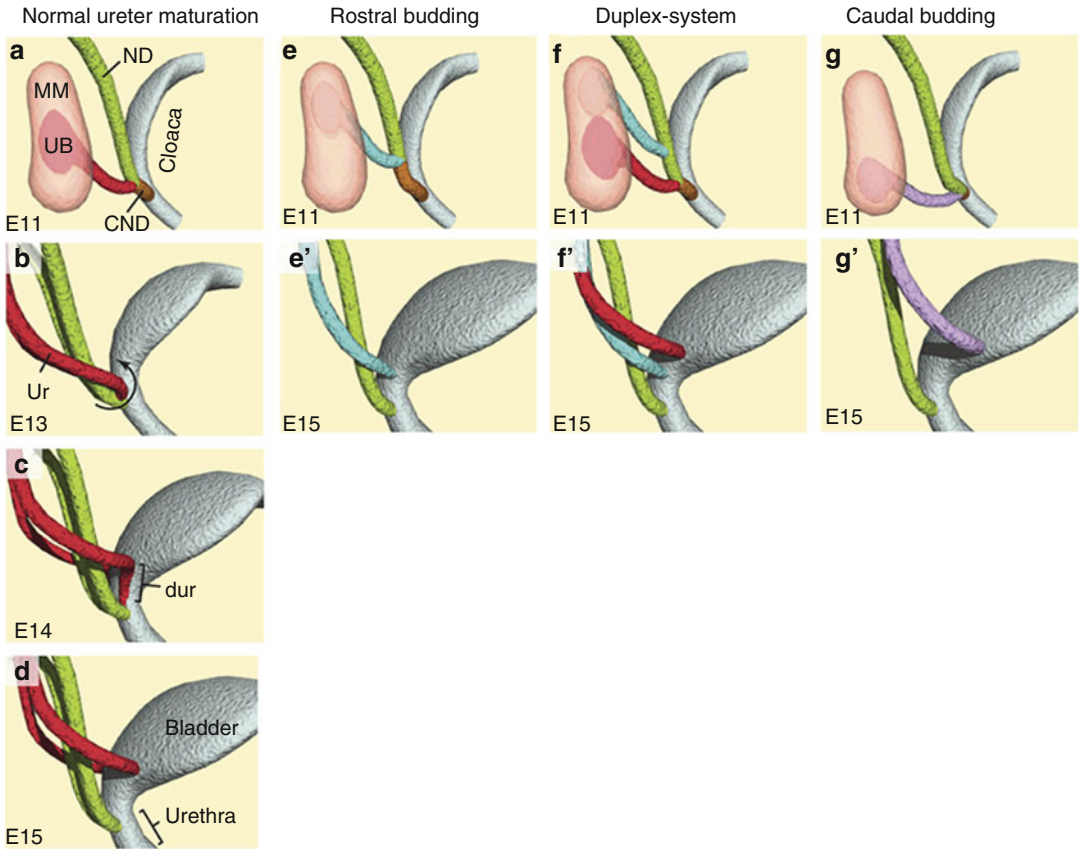

Fig. 1.17 Morphological events in distal ureter maturation and associated lower urinary tract malformations. (**a**)–(**d**) Normal distal ureter maturation and UVJ formation. (**a**) Following invasion of the metanephric mesenchyme (MM) by the newly formed ureteric bud (UB), the common nephric duct (CND; *brown*) is eliminated by apoptosis. (**b**) CND elimination brings the ureter in direct contact with the cloaca, or primordial bladder. Concomitantly, the ureter rotates around the long axis of the nephric duct (ND). (**c**) Ureter rotation orients the distal segment of the ureter towards the developing bladder. The distal-most segment of the ureter (dUr) lies against the exterior bladder surface and is also eliminated by apoptosis. (**d**) A new connection point between the ureter and the bladder is generated. (**e**) A rostral ectopic ureter (*blue*) forms in the absence of primary ureter. In this scenario, the CND is longer than usual. This results in a shorter segment of distal ureter meeting the bladder once ureter rotation takes place, and results (**e'**) in formation of an ectopic ureter that connects in the bladder neck, urethra, or remains attached to nephric duct. (**f**) In duplex systems, a second ureter (*blue*) is induced along the nephric duct, typically at a rostral position in reference to the primary ureter (*red*). (**f'**) Following distal ureter maturation, the rostral ectopic ureter connects below the primary ureter, either in the bladder neck, urethra or remains attached to nephric duct. (**g**) When caudal ureter budding occurs, the connection point between the ureter and nephric duct is very close to the cloaca. In this instance, the length of the CND is very short. (**g'**) The near absence of CND accelerates elimination of the distal ureter and positions the VUJ in an ectopic lateral position (Reproduced with modifications from Uetani and Bouchard [340]. With permission)

reflux when budding occurs ectopically [184]. This principle was put forward by Mackie and Stevens and is based on their pathological studies of urinary tract specimens from fetuses with congenital hydronephrosis. Based on their observations, they proposed that ureters entering the bladder ectopically in the region of the bladder neck would arise as the result of UB formation at a position which is anterior to the usual site of budding. Conversely, they postulated that UB formation at a posterior position would lead to ectopic ureters entering the bladder abnormally

and closer to its apex. This model of abnormal UVJ formation has been validated by in vivo evidence from several mouse models of VUR (reviewed in Murawski et al. [217]) and may be explained by defects in ureter separation and rotation as described above. For example, UB formation at an anterior ectopic site would result in a longer common nephric duct and hence a greater distance between the site of ureteric budding and the primitive bladder (Fig. 1.17e). By increasing the distance between the UB and the growing primitive bladder, ureter rotation and separation from the nephric duct might be delayed and result in a low-lying insertion site [339] (Fig. 1.17e'). This is best exemplified in humans and mice with duplicated ureters which feature an anterior renal moiety connecting with a ureter that inserts ectopically into the bladder neck and which may be associated with hydroureter and hydronephrosis [184] (Fig. 1.17f and f'). Conversely, a posteriorly positioned UB site causes the ureter to reach the primitive bladder prematurely owing to a shorter common nephric duct and thus positions the ureter to inappropriately enter the bladder at a more lateral angle than normal [340] (Fig. 1.17g and g').

The mechanism by which the ureter passes through the trigone is poorly understood. Traditional beliefs on bladder embryology suggested that the intramural passage was generated from ureteral smooth muscle fibers extending into the bladder and stretching across the base of the bladder during trigone formation [327, 357]. This theory has been refuted by a recent study involving smooth muscle cell lineage analysis in transgenic mice, which strongly suggests that ureter-derived tissue plays a minor role in forming the intramural passage [346]. Using two transgenic mouse lines that selectively labeled derivatives of either ureteral mesenchyme or bladder mesenchyme, it was shown that the ureter contributed only a thin sheath of longitudinal smooth muscle fibers to the intramural passage through the trigone. The same report provided evidence that the process involved in forming the intramural tunnel is intrinsic to the bladder and is not dependent on the presence of a ureter. This was revealed in *Pax2* mutant mice, which lack ureters due to agenesis of the posterior nephric duct. In the absence of ureters, *Pax2* mutant mice displayed normal bladder development and showed normal patterns of smooth muscle organization in the trigone. Moreover, the trigone of *Pax2* mutants exhibited gaps in smooth muscle which corresponded to regions where the intramural tunnels would be formed. These gaps contained blood vessels that would normally follow the intramural ureter yet were devoid of ureter epithelium and ureter-derived smooth muscle [346].

1.5.3.1 Genes Implicated in UVJ Formation

Due to the variable penetrance of a number of gene mutations which disrupt early stages of urinary tract development (e.g., nephric duct morphogenesis, ureteric bud outgrowth), there exists the opportunity to define functions for these genes at later stages of development, including UVJ formation [217]. These genes include *Foxc1*, *Foxc2*, *Robo2*, *Bmp4*, and *Spry1*. As described in Sect. 1.3.3.1, *Foxc1*, *Foxc2*, and *Robo2* are involved in repressing mesenchymal *Gdnf* expression and thereby limiting the sites where RET is activated in the nephric duct [107, 166]. *Bmp4* and *Spry1* exert their inhibitory roles on GDNF-RET signaling activity by altering the sensitivity of nephric duct/UB cells to GDNF [14, 197, 206]. Mice with loss-of-function mutations in any one of these five genes exhibit inappropriate activation of GDNF-RET signaling in nephric duct cells which leads to the formation of supernumerary UBs or anterior displacement of the site of UB outgrowth [14, 107, 166, 206]. Ureters arising from anterior ectopic UBs in these mutants are severely dilated and insert below the bladder neck, as predicted by Mackie and Stevens [184]. Although VUR has only been associated with *ROBO2* mutations in humans (OMIM: 602431) [180], evidence from genetic studies in mice strongly support the possibility that other genes involved in regulating GDNF-RET signaling activity may be mutated in humans with VUR [217].

Defective regulation of *Ret* expression in the nephric duct has been shown to associate with VUJ malformation in mice. In the absence of *Ret*

function, mouse kidney and ureter development is arrested and 80 % of mutants display renal and ureter agenesis [303, 304]. The remaining 20 % exhibited severe hydroureter and refluxing ureters that inserted abnormally below the bladder [61]. The defect in these *Ret* mutants is attributed to a failure to connect the nephric duct with the urogenital sinus until after the UB is formed. Morphologic analysis of nephric duct extension in *Ret* mutants revealed a defect in formation of cytoplasmic extensions which are thought to guide cells at the tip of the caudal nephric towards the urogenital sinus. *Ret* expression depends on retinoic acid signaling as revealed in *Aldh2* null mice, which display decreased Ret expression in the nephric duct and show a similar phenotype in nephric duct guidance [61]. In addition, *Ret* expression also depends on the function of *Gata3*. When *Gata3* function is removed in nephric duct cells by conditional deletion, embryos exhibit the same delay in uniting the nephric duct with urogenital sinus [61, 109]. *Gata3* expression is maintained in mice with mutations in genes involved in retinoic acid synthesis (e.g., *Aldh2* mutants), and *Aldh2* expression is unaffected in mutants [61]. Consequently, it is not known how Gata3 and retinoic acid signaling interact to control *Ret* expression.

Thirty percent of mice that carry the *Pax2*^{1Neu} mutation develop VUR which is associated with renal hypoplasia [25]. The *Pax2*^{1Neu} mutation in mice is a nonsense mutation and is orthologous to the *PAX2* mutation in humans with renal coloboma syndrome (OMIM: 120330), which is characterized by optic colobomas and a range of renal anomalies including VUR [295, 299]. Mice heterozygous for the *Pax2*^{1Neu} mutation demonstrate posteriorly placed UBs and ureters which penetrate the bladder aberrantly, resulting in a shortened intramural ureter [95]. The developmental defect in *Pax2*^{1Neu} heterozygous mutant mice is attributed to delayed separation of the ureter from the nephric duct which occurs independent of a defect in nephric duct elongation or *Ret* expression [25]. Moreover, ectopic positioning of the UB occurs without changes in *Gdnf* expression or dysregulated expression of genes which control the *Gdnf* expression domain, such as *Robo2*. Anterior budding is believed to be the primary cause of vesicoureteral reflux (VUR) in humans and mice [215]. However, at least two mouse models of VUR (i.e., *Pax2*$^{1Neu/+}$ mice and the C3H/HeJ mouse strain [216]) have been linked causally to posterior UB positioning resulting in delayed UVJ formation. These studies suggest that strict adherence to the spatiotemporal relationships between ureteric budding and distal ureter morphogenesis may not be universally applied to the pathogenesis of VUR in all instances.

Elimination of the common nephric duct by apoptosis is viewed as a key step in coordinating subsequent remodeling events during distal ureter morphogenesis. Studies in mice show that when common nephric duct degeneration is either delayed or blocked completely, the developing ureter does not establish proper spatial relationships with the primitive bladder and defects in ureter separation and bladder insertion occur [17]. Vitamin A or retinoic acid signaling appears to be necessary to regulate apoptosis and trigger common nephric duct degeneration. Removing retinoic acid signaling genetically results in urinary tract obstruction and dilated ureters which insert ectopically below the bladder. This phenotype is demonstrated in mice with homozygous null mutations in *Aldh2* [17] as well as in compound mutant mice doubly homozygous for null mutations in *Rarα* and *Rarβ* [16], which encode retinoic acid receptors. *Aldh2* mutants showed a marked reduction in common nephric duct apoptosis [17], suggesting that retinoic acid signaling is necessary for elimination of the common nephric duct by regulating apoptosis in this region. As in all retinoic acid pathway mutants, the common nephric duct persists and ureter separation is delayed, leading to the conclusion that the regulatory function of retinoic acid-dependent signals on common nephric duct apoptosis is critical for proper ureter remodeling.

A recent study implicated genes encoding members of the leukocyte antigen-related (LAR) family of phosphatases as strong candidates to activate common nephric duct cell apoptosis. LAR phosphatases function by dephosphorylating activated tyrosine kinase receptors [265], including activated RET [271]. Protein tyrosine

phosphatase, receptor type, F (*Ptprf*) and protein tyrosine phosphatase, receptor type, D (*Ptprd*) encode two LAR family members which are expressed in common nephric duct cells and are shown in vitro and in vivo to induce apoptosis by opposing RET activation [339]. In vivo analyses show that 52 % of mice with compound homozygous inactivating mutations in both *Ptprf* and *Ptprd* exhibited unilateral or bilateral urinary tract obstruction in addition to severe craniofacial abnormalities. Loss of LAR phosphatase function in these mutants suppressed apoptosis of common nephric duct cells and was associated with higher levels of phosphorylated RET as detected with specific antibodies. Detailed analyses of distal ureter morphogenesis in these mutants revealed significant remodeling defects that were associated with abnormal positioning of the UVJ below the bladder. In vitro studies in HEK cells further confirmed the functional relationships between LAR phosphatase activity and RET phosphorylation in the regulation of apoptosis. Since the UVJ phenotype in *Ptprf/Ptprd* double mutants is not fully penetrant, the mechanism for regulating apoptosis during ureter maturation must involve other genes which remain to be defined.

1.6 Human Urinary Tract Malformations and Genetic Associations

A number of urinary tract malformation syndromes display familial patterns of inheritance. For example, heterogeneous renal malformations are reported in 9 % of first-degree relatives of newborns with bilateral renal agenesis or bilateral renal dysgenesis [280]. Siblings of patients with VUR are reported to have a higher incidence of reflux than the normal population [57]. Hence, the occurrence of urinary tract malformations with familial patterns of inheritance in humans favors a genetic etiology. However, the majority of cases with isolated defects occur in patients without a familial pattern [280].

Candidate approaches have been used to screen patients or stillborn fetuses with isolated UT malformations for mutations in genes associated with syndromic malformations. In one study, a large European cohort of children with chronic kidney disease and severe renal hypoplasia and/or dysplasia was screened for mutations in *HNF1β*, *PAX2*, *SALL1*, *EYA1,* and *SIX1* [354]. These genes were selected on the basis of their associations with congenital syndromes that feature renal hypoplasia/dysplasia prominently. For instance, *HNF1β* mutations are known to cause renal cysts and diabetes syndrome (also known as MODY5; OMIM: 137920) [20, 21]. Human *PAX2* mutations are associated with renal coloboma syndrome, an autosomal dominant condition characterized in humans by optic nerve colobomas, hearing defects, and a spectrum of renal malformations including hypoplasia, renal agenesis, dysplasia, and VUR [295, 299]. *SALL1* mutations are found in patients with Townes-Brock syndrome (OMIM: 107480), which features anal, limb, and ear anomalies as well as renal hypoplasia, dysplasia, and VUR [160, 292]. *EYA1* and *SIX1* mutations have been identified in patients with branchio-oto-renal (OMIM: 113650), which presents with branchial arch, otic, and renal anomalies including unilateral or bilateral renal agenesis, hypoplasia, dysplasia as well as ureteral defects such as duplication or absence of ureter and megaureter [1]. In the European cohort study, *HNF1β* and *PAX2* mutations were detected in 15 % of all CKD subjects whereas *SALL1*, *EYA1*, and *SIX1* mutations were rarely detected in patients who did not have syndromic features [354]. Results from this study showed that mutations in *HNF1β* accounted for 25 % of mutations in patients with renal dysplasia or hypoplasia who manifest cysts, leading the authors to conclude that patients with cystic renal dysplasia should be screened for *HNF1β* mutations. Another study involving a multiethnic North American pediatric cohort reported an association for *HNF1β* mutations in 10 % of children with chronic kidney disease associated with unilateral renal agenesis or renal hypodysplasia [332]. The presence of *HNF1β* mutations has also been shown to exacerbate the phenotype of individuals with autosomal dominant polycystic kidney disease who carry mutations in PKD1

(polycystin-1) [19]. From a clinical perspective, it is possible that the presence of an *HNF1β* mutation may impart a higher risk of disease progression in patients with other types of urinary tract malformations.

Attention has been given to looking for mutations in genes encoding components of the GDNF-RET signaling cascade considering the strength of evidence for this pathway in normal urinary tract development in rodents [68]. In humans, heterozygous *RET* mutations cause Hirschsprung's disease (intestinal aganglionosis; OMIM# 142623) [83] and are reported rarely in patients with Hirschsprung's disease who also have renal hypoplasia and/or severe hydronephrosis [254]. A number of studies have reported low detection frequencies of *GDNF*, *RET*, and *GFRα1* mutations in cases of isolated UT malformation. In one study, *RET* mutations were reported in 8 of 29 stillborn fetuses (27.5 %) with unilateral or bilateral renal agenesis whereas no disease-causing mutations in *GDNF* or *GFRα1* were found in these cases [318]. Another study involving 105 fetuses with bilateral renal agenesis or hypodysplasia identified *RET* mutations in less than 7 % of cases and detected no *GDNF* mutations [145]. Similarly, a low detection rate for *GDNF* and *RET* mutations (<5 %) has been reported in a study involving 122 patients with UT malformations other than renal agenesis [53]. The explanation for detecting mutations in GDNF-RET pathway genes with such low frequency is not clear, although it is possible that there may be critical as-yet undefined differences between humans and mice regarding molecular control of UB outgrowth and branching events.

The pace of gene discovery in humans with isolated urinary tract malformations has been accelerated by the development of array-based analyses, whole exome sequencing, and genome-wide association studies (GWAS) [352, 353]. Despite technological advances in bioinformatics and high-throughput sequencing, it is estimated that a monogenic cause is identified in less than 20 % of patients with at least one recognizable urinary tract defect [128, 222, 289, 354]. Oligenic inheritance or gene modifier effects are likely to account for the remaining 80+% cases of children with isolated urinary tract malformations [354]. Table 1.6 lists a number of websites which are searchable databases for information on genetic associations with human disease.

Estimating the true prevalence of gene mutations in humans with severe urinary tract malformations

Table 1.6 Web resources for genetic associations with human disease

OMIM (Online Mendelian Inheritance in Man)	
Web address	http://www.ncbi.nlm.nih.gov/omim
Summary	Curated database cataloguing known gene-disease associations and providing clinical and molecular information on Mendelian disorders
HuGE Navigator (Human Genome Epidemiology Navigator)	
Web address	http://hugenavigator.net/HuGENavigator/home.do
Summary	Updated database in human genome epidemiology including population prevalence for genetic variants and disease-gene associations, gene-gene and gene-environment interactions, and interpretation of gene testing
DECIPHER (Database of Chromosomal Imbalances and Phenotype in Humans using Ensembl Resources)	
Web address	http://decipher.sanger.ac.uk/
Summary	Curated database cataloguing known gene-disease associations and providing clinical and molecular information on Mendelian disorders
GeneTests	
Web address	http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests
Summary	Information center for clinical genetic testing including clinical and research lab directory
CTD (Comparative Toxicogenomics Database)	
Web address	http://ctdbase.org/
Summary	Searchable database providing information on known and predicted gene-disease and chemical-disease associations

is complicated by interactions between genetic, environmental, and nutritional factors. Environmental, drug, or toxin exposure, and maternal nutrition have all been associated with the occurrence of urinary tract malformation in humans and rodents [18, 85, 167, 266]. The strength of genotype-phenotype correlation may be further weakened by incomplete penetrance or variable expressivity, as reported for non-syndromic forms of CAKUT with autosomal dominant inheritance patterns [194, 352]. These factors must be taken into consideration when weighing the benefits of offering genetic testing to patients or relatives.

References

1. Abdelhak S, Kalatzis V, Heilig R, Compain S, Samson DCV, Weil D, Cruaud C, Sahly I, Leibovici M, Bitner-Glindizicz M, Francis M, Lacombe D, Vigneron J, Charachon R, Boven K, Bedbeder P, Van Regemorter N, Weissenbach J, Petit C (1997) A human homologue of the *Drosophila eyes absent* gene underlies Branchio-Oto-Renal (BOR) syndrome and identifies a novel gene family. Nat Genet 15:157–164
2. Airik R, Bussen M, Singh MK, Petry M, Kispert A (2006) Tbx18 regulates the development of the ureteral mesenchyme. J Clin Invest 116(3):663–674
3. Airik R, Kispert A (2007) Down the tube of obstructive nephropathies: the importance of tissue interactions during ureter development. Kidney Int 72(12): 1459–1467
4. Al-Awqati Q, Goldberg MR (1998) Architectural patterns in branching morphogenesis in the kidney. Kidney Int 54:1832–1842
5. Alagille D, Estrada A, Hadchouel M, Gautier M, Odievre M, Dommergues JP (1987) Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. J Pediatr 110(2):195–200
6. Alcaraz A, Vinaixa F, Tejedo-Mateu A, Fores MM, Gotzens V, Mestres CA, Oliveira J, Carretero P (1991) Obstruction and recanalization of the ureter during embryonic development. J Urol 145(2): 410–416
7. Amri K, Freund N, Vilar J, Merlet-Benichou C, Lelievre-Pegorier M (1999) Adverse effects of hyperglycemia on kidney development in rats: in vivo and in vitro studies. Diabetes 48(11): 2240–2245
8. Angrist M, Bolk S, Halushka M, Lapchak PA, Chakravarti A (1996) Germline mutations in glial cell line-derived neurotrophic factor (GDNF) and RET in a Hirschsprung disease patient. Nat Genet 14(3):341–344
9. Barak H, Rosenfelder L, Schultheiss TM, Reshef R (2005) Cell fate specification along the anterior-posterior axis of the intermediate mesoderm. Dev Dyn 232(4):901–914
10. Barbaux S, Niaudet P, Gubler M-C, Grünfeld J-P, Jaubert F, Kuttenn F, Fékété CN, Souleyreau-Therville N, Thibaud E, Fellous M, McElreavey K (1997) Donor splice-site mutations in WT1 are responsible for Frasier syndrome. Nat Genet 17: 467–470
11. Bard J (1996) A new role for the stromal cells in kidney development. Bioessays 18(9):705–707
12. Bard JB (2002) Growth and death in the developing mammalian kidney: signals, receptors and conversations. Bioessays 24(1):72–82
13. Baskin LS, Hayward SW, Young P, Cunha GR (1996) Role of mesenchymal-epithelial interactions in normal bladder development. J Urol 156(5):1820–1827
14. Basson MA, Akbulut S, Watson-Johnson J, Simon R, Carroll TJ, Shakya R, Gross I, Martin GR, Lufkin T, McMahon AP, Wilson PD, Costantini FD, Mason IJ, Licht JD (2005) Sprouty1 is a critical regulator of GDNF/RET-mediated kidney induction. Dev Cell 8(2):229–239
15. Basson MA, Watson-Johnson J, Shakya R, Akbulut S, Hyink D, Costantini FD, Wilson PD, Mason IJ, Licht JD (2006) Branching morphogenesis of the ureteric epithelium during kidney development is coordinated by the opposing functions of GDNF and Sprouty1. Dev Biol 299(2):466–477
16. Batourina E, Choi C, Paragas N, Bello N, Hensle T, Costantini FD, Schuchardt A, Bacallao RL, Mendelsohn CL (2002) Distal ureter morphogenesis depends on epithelial cell remodeling mediated by vitamin A and Ret. Nat Genet 32(1):109–115
17. Batourina E, Tsai S, Lambert S, Sprenkle P, Viana R, Dutta S, Hensle T, Wang F, Niederreither K, McMahon AP, Carroll TJ, Mendelsohn CL (2005) Apoptosis induced by vitamin A signaling is crucial for connecting the ureters to the bladder. Nat Genet 37(10):1082–1089
18. Battin M, Albersheim S, Newman D (1995) Congenital genitourinary tract abnormalities following cocaine exposure in utero. Am J Perinatol 12(6): 425–428
19. Bergmann C, von Bothmer J, Ortiz Bruchle N, Venghaus A, Frank V, Fehrenbach H, Hampel T, Pape L, Buske A, Jonsson J, Sarioglu N, Santos A, Ferreira JC, Becker JU, Cremer R, Hoefele J, Benz MR, Weber LT, Buettner R, Zerres K (2011) Mutations in multiple PKD genes may explain early and severe polycystic kidney disease. J Am Soc Nephrol 22(11):2047–2056
20. Bingham C, Bulman MP, Ellard S, Allen LI, Lipkin GW, Hoff WG, Woolf AS, Rizzoni G, Novelli G, Nicholls AJ, Hattersley AT (2001) Mutations in the

hepatocyte nuclear factor-1beta gene are associated with familial hypoplastic glomerulocystic kidney disease. Am J Hum Genet 68(1):219–224
21. Bingham C, Ellard S, Allen L, Bulman M, Shepherd M, Frayling T, Berry PJ, Clark PM, Lindner T, Bell GI, Ryffel GU, Nicholls AJ, Hattersley AT (2000) Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1 beta. Kidney Int 57(3): 898–907
22. Blomqvist SR, Vidarsson H, Fitzgerald S, Johansson BR, Ollerstam A, Brown R, Persson AE, Bergstrom GG, Enerback S (2004) Distal renal tubular acidosis in mice that lack the forkhead transcription factor Foxi1. J Clin Invest 113(11):1560–1570
23. Bok G, Drews U (1983) The role of the Wolffian ducts in the formation of the sinus vagina: an organ culture study. J Embryol Exp Morphol 73: 275–295
24. Bort R, Signore M, Tremblay K, Martinez Barbera JP, Zaret KS (2006) Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development. Dev Biol 290(1):44–56
25. Boualia SK, Gaitan Y, Murawski I, Nadon R, Gupta IR, Bouchard M (2011) Vesicoureteral reflux and other urinary tract malformations in mice compound heterozygous for Pax2 and Emx2. PLoS One 6(6):e21529
26. Bouchard M, Souabni A, Mandler M, Neubuser A, Busslinger M (2002) Nephric lineage specification by Pax2 and Pax8. Genes Dev 16(22):2958–2970
27. Boyle S, de Caestecker M (2006) Role of transcriptional networks in coordinating early events during kidney development. Am J Physiol Renal Physiol 291(1):F1–F8
28. Boyle S, Misfeldt A, Chandler KJ, Deal KK, Southard-Smith EM, Mortlock DP, Baldwin HS, de Caestecker M (2008) Fate mapping using Cited1-CreERT2 mice demonstrates that the cap mesenchyme contains self-renewing progenitor cells and gives rise exclusively to nephronic epithelia. Dev Biol 313(1):234–245
29. Brenner-Anantharam A, Cebrian C, Guillaume R, Hurtado R, Sun TT, Herzlinger D (2007) Tailbud-derived mesenchyme promotes urinary tract segmentation via BMP4 signaling. Development 134(10):1967–1975
30. Brenner BM, Anderson S (1992) The interrelationships among filtration surface area, blood pressure, and chronic renal disease. J Cardiovas Pharmacol 19(Suppl 6):S1–S7
31. Brenner BM, Garcia DL, Anderson S (1988) Glomeruli and blood pressure. Less of one, more the other? Am J Hypertens 1(4 Pt 1):335–347
32. Bridgewater D, Cox B, Cain J, Lau A, Athaide V, Gill PS, Kuure S, Sainio K, Rosenblum ND (2008) Canonical WNT/beta-catenin signaling is required for ureteric branching. Dev Biol 317(1):83–94
33. Brodbeck S, Englert C (2004) Genetic determination of nephrogenesis: the Pax/Eya/Six gene network. Pediatr Nephrol 19(3):249–255
34. Brophy PD, Ostrom L, Lang KM, Dressler GR (2001) Regulation of ureteric bud outgrowth by Pax2-dependent activation of the glial derived neurotrophic factor gene. Development 128:4747–4756
35. Brunskill EW, Aronow BJ, Georgas K, Rumballe B, Valerius MT, Aronow J, Kaimal V, Jegga AG, Yu J, Grimmond S, McMahon AP, Patterson LT, Little MH, Potter SS (2008) Atlas of gene expression in the developing kidney at microanatomic resolution. Dev Cell 15(5):781–791
36. Burn SF, Webb A, Berry RL, Davies JA, Ferrer-Vaquer A, Hadjantonakis AK, Hastie ND, Hohenstein P (2011) Calcium/NFAT signalling promotes early nephrogenesis. Dev Biol 352(2):288–298
37. Bush KT, Sakurai H, Steer DL, Leonard MO, Sampogna RV, Meyer TN, Schwesinger C, Qiao J, Nigam SK (2004) TGF-beta superfamily members modulate growth, branching, shaping, and patterning of the ureteric bud. Dev Biol 266(2):285–298
38. Cacalano G, Farinas I, Wang LC, Hagler K, Forgie A, Moore M, Armanini M, Phillips H, Ryan AM, Reichardt LF, Hynes M, Davies A, Rosenthal A (1998) GFRalpha1 is an essential receptor component for GDNF in the developing nervous system and kidney. Neuron 21:53–62
39. Cain JE, Hartwig S, Bertram JF, Rosenblum ND (2008) Bone morphogenetic protein signaling in the developing kidney: present and future. Differentiation 76(8):831–842
40. Cain JE, Islam E, Haxho F, Blake J, Rosenblum ND (2011) GLI3 repressor controls functional development of the mouse ureter. J Clin Invest 121(3): 1199–1206
41. Cain JE, Islam E, Haxho F, Chen L, Bridgewater D, Nieuwenhuis E, Hui CC, Rosenblum ND (2009) GLI3 repressor controls nephron number via regulation of Wnt11 and Ret in ureteric tip cells. PLoS One 4(10):e7313
42. Cain JE, Nion T, Jeulin D, Bertram JF (2005) Exogenous BMP-4 amplifies asymmetric ureteric branching in the developing mouse kidney in vitro. Kidney Int 67(2):420–431
43. Cancilla B, Davies A, Cauchi JA, Risbridger GP, Bertram JF (2001) Fibroblast growth factor receptors and their ligands in the adult rat kidney. Kidney Int 60(1):147–155
44. Cano-Gauci DF, Song H, Yang H, McKerlie C, Choo B, Shi W, Pullano R, Piscione TD, Grisaru S, Soon S, Sedlackova L, Tanswell AK, Mak TW, Yeger H, Rosenblum ND, Filmus J (1999) Glypican-3-deficient mice exhibit developmental overgrowth and some of the renal abnormalities typical of Simpson-Golabi-Behmel syndrome. J Cell Biol 146:255–264
45. Cantley LG, Barros EJG, Gandhi M, Rauchman M, Nigam SK (1994) Regulation of mitogenesis, motogenesis, and tubulogenesis by hepatocyte growth

factor in renal collecting duct cells. Am J Physiol 267:F271–F280
46. Cao M, Liu B, Cunha G, Baskin L (2008) Urothelium patterns bladder smooth muscle location. Pediatr Res 64(4):352–357
47. Cao M, Tasian G, Wang MH, Liu B, Cunha G, Baskin L (2010) Urothelium-derived Sonic hedgehog promotes mesenchymal proliferation and induces bladder smooth muscle differentiation. Differentiation 79(4–5):244–250
48. Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP (2005) Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. Dev Cell 9(2):283–292
49. Caruana G, Cullen-McEwen L, Nelson AL, Kostoulias X, Woods K, Gardiner B, Davis MJ, Taylor DF, Teasdale RD, Grimmond SM, Little MH, Bertram JF (2006) Spatial gene expression in the T-stage mouse metanephros. Gene Expr Patterns 6(8):807–825
50. Caubit X, Lye CM, Martin E, Core N, Long DA, Vola C, Jenkins D, Garratt AN, Skaer H, Woolf AS, Fasano L (2008) Teashirt 3 is necessary for ureteral smooth muscle differentiation downstream of SHH and BMP4. Development 135(19):3301–3310
51. Cebrian C, Borodo K, Charles N, Herzlinger DA (2004) Morphometric index of the developing murine kidney. Dev Dyn 231(3):601–608
52. Challen G, Gardiner B, Caruana G, Kostoulias X, Martinez G, Crowe M, Taylor DF, Bertram J, Little M, Grimmond SM (2005) Temporal and spatial transcriptional programs in murine kidney development. Physiol Genom 23(2):159–171
53. Chatterjee R, Ramos E, Hoffman M, Vanwinkle J, Martin DR, Davis TK, Hoshi M, Hmiel SP, Beck A, Hruska K, Coplen D, Liapis H, Mitra R, Druley T, Austin P, Jain S (2012) Traditional and targeted exome sequencing reveals common, rare and novel functional deleterious variants in RET-signaling complex in a cohort of living US patients with urinary tract malformations. Human Genet 131(11):1725–1738
54. Chen L, Al-Awqati Q (2005) Segmental expression of Notch and Hairy genes in nephrogenesis. Am J Physiol Renal Physiol 288(5):F939–F952
55. Cheng HT, Kim M, Valerius MT, Surendran K, Schuster-Gossler K, Gossler A, McMahon AP, Kopan R (2007) Notch2, but not Notch1, is required for proximal fate acquisition in the mammalian nephron. Development 134(4):801–811
56. Cheng W, Yeung CK, Ng YK, Zhang JR, Hui CC, Kim PC (2008) Sonic Hedgehog mediator Gli2 regulates bladder mesenchymal patterning. J Urol 180(4):1543–1550
57. Chertin B, Puri P (2003) Familial vesicoureteral reflux. J Urol 169(5):1804–1808
58. Chi L, Saarela U, Railo A, Prunskaite-Hyyrylainen R, Skovorodkin I, Anthony S, Katsu K, Liu Y, Shan J, Salgueiro AM, Belo JA, Davies J, Yokouchi Y, Vainio SJ (2011) A secreted BMP antagonist, Cer1, fine tunes the spatial organization of the ureteric bud tree during mouse kidney development. PLoS One 6(11):e27676
59. Chi L, Zhang S, Lin Y, Prunskaite-Hyyrylainen R, Vuolteenaho R, Itaranta P, Vainio S (2004) Sprouty proteins regulate ureteric branching by coordinating reciprocal epithelial Wnt11, mesenchymal Gdnf and stromal Fgf7 signalling during kidney development. Development 131(14):3345–3356
60. Chi X, Michos O, Shakya R, Riccio P, Enomoto H, Licht JD, Asai N, Takahashi M, Ohgami N, Kato M, Mendelsohn C, Costantini F (2009) Ret-dependent cell rearrangements in the Wolffian duct epithelium initiate ureteric bud morphogenesis. Dev Cell 17(2):199–209
61. Chia I, Grote D, Marcotte M, Batourina E, Mendelsohn C, Bouchard M (2011) Nephric duct insertion is a crucial step in urinary tract maturation that is regulated by a Gata3-Raldh2-Ret molecular network in mice. Development 138(10):2089–2097
62. Chiba S (2006) Notch signaling in stem cell systems. Stem Cells (Dayton, Ohio) 24(11):2437–2447
63. Clarren SK (1994) Chapter 27: Malformations of the renal system. In: Holliday MA, Barratt TM, Avner ED (eds) Pediatric nephrology, 3rd edn. Williams & Wilkins, Baltimore
64. Clark AT, Young RJ, Bertram JF (2001) In vitro studies on the roles of transforming growth factor-beta 1 in rat metanephric development. Kidney Int 59:1641–1653
65. Clarke JC, Patel SR, Raymond RM Jr, Andrew S, Robinson BG, Dressler GR, Brophy PD (2006) Regulation of c-Ret in the developing kidney is responsive to Pax2 gene dosage. Hum Mol Genet 15(23):3420–3428
66. Constantinou CE (1978) Contractility of the pyeloureteral pacemaker system. Urol Int 33(6):399–416
67. Coppes MJ, Liefers GJ, Higuchi M, Zinn AB, Balfe JW, Williams BR (1992) Inherited WT1 mutation in Denys-Drash syndrome. Cancer Res 52(21):6125–6128
68. Costantini F (2010) GDNF/Ret signaling and renal branching morphogenesis: from mesenchymal signals to epithelial cell behaviors. Organogenesis 6(4):252–262
69. Cui S, Schwartz L, Quaggin SE (2003) Pod1 is required in stromal cells for glomerulogenesis. Dev Dyn 226(3):512–522
70. David SG, Cebrian C, Vaughan ED Jr, Herzlinger D (2005) c-kit and ureteral peristalsis. J Urol 173(1):292–295
71. Deschamps J, van Nes J (2005) Developmental regulation of the Hox genes during axial morphogenesis in the mouse. Development 132(13):2931–2942
72. Dewulf N, Verschueren K, Lonnoy O, Morén A, Grimsby S, Vande Spiegle K, Miyazono K, Huylebroeck D, Ten Dijke P (1995) Distinct spatial and temporal expression patterns of two type 1 receptors

for bone morphogenetic proteins during mouse embryogenesis. Endocrinology 136:2652–2663
73. Donovan MJ, Natoli TA, Sainio K, Amstutz A, Jaenisch R, Sariola H, Kreidberg JA (1999) Initial differentiation of the metanephric mesenchyme is independent of WT1 and the ureteric bud. Dev Genet 24:252–262
74. Douglas-Denton R, Moritz KM, Bertram JF, Wintour EM (2002) Compensatory renal growth after unilateral nephrectomy in the ovine fetus. J Am Soc Nephrol 13(2):406–410
75. Dressler GR (2006) The cellular basis of kidney development. Annu Rev Cell Dev Biol 22:509–529
76. Dressler GR (2009) Advances in early kidney specification, development and patterning. Development 136(23):3863–3874
77. Drews U, Sulak O, Schenck PA (2002) Androgens and the development of the vagina. Biol Reprod 67(4):1353–1359
78. Dreyer SD, Zhou G, Baldini A, Winterpacht A, Zabel B, Cole W, Johnson RL, Lee B (1998) Mutations in LMX1B cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. Nat Genet 19:47–50
79. Dudley AT, Lyons KM, Robertson EJ (1995) A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev 9:2795–2807
80. Dudley AT, Robertson EJ (1997) Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7 deficient embryos. Dev Dyn 208:349–362
81. Dunn NR, Winnier GE, Hargett LK, Schrick JJ, Fogo AB, Hogan BLM (1997) Haploinsufficient phenotypes in Bmp4 heterozygous null mice and modification by mutations in Gli3 and Alx4. Dev Biol 188:235–247
82. Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P, Smith D, Ponder B, Costantini F, Saarma M, Sariola H, Pachnis V (1996) GDNF signalling through the Ret receptor tyrosine kinase. Nature 381:789–792
83. Edery P, Lyonnet S, Mulligan LM, Pelet A, Dow E, Abel L, Holder S, Nihoul-Fekete C, Ponder BAJ, Munnich A (1994) Mutations of the *RET* proto-oncogene in Hirschprung's disease. Nature 367:378–380
84. Edwin F, Anderson K, Ying C, Patel TB (2009) Intermolecular interactions of Sprouty proteins and their implications in development and disease. Mol Pharmacol 76(4):679–691
85. El-Dahr SS, Harrison-Bernard LM, Dipp S, Yosipiv IV, Meleg-Smith S (2000) Bradykinin B2 null mice are prone to renal dysplasia: gene-environment interactions in kidney development. Physiol Genom 3(3):121–131
86. Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, Smith SD, Sklar J (1991) TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 66(4):649–661
87. Emerick KM, Rand EB, Goldmuntz E, Krantz ID, Spinner NB, Piccoli DA (1999) Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. Hepatology 29(3):822–829
88. Enomoto H, Araki T, Jackman A, Heuckeroth RO, Snider WD, Johnson EMJ, Milbrandt J (1998) GFRa 1-deficient mice have deficits in the enteric nervous system and kidneys. Neuron 21:317–324
89. Eremina V, Cui S, Gerber H, Ferrara N, Haigh J, Nagy A, Ema M, Rossant J, Jothy S, Miner JH, Quaggin SE (2006) Vascular endothelial growth factor a signaling in the podocyte-endothelial compartment is required for mesangial cell migration and survival. J Am Soc Nephrol 17(3):724–735
90. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE (2003) Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest 111(5):707–716
91. Erkner A, Gallet A, Angelats C, Fasano L, Kerridge S (1999) The role of Teashirt in proximal leg development in Drosophila: ectopic Teashirt expression reveals different cell behaviours in ventral and dorsal domains. Dev Biol 215(2):221–232
92. Evan AP, Gattone VH II, Schwartz GJ (1983) Development of solute transport in rabbit proximal tubule. II. Morphologic segmentation. Am J Physiol 245(3):F391–F407
93. Fagman H, Andersson L, Nilsson M (2006) The developing mouse thyroid: embryonic vessel contacts and parenchymal growth pattern during specification, budding, migration, and lobulation. Dev Dyn 235(2):444–455
94. Fasano L, Roder L, Core N, Alexandre E, Vola C, Jacq B, Kerridge S (1991) The gene teashirt is required for the development of Drosophila embryonic trunk segments and encodes a protein with widely spaced zinc finger motifs. Cell 64(1):63–79
95. Favor J, Sandulache R, Neuhauser-Klaus A, Pretsch W, Chaterjee B, Senft E, Wurst W, Blanquet V, Grimes P, Sporle R, Schughart K (1996) The mouse Pax (1Neu) mutation is identical to a human PAX2 mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye and kidney. Proc Natl Acad Sci U S A 93:13870–13875
96. Fenske S, Mader R, Scharr A, Paparizos C, Cao-Ehlker X, Michalakis S, Shaltiel L, Weidinger M, Stieber J, Feil S, Feil R, Hofmann F, Wahl-Schott C, Biel M (2011) HCN3 contributes to the ventricular action potential waveform in the murine heart. Circ Res 109(9):1015–1023
97. Fetterman GH, Shuplock NA, Philipp FJ, Gregg HS (1965) The growth and maturation of human glomeruli and proximal convolutions from term to adulthood: studies by microdissection. Pediatrics 35:601–619

98. Fischer E, Legue E, Doyen A, Nato F, Nicolas JF, Torres V, Yaniv M, Pontoglio M (2006) Defective planar cell polarity in polycystic kidney disease. Nat Genet 38(1):21–23
99. Gao F, Maiti S, Sun G, Ordonez NG, Udtha M, Deng JM, Behringer RR, Huff V (2004) The Wt1+/R394W mouse displays glomerulosclerosis and early-onset renal failure characteristic of human Denys-Drash syndrome. Mol Cell Biol 24(22):9899–9910
100. Garrod DR, Fleming S (1990) Early expression of desmosomal components during kidney tubule morphogenesis in human and murine embryos. Development 108(2):313–321
101. Georgas K, Rumballe B, Valerius MT, Chiu HS, Thiagarajan RD, Lesieur E, Aronow BJ, Brunskill EW, Combes AN, Tang D, Taylor D, Grimmond SM, Potter SS, McMahon AP, Little MH (2009) Analysis of early nephron patterning reveals a role for distal RV proliferation in fusion to the ureteric tip via a cap mesenchyme-derived connecting segment. Dev Biol 332(2):273–286
102. Gilpin SA, Gosling JA (1983) Smooth muscle in the wall of the developing human urinary bladder and urethra. J Anat 137(Pt 3):503–512
103. Godin RE, Robertson EJ, Dudley AT (1999) Role of BMP family members during kidney development. Int J Dev Biol 43:405–411
104. Godin RE, Takaesu NT, Robertson EJ, Dudley AT (1998) Regulation of BMP7 expression during kidney development. Development 125:3473–3482
105. Gong KQ, Yallowitz AR, Sun H, Dressler GR, Wellik DM (2007) A Hox-Eya-Pax complex regulates early kidney developmental gene expression. Mol Cell Biol 27(21):7661–7668
106. Grieshammer U, Cebrian C, Ilagan R, Meyers E, Herzlinger D, Martin GR (2005) FGF8 is required for cell survival at distinct stages of nephrogenesis and for regulation of gene expression in nascent nephrons. Development 132(17):3847–3857
107. Grieshammer U, Le M, Plump AS, Wang F, Tessier-Lavigne M, Martin GR (2004) SLIT2-mediated ROBO2 signaling restricts kidney induction to a single site. Dev Cell 6(5):709–717
108. Grote D, Boualia SK, Souabni A, Merkel C, Chi X, Costantini F, Carroll T, Bouchard M (2008) Gata3 acts downstream of beta-catenin signaling to prevent ectopic metanephric kidney induction. PLoS Genet 4(12):e1000316
109. Grote D, Souabni A, Busslinger M, Bouchard M (2006) Pax 2/8-regulated Gata 3 expression is necessary for morphogenesis and guidance of the nephric duct in the developing kidney. Development 133(1):53–61
110. Guillaume R, Bressan M, Herzlinger D (2009) Paraxial mesoderm contributes stromal cells to the developing kidney. Dev Biol 329(2):169–175
111. Guo G, Morrison DJ, Licht JD, Quaggin SE (2004) WT1 activates a glomerular-specific enhancer identified from the human nephrin gene. J Am Soc Nephrol 15(11):2851–2856
112. Guo JK, Menke AL, Gubler MC, Clarke AR, Harrison D, Hammes A, Hastie ND, Schedl A (2002) WT1 is a key regulator of podocyte function: reduced expression levels cause crescentic glomerulonephritis and mesangial sclerosis. Hum Mol Genet 11(6):651–659
113. Hall JG, Pallister PD, Clarren SK, Beckwith JB, Wiglesworth FW, Fraser FC, Cho S, Benke PJ, Reed SD (1980) Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus and postaxial polydactyly–a new syndrome? Part I: Clinical, causal, and pathogenetic considerations. Am J Med Genet 7(1):47–74
114. Hammes A, Guo JK, Lutsch G, Leheste JR, Landrock D, Ziegler U, Gubler MC, Schedl A (2001) Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. Cell 106(3):319–329
115. Hanson J, Gorman J, Reese J, Fraizer G (2007) Regulation of vascular endothelial growth factor, VEGF, gene promoter by the tumor suppressor, WT1. Front Biosci: J Virtual Libr 12: 2279–2290
116. Haraguchi R, Mo R, Hui C, Motoyama J, Makino S, Shiroishi T, Gaffield W, Yamada G (2001) Unique functions of Sonic hedgehog signaling during external genitalia development. Development 128(21): 4241–4250
117. Harding SD, Armit C, Armstrong J, Brennan J, Cheng Y, Haggarty B, Houghton D, Lloyd-MacGilp S, Pi X, Roochun Y, Sharghi M, Tindal C, McMahon AP, Gottesman B, Little MH, Georgas K, Aronow BJ, Potter SS, Brunskill EW, Southard-Smith EM, Mendelsohn C, Baldock RA, Davies JA, Davidson D (2011) The GUDMAP database–an online resource for genitourinary research. Development 138(13):2845–2853
118. Harrison MR, Golbus MS, Filly RA, Nakayama DK, Callen PW, de Lorimier AA, Hricak H (1982) Management of the fetus with congenital hydronephrosis. J Pediatr Surg 17(6):728–742
119. Hartwig S, Bridgewater D, Di Giovanni V, Cain J, Mishina Y, Rosenblum ND (2008) BMP receptor ALK3 controls collecting system development. J Am Soc Nephrol 19(1):117–124
120. Hartwig S, Ho J, Pandey P, Macisaac K, Taglienti M, Xiang M, Alterovitz G, Ramoni M, Fraenkel E, Kreidberg JA (2010) Genomic characterization of Wilms' tumor suppressor 1 targets in nephron progenitor cells during kidney development. Development 137(7):1189–1203
121. Hartwig S, Hu MC, Cella C, Piscione T, Filmus J, Rosenblum ND (2005) Glypican-3 modulates inhibitory Bmp2-Smad signaling to control renal development in vivo. Mech Dev 122(7–8):928–938
122. Hatini V, Huh SO, Herzlinger D, Soares VC, Lai E (1996) Essential role of stromal mesenchyme in kidney morphogenesis revealed by targeted disruption of Winged Helix transcription factor *BF-2*. Genes Dev 10:1467–1478

123. Hendry C, Rumballe B, Moritz K, Little MH (2011) Defining and redefining the nephron progenitor population. Pediatr Nephrol 26(9):1395–1406
124. Higgins JP, Wang L, Kambham N, Montgomery K, Mason V, Vogelmann SU, Lemley KV, Brown PO, Brooks JD, van de Rijn M (2004) Gene expression in the normal adult human kidney assessed by complementary DNA microarray. Mol Biol Cell 15(2):649–656
125. Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, Van Velzen D (1992) The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol 99(4):296–301
126. Hinchliffe SA, Sargent PH, Howard CV, Chan YF, van Velzen D (1991) Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the disector method and Cavalieri principle. Lab Invest 64(6):777–784
127. Hoppe CC, Evans RG, Bertram JF, Moritz KM (2007) Effects of dietary protein restriction on nephron number in the mouse. Am J Physiol Regul Integr Comp Physiol 292(5):R1768–R1774
128. Hoskins BE, Cramer CH 2nd, Tasic V, Kehinde EO, Ashraf S, Bogdanovic R, Hoefele J, Pohl M, Hildebrandt F (2008) Missense mutations in EYA1 and TCF2 are a rare cause of urinary tract malformations. Nephrol Dial Transplant 23(2):777–779
129. Hoy WE, Bertram JF, Denton RD, Zimanyi M, Samuel T, Hughson MD (2008) Nephron number, glomerular volume, renal disease and hypertension. Curr Opin Nephrol Hypertens 17(3):258–265
130. Hu MC, Rosenblum ND (2003) Genetic regulation of branching morphogenesis: lessons learned from loss-of-function phenotypes. Pediatr Res 54(4):433–438
131. Hughson M, Farris AB 3rd, Douglas-Denton R, Hoy WE, Bertram JF (2003) Glomerular number and size in autopsy kidneys: the relationship to birth weight. Kidney Int 63(6):2113–2122
132. Hui CC, Angers S (2011) Gli proteins in development and disease. Annu Rev Cell Dev Biol 27:513–537
133. Hurtado R, Bub G, Herzlinger D (2010) The pelvis-kidney junction contains HCN3, a hyperpolarization-activated cation channel that triggers ureter peristalsis. Kidney Int 77(6):500–508
134. Hyink DP, Tucker DC, St John PL, Leardkamolkarn V, Accavitti MA, Abrass CK, Abrahamson DR (1996) Endogenous origin of glomerular endothelial and mesangial cells in grafts of embryonic kidneys. Am J Physiol 270(5 Pt 2):F886–F899
135. Hynes PJ, Fraher JP (2004) The development of the male genitourinary system. I. The origin of the urorectal septum and the formation of the perineum. Br J Plast Surg 57(1):27–36
136. Iafolla K, Fratkin JD, Spiegel PK, Cohen MM Jr, Graham JM Jr (1989) Case report and delineation of the congenital hypothalamic hamartoblastoma syndrome (Pallister-Hall syndrome). Am J Med Genet 33(4):489–499
137. Iglesias DM, Hueber PA, Chu L, Campbell R, Patenaude AM, Dziarmaga AJ, Quinlan J, Mohamed O, Dufort D, Goodyer PR (2007) Canonical WNT signaling during kidney development. Am J Physiol Renal Physiol 293(2):F494–F500
138. Ishibe S, Karihaloo A, Ma H, Zhang J, Marlier A, Mitobe M, Togawa A, Schmitt R, Czyczk J, Kashgarian M, Geller DS, Thorgeirsson SS, Cantley LG (2009) Met and the epidermal growth factor receptor act cooperatively to regulate final nephron number and maintain collecting duct morphology. Development 136(2):337–345
139. Hutson J (2008) Development of the urogenital system. In: Standring S (ed) Gray's anatomy. 40th edn. Churchill Livingstone Elsevier, Edinburgh/New York, pp 1305–1325
140. Jackson SM, Nakato H, Sugiura M, Jannuzi A, Oakes R, Kaluza V, Golden C, Selleck SB (1997) dally, a drosophila glypican, controls cellular responses to the TGF-ß-related morphogen, Dpp. Development 124:4113–4120
141. James RG, Kamei CN, Wang Q, Jiang R, Schultheiss TM (2006) Odd-skipped related 1 is required for development of the metanephric kidney and regulates formation and differentiation of kidney precursor cells. Development 133(15):2995–3004
142. James RG, Schultheiss TM (2003) Patterning of the avian intermediate mesoderm by lateral plate and axial tissues. Dev Biol 253(1):109–124
143. James RG, Schultheiss TM (2005) Bmp signaling promotes intermediate mesoderm gene expression in a dose-dependent, cell-autonomous and translation-dependent manner. Dev Biol 288(1):113–125
144. Jeanpierre C, Denamur E, Henry I, Cabanis MO, Luce S, Cecille A, Elion J, Peuchmaur M, Loirat C, Niaudet P, Gubler MC, Junien C (1998) Identification of constitutional WT1 mutations, in patients with isolated diffuse mesangial sclerosis, and analysis of genotype/phenotype correlations by use of a computerized mutation database. Am J Hum Genet 62(4):824–833
145. Jeanpierre C, Mace G, Parisot M, Moriniere V, Pawtowsky A, Benabou M, Martinovic J, Amiel J, Attie-Bitach T, Delezoide AL, Loget P, Blanchet P, Gaillard D, Gonzales M, Carpentier W, Nitschke P, Tores F, Heidet L, Antignac C, Salomon R (2011) RET and GDNF mutations are rare in fetuses with renal agenesis or other severe kidney development defects. J Med Genet 48(7):497–504
146. Jenkins D, Bitner-Glindzicz M, Malcolm S, Hu CC, Allison J, Winyard PJ, Gullett AM, Thomas DF, Belk RA, Feather SA, Sun TT, Woolf AS (2005) De novo Uroplakin IIIa heterozygous mutations cause human renal adysplasia leading to severe kidney failure. J Am Soc Nephrol 16(7):2141–2149
147. Jeong HW, Jeon US, Koo BK, Kim WY, Im SK, Shin J, Cho Y, Kim J, Kong YY (2009) Inactivation of Notch signaling in the renal collecting duct causes nephrogenic diabetes insipidus in mice. J Clin Invest 119(11):3290–3300

148. Kang S, Graham JM Jr, Olney AH, Biesecker LG (1997) GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome. Nat Genet 15(3):266–268
149. Karihaloo A, O'Rourke DA, Nickel C, Spokes K, Cantley LG (2001) Differential MAPK pathways utilized for HGF- and EGF-dependent renal epithelial morphogenesis. J Biol Chem 276(12):9166–9173
150. Karner CM, Chirumamilla R, Aoki S, Igarashi P, Wallingford JB, Carroll TJ (2009) Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. Nat Genet 41(7):793–799
151. Kispert A, Vainio S, McMahon AP (1998) Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. Development 125:4225–4234
152. Kispert A, Vainio S, Shen L, Rowitch DH, McMahon AP (1996) Proteoglycans are required for maintenance of *Wnt-11* expression in the ureter tips. Development 122:3627–3637
153. Kitamoto Y, Tokunaga H, Tomita K (1997) Vascular endothelial growth factor is an essential molecule for mouse kidney development: glomerulogenesis and nephrogenesis. J Clin Invest 99(10):2351–2357
154. Klamt B, Koziell A, Poulat F, Wieacker P, Scambler P, Berta P, Gessler M (1998) Frasier syndrome is caused by defective alternative splicing of WT1 leading to an altered ratio of WT1+/-KTS splice isoforms. Hum Mol Genet 7:709–714
155. Klein G, Langegger M, Goridis C, Ekblom P (1988) Neural cell adhesion molecules during embryonic induction and development of the kidney. Development 102(4):749–761
156. Kleinman LI, Lubbe RJ (1972) Factors affecting the maturation of glomerular filtration rate and renal plasma flow in the new-born dog. J Physiol 223(2): 395–409
157. Kobayashi A, Kwan KM, Carroll TJ, McMahon AP, Mendelsohn CL, Behringer RR (2005) Distinct and sequential tissue-specific activities of the LIM-class homeobox gene Lim1 for tubular morphogenesis during kidney development. Development 132(12): 2809–2823
158. Kobayashi A, Valerius MT, Mugford JW, Carroll TJ, Self M, Oliver G, McMahon AP (2008) Six2 defines and regulates a multipotent self-renewing nephron progenitor population throughout mammalian kidney development. Cell Stem Cell 3(2):169–181
159. Kohan DE (2008) Progress in gene targeting: using mutant mice to study renal function and disease. Kidney Int 74(4):427–437
160. Kohlhase J, Wischermann A, Reichenbach H, Froster U, Engel W (1998) Mutations in the SALL1 putative transcription factor gene cause Townes-Brocks syndrome. Nat Genet 18:81–83
161. Koleda P, Apoznanski W, Wozniak Z, Rusiecki L, Szydelko T, Pilecki W, Polok M, Kalka D, Pupka A (2012) Changes in interstitial cell of Cajal-like cells density in congenital ureteropelvic junction obstruction. Int Urol Nephrol 44(1):7–12
162. Kong XT, Deng FM, Hu P, Liang FX, Zhou G, Auerbach AB, Genieser N, Nelson PK, Robbins ES, Shapiro E, Kachar B, Sun TT (2004) Roles of uroplakins in plaque formation, umbrella cell enlargement, and urinary tract diseases. J Cell Biol 167(6):1195–1204
163. Korostylev A, Worzfeld T, Deng S, Friedel RH, Swiercz JM, Vodrazka P, Maier V, Hirschberg A, Ohoka Y, Inagaki S, Offermanns S, Kuner R (2008) A functional role for semaphorin 4D/plexin B1 interactions in epithelial branching morphogenesis during organogenesis. Development 135(20): 3333–3343
164. Kreidberg JA (2003) Podocyte differentiation and glomerulogenesis. J Am Soc Nephrol 14(3): 806–814
165. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenisch R (1993) WT-1 is required for early kidney development. Cell 74:679–691
166. Kume T, Deng K, Hogan BL (2000) Murine forkhead/winged helix genes Foxc1 (Mf1) and Foxc2 (Mfh1) are required for the early organogenesis of the kidney and urinary tract. Development 127:1387–1395
167. Langley-Evans SC, Welham SJ, Jackson AA (1999) Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. Life Sci 64(11):965–974
168. Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C (1994) Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev 8:1875–1887
169. Levinson RS, Batourina E, Choi C, Vorontchikhina M, Kitajewski J, Mendelsohn CL (2005) Foxd1-dependent signals control cellularity in the renal capsule, a structure required for normal renal development. Development 132(3):529–539
170. Levitt MA, Pena A (2007) Anorectal malformations. Orphanet J Rare Dis 2
171. Liang FX, Bosland MC, Huang H, Romih R, Baptiste S, Deng FM, Wu XR, Shapiro E, Sun TT (2005) Cellular basis of urothelial squamous metaplasia: roles of lineage heterogeneity and cell replacement. J Cell Biol 171(5):835–844
172. Lien SC, Usami S, Chien S, Chiu JJ (2006) Phosphatidylinositol 3-kinase/Akt pathway is involved in transforming growth factor-beta1-induced phenotypic modulation of 10T1/2 cells to smooth muscle cells. Cellular Signalling 18:1270–1278
173. Limwongse C, Clarren SK, Cassidy SB (1999) Syndromes and malformations of the urinary tract. In: Barratt TM, Avner ED, Harmon WE (eds) Pediatric nephrology, 3rd edn. Williams & Wilkins, Baltimore, pp 427–452
174. Lin Y, Zhang S, Tuukkanen J, Peltoketo H, Pihlajaniemi T, Vainio S (2003) Patterning parameters associated with the branching of the ureteric bud regulated by epithelial-mesenchymal interactions. Int J Dev Biol 47(1):3–13

175. Lindahl P, Hellström M, Kalén M, Karlsson L, Pekny M, Pekna M, Soriano P, Betsholtz C (1998) Paracrine PDGF-B/PDGF-Rß signaling controls mesangial cell development in kidney glomeruli. Development 125:3313–3322
176. Little M, Georgas K, Pennisi D, Wilkinson L (2010) Kidney development: two tales of tubulogenesis. Curr Top Dev Biol 90:193–229
177. Little MH, Williamson KA, Mannens M, Kelsey A, Gosden C, Hastie ND, van Heyningen V (1993) Evidence that WT1 mutations in Denys-Drash syndrome patients may act in a dominant-negative fashion. Hum Mol Genet 2(3):259–264
178. Liu W, Li Y, Cunha S, Hayward G, Baskin L (2000) Diffusable growth factors induce bladder smooth muscle differentiation. In Vitro Cell Dev Biol 36(7):476–484
179. Lu BC, Cebrian C, Chi X, Kuure S, Kuo R, Bates CM, Arber S, Hassell J, MacNeil L, Hoshi M, Jain S, Asai N, Takahashi M, Schmidt-Ott KM, Barasch J, D'Agati V, Costantini F (2009) Etv4 and Etv5 are required downstream of GDNF and Ret for kidney branching morphogenesis. Nat Genet 41(12):1295–1302
180. Lu W, van Eerde AM, Fan X, Quintero-Rivera F, Kulkarni S, Ferguson H, Kim HG, Fan Y, Xi Q, Li QG, Sanlaville D, Andrews W, Sundaresan V, Bi W, Yan J, Giltay JC, Wijmenga C, de Jong TP, Feather SA, Woolf AS, Rao Y, Lupski JR, Eccles MR, Quade BJ, Gusella JF, Morton CC, Maas RL (2007) Disruption of ROBO2 is associated with urinary tract anomalies and confers risk of vesicoureteral reflux. Am J Hum Genet 80(4):616–632
181. Luyckx VA, Brenner BM (2010) The clinical importance of nephron mass. J Am Soc Nephrol 21(6):898–910
182. Lye CM, Fasano L, Woolf AS (2010) Ureter myogenesis: putting Teashirt into context. J Am Soc Nephrol 21(1):24–30
183. Mackenzie HS, Lawler EV, Brenner BM (1996) Congenital oligonephropathy: the fetal flaw in essential hypertension? Kidney Int 55:S30–S34
184. Mackie GG, Stephens FD (1975) Duplex kidneys: a correlation of renal dysplasia with position of the ureteral orifice. J Urol 114:274–280
185. Maeshima A, Sakurai H, Choi Y, Kitamura S, Vaughn DA, Tee JB, Nigam SK (2007) Glial cell-derived neurotrophic factor independent ureteric bud outgrowth from the Wolffian duct. J Am Soc Nephrol 18(12):3147–3155
186. Majumdar A, Vainio S, Kispert A, McMahon J, McMahon AP (2003) Wnt11 and Ret/Gdnf pathways cooperate in regulating ureteric branching during metanephric kidney development. Development 130(14):3175–3185
187. Manalich R, Reyes L, Herrera M, Melendi C, Fundora I (2000) Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. Kidney Int 58(2):770–773
188. Marose TD, Merkel CE, McMahon AP, Carroll TJ (2008) Beta-catenin is necessary to keep cells of ureteric bud/Wolffian duct epithelium in a precursor state. Dev Biol 314(1):112–126
189. Matsuno T, Tokunaka S, Koyanagi T (1984) Muscular development in the urinary tract. J Urol 132(1):148–152
190. Mauch TJ, Yang G, Wright M, Smith D, Schoenwolf GC (2000) Signals from trunk paraxial mesoderm induce pronephros formation in chick intermediate mesoderm. Dev Biol 220(1):62–75
191. McCright B, Gao X, Shen L, Lozier J, Lan Y, Maguire M, Herzlinger D, Weinmaster G, Jiang R, Gridley T (2001) Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic Notch2 mutation. Development 128:491–502
192. McCright B, Lozier J, Gridley T (2002) A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. Development 129:1075–1082
193. McDill BW, Li SZ, Kovach PA, Ding L, Chen F (2006) Congenital progressive hydronephrosis (cph) is caused by an S256L mutation in aquaporin-2 that affects its phosphorylation and apical membrane accumulation. Proc Natl Acad Sci U S A 103(18): 6952–6957
194. McPherson E, Carey J, Kramer A, Hall JG, Pauli RM, Schimke RN, Tasin MH (1987) Dominantly inherited renal adysplasia. Am J Med Genet 26(4): 863–872
195. Michael L, Davies JA (2004) Pattern and regulation of cell proliferation during murine ureteric bud development. J Anat 204(4):241–255
196. Michos O (2009) Kidney development: from ureteric bud formation to branching morphogenesis. Curr Opin Genet Dev 19(5):484–490
197. Michos O, Cebrian C, Hyink D, Grieshammer U, Williams L, D'Agati V, Licht JD, Martin GR, Costantini F (2010) Kidney development in the absence of Gdnf and Spry1 requires Fgf10. PLoS Genet 6(1):e1000809
198. Michos O, Goncalves A, Lopez-Rios J, Tiecke E, Naillat F, Beier K, Galli A, Vainio S, Zeller R (2007) Reduction of BMP4 activity by gremlin 1 enables ureteric bud outgrowth and GDNF/WNT11 feedback signalling during kidney branching morphogenesis. Development 134(13):2397–2405
199. Michos O, Panman L, Vintersten K, Beier K, Zeller R, Zuniga A (2004) Gremlin-mediated BMP antagonism induces the epithelial-mesenchymal feedback signaling controlling metanephric kidney and limb organogenesis. Development 131(14):3401–3410
200. Miner JH, Li C (2000) Defective glomerulogenesis in the absence of laminin alpha5 demonstrates a developmental role for the kidney glomerular basement membrane. Dev Biol 217(2):278–289
201. Miner JH, Morello R, Andrews KL, Li C, Antignac C, Shaw AS, Lee B (2002) Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation. J Clin Invest 109(8): 1065–1072

202. Miner JH, Sanes JR (1994) Collagen IV alpha 3, alpha 4, and alpha 5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. J Cell Biol 127(3): 879–891
203. Miner JH, Sanes JR (1996) Molecular and functional defects in kidneys of mice lacking collagen alpha 3(IV): implications for Alport syndrome. J Cell Biol 135(5):1403–1413
204. Miyamoto N, Yoshida M, Kuratani S, Matuso I, Aizawa S (1997) Defects of urogenital development in mice lacking *Emx2*. Development 124: 1653–1664
205. Miyazaki Y, Ichikawa I (2003) Ontogeny of congenital anomalies of the kidney and urinary tract, CAKUT. Pediatr Int: Official J Jpn Pediatr Soc 45(5):598–604
206. Miyazaki Y, Oshima K, Fogo A, Hogan BLM, Ichikawa I (2000) Bone morphogenetic protein 4 regulates the budding site and elongation of the mouse ureter. J Clin Invest 105:863–873
207. Miyazaki Y, Oshima K, Fogo A, Ichikawa I (2003) Evidence that bone morphogenetic protein 4 has multiple biological functions during kidney and urinary tract development. Kidney Int 63(3):835–844
208. Miyazaki Y, Tsuchida S, Nishimura H, Pope JC IV, Harris RC, McKanna JM, Inagami T, Hogan BLM, Fogo A, Ichikawa I (1998) Angiotensin induces the urinary peristaltic machinery during the perinatal period. J Clin Invest 102:1489–1497
209. Montesano R, Soriano JV, Pepper MS, Orci L (1997) Induction of epithelial branching tubulogenesis in vitro. J Cell Physiol 173:152–161
210. Moore MW, Klein RD, Farinas I, Sauer H, Armanini M, Phillips H, Reichardt LF, Ryan AM, Carver-Moore K, Rosenthal A (1996) Renal and neuronal abnormalities in mice lacking GDNF. Nature 382:76–79
211. Moritz KM, Mazzuca MQ, Siebel AL, Mibus A, Arena D, Tare M, Owens JA, Wlodek ME (2009) Uteroplacental insufficiency causes a nephron deficit, modest renal insufficiency but no hypertension with ageing in female rats. J Physiol 587(Pt 11):2635–2646
212. Mugford JW, Sipila P, Kobayashi A, Behringer RR, McMahon AP (2008) Hoxd11 specifies a program of metanephric kidney development within the intermediate mesoderm of the mouse embryo. Dev Biol 319(2):396–405
213. Mugford JW, Sipila P, McMahon JA, McMahon AP (2008) Osr1 expression demarcates a multi-potent population of intermediate mesoderm that undergoes progressive restriction to an Osr1-dependent nephron progenitor compartment within the mammalian kidney. Dev Biol 324(1):88–98
214. Mugford JW, Yu J, Kobayashi A, McMahon AP (2009) High-resolution gene expression analysis of the developing mouse kidney defines novel cellular compartments within the nephron progenitor population. Dev Biol 333(2):312–323
215. Murawski IJ, Gupta IR (2006) Vesicoureteric reflux and renal malformations: a developmental problem. Clin Genet 69(2):105–117
216. Murawski IJ, Maina RW, Malo D, Guay-Woodford LM, Gros P, Fujiwara M, Morgan K, Gupta IR (2010) The C3H/HeJ inbred mouse is a model of vesico-ureteric reflux with a susceptibility locus on chromosome 12. Kidney Int 78(3):269–278
217. Murawski IJ, Watt CL, Gupta IR (2011) Vesico-ureteric reflux: using mouse models to understand a common congenital urinary tract defect. Pediatr Nephrol 26(9):1513–1522
218. Murnaghan GF (1958) The dynamics of the renal pelvis and ureter with reference to congenital hydronephrosis. Br J Urol 30(3):321–329
219. Nagata M, Nakayama K, Terada Y, Hoshi S, Watanabe T (1998) Cell cycle regulation and differentiation in the human podocyte lineage. Am J Pathol 153(5):1511–1520
220. Naiche LA, Harrelson Z, Kelly RG, Papaioannou VE (2005) T-box genes in vertebrate development. Annu Rev Genet 39:219–239
221. Nakai S, Sugitani Y, Sato H, Ito S, Miura Y, Ogawa M, Nishi M, Jishage K, Minowa O, Noda T (2003) Crucial roles of Brn1 in distal tubule formation and function in mouse kidney. Development 130(19):4751–4759
222. Nakayama M, Nozu K, Goto Y, Kamei K, Ito S, Sato H, Emi M, Nakanishi K, Tsuchiya S, Iijima K (2010) HNF1B alterations associated with congenital anomalies of the kidney and urinary tract. Pediatr Nephrol 25(6):1073–1079
223. Neiss WF (1982) Histogenesis of the loop of Henle in the rat kidney. Anat Embryol (Berl) 164(3): 315–330
224. Neiss WF, Klehn KL (1981) The postnatal development of the rat kidney, with special reference to the chemodifferentiation of the proximal tubule. Histochemistry 73(2):251–268
225. Nelson RG, Morgenstern H, Bennett PH (1998) Intrauterine diabetes exposure and the risk of renal disease in diabetic Pima Indians. Diabetes 47(9): 1489–1493
226. Netter FH, Cochard LR (2002) Netter's Atlas of human embryology. Icon Learning Systems, Teterboro
227. Newman J, Antonakopoulos GN (1989) The fine structure of the human fetal urinary bladder. Development and maturation. A light, transmission and scanning electron microscopic study. J Anat 166:135–150
228. Nie X, Xu J, El-Hashash A, Xu PX (2011) Six1 regulates Grem1 expression in the metanephric mesenchyme to initiate branching morphogenesis. Dev Biol 352(1):141–151
229. Nievelstein RA, van der Werff JF, Verbeek FJ, Valk J, Vermeij-Keers C (1998) Normal and abnormal embryonic development of the anorectum in human embryos. Teratology 57(2):70–78
230. Niimura F, Labostky PA, Kakuchi J, Okubo S, Yoshida H, Oikawa T, Ichiki T, Naftilan AJ, Fogo A,

Inagami T et al (1995) Gene targeting in mice reveals a requirement for angiotensin in the development and maintenance of kidney morphology and growth factor regulation. J Clin Invest 96:2947–2954
231. Nishimura H, Yerkes E, Hohenfellner K, Miyazaki Y, Ma J, Hunley TE, Yoshida H, Ichiki T, Threadgill D, Phillips JA, Hogan BML, Fogo A, Brock JW, Inagami T, Ichikawa I (1999) Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. Mol Cell 3:1–10
232. Nishinakamura R, Matsumoto Y, Nakao K, Nakamura K, Sato A, Copeland NG, Gilbert DJ, Jenkins NA, Scully S, Lacey DL, Katsuki M, Asashima M, Yokota T (2001) Murine homolog of SALL1 is essential for ureteric bud invasion in kidney development. Development 128:3105–3115
233. Noakes PG, Miner JH, Gautam M, Cunningham JM, Sanes JR, Merlie JP (1995) The renal glomerulus of mice lacking s-laminin/laminin ß2: nephrosis despite molecular compensation by laminin ß1. Nat Genet 10:400–406
234. Nyengaard JR, Bendtsen TF (1992) Glomerular number and size in relation to age, kidney weight, and body surface in normal man. Anat Rec 232:194–201
235. O'Brien LL, Grimaldi M, Kostun Z, Wingert RA, Selleck R, Davidson AJ (2011) Wt1a, Foxc1a, and the Notch mediator Rbpj physically interact and regulate the formation of podocytes in zebrafish. Dev Biol 358(2):318–330
236. Obara-Ishihara T, Kuhlman J, Niswander L, Herzlinger D (1999) The surface ectoderm is essential for nephric duct formation in intermediate mesoderm. Development 126:1103–1108
237. Oh S, Shin S, Janknecht R (2012) ETV1, 4 and 5: an oncogenic subfamily of ETS transcription factors. Biochim Biophys Acta 1826(1):1–12
238. Olatunbosun ST, Bella AF (2000) Relationship between height, glucose intolerance, and hypertension in an urban African black adult population: a case for the "thrifty phenotype" hypothesis? J Natl Med Assoc 92(6):265–268
239. Osafune K, Takasato M, Kispert A, Asashima M, Nishinakamura R (2006) Identification of multipotent progenitors in the embryonic mouse kidney by a novel colony-forming assay. Development 133(1):151–161
240. Osathanondh V, Potter EL (1963) Development of human kidney as shown by microdissection. II. Renal pelvis, calyces, and papillae. Arch Pathol 76:277–289
241. Osathanondh V, Potter EL (1963) Development of human kidney as shown by microdissection.III. Formation and interrelationship of collecting tubules and nephrons. Arch Pathol 76:66–78
242. Osathanondh V, Potter EL (1966) Development of human kidney as shown by microdissection. Arch Pathol 82:391–402
243. Palmer RE, Kotsianti A, Cadman B, Boyd T, Gerald W, Haber DA (2001) WT1 regulates the expression of the major glomerular podocyte membrane protein Podocalyxin. Curr Biol 11(22):1815–1809
244. Park JS, Valerius MT, McMahon AP (2007) Wnt/beta-catenin signaling regulates nephron induction during mouse kidney development. Development 134(13):2533–2539
245. Patek CE, Little MH, Fleming S, Miles C, Charlieu JP, Clarke AR, Miyagawa K, Christie S, Doig J, Harrison DJ, Porteous DJ, Brookes AJ, Hooper ML, Hastie ND (1999) A zinc finger truncation of murine WT1 results in the characteristic urogenital abnormalities of Denys-Drash syndrome. Proc Natl Acad Sci U S A 96(6):2931–2936
246. Pavenstadt H, Kriz W, Kretzler M (2003) Cell biology of the glomerular podocyte. Physiol Rev 83(1): 253–307
247. Pedersen A, Skjong C, Shawlot W (2005) Lim 1 is required for nephric duct extension and ureteric bud morphogenesis. Dev Biol 288(2):571–581
248. Pelletier J, Schalling M, Buckler AJ, Rogers A, Haber DA, Housman D (1991) Expression of the Wilms' tumor gene WT1 in the murine urogenital system. Genes Dev 5:1345–1356
249. Pena A, Levitt MA, Hong A, Midulla P (2004) Surgical management of cloacal malformations: a review of 339 patients. J Pediatr Surg 39(3):470–479, discussion 470-479
250. Pepicelli CV, Kispert A, Rowitch D, McMahon AP (1997) GDNF induces branching and increased cell proliferation in the ureter of the mouse. Dev Biol 192:193–198
251. Perantoni AO, Timofeeva O, Naillat F, Richman C, Pajni-Underwood S, Wilson C, Vainio S, Dove LF, Lewandoski M (2005) Inactivation of FGF8 in early mesoderm reveals an essential role in kidney development. Development 132(17):3859–3871
252. Perriton CL, Powles N, Chiang C, Maconochie MK, Cohn MJ (2002) Sonic hedgehog signaling from the urethral epithelium controls external genital development. Dev Biol 247(1):26–46
253. Pichel JG, Shen L, Sheng HZ, Granholm A-C, Drago J, Grinberg A, Lee EJ, Huang SP, Saarmas M, Hoffer BJ, Sariola H, Westphal H (1996) Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 382:73–76
254. Pini Prato A, Musso M, Ceccherini I, Mattioli G, Giunta C, Ghiggeri GM, Jasonni V (2009) Hirschsprung disease and congenital anomalies of the kidney and urinary tract (CAKUT): a novel syndromic association. Medicine 88(2):83–90
255. Piscione TD, Phan T, Rosenblum ND (2001) BMP7 controls collecting tubule cell proliferation and apoptosis via Smad1-dependent and -independent pathways. Am J Physiol 280:F19–F33
256. Piscione TD, Rosenblum ND (1999) The malformed kidney: disruption of glomerular and tubular development. Clin Genet 56(5):343–358
257. Piscione TD, Waters AM (2008) Structural and functional development of the kidney. In: Geary DF, Schaefer F (eds) Comprehensive pediatric

nephrology, 1st edn. Mosby, Inc., Philadelphia, pp 91–130
258. Piscione TD, Wu MY, Quaggin SE (2004) Expression of hairy/enhancer of split genes, Hes1 and Hes5, during murine nephron morphogenesis. Gene Expr Patterns 4(6):707–711
259. Piscione TD, Yager TD, Gupta IR, Grinfeld B, Pei Y, Attisano L, Wrana JL, Rosenblum ND (1997) BMP-2 and OP-1 exert direct and opposite effects on renal branching morphogenesis. Am J Physiol 273:F961–F975
260. Poladia DP, Kish K, Kutay B, Hains D, Kegg H, Zhao H, Bates CM (2006) Role of fibroblast growth factor receptors 1 and 2 in the metanephric mesenchyme. Dev Biol 291(2):325–339
261. Potter EL (1972) Normal and abnormal development of the kidney. Year Book Medical Publishers Inc, Chicago
262. Povey S, Lovering R, Bruford E, Wright M, Lush M, Wain H (2001) The HUGO Gene Nomenclature Committee (HGNC). Human Genet 109(6):678–680
263. Preger-Ben Noon E, Barak H, Guttmann-Raviv N, Reshef R (2009) Interplay between activin and Hox genes determines the formation of the kidney morphogenetic field. Development 136(12):1995–2004
264. Price KL, Long DA, Jina N, Liapis H, Hubank M, Woolf AS, Winyard PJ (2007) Microarray interrogation of human metanephric mesenchymal cells highlights potentially important molecules in vivo. Physiol Genom 28(2):193–202
265. Pulido R, Krueger NX, Serra-Pages C, Saito H, Streuli M (1995) Molecular characterization of the human transmembrane protein-tyrosine phosphatase delta. Evidence for tissue-specific expression of alternative human transmembrane protein-tyrosine phosphatase delta isoforms. J Biol Chem 270(12):6722–6728
266. Qazi Q, Masakawa A, Milman D, McGann B, Chua A, Haller J (1979) Renal anomalies in fetal alcohol syndrome. Pediatrics 63(6):886–889
267. Qi BQ, Beasley SW, Frizelle FA (2002) Clarification of the processes that lead to anorectal malformations in the ETU-induced rat model of imperforate anus. J Pediatr Surg 37(9):1305–1312
268. Qiao J, Bush KT, Steer DL, Stuart RO, Sakurai H, Wachsman W, Nigam SK (2001) Multiple fibroblast growth factors support growth of the ureteric bud but have different effects on branching morphogenesis. Mech Dev 109(2):123–135
269. Qiao J, Sakurai H, Nigam SK (1999) Branching morphogenesis independent of mesenchymal-epithelial contact in the developing kidney. Proc Natl Acad Sci U S A 96:7330–7335
270. Qiao J, Uzzo R, Obara-Ishihara T, Degenstein L, Fuchs E, Herzlinger D (1999) FGF-7 modulates ureteric bud growth and nephron number in the developing kidney. Development 126:547–554
271. Qiao S, Iwashita T, Furukawa T, Yamamoto M, Sobue G, Takahashi M (2001) Differential effects of leukocyte common antigen-related protein on biochemical and biological activities of RET-MEN2A and RET-MEN2B mutant proteins. J Biol Chem 276(12):9460–9467
272. Quaggin SE, Schwartz L, Cui S, Igarashi P, Deimling J, Post M, Rossant J (1999) The basic-helix-loop-helix protein pod1 is critically important for kidney and lung organogenesis. Development 126: 5771–5783
273. Queisser-Luft A, Stolz G, Wiesel A, Schlaefer K, Spranger J (2002) Malformations in newborn: results based on 30,940 infants and fetuses from the Mainz congenital birth defect monitoring system (1990–1998). Arch Gynecol Obstetr 266(3):163–167
274. Raatikainen-Ahokas A, Hytonen M, Tenhunen A, Sainio K, Sariola H (2000) Bmp-4 affects the differentiation of metanephric mesenchyme and reveals an early anterior-posterior axis of the embryonic kidney. Dev Dyn 217:146–158
275. Ricono JM, Xu YC, Arar M, Jin DC, Barnes JL, Abboud HE (2003) Morphological insights into the origin of glomerular endothelial and mesangial cells and their precursors. J Histochem Cytochem 51(2): 141–150
276. Robboy SJ, Taguchi O, Cunha GR (1982) Normal development of the human female reproductive tract and alterations resulting from experimental exposure to diethylstilbestrol. Human Pathol 13(3):190–198
277. Robert B, St John PL, Hyink DP, Abrahamson DR (1996) Evidence that embryonic kidney cells expressing flk-1 are intrinsic, vasculogenic angioblasts. Am J Physiol 271(3 Pt 2):F744–F753
278. Rodriguez MM, Gomez AH, Abitbol CL, Chandar JJ, Duara S, Zilleruelo GE (2004) Histomorphometric analysis of postnatal glomerulogenesis in extremely preterm infants. Pediatr Dev Pathol 7(1):17–25
279. Rogers SA, Ryan G, Purchio AF, Hammerman MR (1993) Metanephric transforming growth factor-b1 regulates nephrogenesis in vitro. Am J Physiol 264: F996–F1002
280. Roodhooft AM, Birnholz JC, Holmes LB (1984) Familial nature of congenital absence and severe dysgenesis of both kidneys. N Engl J Med 310(21): 1341–1345
281. Ruano-Gil D, Coca-Payeras A, Tejedo-Mateu A (1975) Obstruction and normal recanalization of the ureter in the human embryo. Its relation to congenital ureteric obstruction. Eur Urol 1(6):287–293
282. Rudolph AM, Heymann MA (1970) Circulatory changes during growth in the fetal lamb. Circ Res 26(3):289–299
283. Rumballe BA, Georgas KM, Combes AN, Ju AL, Gilbert T, Little MH (2011) Nephron formation adopts a novel spatial topology at cessation of nephrogenesis. Dev Biol 360(1):110–122
284. Saburi S, Hester I, Fischer E, Pontoglio M, Eremina V, Gessler M, Quaggin SE, Harrison R, Mount R, McNeill H (2008) Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease. Nat Genet 40(8):1010–1015
285. Sadl V, Jin F, Yu J, Cui S, Holmyard D, Quaggin S, Barsh G, Cordes S (2002) The mouse Kreisler

(Krml1/MafB) segmentation gene is required for differentiation of glomerular visceral epithelial cells. Dev Biol 249(1):16–29
286. Sainio K, Hellstedt P, Kreidberg JA, Saxen L, Sariola H (1997) Differential regulation of two sets of mesonephric tubules by WT-1. Development 124: 1293–1299
287. Sainio K, Nonclercq D, Saarma M, Palgi J, Saxen L, Sariola H (1994) Neuronal characteristics in embryonic renal stroma. Int J Dev Biol 38(1):77–84
288. Sainio K, Suvanto P, Davies J, Wartiovaara J, Wartiovaara K, Saarma M, Arumäe U, Meng X, Lindahl M, Pachnis V, Sariola H (1997) Glial-cell-line-derived neurotrophic factor is required for bud initiation from ureteric epithelium. Development 124:4077–4087
289. Saisawat P, Tasic V, Vega-Warner V, Kehinde EO, Gunther B, Airik R, Innis JW, Hoskins BE, Hoefele J, Otto EA, Hildebrandt F (2012) Identification of two novel CAKUT-causing genes by massively parallel exon resequencing of candidate genes in patients with unilateral renal agenesis. Kidney Int 81(2):196–200
290. Sakurai H, Barros EJ, Tsukamoto T, Barasch J, Nigam SK (1997) An in vitro tubulogenesis system using cell lines derived from the embryonic kidney shows dependence on multiple soluble growth factors. Proc Natl Acad Sci U S A 94:6279–6284
291. Sakurai H, Nigam S (1997) Transforming growth factor-beta selectively inhibits branching morphogenesis but not tubulogenesis. Am J Physiol 272(1 Pt 2):F139–F146
292. Salerno A, Kohlhase J, Kaplan BS (2000) Townes-Brocks syndrome and renal dysplasia: a novel mutation in the SALL1 gene. Pediatr Nephrol 14(1):25–28
293. Sanchez MP, Silos-Santiago I, Frisen J, He B, Lira SA, Barbacid M (1996) Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature 382:70–73
294. Sanders KM, Koh SD, Ward SM (2006) Interstitial cells of Cajal as pacemakers in the gastrointestinal tract. Annu Rev Physiol 68:307–343
295. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont ME, Sullivan MJ, Dobyns WB, Eccles MR (1995) Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. Nat Genet 9(4):358–364
296. Sariola H, Ekblom P, Lehtonen E, Saxen L (1983) Differentiation and vascularization of the metanephric kidney grafted on the chorioallantoic membrane. Dev Biol 96(2):427–435
297. Sariola H, Holm K, Henke-Fahle S (1988) Early innervation of the metanephric kidney. Development 104(4):589–599
298. Saxen L (1987) Organogenesis of the kidney. Cambridge University Press, Cambridge
299. Schimmenti LA, Pierpont ME, Carpenter BL, Kashtan CE, Johnson MR, Dobyns WB (1995) Autosomal dominant optic nerve colobomas, vesicoureteral reflux, and renal anomalies. Am J Med Genet 59(2):204–208
300. Schmidt-Ott KM, Yang J, Chen X, Wang H, Paragas N, Mori K, Li JY, Lu B, Costantini F, Schiffer M, Bottinger E, Barasch J (2005) Novel regulators of kidney development from the tips of the ureteric bud. J Am Soc Nephrol 16(7):1993–2002
301. Schmidt IM, Damgaard IN, Boisen KA, Mau C, Chellakooty M, Olgaard K, Main KM (2004) Increased kidney growth in formula-fed versus breast-fed healthy infants. Pediatr Nephrol 19(10): 1137–1144
302. Schonfelder EM, Knuppel T, Tasic V, Miljkovic P, Konrad M, Wuhl E, Antignac C, Bakkaloglu A, Schaefer F, Weber S (2006) Mutations in Uroplakin IIIA are a rare cause of renal hypodysplasia in humans. Am J Kidney Dis 47(6):1004–1012
303. Schuchardt A, D'Agati V, Larsson-Blomberg L, Costantini F, Pachnis V (1994) Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367:380–383
304. Schuchardt A, D'Agati V, Pachnis V, Costantini F (1996) Renal agenesis and hypodysplasia in *ret-k*$^{-}$ mutant mice result from defects in ureteric bud development. Development 122:1919–1929
305. Schwab K, Patterson LT, Aronow BJ, Luckas R, Liang HC, Potter SS (2003) A catalogue of gene expression in the developing kidney. Kidney Int 64(5):1588–1604
306. Seifert AW, Bouldin CM, Choi KS, Harfe BD, Cohn MJ (2009) Multiphasic and tissue-specific roles of sonic hedgehog in cloacal septation and external genitalia development. Development 136(23):3949–3957
307. Seikaly MG, Ho PL, Emmett L, Fine RN, Tejani A (2003) Chronic renal insufficiency in children: the 2001 annual report of the NAPRTCS. Pediatr Nephrol 18(8):796–804
308. Self M, Lagutin OV, Bowling B, Hendrix J, Cai Y, Dressler GR, Oliver G (2006) Six2 is required for suppression of nephrogenesis and progenitor renewal in the developing kidney. EMBO J 25(21): 5214–5228
309. Shakya R, Jho EH, Kotka P, Wu Z, Kholodilov N, Burke R, D'Agati V, Costantini F (2005) The role of GDNF in patterning the excretory system. Dev Biol 283(1):70–84
310. Shakya R, Watanabe T, Costantini F (2005) The role of GDNF/Ret signaling in ureteric bud cell fate and branching morphogenesis. Dev Cell 8(1):65–74
311. Shawlot W, Behringer RR (1995) Requirement for *Lim1* in head-organizer function. Nature 374:425–430
312. Shimizu K, Chiba S, Hosoya N, Kumano K, Saito T, Kurokawa M, Kanda Y, Hamada Y, Hirai H (2000) Binding of Delta1, Jagged1, and Jagged2 to Notch2 rapidly induces cleavage, nuclear translocation, and hyperphosphorylation of Notch2. Mol Cell Biol 20(18):6913–6922
313. Shimizu K, Chiba S, Kumano K, Hosoya N, Takahashi T, Kanda Y, Hamada Y, Yazaki Y, Hirai H

(1999) Mouse jagged1 physically interacts with notch2 and other notch receptors. Assessment by quantitative methods. J Biol Chem 274(46): 32961–32969
314. Shiroyanagi Y, Liu B, Cao M, Agras K, Li J, Hsieh MH, Willingham EJ, Baskin LS (2007) Urothelial sonic hedgehog signaling plays an important role in bladder smooth muscle formation. Differentiation 75(10):968–977
315. Sichieri R, Siqueira KS, Pereira RA, Ascherio A (2000) Short stature and hypertension in the city of Rio de Janeiro, Brazil. Public Health Nutr 3(1):77–82
316. Sims-Lucas S, Argyropoulos C, Kish K, McHugh K, Bertram JF, Quigley R, Bates CM (2009) Three-dimensional imaging reveals ureteric and mesenchymal defects in Fgfr2-mutant kidneys. J Am Soc Nephrol 20(12):2525–2533
317. Skibo LK, Lyons EA, Levi CS (1992) First-trimester umbilical cord cysts. Radiology 182(3):719–722
318. Skinner MA, Safford SD, Reeves JG, Jackson ME, Freemerman AJ (2008) Renal aplasia in humans is associated with RET mutations. Am J Hum Genet 82(2):344–351
319. Solari V, Piotrowska AP, Puri P (2003) Altered expression of interstitial cells of Cajal in congenital ureteropelvic junction obstruction. J Urol 170(6 Pt 1):2420–2422
320. Soriano P (1994) Abnormal kidney development and hematological disorders in PDGF ß-receptor mutant mice. Genes Dev 8:1888–1896
321. Spencer J, Wang Z, Hoy W (2001) Low birth weight and reduced renal volume in Aboriginal children. Am J Kidney Dis 37(5):915–920
322. Spitzer A, Brandis M (1974) Functional and morphologic maturation of the superficial nephrons. Relationship to total kidney function. J Clin Invest 53(1):279–287
323. Stark K, Vainio S, Vassileva G, McMahon AP (1994) Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by *Wnt-4*. Nature 372:679–683
324. Surendran K, Selassie M, Liapis H, Krigman H, Kopan R (2010) Reduced Notch signaling leads to renal cysts and papillary microadenomas. J Am Soc Nephrol 21(5):819–832
325. Tacciuoli M, Lotti T, de Matteis A, Laurenti C (1975) Development of the smooth muscle of the ureter and vesical trigone: histological investigation in human fetus. Eur Urol 1(6):282–286
326. Takemoto M, He L, Norlin J, Patrakka J, Xiao Z, Petrova T, Bondjers C, Asp J, Wallgard E, Sun Y, Samuelsson T, Mostad P, Lundin S, Miura N, Sado Y, Alitalo K, Quaggin SE, Tryggvason K, Betsholtz C (2006) Large-scale identification of genes implicated in kidney glomerulus development and function. EMBO J 25(5):1160–1174
327. Tanagho EA, Meyers FH, Smith DR (1968) The trigone: anatomical and physiological considerations. I. In relation to the ureterovesical junction. J Urol 100(5):623–632
328. Tang MJ, Cai Y, Tsai SJ, Wang YK, Dressler GR (2002) Ureteric bud outgrowth in response to RET activation is mediated by phosphatidylinositol 3-kinase. Dev Biol 243(1):128–136
329. Tang MJ, Worley D, Sanicola M, Dressler GR (1998) The RET-glial cell-derived neurotrophic factor (GDNF) pathway stimulates migration and chemoattraction of epithelial cells. J Cell Biol 142(5):1337–1345
330. Tanigawa S, Wang H, Yang Y, Sharma N, Tarasova N, Ajima R, Yamaguchi TP, Rodriguez LG, Perantoni AO (2011) Wnt4 induces nephronic tubules in metanephric mesenchyme by a non-canonical mechanism. Dev Biol 352(1):58–69
331. Tasian G, Cunha G, Baskin L (2010) Smooth muscle differentiation and patterning in the urinary bladder. Differentiation 80(2–3):106–117
332. Thomas R, Sanna-Cherchi S, Warady BA, Furth SL, Kaskel FJ, Gharavi AG (2011) HNF1B and PAX2 mutations are a common cause of renal hypodysplasia in the CKiD cohort. Pediatr Nephrol 26(6):897–903
333. Torban E, Dziarmaga A, Iglesias D, Chu LL, Vassilieva T, Little M, Eccles M, Discenza M, Pelletier J, Goodyer P (2006) PAX2 activates WNT4 expression during mammalian kidney development. J Biol Chem 281(18):12705–12712
334. Torihashi S, Ward SM, Nishikawa S, Nishi K, Kobayashi S, Sanders KM (1995) c-kit-dependent development of interstitial cells and electrical activity in the murine gastrointestinal tract. Cell Tissue Res 280(1):97–111
335. Torres M, Gomez-Pardo E, Dressler GR, Gruss P (1995) Pax-2 controls multiple steps of urogenital development. Development 121(12):4057–4065
336. Tsang TE, Shawlot W, Kinder SJ, Kobayashi A, Kwan KM, Schughart K, Kania A, Jessell TM, Behringer RR, Tam PP (2000) Lim1 activity is required for intermediate mesoderm differentiation in the mouse embryo. Dev Biol 223(1):77–90
337. Tufro A, Norwood VF, Carey RM, Gomez RA (1999) Vascular endothelial growth factor induces nephrogenesis and vasculogenesis. J Am Soc Nephrol 10(10):2125–2134
338. Tufro A, Teichman J, Woda C, Villegas G (2008) Semaphorin3a inhibits ureteric bud branching morphogenesis. Mech Dev 125(5–6):558–568
339. Uetani N, Bertozzi K, Chagnon MJ, Hendriks W, Tremblay ML, Bouchard M (2009) Maturation of ureter-bladder connection in mice is controlled by LAR family receptor protein tyrosine phosphatases. J Clin Invest 119(4):924–935
340. Uetani N, Bouchard M (2009) Plumbing in the embryo: developmental defects of the urinary tracts. Clin Genet 75(4):307–317
341. van der Putte SC (2005) The development of the perineum in the human. A comprehensive histological study with a special reference to the role of the stromal components. Adv Anat Embryol Cell Biol 177:1–131

342. Van Esch H, Groenen P, Nesbit MA, Schuffenhauer S, Lichtner P, Vanderlinden G, Harding B, Beetz R, Bilous RW, Holdaway I, Shaw NJ, Fryns JP, Van de Ven W, Thakker RV, Devriendt K (2000) GATA3 haplo-insufficiency causes human HDR syndrome. Nature 406(6794):419–422
343. Vega QC, Worby CA, Lechner MS, Dixon JE, Dressler GR (1996) Glial cell line-derived neurotrophic factor activates the receptor tyrosine kinase RET and promotes kidney morphogenesis. Proc Natl Acad Sci U S A 93(20):10657–10661
344. Veille JC, McNeil S, Hanson R, Smith N (1998) Renal hemodynamics: longitudinal study from the late fetal life to one year of age. J Matern Fetal Investig 8(1):6–10
345. Verschueren K, Dewulf N, Goumans MJ, Lonnoy O, Feijen A, Grimsby S, Vande Spiegle K, ten Dijke P, Moren A, Vanscheeuwijck P, Heldin C-H, Miyazono K, Mummery C, Van Den Eijnden-Van Raaij J, Huylebroeck D (1995) Expression of type I and type IB receptors for activin in midgestation mouse embryos suggests distinct functions in organogenesis. Mech Dev 52:109–123
346. Viana R, Batourina E, Huang H, Dressler GR, Kobayashi A, Behringer RR, Shapiro E, Hensle T, Lambert S, Mendelsohn C (2007) The development of the bladder trigone, the center of the anti-reflux mechanism. Development 134(20):3763–3769
347. Wagner CA, Devuyst O, Bourgeois S, Mohebbi N (2009) Regulated acid-base transport in the collecting duct. Pflugers Arch 458(1):137–156
348. Wagner KD, Wagner N, Schedl A (2003) The complex life of WT1. J Cell Sci 116(Pt 9):1653–1658
349. Wagner N, Wagner KD, Xing Y, Scholz H, Schedl A (2004) The major podocyte protein nephrin is transcriptionally activated by the Wilms' tumor suppressor WT1. J Am Soc Nephrol 15(12): 3044–3051
350. Wang GJ, Brenner-Anantharam A, Vaughan ED, Herzlinger D (2009) Antagonism of BMP4 signaling disrupts smooth muscle investment of the ureter and ureteropelvic junction. J Urol 181(1):401–407
351. Watanabe T, Costantini F (2004) Real-time analysis of ureteric bud branching morphogenesis in vitro. Dev Biol 271(1):98–108
352. Weber S (2012) Novel genetic aspects of congenital anomalies of kidney and urinary tract. Curr Opin Pediatr 24(2):212–218
353. Weber S, Landwehr C, Renkert M, Hoischen A, Wuhl E, Denecke J, Radlwimmer B, Haffner D, Schaefer F, Weber RG (2011) Mapping candidate regions and genes for congenital anomalies of the kidneys and urinary tract (CAKUT) by array-based comparative genomic hybridization. Nephrol Dial Transplant 26(1):136–143
354. Weber S, Moriniere V, Knuppel T, Charbit M, Dusek J, Ghiggeri GM, Jankauskiene A, Mir S, Montini G, Peco-Antic A, Wuhl E, Zurowska AM, Mehls O, Antignac C, Schaefer F, Salomon R (2006) Prevalence of mutations in renal developmental genes in children with renal hypodysplasia: results of the ESCAPE study. J Am Soc Nephrol 17(10):2864–2870
355. Weiss JP (1988) Embryogenesis of ureteral anomalies: a unifying theory. Aust N Z J Surg 58(8): 631–638
356. Wellik DM, Hawkes PJ, Capecchi MR (2002) Hox11 paralogous genes are essential for metanephric kidney induction. Genes Dev 16(11):1423–1432
357. Wesson MB (1925) Anatomical, embryological, and physiological studies of the trigone and bladder neck. J Urol 4:280–306
358. White JT, Zhang B, Cerqueira DM, Tran U, Wessely O (2010) Notch signaling, wt1 and foxc2 are key regulators of the podocyte gene regulatory network in Xenopus. Development 137(11): 1863–1873
359. Wiesel A, Queisser-Luft A, Clementi M, Bianca S, Stoll C (2005) Prenatal detection of congenital renal malformations by fetal ultrasonographic examination: an analysis of 709,030 births in 12 European countries. Eur J Med Genet 48(2):131–144
360. Winnier G, Blessing M, Labosky PA, Hogan BLM (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev 9:2105–2116
361. Woodburne RT (1965) The ureter, ureterovesical junction, and vesical trigone. Anat Rec 151: 243–249
362. Woods LL, Weeks DA, Rasch R (2004) Programming of adult blood pressure by maternal protein restriction: role of nephrogenesis. Kidney Int 65(4): 1339–1348
363. Woolf AS, Kolatsi-Joannou M, Hardman P, Andermarcher E, Moorby C, Fine LG, Jat PS, Noble MD, Gherardi E (1995) Roles of hepatocyte growth factor/scatter factor and the met receptor in the early development of the metanephros. J Cell Biol 128(1–2):171–184
364. Woolf AS, Winyard P, Hermanns MH, Welham SJ (2003) 21. Maldevelopment of the human kidney and lower urinary tract: an overview. In: Vize PD, Woolf AS, Bard JB (eds) The kidney: from normal development to congenital disease, 1st edn. Elsevier Science, San Diego, pp 377–393
365. Xiao J, Nguyen TV, Ngui K, Strijbos PJ, Selmer IS, Neylon CB, Furness JB (2004) Molecular and functional analysis of hyperpolarisation-activated nucleotide-gated (HCN) channels in the enteric nervous system. Neuroscience 129(3):603–614
366. Xu P-X, Adams J, Peters H, Brown MC, Heaney S, Maas R (1999) Eya1-deficient mice lack ears and kidneys and show abnormal apoptosis of organ primordia. Nat Genet 23:113–117
367. Xu PX, Zheng W, Huang L, Maire P, Laclef C, Silvius D (2003) Six1 is required for the early organogenesis of mammalian kidney. Development 130(14):3085–3094
368. Yamaguchi OA, Constantinou CE (1989) Renal calyceal and pelvic contraction rhythms. Am J Physiol 257(4 Pt 2):R788–R795

369. Yang Y, Jeanpierre C, Dressler GR, Lacoste M, Niaudet P, Gubler MC (1999) WT1 and PAX-2 podocyte expression in Denys-Drash syndrome and isolated diffuse mesangial sclerosis. Am J Pathol 154(1):181–192
370. Yeger H, Forget D, Alami J, Williams BR (1996) Analysis of WT1 gene expression during mouse nephrogenesis in organ culture. In Vitro Cell Dev Biol 32:496–504
371. Yi SE, Daluiski A, Pederson R, Rosen V, Lyons KM (2000) The type I BMP receptor BMPRIB is required for chondrogenesis in the mouse limb. Development 127:621–630
372. Yosypiv IV (2009) Renin-angiotensin system-growth factor cross-talk: a novel mechanism for ureteric bud morphogenesis. Pediatr Nephrol 24(6):1113–1120
373. Yosypiv IV, Boh MK, Spera MA, El-Dahr SS (2008) Downregulation of Spry-1, an inhibitor of GDNF/Ret, causes angiotensin II-induced ureteric bud branching. Kidney Int 74(10):1287–1293
374. Yu J, Carroll TJ, McMahon AP (2002) Sonic hedgehog regulates proliferation and differentiation of mesenchymal cells in the mouse metanephric kidney. Development 129(22):5301–5312
375. Yu J, Carroll TJ, Rajagopal J, Kobayashi A, Ren Q, McMahon AP (2009) A Wnt7b-dependent pathway regulates the orientation of epithelial cell division and establishes the cortico-medullary axis of the mammalian kidney. Development 136(1):161–171
376. Zhang H, Bradley A (1996) Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. Development 122:2977–2986
377. Zhang S, Lin Y, Itaranta P, Yagi A, Vainio S (2001) Expression of Sprouty genes 1, 2 and 4 during mouse organogenesis. Mech Dev 109(2):367–370
378. Zhang Z, Quinlan J, Hoy W, Hughson MD, Lemire M, Hudson T, Hueber PA, Benjamin A, Roy A, Pascuet E, Goodyer M, Raju C, Houghton F, Bertram J, Goodyer P (2008) A common RET variant is associated with reduced newborn kidney size and function. J Am Soc Nephrol 19(10):2027–2034
379. Zhao H, Kegg H, Grady S, Truong HT, Robinson ML, Baum M, Bates CM (2004) Role of fibroblast growth factor receptors 1 and 2 in the ureteric bud. Dev Biol 276(2):403–415
380. Zimanyi MA, Bertram JF, Black MJ (2002) Nephron number and blood pressure in rat offspring with maternal high-protein diet. Pediatr Nephrol 17(12): 1000–1004