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REVIEW

Genetics of congenital anomalies of the kidney and urinary tract

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Abstract Congenital anomalies of the kidney and urinary tract (CAKUT) occur in 1 in 500 births and are a major cause of morbidity in children. Notably, CAKUT account for the most cases of pediatric end-stage renal disease and predispose the individual to hypertension and cardiovascular disease throughout life. Although some forms of CAKUT are a part of a syndrome or are associated with a positive family history, most cases of renal system anomalies are sporadic and isolated to the urinary tract. Broad phenotypic spectrum of CAKUT and variability in genotype–phenotype correlation indicate that pathogenesis of CAKUT is a complex process that depends on interplay of many factors. This review focuses on the genetic mechanisms (single-gene mutations, modifier genes) leading to renal system anomalies in humans and discusses emerging insights into the role of epigenetics, in utero environmental factors, and micro-RNAs (miRNAs) in the pathogenesis of CAKUT. Common gene networks that function in defined temporospatial fashion to orchestrate renal system morphogenesis are highlighted. Derangements in cellular, molecular, and morphogenetic mechanisms that direct normal renal system development are emphasized as a major cause of CAKUT. Integrated understanding of how morphogenetic process disruptions are linked to CAKUT will enable improved diagnosis, treatment, and prevention of congenital renal system anomalies and their consequences.

Keywords Kidney development · CAKUT · Renal hypodysplasia · Renin-angiotensin

Introduction

Congenital anomalies of the kidney and urinary tract (CAKUT) occur at a frequency of 1 in 500 live births [1, 2]. CAKUT comprise a spectrum of renal tract malformations that occur at the level of the kidney (e.g. hypoplasia, dysplasia), collecting system (e.g. hydronephrosis, megaureter), bladder (e.g. ureterocele, vesicoureteral reflux), or urethra (e.g. posterior urethral valves) (Table 1). Although some forms of CAKUT are a part of a syndrome or are associated with a positive family history, most cases of renal system anomalies are sporadic and isolated to the urinary tract. Notably, CAKUT are the most common cause of renal failure in childhood, accounting for 31% of children with end-stage renal disease (ESRD) in the United States [3]. All children with ESRD require renal replacement therapy, and up to 70% of them develop hypertension [4]. These observations emphasize the need for development of new strategies aimed at decreasing the incidence of CAKUT, preserving renal function, and preventing morbidity from cardiovascular disease. Despite the diverse anatomical and histological spectrum of kidney and urinary tract malformations, all forms of CAKUT stem from faulty renal system development. To understand the basis of

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Table 1 Nonsyndromic human congenital anomalies of the kidney and urinary tract (CAKUT)

Type of malformation	Cause	Anatomical and histological characteristics	Gene	References
Renal agenesis	No interaction between the UB and MM	Absence of the ureter and kidney	*Ret, GDNF*	[118]
Renal hypoplasia	Aberrant interactions among the UB, MM or stroma	Reduced number of UB branches and nephrons that are fully formed, small kidney size	*Pax2, Sall1*	[69]
			Six2, BMP4	[79]
			HNF1β	[61, 69]
			UMOD	[119]
Renal dysplasia	Aberrant interactions among the UB, MM or stroma	Reduced number of UB branches and nephrons. Presence of undifferentiated stromal and mesenchymal cells, cysts or cartilage. Frequently associated with kidney hypoplasia	*Pax2*	[69]
			HNF1β	[61, 69]
			UMOD	[65]
			Nphp1	[120]
			BMP4, *Six2*	[79]
			XPNPEP3	[120]
Polycystic kidneys	Aberrant tubular and collecting duct patterning	Cysts in tubules and collecting ducts Normally formed glomeruli	*Pkd1*, *Pkd2*	[56]
			HNF1β	[61]
			NPHP1	[120]
Multicystic dysplastic kidneys	Aberrant interactions among the UB, MM or stroma	Absence of glomeruli and tubules	*HNF1β*	[61, 69]
		Presence of large cysts	*UPIIIA*	[78]
		Aberrant patterning		
		Poorly formed atretic ureters		
		Small remnant kidney (if organ involutes)		
Medullary cystic kidney disease 2	Aberrant tubular and collecting-duct patterning	Tubular atrophy, interstitial fibrosis, cysts in distal tubules and medullary collecting ducts	*UMOD*	[63]
Duplex ureters	Supernumerary UB budding from the ND	Duplex ureters and kidneys or duplex ureters and collecting systems	*Robo2*	[70]
		May be associated with VUR or obstruction if UB budding is ectopic	*FoxC1*, *FoxC2*	[121]
			BMP4	[79]
Horseshoe kidney	Defects in renal capsule	Kidneys are fused at inferior lobes and located lower than usual	*HNF1*β	[60]

human CAKUT, it is essential to consider how the kidney and urinary tract develop.

Morphologic development of the kidney and urinary tract

Development of the human kidney begins when the nephric duct (ND) is formed from the intermediate mesoderm on embryonic (E) day 22 [5]. The ND extends caudally and induces the adjacent mesoderm to form two transient kidney types, pronephros and mesonephros. Mesonephros gives rise to male reproductive organs and involutes. On E28, ND forms an epithelial outgrowth called the ureteric bud (UB), which invades the adjacent metanephric mesenchyme (MM) to induce permanent metanephric kidney (Fig. 1). Formation of the metanephros occurs via reciprocal inductive interactions between the UB and the mesenchyme [6]. Multiple gene networks have been shown to induce metanephric organogenesis [7–9]. Distal UB will undergo branching morphogenesis to form the renal collecting system (collecting ducts, renal calyces, pelvis, and ureter), whereas MM will give rise to all epithelial cells that form nephrons (from the glomerulus through the distal tubule) [10, 11]. UB branching is completed by 22 weeks of gestation, whereas nephrogenesis continues until 34–36 weeks of fetal life. In the absence of UB branching after 22 weeks of gestation, nephrogenesis continues through the poorly understood process of arcade formation [12]. Initial generations of UB branches are remodeled into the ureter, pelvis, and calyces, whereas subsequent generations give rise to collecting ducts. Linear arrays of inner (medullary) collecting ducts will converge centrally to form the papilla. Following acquisition of the full complement of nephrons, subsequent glomerular development occurs via hypertrophy [13]. The bladder and urethra originate from

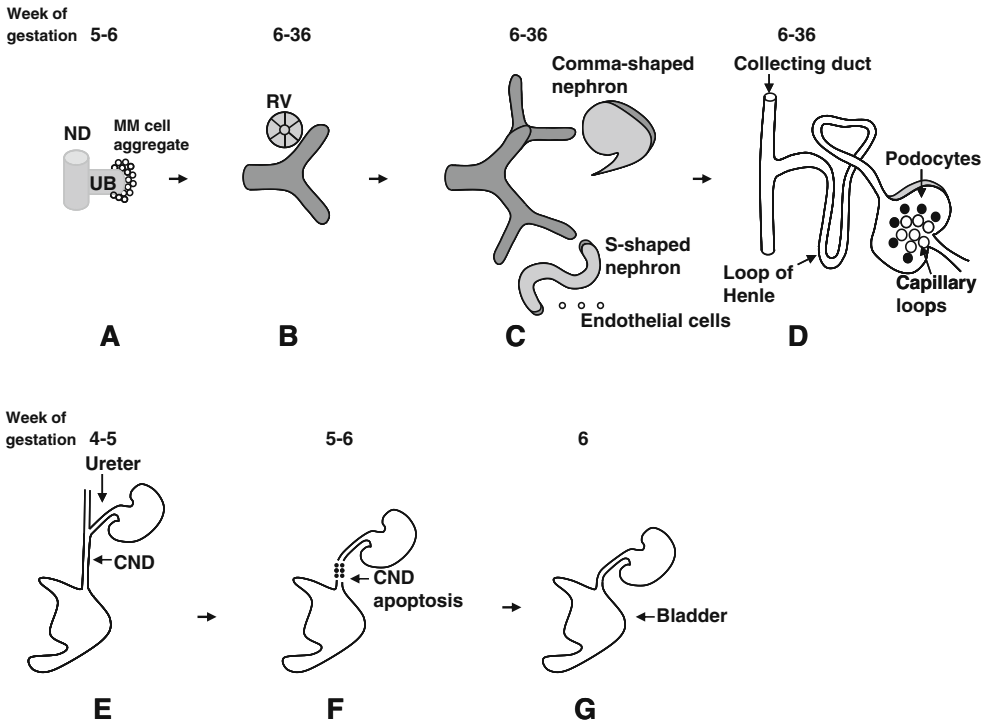

Fig. 1 Normal development of the kidney and urinary tract. **a** Invasion of the metanephric mesenchyme (MM) by the ureteric bud (UB) on weeks 5–6 of gestation induces MM cells to aggregate around the UB tip. **a**–**c** UB outgrowth from the nephric duct (ND), its subsequent iterative branching (branching morphogenesis), and continuous condensation of the MM cells around emerging UB tips are induced primarily by reciprocal interactions among glial-derived neurotrophic factor (*GDNF*), its receptor *c-Ret*, and its coreceptor *GFRα1*. **b** MM cell aggregates undergo mesenchymal-to-epithelial transformation (MET) to form the renal vesicle (RV) on weeks 6–36 of gestation. **c** RV elongates along the proximal–distal axis to form comma-shaped and then S-shaped nephrons. Distal ends of S-shaped nephrons fuse with UB-derived collecting ducts, whereas proximal clefts form glomeruli. Endothelial cells migrate into the proximal cleft. UB branching occurs on weeks 6–22 of gestation. Formation of nascent nephrons and their patterning occur on weeks 6–36 of gestation. **d** Patterning of the S-shaped nephron and UB results in formation of mature nephrons that contain glomerular capillary tuft, podocytes, proximal tubule, loop of Henle, distal tubule, and collecting duct. **e** Ureter becomes patent, and common ND (CND) fuses with cloaca on weeks 4–5 of gestation. **f** Apoptosis of the CND accounts for the positioning of the ureter (derived from proximal UB) in proximity of the urogenital sinus on weeks 5–6 of gestation. **g** Ureter fuses with the bladder by 6 weeks of gestation. Please see text for details

the urogenital sinus. In turn, urogenital sinus is formed from the cloaca. Proximal UB will form the ureter, which will dissociate from the ND to fuse with the bladder epithelium in the trigone, the muscular region located at the base of the bladder [14].

Molecular biology of mammalian kidney and urinary tract development

Genetic manipulations in mice provided critical information on the cellular and molecular mechanisms that orchestrate kidney and urinary tract morphogenesis, yielding new insights into the pathogenesis of CAKUT [6–8, 14]. Organogenesis of the metanephric kidney and lower urinary tract is coordinated by complex interactions among numerous transcription/growth factors and intracellular signaling molecules that may be collectively referred to as renal developmental genes (RDGs) [6–8, 14]. RDGs are expressed in the MM, stroma, angioblasts, UB, and cloaca [6–9, 14]. Here, we review the most significant gene networks that function at multiple stages of renal system development.

UB induction from the ND is mediated by glial-derived neurotrophic factor (*GDNF*) secreted by MM, which interacts with the *c-Ret* receptor tyrosine kinase (RTK) expressed in the UB to induce branching [15, 16] (Fig. 1). *GDNF* levels and its spatial expression are regulated by multiple transcription and growth factors, such as paired box 2 (*Pax2*), eyes absent homolog 1 (*Eya1*), SIX homeobox (*Six)1*, *2*, and *4*, forkhead/winged-helix transcription factor d1 (*FoxC1*), roundabout, axon guidance receptor, homolog 2 (*Robo2*), slit homolog 2 (*Slit2*), wingless-type MMTV integration site family, member 11, (*Wnt11*) [6, 7]. *c-Ret* expression and signaling activity is inhibited by sprouty homolog 1 (*Spry1*) and induced by *Pax2* or retinoic acid [17–19]. Interestingly, other growth factors, such as fibroblast growth factor (FGF) 10, can

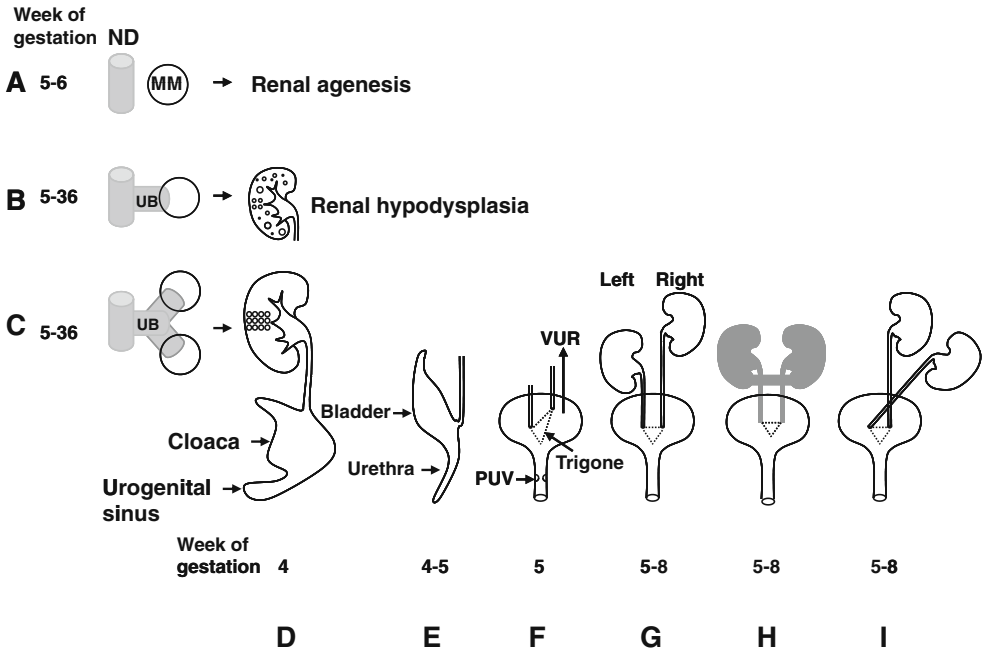

Fig. 2 Normal and abnormal development of the kidney and urinary tract. Reciprocal inductive interactions between the ureteric bud (UB) and metanephric mesenchyme (MM) are essential for normal metanephric kidney development. **a** Absence of UB outgrowth from the nephric duct (ND) on weeks 5–6 of gestation results in renal agenesis. **b** Aberrant interactions between the UB and MM on weeks 5–36 of gestation lead to renal hypodysplasia (small kidney containing normally formed nephrons, immature undifferentiated nephrons or cysts). **c**, **d** Normal interactions of the UB with the MM and cloaca on weeks 5–36 of gestation result in formation of normally structured urinary system. Urogenital sinus separates from the rectum on the 4th week of gestation. **e** Cloaca and urogenital sinus give rise to bladder and urethra on weeks 4–5 of gestation. **f** A UB that originates at proper site of outgrowth from the ND will join the bladder trigone normally (*left*). A UB that outgrows too low from the ND joins the trigone lateral and anterior (higher), causing vesicoureteral reflux (VUR) (*right*). Trigone is formed on the 5th week of gestation. Retention of the remnants of the urogenital membrane lead to formation of posterior urethral valves (PUV). Embryogenesis of PUV and its timing are unknown. **g** Failure of ascent of the left kidney leads to pelvic kidney. Kidney ascent occurs on weeks 5–8 of gestation. **h** Defects in renal capsule cause kidney fusion at lower poles (horseshoe kidney). Due to failed upward migration, horseshoe kidney is located lower than usual. **i** Abnormal migration of the left kidney on weeks 5–8 of gestation leads to crossed ectopia

induce UB branching in the absence of *GDNF* or *c-Ret* RTK [20]. Stromal cells stimulate UB branching and nephrogenesis via (*Foxd1*), retinoic acid, FGF7, bone morphogenetic protein 4 (BMP4), and podocyte 1 (*Pod1*) [21–23]. UB signals to MM by secreting Wnt9b, a soluble growth factor, that acts via canonical β-catenin to induce expression of *FGF8*, LIM homeobox 1 (*Lhx1*), and *Wnt4* in the MM [24–26]. In turn, Wnt4 induces MM cells to undergo mesenchymal-to-epithelial transformation (MET) and differentiate into nephron epithelia [24]. *Wnt9b*-induced MET is regulated by hepatocyte nuclear factor 1β (*HNF1β*) [27]. In addition to its role in MET, *Wnt9b* is also required for later nephron patterning [28]. *Six2*, a homeodomain transcription factor expressed in the MM, maintains MM cells in an undifferentiated state, thereby allowing continued UB branching and nephron formation to proceed [29]. Iterative UB branching and nephron induction are accompanied by their continuous patterning (gradual acquisition of structure characteristic of the mature organ). Examples of patterning include nephron segmentation and UB remodeling into the renal collecting system. With respect to nephrogenesis, pretubular-mesenchymal-cell aggregates are induced at the tips of the UBs and then undergo MET to form the renal vesicle (RV) (Fig. 1). The RV elongates along the proximal–distal axis to form comma-shaped and then mature nephron, which comprises the following distinct regions: glomerulus, proximal tubule, loop of Henle, and distal tubule. Transition form the RV to comma-shaped nephron is induced by transcription factors *Lhx1* and POU class 3 homeobox 3 (*Brn1*) [29]. In turn, comma-shaped nephron elongates to form an S-shaped body. Distal parts of the S-shaped body differentiate into distal tubule segments under control of *Brn1* and Iroquois-class homeodomain (*Irx*) transcription factors *Irx*1–3 [30], whereas proximal tubule formation is controlled by *Notch2* [31]. *Notch2* controls proximal nephron development by stabilizing, rather than specifying, its fate via depletion of *Six-2*-positive progenitor cells [32]. Podocyte differentiation is regulated by transcription factors Wilms tumor 1 (*WT1*), *Pax2*, LIM homeobox transcription factor 1β (*Lmx1β*), and semaphorin3a (Sema3a) [33–35]. Podocytes induce migration of vascular endothelial cells into the

vascular cleft by releasing vascular endothelial growth factor (VEGF) [36]. In turn, endothelial cells promote differentiation of mesangial cells via production of platelet-derived growth factor (PDGF) [37]. Dissociation of the ureter from the ND and its fusion with the bladder is regulated by retinoic acid-, c-Ret-, and leukocyte antigen-related (LAR)-family protein tyrosine phosphatase-dependent apoptosis of the common ND [38–40]. Despite new insights into the molecular basis of kidney and urinary tract development, integrated understanding of this process is far from complete. Continued use of model organisms complemented by new technologies will enable identification of novel genes and factors that act in concert to form a kidney.

Developmental origin of CAKUT

Animal model systems have provided evidence for an association between interruption of specific molecular pathways and CAKUT phenotype. For example, absence of UB outgrowth from the ND observed in the presence of *GDNF* or *Ret* mutations will lead to renal agenesis [6] (Table 1, Fig. 2). Notably, unilateral renal agenesis occurs in 0.008% of fetuses and in 1 in 5,000 newborns and bilateral in 0.013% fetuses and in 1 in 30,000 newborns [41, 42]. Ectopic UB outgrowth from the ND may lead to obstruction, vesicoureteral reflux (VUR), hydronephrosis, duplex ureters, renal collecting systems, or kidneys [43, 44]. Congenital hydronephrosis has an overall prevalence of 1 in 1,000 live births [45]. Molecular mechanisms leading to hydronephrosis/hydroureter in the absence of anatomic obstruction include T-box transcription factor 18 (*Tbx18*) or *Six-1*-dependent delay in differentiation of ureteral smooth muscle cells [46, 47].

Aberrant interactions between the UB and MM or stroma result in renal hypodysplasia, which is observed in 0.027% of fetuses by ultrasonography [42] (Fig. 2). Importantly, renal hypodysplasia due to decreased nephron endowment is causally linked to hypertension and eventual progression to chronic renal failure [48, 49]. Horseshoe kidney, which occurs in 1 in 1,000 newborns, may be caused by defects in renal capsule and failed migration due to aberrant stromal *Foxd1* signaling [50, 51]. Nephron patterning defects may be due to aberrant cell proliferation, apoptosis, cell movements, or other causes. In this regard, altered balance of cell proliferation and apoptosis is responsible for abnormal patterning of the medulla in Beckwith–Wiedemann syndrome due to mutation in $p57^{KIP2}$, an inhibitor of cell proliferation [52]. Polycystic kidney disease (PKD), in which cysts arise from tubules that were formed normally in the context of development, represents another example of patterning defect. Here, increased tubular cell proliferation together with decreased integration of cells into the plane of tubular epithelium or loss of oriented cell division may account for postnatal cyst formation [53]. PUV, observed in 0.003% of fetal ultrasounds, are due to aberrant cloacal development [42]. A concerted effort to provide new insights into pathologic processes underlying the development of CAKUT is in progress within the Genitourinary Development Molecular Atlas Project (GUDMAP) [54].

Genetic mutations associated with human renal hypodysplasia

It is well appreciated that many instances of human CAKUT have a genetic basis and are associated with hereditary syndromes (Supplemental Table 1) [55]. The inheritance pattern of some nonsyndromic cases of CAKUT (e.g. autosomal-dominant or -recessive polycystic kidney disease) is well known [56]. Evidence is accumulating to suggest that mutations in select RDGs may be causally linked to the pathogenesis of nonsyndromic forms of CAKUT in humans (Table 1). Interestingly, mutations in a given RDG may be associated with a spectrum of urinary system anomalies. For example, Branchio-oto-renal (BOR) syndrome due to *Eya1* mutations may be accompanied by renal agenesis or dysplasia, hypoplasia, or renal collecting system anomalies [57]. It is therefore conceivable that *Eya1* plays an important role at multiple stages of renal system morphogenesis. One of the mechanisms accounting for stage-specific impact of *Eya1* mutation on renal system phenotype may involve cell-type-dependent interplay of up- or downstream regulators of *Eya1*. This possibility is supported by the findings that *HNF1β,* developmentally regulated transcription factor induces expression of uromodulin (*UMOD*) [58]. Although *HNF1β* or *UMOD* mutations in humans cause renal hypodysplasia, the spectrum of disease phenotypes and age of their manifestations differ. *HNF1β* mutations manifest earlier in childhood and are associated with glomerulocystic kidney disease, unilateral multicystic dysplastic kidney (MCDK), uni- or bilateral cystic kidneys, renal hypodysplasia without cysts, horseshoe kidney, and oligomeganephronia [59–62]. *HNF1β* mutations are detected in 33% of children with nonsyndromic MCDK or renal hypodysplasia [61]. Most genetic alterations (64%) in this study were due to complete deletion of the gene, followed by truncating and nonsense mutations. No correlation between type of mutation and phenotype was observed. Interestingly, in 53% of probands, *HNF1β* mutations occurred de novo [61]. These findings suggest a weak probability of genetic inheritance.

UMOD mutations are identified in medullary cystic kidney disease 2 (MCKD2) and familial juvenile hyper-

uricemic nephropathy, diseases that manifest later in life [63]. In fact, no known *UMOD* mutations were identified in children with diverse forms of nonsyndromic CAKUT in a study by Wolf et al. [64]. The authors proposed that unknown *UMOD* mutations may be causally linked to CAKUT in children. This possibility is supported by recent identification of a novel *UMOD* sequence variant in a child with decreased kidney function and hyperuricemia accompanied by histologic evidence of immature renal structures [65]. In contrast to the impact of *HNF1β* or *UMOD* mutations on renal disease phenotype, *Pax2*, a transcription factor present in the metanephric mesenchyme, but not *GDNF*, polymorphism is associated with reduced kidney size in neonates [66, 67]. GDNF/c-Ret pathway is critical for metanephric development and *GDNF* expression is activated by *Pax2* [68]. Again, differential effects of *Pax2* and *GDNF* polymorphism on renal phenotype may be due, in part, to unidentified cell-specific cofactors that regulate gene expression. Interestingly, RDG mutations associated with syndromal CAKUT [*HNF1β*, *Eya1*, *Six1*, sal-like 1 (*Sall1*), *Pax2*] are identified only in 5–15% of children from the European population with nonsyndromic CAKUT [69]. Another recent study that examined children from the European population found no association of primary VUR with mutations in *Pax2*, *HNF1β*, *c-Ret* or *Robo2* [70].

Several studies report a discrepancy in the impact of genetic mutations on renal-system phenotype between mice and humans. For example, despite severe renal phenotype observed in *ROBO2/SLIT2*-mutant mice, which includes formation of supernumerary ureters [44], these gene mutations are very rarely associated with familial nonsyndromic VUR in children [71, 72]. Similarly, mutations in homeobox A11 and D11(*HoxA11*/*HoxD11*), which cause renal hypoplasia in mice [73], are not associated with CAKUT in children [74]. In addition, uroplakin (UP) *UPII* or *UPIIIA* mutations, which, respectively, cause hydronephrosis or VUR in mice [75], are not detected in the majority of children with nonsyndromic cases of CAKUT. However, *UPII* or *UPIIIA* mutations may account for CAKUT (e.g. MCDK or VUR) in a minority of these children [76–78]. Functionally, UPs account for impermeability of bladder epithelia to water. Mutations in *Six2* and *BMP4*, which are expressed in the fetal kidney on weeks 9 and 12 of gestation, are identified in children with a broad spectrum of CAKUT ranging from unilateral or bilateral hypodysplasia to cystic dysplasia with or without VUR [79]. Moreover, detected *BMP4* mutations are associated with decreased *BMP4* messenger RNA (mRNA) levels and enhanced protein degradation [80]. Collectively, these data indicate that aberrant expression of RDGs is associated with diverse forms of CAKUT in humans. Interestingly, RDG mutations are detected in a minority of these patients [68]. These observations point to the potential role for other mechanisms in the developmental origin of CAKUT.

Insights into the mechanisms controlling CAKUT phenotype

Although mutations in many different single RDGs are associated with broad phenotypic spectrum of CAKUT ([6–9, 14], Tables 1 and 2, Supplemental Table 1), variability in genotype–phenotype correlation in renal system anomalies points to the essential roles of other factors. Phenotypic heterogeneity of CAKUT may result from differences in genetic background (variation of gene expression between alleles) [81], genetic [47, 82, 83] or epigenetic modifiers [84–86], and environment [87, 88]. In monogenic diseases, phenotypic outcome and disease severity depend on the mode of inheritance and type of mutation. Recessive diseases, such as autosomal recessive polycystic kidney disease, usually manifest full penetrance and present earlier in life. Disease course is more severe in truncating than missense polycystic kidney and hepatic disease 1 (*PKHD1*) mutations [89]. The strength of genotype–phenotype correlation is reduced in autosomal dominant nonsyndromic types of CAKUT [90]. This may be due to incomplete

Table 2 Renin–angiotensin system (*RAS*) gene mutations in human congenital anomalies of the kidney and urinary tract (CAKUT)

Gene mutation	Renal system phenotype	References
Angiotensinogen	Renal tubular dysgenesis: reduced number of proximal tubules, short proximal tubules without brush border, atrophic loops of Henle and collecting ducts, closely packed glomeruli, marked thickening and disorganization of interlobular and preglomerular arteries	[111, 112]
Renin	Renal tubular dysgenesis	[111, 112]
ACE	Renal tubular dysgenesis	[111, 112]
	Renal hypodysplasia due to posterior urethral valves	[117]
AT_1 receptor	Renal tubular dysgenesis	
AT_2 receptor	UPJ stenosis, megaureter, MCDK, hydronephrosis, PUV	[113–116]

UPJ ureteropelvic junction, *MCDK* multicystic dysplastic kidney, *PUV* posterior urethral valves. Please see Supplemental Table 2 for glossary of genes

penetrance (lack of disease manifestation in the presence of gene mutation) or variable expression (variation in type and severity of disease between individuals with the same gene mutation). Variability in genotype–phenotype correlation in CAKUT may also result from a modifier gene effect. Here, mutation in one gene will cause CAKUT or alter the phenotype only in the presence of genetic change in another gene (epistatic gene interactions). In this regard, nephronophthisis 1 (*NPHP1*) mutation causes isolated nephronophthisis, whereas *NPHP6* mutation alone does not lead to disease. In contrast, a combination of the same mutations in *NPHP1* and *NPHP6* causes an additional extrarenal disease phenotype [91]. Other RDGs that interact genetically and may influence renal phenotype include *Pax2* and *LMX1B* or *Six1* and *Tbx18* [47, 82]. Noteworthy, two missense mutations in *Six1* identified in patients with BOR syndrome reduce *Six1-Tbx18* complex formation [47].

In polygenic diseases, mutations in multiple genes act in concert with environmental effects to cause a phenotype. With respect to renal system anomalies, intrauterine environment and fetal programming have been linked to CAKUT. Maternal low-protein diet initiated at onset of pregnancy reduces nephron number and alters gene expression in the embryonic metanephros in mice [87]. Recent data indicate that environmental factors may act in concert with single-gene mutations to cause CAKUT. In this regard, renal hypodysplasia is caused by salt loading during embryonic development in bradykinin B2 receptor-deficient, but not in wild-type, mice [88]. Exposure to cocaine or alcohol during gestation results in CAKUT in children [92, 93]. Importantly, the mechanistic basis for CAKUT associated with altered intrauterine environment remains to be elucidated.

There is a growing appreciation of the role of epigenetics in the regulation of gene expression and disease causality. The major players in epigenetic mechanisms of gene regulation are DNA or chromatin protein methylation, acetylation, and chromatin remodeling. Posttranslational modifications of histones such as histone acetylation and methylation may affect chromatin function and alter RDG expression in the absence of changes in DNA sequence [94, 95]. Relevant to pathogenesis of CAKUT, a recent study demonstrated a link between Pax2, a transcription factor critical for renal morphogenesis, and chromatin methylation [84]. Gene expression may also be regulated at the posttranscriptional level by noncoding micro-RNAs (miRNAs). A critical role for miRNAs in kidney development is evident from the observation that targeted genetic inactivation of Dicer, the miRNA-processing enzyme, in UB in mice results in hydronephrosis [96]. Collectively, these observations indicate that pathogenesis of CAKUT is a complex process that depends on the interplay of many factors. Moreover, despite advances in elucidation of the cellular and molecular mechanisms linking kidney organogenesis to CAKUT, our understanding of its cause in an individual patient is still too limited.

Renin–angiotensin system and CAKUT

The renin–angiotensin system (RAS) plays a major role in controlling blood pressure, fluid, and electrolyte homeostasis. The developing mammalian metanephros expresses all components of the RAS [9]. Mutations in the genes encoding components of the RAS in mice cause diverse forms of CAKUT, which include hydronephrosis, hypoplastic medulla and papilla, marked thickening of renal arterial walls, duplex ureters, and vesicoureteral reflux [9, 97, 98]. Genetic manipulations in mice provided essential information regarding the mechanisms by which an intact RAS controls proper renal system development. The mechanistic basis of CAKUT due to *RAS* mutations may involve hypoplastic ureteral smooth-muscle layer, which exhibits impaired peristalsis [99], inhibition of *Pax2* [100], *GDNF*/*c-Ret* [101, 102], or epidermal growth factor (EGF)-receptor [103] signaling, induction of *BMP4* [104], decreased UB cell proliferation/survival resulting in reduced UB branching [101, 104] and reduced glomerular size [100]. Reduction in *EGF* and *PDGF* expression in the medulla may account for renal papillary hypoplasia observed in angiotensinogen (*AGT*)-null mice [105]. In addition, increased *PDGF* expression in endothelial cells may be responsible for thickening of the renal arteries observed in these mice [105].

With respect to epigenetic modifications of the *RAS* genes and its impact on CAKUT, recent studies in mice demonstrated that genetic inactivation of histone acetyltransferases such as cyclic adenosine monophosphate (cAMP)-response element-binding protein [CREB; (CBP)] and p300 alters kidney structure and results in a decreased number of renin-positive cells [86]. Notably, kidneys of CBP/p300-null mice exhibit interstitial fibrosis, disorganized vasculature, and medullary and cortical cysts [86]. In addition, targeted genetic inactivation of Dicer in juxtaglomerular cells causes a decrease in renin expression accompanied by striped fibrosis, a decrease in the number of renal vessels, and glomerular sclerosis [106]. These data demonstrate that epigenetic modifications of the RAS and miRNAs play an important role in maintaining renal structural integrity.

Impact of *RAS* gene mutations on CAKUT in humans

All components of the RAS are expressed in the embryonic kidney as early as the fifth week of gestation [107, 108]

when metanephric organogenesis is initiated [5]. Initial evidence to indicate the critical role of the RAS in renal system organogenesis in humans was provided by the findings that the use of angiotensin-converting enzyme (ACE) inhibitors or AT_1-receptor antagonists causes fetal anuria, leading to oligohydramnios [109, 110]. Evidence is emerging to suggest that mutations in the *RAS* genes may be causally linked to the pathogenesis of CAKUT in humans. In this regard, homozygous or compound heterozygous mutations in the genes encoding for AGT, renin, ACE, or AT_1 receptors in French children are associated with renal tubular dysgenesis (RTD) [111]. Detected DNA mutations include the following nucleotide alterations: 1124G>A (AGT), 145C>T, 689G>A, 310G>A (renin), 798C>G (ACE), 845C>T (AT_1R). In RTD, the kidneys are usually of normal size. Renal cortex exhibits a paucity of proximal tubules. In the medulla, collapsed collecting ducts and abundant interstitial fibrosis are observed [112]. Vascular changes involve thickening of arterial walls. Glomerular number may be normal, low, or increased, whereas glomerular size is normal or enlarged. In addition, all affected individuals exhibit hypotension. Importantly, the majority of children with RTD die in the perinatal period due to anuria combined with pulmonary hypoplasia. Few patients survive beyond the neonatal period. However, no correlation has been identified so far between the type of mutation and clinical course.

Association of single polymorphism in the human AT_2R gene, 1332A>G transition, with CAKUT have been reported in several, but not all, studies. This alteration in the AT_2R is described in American and German Caucasian males with ureteropelvic junction (UPJ) stenosis, megaureter, and MCDK [98, 113]. The same transition was detected in Korean children with unilateral MCDK, renal agenesis, and hydronephrosis [114]; in Italian children with VUR, hypoplastic kidney, UPJ stenosis, nonobstructive megaureter, and PUV [115], and in Serbian children with UPJ, VUR, or megaureter [116]. In contrast, there was no association of primary VUR with mutations in AT_2R in a recent European study [70]. This difference may be due to an insufficient number of patients studied, limitations in study design, low penetrance of AT_2R mutation, contribution of modifier genes, or other factors. *ACE* polymorphism is associated with kidney hypodysplasia caused by posterior urethral valves [117]. Collectively, emerging evidence points to an important role for the intact RAS in structural maturation of the kidney and urinary tract in humans.

The reasons for different renal phenotypes, particularly in papillary hypoplasia and hydronephrosis observed in mice but not in humans with *RAS* gene mutations are not clear. Additional discrepancy includes the presence of severe proximal tubular damage in humans and apparent absence of these changes in mice. One potential mechanism to explain these differences may include timing in completion of nephrogenesis. In this regard, new nephron formation ceases at 36 weeks of gestation in humans but continues for 2 weeks after birth in mice. It is conceivable that postnatal rise in renal blood flow (RBF) contributes to normal proximal tubule patterning in mice. In addition, rising RBF may enhance the postnatal urine flow rate, which, together with decreased ureteral peristalsis [99], will cause hydronephrosis.

Conclusions

Given the broad spectrum of human CAKUT and variable clinical impact of different forms of renal system anomalies ranging from mild asymptomatic cases to severe kidney injury manifesting before or after birth, each patient with CAKUT requires individualized clinical management. Because morbidity in mild cases of CAKUT may not manifest until later in life, these patients should be closely followed throughout life. Medical monitoring should include diet, nutritional status, growth, blood pressure, renal function, proteinuria, and urinary tract imaging. New biomarkers are needed to better assess disease progression and therapeutic strategies (e.g. prenatal correction of obstruction or use of renin system inhibitors to slow progression of renal injury to preserve renal function). Genetic counseling is recommended for all patients with familial cases of CAKUT or newly diagnosed forms of CAKUT that suggest presence of genetic anomalies. Introduction of more sensitive array-based methods that allow screening for multiple gene mutations and unravel a complex network of molecular interactions will help determine and predict occurrence and consequences of CAKUT. Finally, establishment of shared large biorepositories of patients with CAKUT for molecular, genetic, and translational studies will have a major impact on designing novel strategies to prevent and manage CAKUT.

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