Chapter 5

Mouse models of congenital kidney anomalies

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Overview

Congenital anomalies of the kidney and urinary tract (CAKUT) are common birth defects, which cause the majority of chronic kidney diseases in children. CAKUT covers a wide range of malformations that derive from deficiencies in embryonic kidney and lower urinary tract development, including renal aplasia, hypodysplasia, hypoplasia, ectopia, and different forms of ureter abnormalities. The majority of the genetic causes of CAKUT remain unknown. Research on mutant mice has identified multiple genes that critically regulate renal differentiation. The data generated from this research have served as an excellent resource to identify the genetic bases of human kidney defects and have led to significantly improved diagnostics. Furthermore, genetic data from human CAKUT studies have also revealed novel genes regulating kidney differentiation.

Introduction

Congenital anomalies of the kidney and lower urinary tract (CAKUT) are caused by inborn defects in the differentiation of the organs, including the kidneys as well as the urine excretion organs. In order to understand the potential genetic, molecular, and cellular bases of these malformations, it is essential to comprehend how these organs normally develop.

A large part of our understanding of mammalian kidney formation comes from the early inductive studies carried out by Clifford Grobstein and Lauri Saxén. It rapidly became evident that the development of the kidney is controlled through reciprocal inductive tissue interactions (1-3). Since then, we have learned that the interacting tissues in the kidney rudiment are the ureteric bud (UB) epithelium, derived from the Wolffian duct (WD, also known as nephric duct), the metanephric mesenchyme (MM, also called renal mesenchyme) in close proximity to the UB, and the stromal cells surrounding the MM. The inductive interplay between these tissues results in developmental changes in the nascent tissue, which reciprocally affects the signal-sending tissue. These interactions are largely mediated by direct cell-cell contacts, secreted paracrine morphogens, and other as-yet-unknown mechanisms.

Renal differentiation

Origin of the kidneys

The kidneys originate from the intermediate mesoderm (IM). Recent advances in understanding the early specification of kidney precursors revealed that IM is temporally regionalized, and the interacting tissues of the developing kidney are derived from its distinct subpopulations (4, 5). The most anterior IM is the first to form and gives rise to the WD and its derivatives, whereas the posterior IM differentiates into MM and stroma. Initially, IM undergoes mesenchyme-to-epithelium transition (MET) to generate the WD, which begins to grow posteriorly simultaneously to MM specification in the leading front of the WD.

Mammalian kidney differentiation involves the formation of three sets of sequentially formed distinct kidneys (6, 7). During the posterior growth of the WD, the first kidneys to form are the pronephroi, which are transient organs with no known function in mammals (**Figure 5.1A**). The next kidneys to develop are the mesonephroi. Each mesonephos in humans contains a total of 34 mesonephric tubules all connected to the WD, and is functional during the early phases of fetal life (**Figure 5.1B**). They secrete urine and form aorta-gonad-mesonephros region, which hosts hematopoietic stem cells (8). In mouse, where the mesonephroi are non-functional organs, there are two distinct sets of mesonephric tubules of which the 4-6 cranial pairs are connected to the WD while the majority of the caudal tubules remain without direct connection to the WD (9). The last kidneys are the metanephroi, the permanent kidneys, which start functioning after mid-gestation and play an important role in fetal health by excreting primary urine to become the amniotic fluid (**Figure 5.1C**). In fact, one phenotypic change in newborns with defective kidney function is the so-called Potter`s sequence (oligohydramnion sequence). Here, a reduced amount of amniotic fluid caused by minimal urine production gives rise to the characteristic fetal features including flattened nose and abnormalities around the eyes and ears, club-feet, and small lungs (pulmonary hypoplasia) resulting in respiratory insufficiency.

Development of the permanent kidney (metanephros)

The development of the permanent kidney, the metanephros, begins at approximately embryonic day 30-32 (E30-32) in humans and at E10.5 in mice when the caudal end of the WD has made contact with the cloaca, the future bladder, and bulges towards the medial-dorsally located MM (10). The initial bulge then rapidly elongates and invades the MM to form the UB. The bud at this stage is already divided into the tip and trunk regions, which bear divergent molecular signatures and show different cellular behaviors (**Figure 5.2**). The UB tip, which is intimately surrounded by MM, is highly proliferative, includes actively moving individual cells, and is surrounded by discontinuous and scarce extracellular matrix. The UB trunk, on the other hand, shows a significantly lower rate of cell division as its elongation is dominated by convergent extensions. It also begins to build up distinct, continuous extracellular matrix characteristic of the collecting ducts and ureter.

Branching morphogenesis

The UB tip is surrounded by the MM, which is more tightly packed near the UB epithelium than the more distally located stromal mesenchyme in the outer most layer of the developing kidney (**Figure 5.2**). After its formation, the UB starts dichotomous branching morphogenesis, instructed by signals derived from the MM (11). First, an existing tip balloons to form an ampulla, which then bifurcates into two new tips that thereafter elongate to generate new trunks (12, 13). Genetic labeling experiments together with live-imaging of cultured kidneys at the single-cell level have revealed that some tip cells are maintained within the tips while others are left behind in trunk regions (14-17). This has led to a relatively well-proven model where the UB tips host progenitor cells for the entire collecting duct, composed of intercalated and principal cells. Currently, the molecular regulation and cell-specific gene signatures to distinguish progenitors from the cells destined for differentiation are missing, but improvements in single-cell transcriptomic techniques together with the possibility to spatially map multiple gene expressions simultaneously are expected to shed light on this currently stochastic-seeming process (18, 19). Finally, it has been estimated that the mouse UB undergoes some 11-12 rounds of repeated branching events to finally generate the shape of the organ with collecting ductal system of an appropriate complexity (20). In addition to branching, the collecting ducts undergo complex reorganization at mid-gestation to form pelvis and distinct medulla-cortex compartmentalization (21).

Nephrogenesis

Nephron differentiation begins simultaneously to the start of branching morphogenesis (**Figure 5.2**). Nephrons are derived from the subpopulation of metanephric mesenchyme called cap mesenchyme, which is defined by its tight and oriented arrangement of mesenchymal cells around UB tips (13, 22-24). These nephron progenitors both self-renew to maintain the cap mesenchyme until cessation of nephrogenesis and differentiate through MET into specialized epithelial cells of all nephron segments (25-27). Nephron progenitors (NPs) are maintained in the cap mesenchyme surrounding each newly formed UB tip, while their differentiation takes place in the nascent mesenchyme of same tip. The molecular regulation of NP specification and maintenance involves both intrinsic (cell autonomous) and UB-derived paracrine pathways, which merge to balance between self-renewal versus differentiation. Nephrogenesis begins by the compaction of NP cells into the armpits of the T-shaped UB, after which this so-called pretubular aggregate epithelializes

via the characteristic morphological stages of renal vesicle, comma-shaped and S-shaped bodies to form a secretory nephron (**Figure 5.2**). Thus, the final nephron number in each individual closely reflects the extent of UB branching (3, 20). Each segment of nephron, namely the podocytes of glomeruli, Bowman's capsule, the proximal tubules, the loop of Henle, the distal tubules, and the connecting piece joining the nephron to the collecting duct system derives from the same NP pool. The causes of NP exhaustion and the end of nephrogenesis at late gestation in humans (week 36) or at early postnatal days in mice (P3) (28, 29) are not known.

Mouse as a mannequin of renal disease in man – inborn errors and effective dissimilarities

Different animals have been used to aid the understanding of the mechanisms guiding differentiation since the early days of developmental biology studies. Modern research largely relies on rodents as mammalian models, and due to the development of genetic manipulation techniques (see below) specific for house mouse, *Mus Musculus*, it has become the predominant animal model in kidney development.

The mouse as an experimental animal comes in various different flavors. The fact that over 450 inbred mouse strains exist translates into a wealth of different genotypes and phenotypes for those utilizing the mouse as a model of diseases in human (30). Moreover, laboratory mice are also maintained as outbred strains, expanding the complexity even further. In reality, researchers worldwide focus on using many of the same inbred strains, which are employed in experiments with genetically modified mouse models (see below). For the purpose of renal diseases, it is important to remember that both gender and genetic variations in the inbred strains very much influence e.g. predisposition to kidney damage. In other words, some strains, like C57BL/6, are rather resistant to kidney damage and develop proteinuria, glomerulosclerosis, and/or hypertension only when highly damaged (31). This likely derives from the different accumulation of genetic aberrations between the strains as e.g. C57BL/6 only has one functional gene for renin, resulting in a decreased renin-angiotensin-aldosterone system as compared to e.g. 129/Sv substrains (32). Moreover, female mice in general better tolerate ischemic renal damage and chemically-induced diabetic nephropathy than male mice.

Spontaneous mouse models

Mice with inborn errors in kidney differentiation resulting in renal aplasia (33), lupus nephritis, polycystic kidney disease, interstitial disease, hypertension, and diabetes-induced nephropathy have greatly facilitated our understanding of the pathogenesis and mechanisms of renal diseases (34). Classical mutations such as *Danforth's short tail* (*Sd*) and *limb deformity* (*ld*) cause renal aplasia due to failure to induce UB formation and growth (35). The first model of autosomal polycystic kidney disease, the *cpk*/*cpk* mouse, represents an aggressive, early-onset renal disease, especially in the DBA/2J strain(36). Similarly, *bpk*/*bpk* and *pcy*/*pcy* mice show inherited proximal tubule cystogenesis, which eventually spreads into all nephron segments. Though great models for diabetic nephropathy are still to come, NOD mice have been widely used for type I

diabetes-associated nephropathy, while the corresponding model for type II diabetic nephropathy are the *db*/*db* mice (37).

Genetically modified mouse models

The mouse became the most popular species in genetic engineering due to the early availability of its genome sequence, the possibility to derive and successfully culture its embryonic stem (ES) cells, and its relative similarity to human physiology. Previously, the possibilities to manipulate mammalian genomes were limited to chemically-induced random mutagenesis (forward genetic approach such as ENU mutagenesis), radiation, and non-targeted integration of foreign DNA (transgenesis). Though rather imperfect, these strategies have provided not only the basis for the more modern techniques currently in use but also advanced our understanding of many complex biological processes. Due to the current predominance of targeted genome editing approaches (reverse genetics), these techniques, which are utilized to produce point mutations, large deletions, and conventional and conditional knockout mouse lines, are shortly described below.

Targeting in ES cells

Genetic engineering of mouse ES cells in combination with the techniques allowing aggregation of mutated and wild-type ES cells to generate chimeric embryos has been the basis for the generation of gene knockouts and conditional alleles for the last two decades (38). The key requirement is that the locus of the gene-of-interest is known and cloned, as this is utilized to build a targeting vector, which includes not only the disrupted gene-of-interest, but also 5' and 3' sequences around it. The surrounding sequences, known as homology arms, anneal to the corresponding region of the genome in ES cells and allow replacement of the endogenous gene through homologous recombination.

Classically, a neomycin resistance gene in the targeting vector is used to inactivate the gene-of-interest. Alternatively, the generation of point mutations and conditional alleles requires insertion of a desired genetic alteration, e.g. the nucleotide change or loxP sequences, to the targeting vector (39). As homologous recombination is a rare event in any cell, neomycin resistance and thymidine kinase genes in the targeting vector are used to identify those ES cells where the endogenous gene is replaced with the targeted gene (40). After confirming the successful targeting by sequencing, the ES cells are introduced into wild-type morulae or blastocysts by aggregation or injections, respectively. Host embryos typically originate from a different mouse strain than the targeted ES cells, allowing identification of the genetically engineered pups by their chimeric coat color. The final requirement for the establishment of a new genetically engineered mouse line is that the chimeric founder transmits the desired gene editing to its offspring. In general, the traditional targeting strategy is a lengthy and expensive procedure where the generation of gene-modified mouse line typically takes 18-24 months.

CRISPR/Cas9-mediated targeting

The revolution of genetic engineering began with the development of programmable, highly specific DNA nucleases of which the first ones, zinc finger nucleases (ZFN) and transcription

activator-like effector nucleases (TALEN), were based on modifiable DNA domains that guide the Fok1 nuclease to a specific genomic location (41). The next generation genome editing now utilizes short RNA guides to specifically locate CAS9 endonuclease (originating from the bacterial immune system) to the desired site in the genome. All these editing systems are ideologically similar as they are based on nuclease function, which cuts DNA to generate double strand breaks that activate the endogenous repair systems in any given cell type (42). The predominant repair system is non-homologous end joining, which is error prone and results in inactivation of the gene-of-interest by introducing small nucleotide insertions and deletions that disrupt the normal reading frame. In animal model generation, this is achieved through injection of RNA guides together with CAS9 (mRNA or protein) into the fertilized oocyte. Injected zygotes then develop into conventional knockout founders, each with a slightly different disruption in the gene-of-interest. The CRISPR/CAS9-based gene inactivation is applicable not only in mice but also in many other species. It has proven to be an efficient and inexpensive method that is an extremely attractive and useful tool for genome editing in development of different disease models.

Opposed to the ease of generating knockouts with CRISPR/CAS9, targeted insertion of additional genetic material into the host genome is more difficult. Targeted insertion is necessary when the goal is to faithfully mimic human diseases, like those caused by congenital kidney defects as the result of point mutations or short deletions. Similar to ES cell targeting, this requires the engagement of homologous recombination, which is inefficient due to its uncommon occurrence. Additional challenges in the generation of knockin models come from the fact that CRIRSP/CAS9 is particularly efficient in genome editing through non-homologous end joining, which is activated simultaneously to homologous recombination. This unfortunately results in additional, undesired editing near the point mutation containing template DNA. Careful design, use of control animals, and genotyping of the founder animals as well as F1 offspring by sequencing are needed to ascertain that possible phenotypic alterations derive from the anticipated edit and not from the extra editing in an undesired locus (43).

Common renal malformations in human fetal autopsies and their analogous mouse models

Oligohydramnion sequence

The founder of pediatric pathology in the USA, Edith Potter (1901-1993), first described the oligohydramnion sequence. It is caused by the lack of the amniotic fluid, a condition called oligohydramnion. Amniotic fluid is predominantly urine, and oligohydramnion is thus caused by the lack of the kidneys (bilateral renal aplasia), urethral valve (thin membrane blocking the urethra) preventing urination, or premature rupture of the fetal membranes (PROM). Placental insufficiency, for instance in pre-eclampsia, can also cause oligohydramnion. Amniotic fluid contains a number of growth factors, and as the fetus inhales amniotic fluid, the factors promote the growth and maturation of the lungs. Oligohydramnion sequence is characterized by typical external features and small lungs (pulmonary hypoplasia), which can cause postnatal respiratory insufficiency and high neonatal mortality rates.

Aplasia and hypoplasia

Lack of one or both kidneys is referred to as uni- or bilateral renal aplasia. If the kidneys are smaller than expected for the developmental or newborn stage, they are hypoplastic. The genetic causes of renal aplasia have been only partially resolved, but a common environmental cause for renal hypoplasia is the lack of vitamin A (44, 45). The biologically inactive vitamin A is locally activated to become retinoic acid by the retinaldehyde dehydrogenase 2 enzyme (RALDH2 also known ALDH2) synthesized by the renal interstitial or stromal cells (46).

Mouse models of renal aplasia

Analysis of spontaneous and knockout mouse models has revealed several causes of renal aplasia and thus has helped understanding of the mechanisms leading to congenital absence of kidneys in humans (33). Among the first aplasia models are *Danforth's short tail* (*sd*) spontaneous mouse mutant, which is caused by disruption of *pancreas specific transcription factor, 1*a (*Ptf1a*) with transposon insertion (47), and *formin* mutation resulting in *limb deformity* (*ld*) model (48). Genetic inactivation of a single mouse gene, such as the transcription factor *Pax2* (49), *Osr1 (50, 51)*, *Gata1 (52)*, *Lhx1* (53, 54), *Wt1* (55), *Hox11* (56, 57), *Eya1* (58), *Six1* (59), or *Sall1* (60) arrests renal differentiation due to defects in early specification of intermediate mesoderm (*Osr1*), formation of WD (*Gata3* and *Lhx1*), or induction of the kidney itself. The lack of kidney induction is typically caused by either the failure to induce primary UB formation (*Eya1*, *Hox11*, *Pax2*, *Six1*, *Sall1*) leading to subsequent apoptosis of the MM, or the inability of the MM to survive (*Wt1*).

In addition to transcription factors, deletion of the specific components in key growth factor signaling pathways causes renal aplasia (25). Characterization of mouse mutants lacking genes for glial cell line derived neurotrophic factor (*Gdnf* (61-63)) or its receptor complex (*Ret* (64, 65) and co-receptor *Gfra1* (66, 67)) revealed renal aplasia due to impaired UB formation (68). Also, simultaneous deletion of *Etv4* and *-5*, the first identified GDNF/RET-dependent transcription factors, causes renal aplasia (16, 69). GDNF belongs to transforming growth factor beta (TGFβ) superfamily, and unlike the TGFβ genes themselves, another TGFβ superfamily member, growth/differentiation factor 11 (GDF11) is necessary for normal UB formation (70-73). Deletion of *Gdf11* results in a spectrum of renal abnormalities with bilateral aplasia being the most frequent (70). Interestingly, several genetic studies in mice imply that the level of bone morphogenetic protein (BMP) signaling, also belonging to TGFβ superfamily, is essential for normal UB outgrowth and early organogenesis of the kidney. Not only is too little or missing signaling harmful, but enhanced levels also result in abnormalities, as shown by renal aplasia in mice with knockout of BMP antagonist *Gremlin1* (74-76).

The fibroblast growth factor (FGF) cascade works parallel to GDNF/RET signaling as shown by the conditional deletion of FGF receptors 1 and 2 with Pax3Cre (77). The UB forms in the absence of the FGF receptors, but its subsequent elongation and branching are arrested leading to renal aplasia. Many FGF ligands are expressed in the developing kidney, suggesting redundancy in their signaling. Accordingly, inactivation of one FGF receptor, but not a single FGF ligand, causes renal aplasia, supporting the essential functional importance of FGF signaling for kidney differentiation (78). Furthermore, simultaneous disruption of *Fgf9* and *-20* results in renal aplasia, likely due to deficiency in UB formation; although, this is not experimentally confirmed (79).

Inactivation of wingless-type MMTV integration site family (WNT) signaling by deleting *Wnt9b* results in renal aplasia in mouse (80). Unlike many other aplasia models with early UB arrest,

the loss of *Wnt9b* primarily affects the MM, which fails to maintain nephron progenitors and induce them for differentiation (81, 82). Canonical WNT signaling is mediated by inactivation of glycogen synthase 3 (GSK3) and causes stabilization of cytoplasmic β-catenin, which leads to activation of TCF/LEF1 transcription factors. Numerous experiments have demonstrated that canonical WNT signaling is essential for UB branching, collecting duct morphogenesis, and nephron induction as well as differentiation (83) (for details, see the section on Mouse models of hypoplasia). Of note, simultaneous deletion of *Gsk3a* and *–b* specifically in the collecting duct results in renal aplasia (ASN Kidney Week 2011, https://www.asn-online.org/abstracts/), unlike similar deletion of *b-catenin*, which usually causes hypodysplasia (84).

Genes encoding essential structural proteins, such as those related to cell adhesion and extracellular matrix, are also vital for early kidney specification and induction. A good example is integrin-linked kinase (ILK), which binds the cytoplasmic domains of b-integrin to regulate actin dynamics and when deleted, results in renal aplasia (85). However, a single gene deletion of a structural protein often appears without a phenotype, probably because of compensation or redundancy by another family member, or results in an incompletely penetrant renal aplasia (86). This is exemplified by inactivation of the Fraser syndrome gene, Fraser extracellular matrix complex subunit 1 (*Fras1*) (87, 88), integrin alpha 8 (Itga8) (89), and nidogens 1 and 2 (simultaneously) (90), which all cause incompletely penetrant kidney development failure.

Mutations identified in human renal aplasia

Sequencing of human CAKUT samples has confirmed that several genes identified as essential regulators of early kidney induction in mice are also mutated in human patients (91). As in mice, mutations in transcription factors *HOX11* (92), *PAX8* (93), *SALL1* (94), *WT1* (95), as well as in signaling pathways such as *FGFR1* (96) (syndromic; renal agenesis in Kallman syndrome), *FGF20* (79) *GREM1* (97), and *RET* (94, 98), cause renal aplasia in humans. Moreover, *FRAS1* (88) (syndromic; renal agenesis in Fraser syndrome) and Fras1-related extracellular matrix protein 2 (*FREM2*) (92, 99), as well as integrin alpha 8 (*ITGA8*) (100) mutations are associated with renal aplasia in CAKUT patients. Other genes not previously identified in animal models cause not only renal aplasia but also other kidney defects in humans. These include WAP four-disulfide core domain 18 (101, 102) (also known as *KAL1*), cell division cycle 5 like (*CDC5L*) (94), establishment of sister chromatid cohesion N-acetyltransferase 2 (*ESCO2*) (103) (syndromic; included in the Roberts syndrome spectrum), and dual serine/threonine and tyrosine protein kinase (*DSTYK*), which is a positive regulator of extracellular signal regulated kinase (ERK) that co-localizes with FGF receptors in the UB and MM and may perturb FGF signaling (104).

The vast majority of CAKUT-causing gene mutations are heterozygous. In general, homozygous mutations of key regulators may result in early miscarriage or abortion of the fetus rather than cause a disease syndrome or single organ defect. It is also possible that multiplied members of a gene families in humans can substitute for the loss of a single member (redundancy), or that modifier aberrations in other genes are required for CAKUT manifestation.

Mutations in human renal hypoplasia and hypodysplasia

Many genes causing renal aplasia in mice are not associated with renal aplasia in human, but rather when mutated in human, cause an explicitly less severe renal phenotype, such as hypoplasia (small kidney with normal morphology) or hypodysplasia (small kidney with abnormal differentiation). Such genes include *EYA1* (94, 105), *OSR1* (106), *PAX2* (94, 107), *SALL1* (94, 105), *RET*

(108) and *GRFα1* (109). Mutations in additional genes associated with human renal hypoplasia or hypodysplasia are transcription factors hepatocyte nuclear factor 1 B (94, 110) (*HNF1B*) and sine oculis-related homeobox 2 (111) (*SIX2*), signaling molecules uroplakin 3A (112) (*UPK3A*), *WNT4* (94), and *BMP4* (113); and planar polarity gene EGF LAG seven-pass G-type receptor 1 (*CELSR1*) (114). It is likely that renal hypoplasia is an underdiagnosed sub-category of CAKUT as patients with hypoplastic kidneys, which mostly function normally and do not develop renal disease, remain unrecognized unless the individual does develop related complications like hypertension. This is different in genetically modified experimental models, which are carefully analyzed for renal defects both during embryogenesis and adulthood (115).

Mouse models of hypo(dys)plasia

A huge number of mouse mutants display either hypoplasia only or in combination with dysplasia. Due to the scope of this chapter, we focus on genes that associate with human CAKUT genes and their signaling cascades.

Mutations in transcription factors HNF1B and PAX2 are the most common causes of renal hypodysplasia in children (105, 116). This is reflected in mouse where the full inactivation of *Pax2* causes aplasia, while its heterozygous deletion alone causes no phenotype, but simultaneous compound heterozygosity for *Hnf1b* results in renal hypodysplasia (117). Homozygous deletion of *Hnf1b* on the other hand results in embryonic lethality before gastrulation (118). However, tetraploid embryo complementation and tissue-specific inactivation studies have demonstrated the requirement of *Hnf1b* for at least growth and integrity of the UB, early nephrogenesis, differentiation of the proximal nephron segment, and differentiation of the renal medulla (119-121). In addition, deletion of transcription factor *Etv4* or *-5* results in renal hypodysplasia though their distinct and joint tissue-specific functions are not fully revealed (16, 69). Also Sox8 and -9 act together downstream of GDNF/RET signaling, and their inactivation in the developing kidney causes renal defects varying from aplasia to mild hypoplasia (122). Interestingly, SOX9 appears to mediate important responses to acute kidney injury, at least as identified in mouse with ischemia reperfusion-induced injury (123, 124).

Renal hypodysplasia in mice without *B cell leukemia/lymphoma 2* (*Bcl2*) factor is caused by an unusual mechanism as its inactivation allows normal renal differentiation until embryonic day 13, after which the nephron progenitors disappear due to increased apoptosis (125). Similarly, inactivation of *Six2* causes renal hypoplasia due to early loss of nephron progenitors, but in this case through their premature differentiation and exhaustion at mid-gestation (22, 126).

Vitamin A deficiency has been experimentally shown to control nephron number as mice with inactivated *Raldh1a2* (also known as Aldh1a1) have small kidneys due to a UB branching defect (127). Genetic experiments have also revealed strong involvement of BMP- and WNT signaling in guiding embryonic kidney growth and differentiation (128-131). The involvement of BMP signaling is exemplified by deletion of *Bmp7*, which results in severe renal hypoplasia with virtually no glomeruli due to a defect in survival of nephron progenitors (132-135). Further studies have shown redundant functions for BMP4 and -7; genetic substitution of *Bmp7* by *Bmp4* in mice rescues renal defects(136). Furthermore, deletion of either ligand in a *Grem*1-null background, which causes renal aplasia on its own (75), restores normal kidney differentiation (137). High redundancy in BMP signaling, as suggested by ligand deletions, is also obvious at the receptor level because deletion of BMP receptors has either no impact on kidney development or results in hypodysplasia in the renal medulla (*Bmpr1a*) (128, 138). The phosphatase *Dullard*, a recently identified negative BMP signaling

modulator, on the other hand is required for maintenance of postnatal nephrons (139). Deletion of another BMP signaling modulator, *Crossveinless2*, which likely amplifies signal strength, results in multi-organ defects including renal hypoplasia due to impaired nephrogenesis (140, 141). Signaling downstream of BMP receptors phosphorylates Smad1/5/8 and/or induces TAK1, which can activate p38/mitogen-activated protein kinase (MAPK), JNK/c-Jun, and NF-kB cascades. Inactivation of either *Tak1* or *Jun* tissue-specifically in nephron progenitors causes their premature depletion and results in mild renal hypoplasia (142).

The importance of WNT signaling for renal differentiation, and especially for nephron induction, was originally revealed by classical induction experiments performed with isolated mouse MM in tissue culture. Initially, these experiments showed that in the absence of a heterologous inducer, such as spinal cord, the MM underwent rapid apoptosis and failed to survive (1, 143). Soon it was discovered that embryonic spinal cord is rich in WNT proteins. When WNT proteins were expressed in cultured cells and placed in contact with MM, they also induced tubulogenesis (144). Inactivation of *Wnt4* in mouse verified the essential function of WNT activation as a nephron inducer, as nephrogenesis failed to progress beyond the pretubular aggregate stage in this model (145). Consequent tissue-specific deletion (146) and activation (147) of β-catenin in the MM verified the requirement for the canonical WNT pathway. Similarly, β-catenin is required for keeping the UB actively branching as shown by the loss- (84, 148) and gain-of-function (149) strategies that both result in renal hypoplasia due to defects in maintaining the balance of self-renewal and differentiation.

As shown by deletion of receptor tyrosine kinases *Ret* or *Fgf* receptors and their ligands, these signaling pathways are essential for initiation of kidney development, and when fully inactivated, most often cause renal aplasia (150). However, mutations introduced in the specific tyrosine domains of *Ret* responsible for the distinct intracellular pathway activation cause phenotypes varying from mild hypoplasia to severe hypodysplasia (151-154). These models together with chemical inhibition experiments in cultured mouse kidney explants have demonstrated that MAPK/ERK, PI3K/AKT, and PLCγ cascades mediate the downstream effects of RTK signaling in developing kidneys (12, 155, 156). Furthermore, tissue-specific inactivation of tyrosine phosphatase *Shp2*, which functions downstream of RTK and hormone receptors, as well as MAPK/ERK in the UB, results in renal hypodysplasia (157, 158). The latter model also revealed a specific requirement for MAPK/ERK activity in kidney growth where it promotes UB arborization via novel branch formation (158). Inactivation of MAPK/ERK activity in the MM, on the other hand, results in slightly less severe renal hypodysplasia, and demonstrates that the MAPK/ERK pathway maintains nephron progenitors while also being crucial for their normal differentiation beyond the comma-shaped body stage (23). UB-specific genetic disruption of another intracellular cascade, the PI3K/AKT pathway, suggests that it is important for shaping the UB branching pattern (159).

Hippo signaling regulates the growth and size of organs in insects and mammals, and has recently been implicated in the guidance of kidney morphogenesis (160). In mammals, the Hippo pathway involves the kinases MST1/2 and LATS1/2, and their functions are mediated by the transcriptional co-activators YAP and TAZ. Genetic studies have revealed that *Lats1* and *-2* are critically required for UB branching as their tissue-specific deletion results in renal aplasia (161). Interestingly, experiments with the mutant UB also revealed an unexpected interaction between *Nf2* (also known as Merlin) and Hippo signaling, as genetic overexpression of either *Yap* or *Taz* fully rescues severe renal hypodysplasia in the kidneys with UB-specific *Nf2* deletion (161). In the MM, *Lats1* and *-2* together promote differentiation of nephron progenitors (162), while *Yap* and *Taz*

appear to have distinct functions in the developing mouse kidney. Similarly to the upstream kinases, *Yap* promotes nephrogenesis (163) while *Taz* is needed for prevention of cyst formation (164, 165).

Cell polarity is an important feature of the functional kidney. Inactivation of either of the planar cell polarity genes *Celsr1 (114)* or *Vangl1 (166)* causes renal hypodysplasia in mice as both genes are required for normal growth and branch patterning in the developing kidney. Tissue-specific deletion of structural proteins, such as actin remodeling factors destrin (*Dstn*) and cofilin (*Cfl1*) (167), and adhesion proteins like p120 (168), also serve as excellent models of renal hypodysplasia. Instead of halting organogenesis at its earliest stage, these models allow formation of the UB, some degree of branching, and induction of nephrogenesis, thus enabling experimentation that facilitates understanding of kidney differentiation at the cellular level.

Ectopia

The kidneys are normally located in the retroperitoneal space (behind the abdominal cavity) at the level of the stomach. The most common ectopic location of the kidney is in the pelvis. Ectopia can be associated with vesicourinary reflux and mislocation of the ureteric opening in the wall of the urinary bladder, where the normal location is close to the urethral orifice in the so-called triangle area of the urinary bladder.

Mouse models of ectopia

Experimental models of ectopia are rare despite its relatively common prevalence in humans. The etiology of ectopia was originally hypothesized by Mackie and Stephens (1975) to derive from aberrant initial ureteric budding site (169) and has since been proven by e.g. genetic reduction of *Bmp4* dosage, which results in hydroureters and ectopia of the ureterovesical orifice (170). Similarly, defective RET signaling results in ectopic connection of the ureter to bladder (46, 171-173), and recent findings show that dosage of *Gdnf* critically regulates the initial UB budding site and width. As shown in recent study, GDNF positively regulates self-renewal of collecting duct progenitors, which in the genetic excess of *Gdnf* expand abnormally resulting in failure of UB trunk elongation *(174, 175)*, Consequently, a phenotype very much resembling human pelvic kidney develops due to short ureter length caused by imbalanced progenitor self-renewal and differentiation. Kidney ectopia may also rise through a different mechanism involving deficiency in retinoic acid receptors (176) and defects in the differentiation of smooth muscle cells around the ureter itself as seen in mice with deleted transcription factors *Sox9* (177), *-11 (178),* or *Tbx18 (179)* that all have abnormally positioned kidneys.

Mutations identified in human renal ectopia

Aberrations in *SOX11* (178) and *TBX18* (180) have been identified in patients with varying renal defects that include posterior urethral valves, ureteropelvic junction obstructions, ureterovesical junction obstruction, and vesicoureteral reflux. Rare mutations in genes encoding *RET* and *GFRa1* were also identified in CAKUT fetuses and patients with urinary tract malformations, while only one patient with combined unilateral agenesis and ectopic kidney so far has been identified with a *GDNF*-only mutation (109, 181).

Ureteral duplication

Ureteral duplication is a common form of CAKUT. It can be complete, resulting in two pelvises and two separate ureters in one kidney. Alternatively, one ureter can be divided into two branches with two distinct orifices in the urinary bladder. Ureteral duplication is associated with many complications of which vesicoureteral reflux and ureteropelvic junction obstruction are most often found in patients with incomplete duplication. Complete duplication, which most often associates with vesicoureteral reflux, ectopic ureterocele, or ectopic ureteral insertion, shows a gender bias as all of the associated complications are more common in girls than in boys (182).

Mouse models of ureteral duplication

Supernumerary ureteric buds are experimentally promoted in mouse by transgenic GDNF overexpression (183) or in kidney culture by GDNF-releasing beads (184). Inactivation of genes that restrict *Gdnf* expression (*Robo2*/*Slit2* (185, 186), *FoxC* (187)) or regulate GDNF/RET signaling activity (*Sprouty1* (188, 189)) cause extra UB formation and exhibit defects commonly associated with duplicated ureters. Interestingly, endogenous overexpression of *Gdnf* by disruption of its 5' untranslated region does not induce extra ureteric budding even though both mRNA and protein levels are significantly increased (174). Quite illogically, inactivating mutations in the RET docking site responsible for PLCϒ activation also cause supernumerary budding similar to that seen with exogenous GDNF (151, 184). These somewhat confusing results from different genetic mouse models of the same signaling pathway can be interpreted in a way that a single UB formation is an absolutely essential requirement for normal renal morphogenesis. Thus, multiple molecular mechanisms, several different genes, and many pathways at distinct levels are engaged to secure the single UB formation. In support of this are the findings demonstrating that simultaneous inactivation of *Spry1* and *Gdnf* or *Ret* allows functional kidney formation, but additional deletion of a single allele of *Fgf8* results in failure to form kidneys (190, 191). Furthermore, exogenous application of FGF together with activin A induces supernumerary ureter budding in cultured kidneys even in the absence of GDNF/RET signaling (192).

Disruption of other genes not directly linked to GDNF or FGF signaling also cause ureteral duplication. Deletion of leucine zipper putative tumor suppressor 2 (*Lzts2*), which modulates transcription and regulates cell cycle, results in ureteral duplication due to ectopic UB formation (193). Genetic experiments have shown that BMP4 is an essential molecule assuring that a single UB forms as mice heterozygous for *Bmp4* loss show multiple UBs (111). Conditional deletion of transcription factor *Islet1* resulted in reduced *Bmp4* expression and multiple UBs mimicking the *Bmp4* heterozygote phenotype (194).

Mutations identified in human ureteral duplications

Roughly the same genes are involved in ureteral duplication as in renal ectopia. In addition to those mentioned previously for ectopic kidneys, mutations in *BMP4* (111, 195), *SIX2* (111), *SOX17* (196), glypican 1 (*GPC1*) (197), and variations in *ROBO2* associate with vesicoureteral reflux and/or duplex kidneys (198-200).

Horseshoe kidneys/renal fusion

Fusion of the lower parts of two kidneys results in a horseshoe kidney that is commonly seen in trisomy 18 (also known as Edward's syndrome, see **Figure 5.3**) and Turner syndrome patients. Its

incidence is likewise slightly increased in trisomy 21 (Down's syndrome). Horseshoe kidneys furthermore occur sporadically without any associated syndromes or functional disturbances. Etiology of this anomaly derives from abnormal migration of nephrogenic cells across the primitive streak and general structural changes caused by midline fusion, flexion/rotation of the caudal spine, and narrowed arterial forks during migration. Fusion typically occurs between weeks four and six of human development. The rare late fusions occur through fibrous isthmus rather than renal parenchyma (201).

Mouse models of horseshoe kidney

Disruption of functional Sonic hedgehog (Shh) signaling causes midline defects and inactivation of *Shh* specifically in the notochord and the floor plate causes fusion of the kidneys (202). Similarly, horseshoe kidneys are seen in mice with mild aberrations in retinoic acid signaling, such as those caused by deletion of *Cyp26a*, which is required for the inactivation of retinoic acid (203, 204). Deletion of transcription factor *FoxD1* results in a range of renal defects, which include fusion of maturing kidneys that remain in the pelvic position (205). A follow-up study focused on analyzing causes of kidney fusion in *Foxd1* mutant kidneys. This revealed the presence of ectopic cell types in the renal capsule, which leads to its maturation defect and pelvic horseshoe kidneys (206).

Mutations identified in human horseshoe kidneys

Regardless of familial clustering of horseshoe kidney in certain cases, its genetic causes remain largely unidentified. Potential deleterious variations have been identified in Dachsous cadherin-related 2 (*DCHS2*) and leucine-rich repeat-containing G protein-coupled receptor 4 (*LGR4*) (207). Genome wide association studies identified *CYP26A* together with *CYP24A* and *BMP4* as potential disease-causing mutations in CAKUT, but their association specifically to horseshoe kidney is not reported (208). Thus, according to current knowledge, large chromosomal aberrations rather than single gene mutations associate with fused kidney in humans.

Renal multi-cystic dysplasia and polycystic kidney disease

Renal multi-cystic dysplasia is characterized by variable numbers of cysts in either one or both kidneys or only in a segment of a kidney (**Figure 5.3**). The pathogenesis is not yet completely resolved, but experimental studies with rabbits have shown that ligation of the ureter causes similar cyst formation and differentiation defect (209).

There are two types of polycystic kidney diseases (PKD), the infantile (early onset recessive) and adult (late onset dominant) polycystic kidney diseases. Infantile PKD manifests at birth or soon after and is associated with high mortality if renal transplantation is not performed. Adult PKD manifests at any age of adulthood and has a less severe outcome. These two most common forms of PKD show autosomal recessive and autosomal dominant inheritance, respectively. The later onset autosomal dominant PKD is one of the most common genetic diseases with an incidence of 1:500 – 1:1000, while the early onset autosomal recessive form is much rarer (210).

Mutations identified in humans with renal cysts

Although renal multi-cystic dysplasia is less abundant in humans than PKD, mutations in BicC family RNA binding protein 1 (*BICC1*) (97, 211), *CDC5L (212)*, dachshund family transcription factor 1 (*DACH1*) (213), *HNF1B* (110, 214, 215), and upstream transcription factor 2 (*USF2*) (216) all associate with cystic dysplasia. Autosomal dominant PKD mainly derives from mutations in polycystin (*PKD*) genes (217). The majority of the mutations (~85%) are in *PKD1*, which encodes a large receptor-like protein (polycystin 1) that interacts with *PKD2* gene product polycystin 2 (218). Mutations in *PKD2* account for ~15% of autosomal dominant PKD cases, while recently a new causative gene, glucosidase II alpha subunit (*GANAB*), was identified in rare patients who are negative for *PKD1* and *-2* mutations (217, 219). Autosomal recessive PKD was long considered a genetically homogenous disease. Mutations in *PKHD1*, which encodes fibrocystin localizing to the primary cilia to modulate Shh and Wnt activities, cause the majority of these cases (220). Aberrations in yet another ciliary protein encoded by *DZIP1L* (221), were recently identified in patients with moderate clinical symptoms of autosomal recessive PKD, affecting also liver function. Mutated *HNF1B* in some patients also causes cystic kidneys, while its aberrations are associated with hypodysplasia and diabetes syndrome (110, 222).

Mouse models of cystic kidney and polycystic kidney disease

The mouse models of polycystic kidney disease have revealed that defects in genes encoding primary cilia-located proteins are responsible for abnormal cellular proliferation and fluid accumulation that jointly lead to the formation of numerous cysts in renal tubules. Deletion of either *Pkd1* (223) or *Pkd2* (224, 225) best models the human PKD, while inactivation of *Pkhd1* (226) in rats and inhibition of ciliogenesis by loss of *Kif3a* in mouse (227) also result in a phenotype very reminiscent of human PKD. In mouse models of inactivated *glucosidase IIβ* and *Sec63p*, which exhibit mild kidney cysts on their own, reduced *Pkd1* sensitizes kidneys for cystogenesis further highlighting its essential role in PKD pathogenesis (228).

Genetic disruption of genes regulating ureter morphogenesis and/or connection to the bladder may cause renal multicystic dysplasia. As examples, atypical cadherin *Fat4 (229)*, its interacting partner four-jointed box 1 (*Fjx1*) (229), cadherin-related dachsous genes (*Dchs1* and *-2*) (229, 230), and other adhesion-related molecules like *Dlg5*, which is required for the delivery of adhesome complex components(231), *Frem2* (99) and *Glc3* (232), all cause cystic kidneys when inactivated in mice. Also, *Lzts2* mutant embryos, which exhibit duplex ureters, show other defects such as hydroureters and hydronephrosis accompanied with renal multicystic dysplasia (193).

Congenital nephrosis

Congenital nephrosis is a condition under which the glomeruli leak protein into the urine during pregnancy. It is caused by the defects of the glomerular filtration barrier, which is composed of the slit diaphragm connecting adjacent podocyte foot processes, the glomerular basement membrane, and the fenestrated endothelium of the capillary loops. Congenital nephrosis is characterized by excess of amniotic fluid or polyhydramnion and enlarged placenta, which weighs more than 50 percent of the fetal weight. Renal symptoms are typically caused by impaired podocyte physiology that derives from mutations in gene encoding proteins of the slit diaphragm (233). The congenital nephrotic syndrome is a rare form of nephrosis, which is highly enriched in the Finnish population with occasional cases reported all over the world (234).

Mutations causing congenital nephrotic syndrome in humans

Kestilä *et al* identified the causative mutation for congenital nephrotic syndrome of the Finnish type in a gene encoding the slit diaphragm protein nephrin (*NPHS1*) (235). Originally, two founder mutations were identified but since then more than 20 additional mutations have been shown to cause this syndrome. Although approximately 98% of Finnish and 38-90% of non-Finnish congenital nephrotic syndrome patients carry mutations in *NPHS1* (236-238), mutations in other genes encoding podocin (*NPHS2*), phospholipase C epsilon-1 (*PLCE1*), *WT1*, and laminin beta 2 (*LAMB2*) have been identified. Genetic diagnosis is important for designing patient treatment plans, as steroid-resistant nephrotic syndrome typically derives from the defects in *NPHS2 (238, 239)*. Moreover, mutations in *PLCE1* (240) and *WT1* (238, 241), like in *FAT1* (242), cause a more diffuse mesangial sclerosis-type of phenotype while *LAMB2* (238, 243) mutations give rise to microcoria. Finally, mutations in genes encoding collagen IV (*COL4A3*, *-A4* and *-A5*), which is the major form of collagen present in the glomerulus, also cause congenital nephrosis due to characteristic abnormalities in the glomerular basement membrane (244).

Mouse models of congenital nephrotic syndrome

Characteristic of congenital nephrotic syndrome studies is that the human mutations were often identified prior to the generation of their genetic animal models. The mouse models where the major syndrome causing genes, *Nphs1* (245), *-2* (246), *Lamb2* (247, 248), or *Col4a3* (249) were knocked out recapitulate the renal symptoms quite well and have been valuable tools for understanding the cellular and molecular mechanisms of these pathologies. Manipulation of other genes encoding slit diaphragm components, like inactivation of CD2-associated protein (*Cd2ap*) (250), often compromises podocyte function and may recapitulate specific features of congenital nephrosis (251).

Nephroblastomatosis (nephrogenic rests) and Wilms´ tumor

Nephroblastomatosis is a condition where islands of the nephrogenic blastema remain in the kidney postnatally (**Figure 5.4**). These islands are also called nephrogenic rests and may reside either intra- or perilobarly. Intralobar rests arise early in renal development, while perilobar rests are in the renal cortex, which is the active differentiating zone. They either spontaneously regress or progress and finally become malignant tumors called nephroblastomas, also known as Wilms´ tumors (**Figure 5.5**). It is impossible to distinguish between regressive and progressive nephoblastoma by any morphological or immunohistochemical means.

Mutations and syndromes associated with Wilms' tumor in humans

Despite being the most common pediatric renal cancer, the prevalence of Wilms' tumor is generally low (1:10,000 children). Its incidence is significantly increased in syndromes like Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR), Beckwith-Wiedemann (BWS), hemihypertrophy, Denys-Drash, and Perlman. Hemihypertrophy is a condition where the left and right sides of the body show clear growth differences, while Beckwith-Wiedemann syndrome is a congenital overgrowth syndrome, where the organs are bigger than expected for the age of the child. Tuberous sclerosis, a highly variable disorder typically diagnosed soon after birth, associates with the development of benign tumors in different organs, including the kidneys (252). Although

otherwise harmless, the benign tumors may interfere with the normal function of the affected organ. Tuberous sclerosis causes cystic kidneys in approximately 20-30% of individuals, but their renal function is rarely compromised.

The most commonly altered genetic locus causing Wilms' tumor is the one disturbing *insulin-like growth factor 2* (*IGF2*), which is one of the imprinted genes and normally expressed only when paternally derived. In approximately 70% of all Wilms' tumors, biallelic IGF2 expression results in tumorigenesis (253). Genetic analysis of WAGR and Denys-Drash revealed an association of germline mutations in *WT1*, and later, somatic mutations were identified in many Wilms' tumors (254). These somatic-inactivating mutations associate with intralobar nephrotic rests and thus could be responsible for early defects in renal differentiation leading to tumor development (255). Other mutations have been identified in the β-catenin encoding *CTNNB1* gene (256), often together with a WT1 mutation (257), and in Wilms' tumor on chromosome X (WTX) gene (258), which couples WT1 to β-catenin as it negatively regulates Wnt signaling and participates in the control of *WT1* transcription (259, 260). Also, mutations in the microRNA-processing genes that derepress IGF2 levels via its regulator PLAG1 have been identified. This further underlines the importance of the IGF2 signaling dosage in tissue homeostasis during kidney differentiation (261). Interestingly, a long-sought Wilms' tumor cancer stem cell, double positive for NCAM1 and RALDH1, was identified (262). This may help the future aims of revealing the relationship between cancer stem cells and other stem cell types in the kidney, namely nephron and collecting ductal progenitors.

Mouse models of nephroblastomatosis and Wilms' tumor

The genes associated with Wilms' tumor in humans are required for the early steps of embryogenesis and renal development. *Wt1* deletion in mouse results in complete failure of kidney induction (55, 263). *Ctnnb1* expressed by the UB is required for branching morphogenesis and in the MM for normal nephrogenesis (84, 149). Thus, the proteins encoded by these genes are crucial for normal organogenesis, and their null mutant mice fail to serve as models for nephrogenic rests or Wilms' tumor. Mosaic inactivation of *Wt1* together with constantly increased *Igf2* rescues mice from perinatal death caused by complete inactivation of *Wt1* and results in nephron progenitor-derived renal tumors that resemble a certain subtype of human tumors (264, 265). Moreover, inactivation of *Wt1* at different nephron differentiation stages and comparison of the resulting RNA signature in mutant kidneys with different subtypes of human Wilms' tumor revealed a correlation supporting the view that the disturbed nephrogenesis is an important contributor of tumorigenesis (263). Interestingly, kidneys in the mouse model of excess endogenous *Gdnf* (174) show structures reminiscent of perilobar nephrogenic rests, but these disappear by spontaneous differentiation during early postnatal life. To our knowledge, the field still lacks a proper genetic model of pediatric renal cancer that could serve as a preclinical model and facilitate understanding of the mechanisms of tumor growth and the role of kidney cancer stem cells.

Renal disease in syndromes

In addition to the syndromes mentioned in the previous sections, Alagille, Branchio-oto-renal (BOR), Fraser, Kallmann, Meckel, Pallister-Hall, renal coloboma, and Townes Brocks syndromes are all associated with renal anomalies.

Alagille syndrome is caused by defective Notch signaling. It is characterized by defects in the liver, heart, skeleton, eyes, and kidneys. The kidneys are most often affected in individuals with NOTCH2 mutations, and include hypodysplasia and cysts, which compromise renal function (266). The majority of Alagille patients, though, have mutations in NOTCH ligand Jagged1 (JAG1). Despite a suggested function in the maintenance of UB tip identity, very little is known about the function of *Jag1* in renal differentiation (267). Inactivation of *Notch2* in mouse recapitulates renal defects and has revealed the importance of this signaling pathway in fate determination of distinct nephron segments (268, 269).

Branchio-oto-renal syndrome is an inherited autosomal dominant disorder, which according to its name, exhibits defects in a variety of organs, including a spectrum of kidney defects ranging from mild to very severe (aplasia). The causative mutations of BOR have been identified in *EYA1*, *SIX5,* and *SIX1* genes, along with *SALL1* mutations, which cause a BOR-related disorder, Townes Brocks syndrome. See section **Aplasia and hypoplasia** for details of these genes and their functions.

Fraser syndrome The spectrum of renal defects in Fraser syndrome include hypo(dys)plasia and uni- or bilateral agenesis. Human mutations have been identified in *FRAS1* and *FREM2*, which were discussed previously (Aplasia and hypoplasia), as well as in glutamate receptor-interacting protein 1 (*GRIP1*) gene (270, 271).

Kallmann syndrome The most typical feature of Kallmann syndrome is delayed or absence of sexual development. The renal manifestation is relatively rare in Kallmann patients, but it may appear as the absence of one kidney. Kallmann syndrome mutations have been identified in *FGFR1*, *FGF8* (discussed earlier), WAP four-disulfide core domain 18 (*WFDC18*, also known as *ANOS1* and *KAL1*) (272), *SOX10* (273), and semaphorin *SEMA3A* (274).

Meckel syndrome (also known as Grubel-Meckel syndrome) consists of a posterior encephalocele, where the dorsal parts of the brain expand backwards and are only covered by skin. The patients also show postaxial polydactyly (duplicated little finger and/or toe) and cystic renal disease that somewhat morphologically resembles polycystic renal disease with liver cysts.

Meckel syndrome, similar to congenital nephrosis of the Finnish type, is greatly enriched in the Finnish population due to the founder effect, which enriches disease inheritance and manifestation in isolated populations typically due to a major mutation distribution among small number of individuals living within the population. Identification of mutations in Meckel syndrome, type 1 (MKS1) gene, encoding a protein required for the normal function of primary cilia (275), was a landmark for understanding the cellular mechanisms leading to Meckel syndrome and other ciliopathies. Since then, mutations in at least 12 more genes have been associated with Meckel syndrome (276). Despite an obvious genetic heterogeneity, the major Meckel syndrome-causing mutations are in *MKS1*, *MKS3/TMEM67*, and *MKS6/CC2D2A*.

Pallister-Hall syndrome derives from defects in Hedgehog signaling manifested as mutations in *GLI3* effector gene (277). The many-fold abnormalities in Pallister-Hall patients are often not life-threatening, unlike e.g. deletion of *Gli3* in mice, which causes renal aplasia (278). Associated kidney abnormalities of Pallister-Hall syndrome are rare, but typically manifest as renal dysplasia.

Renal coloboma syndrome The characteristic defects of renal coloboma syndrome, also known as papillorenal syndrome, include optic nerve dysplasia and renal hypodysplasia. It is a rare autosomal dominant disorder, which is caused by mutations in *PAX2* (279). Defects caused by *Pax2* inactivation in mouse have been discussed in the Aplasia and hypoplasia section above.

Conclusions

Mouse models have greatly facilitated our understanding of renal differentiation and its molecular regulation. The benefit for genetics and diagnosis of congenital kidney anomalies is also obvious. However, the majority of human CAKUT cases are idiopathic, and their causes remain unknown despite the strong familiar aggregation in approximated 15% of patients (91, 280). In addition to genetic causes, environmental and epigenetic factors are also involved, while only approximately 5-20% of CAKUT are considered monogenic disease (281-283). The two challenges in modeling congenital kidney anomalies in experimental animals are the lack of knowledge of causative gene mutation and use of inappropriate strategy to mimic given human disease in mouse. The first of these may derive from the fact that large portions of genome (GC-rich and other repetitive or difficult-to-reach regions) are not covered in whole-genome sequencing experiments. Potential aberrations in these non-coding genomic regions may be considered epigenetic causes of CAKUT, and they remain unexplored until better knowledge and methodologies are available for their exploration. The second challenge refers to the fact that mouse models of CAKUT so far have largely been made by complete inactivation of the entire gene. The next task for the scientific community is to utilize CRISPR/CAS9 methodology to mimic specific human CAKUT mutations in mice, a strategy which is expected to much better phenocopy the given anomaly it associates with in human patients.

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Figure legends

Figure 5.1. Illustration of the three developmental stages of kidney morphogenesis. A) Pronephros, the first kidney to form, is present in the fetus before the Wolffian duct (WD) makes the connection to cloaca (C), approximately at embryonic day 9.5 (E9.5). Nephrogenic cord (NC) is formed together with the WD and is depicted in light gray. **B)** Mesonephros development follows the pronephros and takes place concomitantly with WD fusion to cloaca (C). **C)** The last kidney to form is the metanephros or permanent kidney, which begins to develop when the NC has differentiated into metanephric mesenchyme (light green) and induces WD epithelium to bulge and form the ureteric bud (brown).

Figure 5.2. Schematic view of metanephros development A) Ureteric bud (UB) is divided into the trunk (dark brown) and tip (light brown) regions, which are already defined at the initial bud stage. The UB is surrounded by the metanephric mesenchyme (MM) while spindle cell stroma (S) encircles the MM. **B)** The UB branching begins by the formation of an ampulla at the tip of the UB. Simultaneously, the UB induces the MM to condense and form a pretubular aggregate (left of the UB tip, light green cells), which begins epithelialization into the nephron by forming the renal vesicle (right of the UB tip, green vesicle). **C)** The UB ampulla extends to develop two new tips while the renal vesicle further differentiates into comma-shaped body (right of the UB). **D)** UB tips continue to branch out with concurrent further connection of the comma-shaped body to the UB tips. Nephrogenesis continues by differentiation of the comma-shaped body into the S-shaped body (right of the UB). These events are reiterated until the end of branching morphogenesis when the final nephron differentiation burst uses up all remaining nephron progenitors in the metanephric mesenchyme, and in this way, each kidney attains its final nephron count.

Figure 5.3. Horseshoe kidney/renal fusion and multi-cystic dysplasia. A) A human horseshoe kidney at gestational week 13 (equal to 11 weeks of fetal development). The genetic cause for the horseshoe kidney in this fetus is trisomy 18. Fusion is always seen in the caudal part of the kidneys (arrow). **B)** Multicystic renal dysplasia is characterized by large cysts surrounded by

clear cytoplasmic stromal cells. The cysts can be either in a segment of a kidney, in one whole kidney, or in both kidneys. Abbreviations; A, adrenal gland.

Figure 5.4. Nephroblastomatosis/Nephrogenic Rests. A) Normal human kidney at gestational week 12. Arrows point to differentiating nephron precursors. **B)** Nephrogenic rests are the dark blue, highly cellular islands in the subcortical or perilobar areas. They represent persistent foci of nephrogenic blastema in the postnatal kidney in a 1 year old child. Because he had multiple foci of nephrogenic rests (white asterisks), the condition is called nephroblastomatosis. Abbreviations; G, glomerulus; U, ureteric bud; S, stroma.

Figure 5.5. Wilms' tumor or nephroblastoma. A) Normal human kidney at gestational week 12. Arrow points to a ureteric bud, B marks blastema (the metanephric mesenchyme), and S is the stroma. **B)** The morphology of the classic Wilms' tumor (also called nephroblastoma) mimics that of embryonic kidneys showing blastemal (B), stromal (S), and epithelial cells (arrow) but in a disorganized manner.