



Disorders of Kidney Formation

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Introduction

Congenital anomalies of the kidneys and urinary tract, otherwise known as CAKUT, are classical disorders of development that are the most common cause of renal failure in children [1–3]. These disorders encompass a spectrum of entities including renal agenesis, renal hypodysplasia (RHD), multicystic kidney dysplasia, duplex renal collecting systems, ureteropelvic junction obstruction (UPJO), ureterovesical junction obstruction, megaureter, posterior urethral valves and vesicoureteral reflux (VUR). While congenital disorders like autosomal recessive and autosomal dominant polycystic kidney disease (PKD), nephronophthisis, and heritable nephrotic syndrome could also be considered as disorders of kidney formation, these generally occur later in kidney development as part of terminal cell dif-

ferentiation events. However, some of the genes that cause nephronophthisis are also associated with CAKUT. In this chapter, we discuss disorders that arise during the early inductive events that lead to the formation of the kidneys and the urinary tracts. Both tissues arise from a common primordial tissue known as the mesonephric duct and thus, congenital kidney and urinary tract malformations commonly co-occur. In this chapter, we will focus on renal disorders encompassed within CAKUT and discuss their etiology, clinical manifestations and management. Other disorders within CAKUT with significant urinary tract pathology like UPJO, ureterovesical junction obstruction, megaureter, posterior urethral valves and VUR are discussed in other chapters.

Classification and Definition of Renal Malformations

Congenital malformations of the kidney can be defined at the macroscopic level by changes in size, shape, location, or number or microscopically by changes within specific lineages like the ureteric bud, the metanephric mesenchyme, or combinations of both [4]. In clinical practice, most congenital renal malformations are defined grossly using imaging methods like ultrasound and nuclear medicine scans. Sometimes renal tissue is obtained from biopsies or from nephrectomies, and in these cases, histological definitions

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of renal hypoplasia, renal dysplasia, and multicystic renal dysplasia can be utilized for classification. One can group congenital malformations of the kidney as follows:

Changes in size

- Renal hypoplasia
- Renal dysplasia

Changes in shape

- Multicystic dysplastic kidney (MCDK)
- Renal fusion

Changes in location

- Renal ectopia
- Renal fusion

Changes in number

- Renal duplication
- Renal agenesis

When induction events do not occur at the right time or location during embryogenesis, the kidneys may fail to form (agenesis, hypoplasia, dysplasia), the kidneys may form, but in the wrong location (ectopia \pm hypoplasia/dysplasia), the kidneys may fail to migrate to the correct location (fusion \pm hypoplasia/dysplasia) or there may be multiple induction events that arise (duplication). Malformations can be either unilateral or bilateral. Importantly, from animal models and human studies, disorders of renal formation are frequently observed with concurrent lower urinary tract malformations. In these cases, it is not clear if the impairment in induction of the kidney is primary or secondary to

urinary tract obstruction. Renal agenesis refers to congenital absence of the kidney and ureter. Typically, renal malformations defined as renal hypoplasia or dysplasia are grossly small in size, defined as less than 2 SD below the mean for kidney length or weight [4–6]. Usually, renal hypoplasia or dysplasia is defined based on the presence of a small hyperechogenic kidney from ultrasound imaging [7]. Simple renal hypoplasia is defined as a small kidney with a reduced number of nephrons and normal architecture. Renal dysplasia is defined by the presence of malformed kidney tissue elements. Characteristic microscopic abnormalities include abnormal differentiation of mesenchymal and epithelial elements, a decreased number of nephrons, loss of corticomedullary differentiation and the presence of dysplastic elements including cartilage and bone (Fig. 8.1). As stated, dysplastic or hypoplastic kidneys are typically small, but can range in size and appear normal or even large due to the presence of multiple cysts or coincident urinary tract obstruction with hydronephrosis. The MCDK is an extreme form of renal dysplasia and is defined grossly as a non-reniform collection of cysts.

In addition to defects in renal formation that affect size, shape, location or number, and tissue patterning, congenital anomalies of the kidneys also encompass defects in the number of nephrons formed. Nephron number in the lower range of that normally observed, but not so low that it would result in renal insufficiency during childhood and/or adolescence, typically manifests in adulthood with hypertension and/or chronic kidney disease (CKD) [8–10].

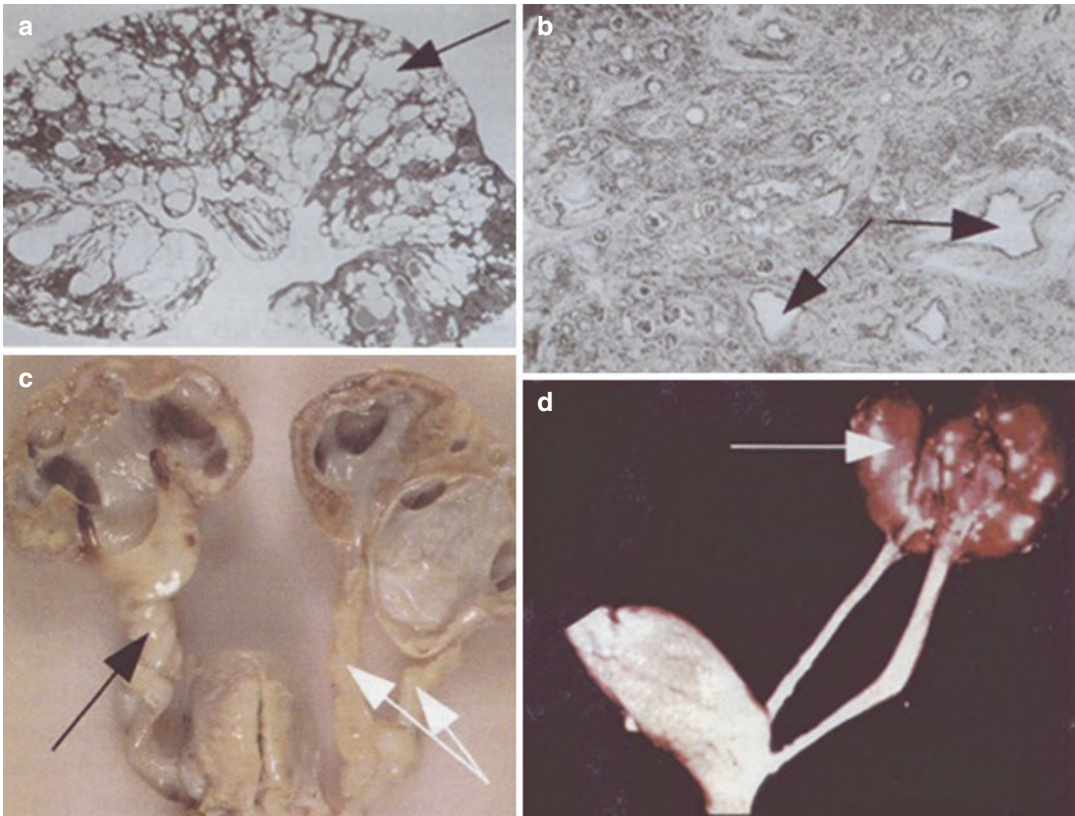


Fig. 8.1 Anatomical features of human renal and lower urinary tract malformations. (a) MCDK characterized by numerous cysts (arrow) distorting the renal architecture. (b) Dysplastic renal tissue demonstrating lack of recognizable nephron elements, dilated tubules, large amounts of stromal tissue and primitive ducts (arrows) character-

ized by epithelial tubules with fibromuscular collars. (c) Ureteral duplication (right, white arrows) and dilated ureter (left, black arrow) associated with a ureterocele. All ureters are obstructed at the level of the bladder and are associated with hydronephrosis. (d) Crossed fused ectopia with fused orthotopic and heterotopic kidneys (arrow)

Epidemiology and Longterm Outcomes of Renal Malformations

The prevalence of renal and urinary tract malformations is 0.3–17 per 1000 liveborn and stillborn infants [11, 12]. Due to their common embryonic origin from the mesonephric duct, lower urinary tract abnormalities are found in about 50% of patients with renal malformations and include VUR (25%), UPJO (11%), and ureterovesical junction obstruction (11%) [13, 14]. Renal malformations are commonly detected in the antenatal period and account for 20–30% of all anomalies detected [15]; upper urinary tract dilatation is the most frequent abnormality that is observed. All major organs are formed between

the 4th and 8th week of gestation: the neural tube closes, the aortic arches undergo transformation, the cloacal membrane ruptures, and the kidneys begin to form. Renal malformations are therefore observed in association with non-renal malformations in about 30% of cases [12]. Indeed, there are over 100 multiorgan syndromes associated with renal and urinary tract malformations [16, 17] (Table 8.1).

Bilateral renal agenesis occurs in 1:3000–10,000 births and males are affected more often than females. Unilateral renal agenesis has been reported with a prevalence of 1:1000 autopsies. The incidence of unilateral hypoplasia/dysplasia is 1 in 3000–5000 births (1:3640 for the MCDK) compared to 1 in 10,000 for bilateral dysplasia

Table 8.1 Most frequent syndromes, chromosomal abnormalities and metabolic disorders with renal or urinary tract malformation

Syndromes
Beckwith-Wiedemann
Cerebro-oculo-renal
CHARGE
DiGeorge
Ectrodactyly, ectodermal dysplasia and cleft/lip palates
Ehlers Danlos
Fanconi pancytopenia syndrome
Fraser
Fryns
Meckel
Marfan
MURCS Association
Oculo-auriculo-vertebral (Goldenhar)
Oculo-facial-digital (OFD)
Pallister-Hall
Renal Cyst and Diabetes
Simpson-Golabi-Behmel (SGBS)
Tuberous sclerosis
Townes Brock
VATER
WAGR
Williams Beuren
Zelweger (cerebrohepatorenal)
Chromosomal abnormalities
Trisomy 21
Klinefelter
DiGeorge, 22q11
45, X0 (Turner)
(XXY) Klinefelter
Tri 9 mosaic, Tri 13, Tri 18, del 4q, del 18q, dup3q, dup 10q
Triploidy
Metabolic disorders
Peroxisomal
Glycosylation defect
Mitochondriopathy
Glutaric aciduria type II
Carnitine palmitoyl transferase II deficiency

[18]. The male to female ratio for bilateral and unilateral renal hypo/dysplasia is 1.32:1 and 1.92:1, respectively [19]. Nine percent of first degree relatives of patients with bilateral renal agenesis or bilateral renal hypoplasia/dysplasia have some type of renal malformation [20]. The incidence of renal ectopia is 1 in 1000 from autopsies, while from clinical studies it is estimated to be less frequent at 1 in 10,000 patients [21]. Males and females are equally affected.

Renal ectopia is bilateral in 10% of cases; when unilateral, there is a slight predilection for the left side. The incidence of fusion anomalies is estimated to be about 1 in 600 infants [22].

While congenital renal malformations are relatively frequent birth defects, they become clinically evident at variable times during life and comprise a wide spectrum of outcomes ranging from no symptoms at all to CKD, which causes early mortality. The range in phenotypic severity makes it extremely difficult to counsel patients with certainty. Melo et al. reported a prevalence of CAKUT of 1.77 per 100 live births (524 cases of CAKUT in 29,653 newborns) in a tertiary care unit and a mortality rate of 24% in those affected (126/524) [11]. Amongst the 524 cases, risk factors for early mortality were the co-existence of non-renal and non-urinary tract organ disease, prematurity, low birth weight, oligohydramnios, and renal involvement (renal agenesis, RHD, multicystic renal dysplasia). Quirino et al. reported on the clinical course of 822 children with prenatally detected CAKUT that were followed for a median time of 43 months [23]. Their results demonstrate that most affected children do well: 29% of the children had urinary tract infection, 2.7% had hypertension, 6% had CKD, and 1.5% died during follow-up. Celedon et al. studied 176 children with chronic renal failure secondary to renal dysplasia, reflux nephropathy or urinary tract obstruction with a minimum of 5 years of follow-up [24]. They noted that patients with a urine albumin to creatinine ratio greater than 200 mg/mmol deteriorated faster compared to those with less than 50 mg/mmol (-6.5 mL/min/1.73 m² year vs. -1.5 mL/min/1.73 m² year change in estimate glomerular filtration rate [eGFR]). They also observed that those children with more than two febrile urinary tract infections deteriorated faster than those with fewer than two infections (median -3.5 mL/min/1.73 m² vs. -2 mL/min/1.73 m² year change in eGFR). Similar differences were noted for children with hypertension when compared to those without. Finally, they noted that the rate of decline in eGFR was greater during puberty (-4 mL/min/1.73 m²/year vs. -1.9 mL/min/1.73 m²/year change in eGFR). They noted

no differences in deterioration of eGFR when comparing children with one or two functioning kidneys. In contrast, Sanna-Cherchi et al. examined the risk of progression to end-stage kidney disease (ESKD) in patients with CAKUT. They found that by 30 years of age, 58 out of 312 patients had initiated dialysis. They also noted that the risk for dialysis was significantly higher for patients with a solitary kidney [25]. The same group reported that patients with bilateral hypodysplasia, solitary kidney, or posterior urethral valves with RHD had a higher risk of dialysis requirement at 30 years when compared to patients with unilateral RHD or horseshoe kidney, and the risk was even higher if there was coexistence of VUR. Wuhl et al. compared patients with CAKUT to age-matched patients with other causes of renal failure who were receiving some form of renal replacement therapy (RRT) and registered within the European Dialysis and Transplant Association Registry [26]. Of 212,930 patients ranging in age from 0 to 75 years who commenced RRT, only 2.2% had renal failure secondary to CAKUT. Importantly, the median age for requirement of RRT was 31 years in the CAKUT cohort versus 61 years in the non-CAKUT cohort, suggesting that most children are likely to require dialysis and/or transplantation as adults. CAKUT was the most frequent cause of need for RRT in all pediatric age groups and peaked in incidence in the 15–19-year-old group.

Low birth weight and prematurity are associated with low nephron number and have therefore been studied as surrogate markers of low nephron number. The Helsinki Study followed approximately 20,000 people born between 1924 and 1944 until death or age 86 years and established that low birth weight was a risk factor for CKD in males, whereas prematurity (birth before 34 weeks of gestation) was a risk factor for CKD in females [9]. Similarly, Crump et al. demonstrated that preterm birth, defined as <37 weeks, and extreme preterm birth, defined as <28 weeks, were strongly associated with an increased risk of CKD in childhood and in adulthood [8]. Keller et al. demonstrated that low nephron number is a risk factor for hypertension in middle-aged adults

compared with age-, sex-, and race-matched controls without hypertension [10].

Taken together, many questions remain in understanding the long-term outcome of CAKUT, but clearly most children are surviving into adulthood, and thus there is a need for adult nephrologists to understand these disorders.

Abnormal Molecular Signaling in the Malformed Kidney

Human renal development is complete by 34 weeks gestation [4]. Thus, by definition, renal malformation is a problem of disordered renal embryogenesis. The morphologic, cellular, and genetic events that underlie normal renal development are reviewed in Chap. 7. During human kidney development, two primordial tissues, the ureteric bud and the metanephric mesenchyme, undergo epithelial morphogenesis to form the final metanephric kidney [27]. The kidneys and the ureters arise from two epithelial tubes that extend along the length of the embryo, the mesonephric ducts. An epithelial swelling emerges from the mesonephric duct and is known as the ureteric bud. The ureteric bud invades the adjacent undifferentiated mesenchyme and induces the formation of the metanephric mesenchyme. Reciprocal signaling between the ureteric bud and the metanephric mesenchyme induces the ureteric bud to elongate and bifurcate in a process known as branching morphogenesis that ultimately gives rise to the collecting duct system of the adult kidney. The process of ureteric bud branching morphogenesis is critical for kidney development: each ureteric bud tip induces the adjacent ventrally located metanephric mesenchyme to undergo mesenchymal-to-epithelial transition and this determines the final number of nephrons formed *in utero*. Perturbations in ureteric bud outgrowth, branching morphogenesis and mesenchymal-to-epithelial transition are thought to underlie the majority of the malformations described in humans.

Failure of ureteric bud outgrowth and invasion of the metanephric blastema are events antecedent to renal agenesis or severe renal dysplasia.

Studies in the mouse embryo, a model of human renal development, have identified genes that control ureteric bud outgrowth, ureteric bud branching morphogenesis, and mesenchymal-to-epithelial transition. Some of these genes are mutated in human renal malformations also characterized by agenesis or severe dysplasia (reviewed by [28]). If the ureteric bud fails to emerge, the ureter and the kidney do not develop, while if the ureteric bud emerges from an abnormal location, the ureter that forms will not connect to the bladder properly and potentially result in obstruction and/or VUR with a malformed kidney. Indeed, a pathogenic role for abnormal ureteric bud outgrowth from the mesonephric duct was first hypothesized based on the clinical-pathological observation that abnormal insertion of the ureter into the lower urinary tract is frequently associated with a duplex kidney. Moreover, the renal parenchyma associated with the ureter with ectopic insertion into the bladder is frequently dysplastic [29]. The local environment of transcription factors and signalling pathways is therefore critical to the successful formation of an intact kidney and urinary tract. While a large number of transcription factors and ligand-receptor signalling pathways have been identified that regulate kidney development [28, 30], we will focus on the function of a few selected molecules that have been implicated in human congenital renal malformations: *Gdnf-Ret*, *EYA1*, *Six1*, *Sall1*, *Pax2*, *HNF1b*, *Shh*, and components of the renin-angiotensin-system (RAS).

The central ligand-receptor signalling pathway that leads to the outgrowth of the ureteric bud from the mesonephric duct is the GDNF-GFR α 1-RET signalling pathway. Glial cell derived neurotrophic factor (GDNF) is a ligand expressed by the metanephric blastema that interacts with the tyrosine kinase receptor, RET, and its co-receptor GFR α 1, both expressed on the surface of the mesonephric duct, to initiate outgrowth of the ureteric bud. Mutational inactivation of *Gdnf*, *Gfra1*, or *Ret* in mice causes bilateral renal agenesis due to failure of ureteric bud outgrowth, demonstrating the importance of this pathway [31–34]. Similarly, when GDNF-

soaked beads are positioned adjacent to cultured murine mesonephric ducts, multiple ectopic ureteric buds emerge, demonstrating the potency of this signalling pathway [35]. The expression domain of GDNF is therefore tightly regulated in the nephrogenic mesenchyme and the metanephric mesenchyme.

A network of transcription factors promotes *Gdnf* expression: *Eya1*, *Six1*, *Sall1*, and *Pax2*, while *Foxc1* restricts *Gdnf* expression [36]. Another ligand-receptor complex that limits the domain of *Gdnf* expression is the secreted factor SLIT2 and its receptor ROBO2 [37]. *Slit2* is expressed in the mesonephric duct, while *Robo2* is expressed in the nephrogenic mesenchyme. Bone morphogenetic protein 4 also negatively regulates the expression domain of *Gdnf* such that the ureteric bud emerges in the correct location [38].

EYA1 is expressed in metanephric mesenchymal cells in the same spatial and temporal pattern as GDNF. Mice with EYA1 deficiency demonstrate renal agenesis and failure of GDNF expression [39]. EYA1 functions in a molecular complex that includes SIX1 and together they translocate to the nucleus to regulate GDNF expression. Therefore, mutational inactivation of *Six1* in mice also results in renal agenesis or severe dysgenesis [40]. Like GDNF, SIX1 and EYA1, SALL1 is expressed in the metanephric mesenchyme prior to and during ureteric bud invasion. Mutational inactivation of *Sall1* in mice causes renal agenesis or severe dysgenesis and a marked decrease in GDNF expression [41]. Thus, EYA1, SIX1 and SALL1 function upstream of GDNF to positively regulate its expression, thereby controlling ureteric bud outgrowth.

PAX2 is another transcription factor that is expressed in the mesonephric duct, the ureteric bud and in metanephric blastema cells induced by ureteric bud branch tips [42]. Mice with a *Pax2* mutation identical in type to that found in humans with renal coloboma syndrome (RCS) exhibit decreased ureteric bud branching and renal hypoplasia. Investigation of the mechanisms controlling abnormal ureteric bud branching in a murine model of RCS (*Pax2*^{1^{Neu}}) revealed that increased ureteric bud cell apoptosis

decreases the number of ureteric bud branches and glomeruli formed. Remarkably, rescue of ureteric bud cell apoptosis normalizes the mutant phenotype [43]. *Pax2* appears to function upstream of *Gdnf* since in *Pax2* null mice no *Gdnf* expression is detected and the PAX2 protein can activate the *Gdnf* promoter [44].

PAX2 and HNF1 β , another transcription factor, are co-expressed in the mesonephric duct and the ureteric bud lineage. Constitutive inactivation of HNF1 β is embryonic-lethal in the mouse at gastrulation prior to the formation of the kidneys, but by using tetraploid and diploid embryo complementation, homozygous mutant embryos were able to proceed past gastrulation. The latter study demonstrated that HNF1 β is critical for mesonephric duct integrity, ureteric bud branching morphogenesis, and early nephron formation [45]. Another group conditionally inactivated HNF1 β in the proximal tubule, loop of Henle and collecting ducts and noted that null mice had cystic kidneys with cysts arising predominantly from collecting duct and loop of Henle segments [46]. The renal phenotype was severe, leading to death from renal failure in the newborn period. Importantly, cystic kidneys from null animals demonstrated downregulation of uromodulin, *Pkd2*, and *Pkhd1*, suggesting that HNF1 β may regulate genes associated with cyst formation [46]. Compound heterozygous mice bearing null alleles for *Pax2* and *Hnf1 β* show severe CAKUT phenotypes, including hypoplasia of the kidneys, caudal ectopic aborted ureteric buds, duplex kidneys, megaureters and hydronephrosis [47]. These phenotypes were much more severe than *Pax2* heterozygous null or *Hnf1 β* heterozygous null mice, strongly suggesting that *Pax2* and *Hnf1 β* genetically interact in a common kidney developmental pathway.

Sonic hedgehog (SHH) is a secreted protein that controls a variety of critical processes during embryogenesis. In mammals, SHH acts to control gene transcription via three members of the GLI family of transcription factors, GLI1, GLI2 and GLI3. A pathogenic role for truncated Gli3 was demonstrated in mice engineered such that the normal *GLI3* allele was replaced with the truncated isoform. These mice are characterized

by renal agenesis or dysplasia similar to humans with Pallister Hall Syndrome (PHS) [48]. Subsequent analysis of renal embryogenesis in mice deficient in SHH suggests that the truncated form of GLI3 represses genes like *Pax2* and *Sall1* that are required for the initiation of renal development [49]. Loss of Hedgehog signalling has also been implicated in nonobstructive hydronephrosis and urinary pacemaker dysfunction in mice [50]. *Shh* is expressed in the epithelium of the ureter during embryonic development and signals to the developing ureteric mesenchyme. *Shh* deficiency results in lack of smooth muscle cell differentiation in the ureter and nonobstructive hydronephrosis [51]. Hedgehog signaling is also active in the embryonic metanephric mesenchyme; genetic deficiency of Hedgehog signaling in this spatial domain also results in nonobstructive hydronephrosis but by a different mechanism. In mice so affected, there is loss of pacemaker cell activity in the renal pelvis and ureter [50]. The observation that constitutive expression of GLI3 repressor also causes nonobstructive hydronephrosis and that genetic deficiency of *Gli3* in mice with decreased Hedgehog rescues hydronephrosis demonstrates the critical role of GLI3 repressor downstream of Hedgehog signaling. Interestingly, some patients with Pallister Hall Syndrome manifest hydronephrosis, although the underlying mechanisms in these patients are unclear.

Analysis of mice with constitutive activation of Hedgehog signaling and human ureter tissues, implicates Hedgehog signaling in the pathogenesis of UPJO. Mice with deficiency of *Patched1*, a cell surface receptor that inhibits Hedgehog signaling, in renal progenitors that give rise to both nephrogenic and stromal cells are characterized by UPJO due to formation of an ectopic cluster of stromal cells that block the UPJ. Analysis of obstructing ureteric tissue in infants and children with congenital UPJO demonstrated upregulation of hedgehog signaling effectors and stromal genes, suggesting that increased hedgehog signaling may contribute to the pathogenesis of human UPJO, as well [52].

In postnatal renal physiology, the RAS plays a critical role in fluid and electrolyte homeostasis

and in the control of blood pressure. Renin cleaves angiotensinogen (AGT) to generate angiotensin (Ang) I which is cleaved by angiotensin-converting enzyme (ACE) to yield Ang II. Ang II is the main effector peptide growth factor of the RAS and acts on two major receptors: AT1R and AT2R. The role of the RAS during kidney development appears to differ somewhat in humans versus rodents, but the metanephric kidney expresses all components of the pathway in both species. Ang is expressed in the ureteric bud lineage and the stromal mesenchyme, while renin is expressed by mesenchymal cells destined to form vascular precursors in the kidney. ACE is expressed slightly later during kidney development in differentiated mesenchymal structures including glomeruli, proximal tubules and collecting ducts. The receptors AT1R and AT2R are expressed in the ureteric bud lineage and in metanephric mesenchymal cells [53]. Mutations of *AGT*, *renin*, *ACE*, or *AT1R* all result in CAKUT phenotypes in the mouse that are characterized by renal malformations with hypoplasia of the medulla and the papillae and hydronephrosis [54]. Mice with mutations in *ATR2* also exhibit CAKUT, but a wider range of phenotypes is observed that includes renal hypodysplasia, duplicated collecting systems, VUR, and hydronephrosis [55]. Importantly, genetic inactivation of the RAS pathway in mice does not result in renal tubular dysgenesis (RTD) as observed in humans with similar mutations. It is postulated this may be due to differences between the species: in humans, RAS activity (renin and ANG II levels) peaks during fetal life while nephrogenesis is occurring, while in rodents, RAS activity peaks postnatally from weeks 2–6, when nephrogenesis has ceased. These temporal differences likely explain the lack of concordance between genetic mouse models and affected humans [56].

Ureteric bud branching and modelling of the lower urinary tract with its insertion into the bladder is also controlled by vitamin A and its signaling effectors [57, 58]. Expression of RET, the receptor for GDNF, is controlled by members of the retinoic acid receptor family of transcription factors that function in the vitamin A signal-

ing pathway. These members, including RAR alpha and RAR beta2, are expressed in stromal cells surrounding *Ret*-expressing ureteric bud branch tips [58, 59]. Mice deficient in these receptors exhibit fewer ureteric bud branches and diminished expression of *Ret*. These observations are consistent with the finding that vitamin A deficiency during pregnancy causes renal hypoplasia in the rat fetus [60]. A similar observation has been noted in a human study where maternal vitamin A deficiency was associated with congenital renal malformation [61].

In summary, genetic and nutritional factors like vitamin A and folic acid [61–63] interact to control ureteric bud outgrowth, ureteric bud branching, nephrogenesis, and ureter formation. The number of nephrons is likely determined by a complex combination of factors including genetic variants, environmental events and stochastic factors. This could explain the variable number of nephrons in humans, ranging from approximately 230,000 to 1,800,000 [64]. Loss-of-function mutations in developmental genes can impair nephron formation *in utero* and depending on the magnitude of this effect, renal insufficiency may present at birth, childhood, adolescence or adulthood. Despite evidence in animals that depletion of protein, total calories or micronutrients causes renal hypoplasia, their contribution to human CAKUT remains unclear and an important area of future investigation.

Human Renal Malformations with a Defined Genetic Etiology

In humans, congenital renal malformations are more frequently sporadic than familial in occurrence. This may be due to the fact that infants with severe renal malformations have only recently survived; prior to the late 1970s, chronic dialysis was not offered as a therapy for children, and this continues to be the case in much of the developing world because of a lack of resources. Therefore, it is only in the past 30 years that children with congenital renal malformations have survived and been able to reproduce and potentially transmit deleterious gene mutations.

Therefore, congenital renal malformations appear as sporadic events over time. Genetic haploinsufficiency for many of the aforementioned transcription factors (*EYA1*, *SIX1*, *PAX2*, etc.) can result in a severe renal developmental phenotype, therefore *de novo* heterozygous mutations continue to arise. However, as reported by others, incomplete penetrance with variable expressivity is frequently observed in genetic studies of CAKUT, especially in relation to many of the transcription factors described previously [65]. Congenital renal malformations can occur in isolation, as part of CAKUT, or as part of a syndrome with organ malformations. Importantly, familial cases and extra-renal symptoms are sometimes unrecognized if carefully phenotypic

ing is not performed. A careful evaluation of family history reveals a clustering of isolated or syndromic urinary tract and renal malformations in more than 10% of the cases [66]. Knowledge of the most frequent syndromes, a careful clinical examination and appropriately selected investigations are critical to the clinical approach to these disorders.

Mutations in more than 30 genes have been identified in children with renal development anomalies, generally as part of a multiorgan syndrome (Table 8.2). Some of these syndromes and their associated genes are described here or in some recent reviews [16, 17]. The most frequent syndromes in which renal malformations are encountered are listed in Tables 8.1 and 8.2. For a

Table 8.2 Human gene mutations exhibiting defects in renal morphogenesis

Primary disease	Gene(s)	Kidney phenotype	References
Alagille syndrome	<i>JAGGED1</i> , <i>NOTCH2</i>	Cystic dysplasia	[177–179]
Apert syndrome (overlaps with Pfeiffer syndrome and Crouzon syndrome)	<i>FGFR2</i> , <i>FGFR1</i>	Hydronephrosis, VUR	[180, 181]
Beckwith-Wiedemann syndrome	<i>CDKN1C</i> (<i>p57^{KIP2}</i>), <i>H19</i> , <i>LIT1</i> , <i>NSD1</i>	Medullary dysplasia, nephromegaly, collecting duct abnormalities, cysts, VUR, hydronephrosis, Wilms tumor	[182, 183]
Branchio-Oto-Renal (BOR) syndrome	<i>EYA1</i> , <i>SIX1</i> , <i>SIX5</i>	Unilateral or bilateral agenesis/dysplasia, hypoplasia, collecting system anomalies	[79, 80, 184]
Campomelic dysplasia	<i>SOX9</i>	Dysplasia, hydronephrosis	[185, 186]
Duane Radial Ray (Okhiro) syndrome	<i>SALL4</i>	UNL agenesis, VUR, malrotation, cross-fused ectopia, pelviectasis	[187]
Fraser syndrome	<i>FRAS1</i> , <i>GRIP1</i> , <i>FREM2</i> , <i>FREM1</i>	Agenesis, dysplasia, CAKUT	[188, 189–191]
Hypoparathyroidism, sensorineural deafness and renal anomalies (HDR) syndrome	<i>GATA3</i>	Dysplasia, VUR, CAKUT, mesangioproliferative glomerulonephritis	[192, 193]
Kallmann syndrome	<i>KAL1</i> , <i>FGFR1</i> , <i>FGF8</i> , <i>PROK2</i> , <i>PROK2R</i> , <i>CHD7</i> , <i>NELF</i> , <i>HS6ST1</i>	Agenesis	[133, 194]
Mammary-Ulnar syndrome	<i>TBX3</i>	Dysplasia	[195]
Pallister-Hall syndrome	<i>GLI3</i>	Dysplasia	[49, 196]
Renal-Coloboma syndrome	<i>PAX2</i>	Hypoplasia, vesicoureteral reflux	[109]
Renal tubular dysgenesis	RAS components, <i>REN</i> , <i>AGT</i> , <i>AGTR1</i> , <i>ACE</i>	Tubular dysplasia	[139]
Renal cysts and diabetes syndrome	<i>HNF1β</i>	Dysplasia, hypoplasia	[197]
Simpson-Golabi Behmel syndrome	<i>GPC3</i>	Medullary dysplasia	[198]
Smith Lemli Opitz syndrome	<i>DHCR7</i>	Agenesis, dysplasia	[199]
Townes-Brock syndrome	<i>SALL1</i>	Hypoplasia, dysplasia, VUR	[91]
Zellweger syndrome	<i>PEX1</i>	VUR, cystic dysplasia	[200]

complete list of syndromes featuring renal malformations, the reader is referred to McKusick's Online Mendelian Inheritance in Man.¹

For most children with renal malformations, neither a syndrome nor a Mendelian pattern of inheritance is obvious. However, genetic studies incorporating chromosomal microarrays, targeted gene panels or whole exome sequencing (WES) have identified a genetic cause for the renal malformation in anywhere from 5% to 30% of cases [16]. One of the first such studies of 100 patients with RHD and renal insufficiency demonstrated that 15% had mutations in two transcription factors [65]: *TCF2* (HNF1 β) (especially in the subset with kidney cysts) and *PAX2*. *EYA1* and *SALL1* mutations were found in single cases. Some of the mutations that were identified in these genes were *de novo* mutations explaining the sporadic appearance of RHD. Careful analysis of patients with *TCF2* and *PAX2* mutations revealed the presence of extrarenal symptoms in only half, supporting previous reports that *TCF2* and *PAX2* mutations can be responsible for isolated renal tract anomalies or at least CAKUT malformations with minimal extrarenal features [67, 68]. This study demonstrates that subtle extrarenal symptoms in syndromal RHD can easily be missed. Genetic testing in children with RHD should be preceded by a thorough clinical evaluation for extrarenal symptoms, including eye, ear, and metabolic anomalies. The presence of nonrenal anomalies increases the likelihood of detecting a specific genetic abnormality (Table 8.5). In addition, mutations in genes that are usually associated with syndromes can occur in patients with isolated RHD.

The GDNF/RET Signaling Pathway

The proto-oncogene *RET*, a tyrosine kinase receptor, and its ligand, GDNF, play a pivotal role during early nephrogenesis and enteric nervous system development. Activating *RET* mutations cause multiple endocrine neoplasia, whereas inactivating mutations lead to

Hirschsprung disease. A number of human studies have demonstrated that patients with CAKUT have mutations in the RET/GDNF signaling pathway [69–72]. A study of 122 patients with CAKUT identified heterozygous deleterious sequence variants in *GDNF* or *RET* in 6/122 patients, 5%, while another group screened 749 families from all over the world and identified 3 families with heterozygous mutations in *RET* [69, 73]. Similar findings have been reported in studies of fetuses with bilateral or unilateral renal agenesis [70, 71].

Branchio-Oto-Renal Syndrome

The association of branchial (B), otic (O) and renal (R) anomalies was first described by Fraser and Melnick [74, 75]. Major diagnostic criteria consist of hearing loss (95%), branchial defects (49–69%), ear pits (83%) and renal anomalies (38–67%) [76, 77]. The association of these three major features defines the classical BOR syndrome (OMIM # 113650). Yet, many patients have only one or two of these major features in association with other minor features such as external ear anomalies, preauricular tags or other facial abnormalities (Table 8.3). Hearing loss can be conductive, sensorineural, or mixed.

The frequency of BOR syndrome has been estimated to be 1 in 40,000 births [78]. The transmission is autosomal dominant with incomplete penetrance and variable expressivity. Renal malformations include unilateral or bilateral renal agenesis, hypodysplasia as well as malformation of the lower urinary tract including VUR, pyeloureteral obstruction, and ureteral duplication. Different renal malformations can be observed in

Table 8.3 Major and minor criteria for the diagnosis of BOR syndrome

Major features	Minor features
Deafness	External ear anomalies
Branchial anomalies	Preauricular tags
Preauricular pits	Other facial anomalies
Renal malformations	Cataracts
	Lacrimal duct stenosis

¹<http://www.ncbi.nlm.nih.gov/>.

the same family; moreover, some individuals have normal kidneys (BO syndrome, OMIM 120502). Other infrequent abnormalities have been described in patients with the BOR syndrome. These include aplasia of the lacrimal ducts, congenital cataracts and anterior segment anomalies [74, 75]. Characteristic temporal bone findings include cochlear hypoplasia (4/5 of normal size with only 2 turns), dilation of the vestibular aqueduct, bulbous internal auditory canals, deep posterior fossae, and acutely angled promontories [77].

Approximately 40% of patients with BOR syndrome have a mutation in *EYA1* [76]. Mutations in *SIX1*, *SIX5*, and *SALL1* have also been identified in patients with BOR syndrome, but at lower frequencies [79–81]. Both *EYA1* and *SIX1* are co-expressed in the developing otic, branchial and renal tissue, where they function in a transcriptional complex that regulates cell proliferation and cell survival [82, 83]. *EYA1* and *SIX1* control the expression of *PAX2* and *GDNF* in the metanephric mesenchyme [84]. The *EYA1* protein contains a highly conserved region called the *eyes absent* homologous region encoded within exons 9–16, which is the site of most mutations identified to date.

A reasonable approach is to perform genetic analysis in families in which at least one member fulfils the criteria for classical BOR syndrome (Table 8.3). Investigations should include a family history, and examination of relatives to look for preauricular pits, lacrimal duct stenosis, and branchial fistulae and/or cysts. Hearing studies and renal ultrasound should be done in all first-degree relatives.

Molecular testing can confirm the diagnosis and provide genetic recurrence risk information to families. However, variability of the phenotype even with the same mutation does not permit accurate prediction of the disease severity. Within the same family, a given mutation may be associated with renal malformation in some individuals, but not in others. This discrepancy might be explained by stochastic factors that impact the formation of the kidneys or by other unlinked genetic events that may act in synergy with the *EYA1* protein during nephrogenesis.

Townes-Brocks Syndrome and VATER/VACTERL Associations

Townes-Brocks syndrome (TBS) is an autosomal dominant malformation syndrome usually defined by a triad of anomalies including imperforate anus, dysplastic ears, and thumb malformations [85]. A wide spectrum of additional features includes renal malformations, congenital heart defects, hand and foot malformations, hearing loss, and eye anomalies [86, 87]. Intelligence is usually normal. REAR Syndrome (renal-ear-anal-radial) has also been used to describe this condition [88]. Its incidence is reported to be 1:250,000 live births [89]. The presentation of TBS is highly variable within and between affected families. Importantly, *SALL1*, is the only gene implicated in TBS and it encodes a C₂H₂ zinc finger transcription factor that is required for the normal development of the limbs, nervous system, ears, anus, heart and kidneys [90, 91].

The detection rate of *SALL1* mutations in patients with TBS appears to be higher when malformations of the hands, the ears, and the anus are present [92]. However genetic testing is further complicated by the fact that the phenotypic features of TBS can resemble other disorders like VACTERL association, Goldenhar syndrome, Oculo-Auriculo-Vertebral spectrum, Pallister-Hall syndrome and even BOR syndrome. TBS features overlap those seen in the VACTERL association (anal, radial and renal malformations). In contrast to VACTERL association, TBS is associated with ear anomalies and deafness and it is not characterized by tracheo-oesophageal fistula or vertebral anomalies.

VACTERL association is defined by the presence of at least three of the following congenital malformations: vertebral anomalies, anal atresia, cardiac defects, tracheo-esophageal defects, renal malformations, and limb anomalies [93]. It is reported to occur in 1:10,000–40,000 of all live births. Renal anomalies are reported in 50–80% of patients and include unilateral or bilateral renal agenesis, horseshoe kidney, cystic kidneys, and dysplastic kidneys; they can be accompanied by urinary tract and genital defects [93, 94]. Ninety percent of VACTERL cases appear to be

sporadic with little evidence of heritability [93]. In a subset of patients there is evidence of heritability [93, 95, 96], and genes that interact with the Sonic Hedgehog pathway have been implicated [97, 98]. The presence of a single umbilical artery on ultrasound has been associated with a variety of congenital birth defects, including VACTERL syndrome [99]. It has been hypothesized that the single umbilical artery is a risk factor for a placental defect that may affect nutrient supply for multiple organs simultaneously during development [100].

An important diagnosis to consider in patients suspected to have VACTERL syndrome is Fanconi's anemia. Patients with Fanconi's anemia can phenocopy VACTERL syndrome, but also exhibit bone marrow failure manifest as pancytopenia. They can also develop malignancies like acute myelogenous leukemia secondary to their propensity for chromosomal instability manifest as spontaneous cytogenetic aberrations. Patients with Fanconi's anemia also frequently demonstrate skin pigmentation (café au lait spots), microcephaly, growth retardation and microphthalmia. There are at least nine different gene mutations implicated in Fanconi's anemia and they are inherited as X-linked or recessive disorders. It has been reported that approximately 5% of patients with confirmed Fanconi's anemia have features consistent with VACTERL syn-

drome [101]. Therefore, the diagnosis of Fanconi's anemia needs to be carefully considered in all patients with VACTERL syndrome and confirmed if needed by performing chromosomal breakage studies [101].

Renal-Coloboma Syndrome

Renal Coloboma Syndrome (RCS) (also named papillo-renal syndrome) is an autosomal dominant disorder characterized by the association of renal hypoplasia, VUR and optic nerve coloboma from a mutation in *PAX2* [102]. The prevalence of the syndrome is unknown, but approximately 100 families have been reported [103]. A wide range of renal malformations are observed in RCS. Oligomeganephronic hypoplasia, renal dysplasia and VUR are the most frequent malformations, but multicystic dysplasia [104] and UPJO have also been described [104]. Similarly, the ocular phenotype is extremely variable. The most common finding is an optic disc pit associated with vascular abnormalities and cilio-retinal arteries, with mild visual impairment limited to blind spot enlargement, the "morning glory" anomaly [105]. In other cases, the only ocular anomaly is optic nerve dysplasia with an abnormal vessel pattern and no functional consequence (Fig. 8.2). In contrast, a large coloboma of the

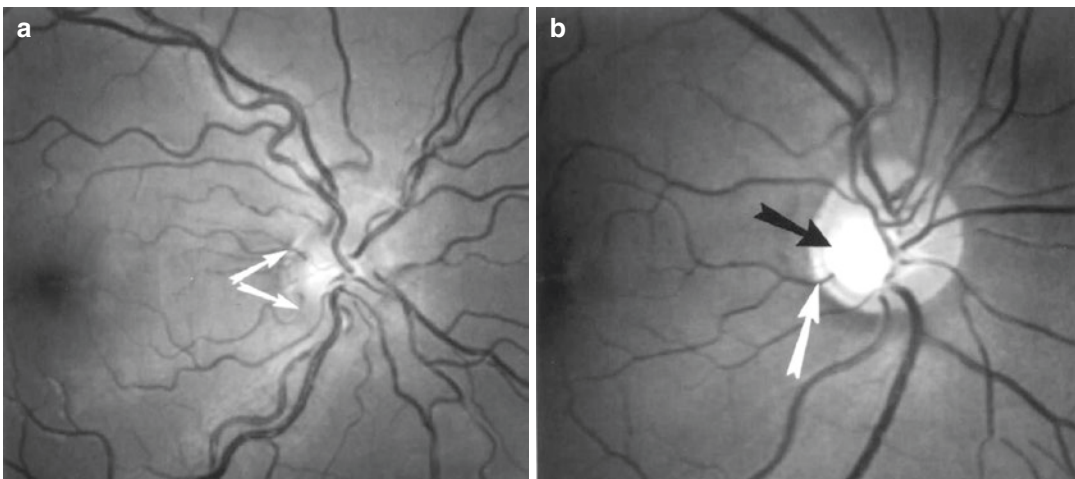


Fig. 8.2 Optic disc appearance in two patients with Renal Coloboma Syndrome and *PAX2* mutations: (a) Characteristic features of optic disk coloboma with a deep

temporal excavation (arrowheads). (b) The optic disk is dysplastic with thickening (arrow) and emergence of abnormal vessels ("morning glory anomaly")

optic nerve or of the choroid and retina and the morning glory anomaly can be responsible for a severe visual impairment [106]. Coloboma and the related anomalies are probably the consequence of an incomplete closure of the embryonic fissure of the optic cup. Other extrarenal manifestations can include sensorineural hearing loss, joint laxity, Arnold-Chiari malformation and seizures of unknown cause [107, 108]. In addition to its expression in the developing kidney and in the optic fissure, *PAX2* is also expressed in the hindbrain during its development. However, neurological symptoms are not usually present in RCS.

PAX2 is a transcription factor of the paired-box family of homeotic genes that is expressed in the mesonephros and in the metanephros during renal development. In 1995, Sanyanusin et al reported heterozygous mutations in two RCS families [109]. Since then, more than 30 mutations have been reported, most of them lying in exons 2–4 that encode the paired domain that binds to DNA or in exons 7–9 that encode the transactivation domain [103]. Other gene(s) are probably also responsible for this syndrome since *PAX2* mutations are not found in approximately 50% of RCS patients. Importantly the RCS phenotype is highly variable, even in patients harboring the same *PAX2* mutation, suggesting that modifier genes might be implicated.

Optic nerve coloboma occurs frequently as an isolated anomaly or as a feature of many other multiorgan syndromes such as the CHARGE association, the COACH syndrome and the acrorenal-ocular syndrome. As optic nerve coloboma and the related disorders can be easily misdiagnosed, it is likely that the prevalence of RCS is underestimated. It is wise to examine the fundus in every patient with RHD, and conversely to perform renal ultrasound and serum creatinine in every patient with optic nerve coloboma.

Even in the absence of optic nerve colobomas, mutations in *PAX2* are one of the more common genetic causes of RHD [65] and they also appear to be associated with low nephron number. Barua et al. described families diagnosed with FSGS anywhere from 7 to 68 years of age due to dominantly inherited mutations in *PAX2*. One patient

had a kidney biopsy sample that exhibited glomerulomegaly, which could be secondary to low nephron endowment at birth [110]. Some of the affected individuals had imaging studies that revealed other CAKUT phenotypes including small kidneys and hydronephrosis. Vivante et al. identified heterozygous mutations in *PAX2* in three families and one child with steroid-resistant nephrotic syndrome [111]. The patients developed their steroid-resistant nephrotic syndrome or FSGS either during infancy or in adolescence. Here again, the FSGS lesion could be secondary to low nephron endowment at birth.

Renal Cyst and Diabetes Syndrome

Mutations in the *TCF2* gene encoding the transcription factor HNF1 β were initially found in patients with maturity onset diabetes of the young, type 5 (MODY5), an autosomal dominant disorder [112, 113]. Diabetes mellitus is present in approximately 60% of all the cases reported, usually occurs before 25 years of age, and is often associated with pancreatic atrophy [114–116]. In some patients, a subclinical deficiency of pancreatic exocrine functions has been demonstrated. Additional features have been described, including a wide spectrum of renal phenotypes (Table 8.4). The presence of cysts is the most consistent feature of the renal phenotype, leading

Table 8.4 Renal cyst and diabetes syndrome

Main features ^a
Fetal large hyperechoic kidneys
Renal hypodysplasia with cortical microcysts
Diabetes mellitus (MODY type 5)
Occasional features
Genital malformations
Female: vaginal aplasia, rudimentary or bicornuate uterus
Male: epididymal cysts, atresia of the vas deferens, asthenospermia, hypospadias
Hyperuricemia, rarely gout (reduced uric acid fraction excretion)
Hypomagnesemia
Moderate elevation of liver enzymes
Subclinical defect of exocrine pancreatic functions

^a Age at onset and severity of these symptoms are highly variable

to the name, “Renal Cysts and Diabetes (RCAD) Syndrome”. The cysts are usually cortical, bilateral, and small [68]. Mutations in the *TCF2* gene have also been found in association with a variety of isolated renal development disorders such as RHD, MCDKs, renal agenesis, horseshoe kidneys, UPJO as well as clubbing and tiny diverticula of the calyces [117–119]. The most specific finding when histology is available is the presence of cortical glomerular cysts with dilatation of the Bowman spaces (glomerulocystic dysplasia) [120]. Other nonspecific lesions such as cystic renal dysplasia, interstitial fibrosis or oligomeganephronia have also been reported. Antenatal presentations with enlarged hyperechoic kidneys or macroscopic cysts can occur [118, 121].

Various genital tract malformations have been reported mostly in females. These include vaginal aplasia, rudimentary uterus, bicornuate uterus, uterus didelphys and double vagina. In males, hypospadias, epididymal cysts, and agenesis of the vas deferens have been reported [114]. These genital anomalies have been described in approximately 10–15% of patients with *TCF2* mutations, but these malformations might be underestimated especially in paediatric reports. Reduced fractional excretion of uric acid (<15%) and moderate hyperuricemia is observed in some cases and is usually asymptomatic. The hyperuricemia is thought to reflect altered urate transport by the kidney and impaired glomerular filtration [114]. Serum hypomagnesemia has also been reported and this may be due to the fact that HNF1b regulates *FXRD2* that is needed for distal tubule reabsorption of magnesium [122]. A similar mechanism may explain the altered urate transport observed in these patients since HNF1b can activate the promoter of *URAT1* that regulates urate transport in the proximal tubule [123]. Moderate elevation of liver enzymes is a common finding, but severe hepatopathy has not been reported.

HNF1 β is a homeobox-containing basic helix-turn-helix transcription factor, which is involved in the development of the pancreas, the kidneys, the liver and intestine. More than 50 mutations have been reported, most of which are located in the first four exons that encode the DNA-binding

domain. In more than one-third of the cases, the gene is entirely deleted [68, 115, 123]. Such alterations are not detected by conventional amplification and screening methods. Importantly, deletions are infrequently transmitted by the parents but appear *de novo* in the proband. Analysis of *TCF2* can thus be recommended not only in patients with a family history of RCAD syndrome but also in cases with renal cysts when polycystic disease or nephronophthisis are unlikely. The presence of cortical bilateral cysts is probably the most typical finding. Reduced uric acid fractional excretion, elevation of liver enzymes, hypomagnesemia, glucose intolerance and abnormalities of the genital tract should be systemically sought and *TCF2* analyzed if one of these symptoms is present. As observed in other syndromes, phenotypic variability can be observed between families and also in family members with the same mutation, suggesting a role for environmental and genetic factors.

Longitudinal follow-up of genetically proven HNF1 β nephropathy has been reported in a group of 62 children and adolescents, of whom 87% were diagnosed with bilateral renal dysplasia. Among these patients, 74% and 16% had visible bilateral or unilateral cysts, respectively, at the end of an average of 4 years of follow-up [124]. During this period, 28% of patients had an increase in cyst number, which was associated with a greater decline in GFR compared to patients without an increase in cyst number. Eight percent of patients developed ESKD at a median age of 15 months. Hypomagnesemia was present in 19 of 52 patients evaluated by a median age of 1 year, while recurrent hyperglycemia was observed in 4 of 50 evaluated patients. Increased uric acid was detected in 37% of patients. HNF1 β mutations were varied in type, were familial or *de novo*, and were not correlated with phenotype. The issue of which patients should be screened for a mutation in *HNF1 β* has been investigated in a cohort of 433 pediatric patients with known *HNF1 β* status and CAKUT using a 17-item weighted score inclusive of abnormalities in renal, pancreatic, genital, electrolyte, and liver function as well as family history. A score of ≥ 8

was reported to have a sensitivity of 98.2%, specificity of 41.1%, positive predictive value of 19.8% and a negative predictive value 99.4% [125]. The possible utility of this scoring system, particularly in predicting absence of a HNF1 β mutation, needs to be validated in other cohorts with HNF1 β -associated disease.

Kallmann Syndrome

Kallmann syndrome (KS) is defined by the presence of hypogonadotropic hypogonadism and deficiency of the sense of smell (anosmia or hyposmia) [126]. Some affected individuals exhibit unilateral renal agenesis, cleft lip and/or palate, selective tooth agenesis, bimanual synkinesis and hearing impairment [127]. Other CAKUT phenotypes including duplex systems, hydronephrosis, and VUR have been rarely reported. Anosmia/hyposmia is related to the absence or hypoplasia of the olfactory bulbs and tracts. Hypogonadism is due to a deficiency in gonadotropin-releasing hormone (GnRH). The GnRH-synthesizing neurons migrate during development from the olfactory epithelium to the forebrain along the olfactory nerve pathway [128]. KS is genetically heterogeneous with at least 8 genes reported including *KAL1*, an X-chromosome encoded gene that gives rise to the extracellular matrix protein anosmin-1 [129], *FGF8* (Fibroblast growth factor 8) [130], *FGFR1* (Fibroblast Growth Factor Receptor 1) [131], *PROK2* (prokinectin-2) and *PROKR2* (prokinectin-2 receptor) [132], *CHD7*, *NELF*, and *HS6ST1* [133]. Chromodomain helicase DNA-binding protein 7 (*CHD7*) is a transcriptional regulator that binds to enhancer elements in the nucleus. It is implicated in CHARGE syndrome, that is characterized by choanal atresia, malformations of the heart, the inner ear, and the retina, and in Kallmann syndrome. In the largest study to date of 219 patients with Kallmann syndrome, mutations were most commonly observed in the FGF pathway (either *FGF8* or *FGFR1*), in *KAL1*, in the *PROK2*/*PROKR2* pathway and in *CHD7* [133]. Importantly in this study, unilateral renal agenesis was only observed in patients with

KAL1 mutations (reported in 18%, 3/17), or in patients with no mutation in the above-mentioned 8 genes, where the frequency was similar at 17% (4/23). Patients with *KAL1* mutations are typically male since the disorder is X-linked and they demonstrate a much more severe reproductive phenotype compared to patients with other mutations with small testes, absent puberty, and micropenis. Females with *KAL1* mutations typically present with partial pubertal development manifesting as spontaneous breast development in the absence of hormonal treatment. *KAL1* is expressed in the developing human metanephric kidney at 11 weeks of gestation [134].

Renal Tubular Dysgenesis and Mutations of RAS System Elements

The differential diagnosis of oligohydramnios with neonatal renal failure includes a spectrum of diagnoses including bilateral renal dysplasia, posterior urethral valves, and PKD. All of these diagnoses are detectable and distinguishable on antenatal ultrasound imaging of the kidneys and the urinary tracts. The presence of normal kidneys on antenatal ultrasound in combination with oligo- or anhydramnios should strongly suggest the diagnosis of RTD [135]. RTD is a severe perinatal disorder characterized by absence or paucity of differentiated proximal tubules, early severe oligohydramnios, and perinatal death. The latter is usually due to pulmonary hypoplasia and skull ossification defects [136]. This condition has also been described in clinical conditions associated with renal ischemia, including the twin–twin transfusion syndrome, major cardiac malformations, severe liver diseases, fetal or infantile renal artery stenosis [137] and in fetuses that are exposed *in utero* to ACE inhibitors, Ang II receptor antagonists [138] or non-steroidal anti-inflammatory medications [135]. All of these environmental insults are postulated to lead to chronic hypoperfusion of the fetal kidneys with upregulation of the RAS. The absence or paucity of proximal tubules is believed to be secondary to chronic renal hypoperfusion [56]. Mutations in

the genes which encode components of the RAS have been identified in some families [139]. Mutations in the *ACE* gene are seen in 65.5 % of cases, while mutations in the *renin* (*REN*) are observed in 20 % of cases. Mutations in *AGT* and in the *AGT type I receptor* (*ATRI*) occur much less frequently [56]. It has been suggested that if there is no expression of the renin protein on immunohistochemistry of the kidneys, then the *renin* gene should first be assessed. Similarly, the plasma renin activity should be measured in the newborn with suspected genetic RTD and if elevated, this should prompt an analysis of genes downstream of the *REN* gene [140].

CHD1L, CHD7 and CHARGE Syndrome

Chromodomain helicase DNA binding protein 1-like protein, CHD1L, belongs to the Snf2 family of helicase-related ATP-hydrolyzing proteins and contains a helicase-like region that is similar to other family members, such as CHD7 which is the major gene that causes CHARGE syndrome. CHARGE syndrome is characterized by Colobomas, Hear defects, choanal Atresia, Retarded growth and development, Genital hypoplasia, and Ear anomalies with deafness. CHARGE syndrome is associated with CAKUT phenotypes including horseshoe kidneys, renal agenesis, VUR and renal cysts [141]. Chromatin-remodelling and -modifying enzymes like CHD1L and CHD7 are predicted to play key roles in differentiation, development and tumour pathogenesis via effects on chromatin structure and accessibility. Brockeschmidt et al. screened 85 patients with CAKUT and identified 3 patients with heterozygous missense variants in CHD1L [142]. The same paper reported that CHD1L was expressed in early ureteric bud and comma- and S-shaped structures during human kidney development. In the postnatal human kidney, CHD1L was expressed in the cytoplasm of tubular cells in all nephron segments. Similarly, Hwang et al. reported that 5 out of 650 families had heterozygous mutations in CHD1L: the affected individuals had a spectrum of CAKUT phenotypes including renal dysplasia, posterior urethral

valves, UVJ obstruction and horseshoe kidneys [73]. It is not yet known if these patients will also be at greater risk for malignancies given that CHD1L is known to be an oncogene in hepatocellular carcinoma [143].

DSTYK and CAKUT

DSTYK is a dual serine-threonine and tyrosine protein kinase that is co-expressed with fibroblast growth factor receptors in the developing mouse and human kidney in both metanephric mesenchyme and ureteric bud cells. Sanna-Cherchi et al. discovered that 7/311 patients with CAKUT had heterozygous mutations in this gene [144]. The CAKUT phenotypes observed in these patients included UPJO, VUR, and RHD.

Copy Number Variants, CAKUT and Neuropsychiatric Disorders

Copy number variants are stretches of DNA that are larger than 1 kb in length with the potential to contribute to functional variation and disease. Rare CNVs have been implicated in neuropsychiatric and craniofacial syndromes, and in syndromes with CAKUT [145, 146]. Sanna-Cherchi et al. examined the burden of rare CNVs in individuals with congenital renal malformations and identified disease-causing CNVs and potentially pathogenic CNVs in 10% and 6%, respectively, of the 522 affected individuals analyzed. This burden of CNVs in CAKUT was compared to 0.2% in population controls [146]. A subsequent analysis of 2824 individuals with CAKUT highlighted an increased prevalence of large, rare, exonic CNVs compared to population-based controls [147]. In this study, genomic abnormalities were identified in 4% of patients with the majority within six pathogenic loci including chromosome 17q12 (RCAD Syndrome), 22q11.2 (DiGeorge Syndrome), 16p11.2, 1q21.1, 4p (Wolf-Hirschhorn Syndrome), and 16p13.11. In addition, 90% of the CNVs associated with congenital renal malformations were previously reported to predispose to developmental delay or

neuropsychiatric disease, suggesting that there are shared pathways implicated in renal and central nervous system development. Similarly, Handrigan et al. demonstrated that copy number variants at chromosome 16q24.2 are associated with autism spectrum disorder, intellectual disability, and congenital renal malformations [145].

Environmental Factors and Renal Malformations

As mentioned earlier in this chapter, genetic causes of CAKUT can be identified in at most 30% of all cases, which suggests that environmental factors or epigenetic factors explain the remaining cases (Table 8.5). Epigenetics refers to changes in gene expression rather than changes in the gene sequence itself and usually arises from DNA methylation and histone modifications that can silence or enhance gene expression. Maternal obesity and diabetes are major risk factors for CAKUT. From questionnaire data of 562 parents of children with CAKUT, maternal obesity was more highly associated with duplex kidneys and VUR, while maternal diabetes was particularly associated with posterior urethral

valves [148]. Dart et al. demonstrated that pre-gestational diabetes was significantly associated with CAKUT (odds ratio, 1.67; 95% confidence interval, 1.14–2.46), which implies a 67% increased chance of CAKUT in the children of mothers with pregestational diabetes compared to the general population (8.3 vs. 5.0 per 1000 births, respectively) [149]. These findings in humans are strongly supported by animal models in which the pregnant dam has diabetes. Animal models have also shown that maternal undernutrition and uteroplacental insufficiency are risk factors for impaired nephrogenesis and CAKUT. Maternal use of medications, alcohol and illicit drugs like cocaine are also risk factors for impaired nephrogenesis and CAKUT. Maternal use of RAS inhibitors such as ACE inhibitors, Ang II receptor blockers and direct renin inhibitors during pregnancy have been linked to an increased risk of fetopathy in humans. The majority of children manifest hyperechogenic and enlarged kidneys with proximal tubular dysplasia, thickening of arterial walls and multiple small cysts [150, 151]. Maternal supplementation with folate may also decrease the risk of CAKUT. CAKUT phenotypes have been observed in infants exposed to folic acid antagonists *in utero*, such as carbamazepine, phenytoin, primidone, phenobarbital or valproic acid, suggesting that folate is important for kidney and urinary tract development [63, 152]. Fertility treatment with in vitro fertilization or intrauterine semination are also risk factors for CAKUT, possibly through epigenetic effects on the developing zygote. Identifying the environmental risk factors that predispose to CAKUT needs to be addressed in future research.

Table 8.5 Clinical indications to search for a renal anomaly

Exposure to teratogens
ACE inhibitors and Angiotensin receptor blockers
Alcohol
Alkylating agents
Cocaine
Folic acid antagonists
Vitamin A congeners
Maternal diabetes
Findings on physical examination
High imperforate or anteriorly positioned anus
Abnormal external genitalia
Supernumerary nipples
Preauricular pits and ear tags, cervical cysts or fistula
Hearing loss
Aniridia
Coloboma or optic disc dysplasia
Hemihypertrophy
Single umbilical artery
Other
Hyperglycemia

Clinical Approach to Renal Malformation

The majority of renal malformations are now diagnosed antenatally, largely because of the widespread use and sensitivity of fetal ultrasound. The sensitivity of prenatal ultrasound screening for renal malformations is about 82% and the mean time at which these malformations

are detected is 23 weeks gestation [12]. In general, urinary tract malformations detected antenatally are isolated and present as mild hydronephrosis with no therapeutic consequences. Parents should be reassured. In contrast, bilateral forms of renal agenesis, severe dysgenesis, bilateral ureteric obstruction, or obstruction of the bladder outlet or the urethra can cause severe oligohydramnios as early as 18 weeks. Because amniotic fluid is critical to lung development, oligohydramnios as early as the second trimester can result in lung hypoplasia, a potentially fatal disorder. The oligohydramnios sequence, termed Potter's syndrome, in its most severe form consists of a typical facial appearance characterized by epicanthal folds, recessed chin, posteriorly rotated, flattened ears and flattened nose, as well as decreased fetal movement, musculoskeletal features including clubfoot and clubhand, hip dislocation, joint contractures and pulmonary hypoplasia. The renal prognosis can be evaluated antenatally. Poor outcome can be predicted when there is severe oligohydramnios, and small and hyperechogenic kidneys. Normative data on kidney dimensions including kidney length from antenatal ultrasound imaging is available from the 15th week of gestation and can be used to determine if a kidney is small, suggesting some type of renal dysplasia, or increased in size, as observed in autosomal recessive PKD (ARPKD) or autosomal dominant PKD (ADPKD) [153]. Indeed, fetal renal hyperechogenicity with renal cysts suggests the fetus may have ARPKD, ADPKD or a mutation in *HNF1B*. If there is concurrent severe oligohydramnios, then ARPKD is the most likely diagnosis. Amniotic fluid analysis may be of help in some cases if the fetus is suspected to have a trisomy. Trisomy 21, 18, and 13 are all associated with CAKUT [154–156]. Antenatal diagnosis and assessment of the renal prognosis are important for consideration of early termination in cases of fatal (or eventually severe renal disease) and to prepare parents and medical staff for the likelihood of neonatal renal insufficiency. Other organ malformations should be sought carefully and, if detected, a karyotype should be done. Some authors have suggested that fetal urine analysis may be helpful to deter-

mine fetal renal prognosis and to decide on *in utero* therapy if congenital lower urinary tract obstruction is noted. Morris et al. performed a systematic review of the literature on fetal urine analysis and concluded that none of the analytes examined had sufficient accuracy to predict poor postnatal renal function [157].

Oligo/anhydramnios from CAKUT is associated with a high incidence of fetal death *in utero*, severe pulmonary hypoplasia, umbilical cord compression and perinatal asphyxia. The earlier that renal oligohydramnios (ROH) is identified in the pregnancy, the more severe the pulmonary hypoplasia [158, 159]. Pulmonary hypoplasia is defined as deficiency in the number of lung cells, airways and alveoli, leading to a reduced surface area for gas exchange. Postnatally, pulmonary hypoplasia is suspected in cases of ROH when there is respiratory failure with the need for high ventilatory support and the chest x-ray reveals a bell-shaped chest, an elevated diaphragm, and/or pneumomediastinum or pneumothorax. Ultrasound and MRI have been used to assess lung volumes antenatally, but there is no consensus on whether they are reliable predictors of pulmonary hypoplasia postnatally. Therefore, it remains difficult to predict pulmonary prognosis postnatally.

When isolated CAKUT or CAKUT with other organ defects is diagnosed in the fetus, genetic counseling and referral to a multidisciplinary team should be offered. An individualized approach that is in accordance with the parental wishes for more information is advised [160]. A screen for gross chromosomal abnormalities can be performed using a chromosomal microarray from the amniotic fluid (if less than 25 weeks) or from fetal blood (greater than 25 weeks or severe oligohydramnios). If the chromosomal microarray is normal, then a targeted gene panel or WES on the parents and the fetus could be considered. Given the incomplete penetrance and variable expressivity of many mutations implicated in CAKUT, it remains to be demonstrated whether targeted gene panels or WES are beneficial for antenatal decision-making.

The clinical presentation of renal malformation in the postnatal period is dependent on the

amount of functioning renal mass, the presence of bilateral urinary tract obstruction and the occurrence of urinary tract infection. Bilateral renal agenesis or severe dysplasia is likely to present soon after birth with decreased renal function. This may be accompanied by oliguria. Alternatively, patients may present with a flank mass or an asymptomatic abnormality detected by renal imaging.

A detailed history and careful physical examination should be carried out on all infants with an antenatally detected renal malformation. An early (within 24 h of life) renal ultrasound is recommended for newborns with a history of oligohydramnios, progressive antenatal hydronephrosis, distended bladder on antenatal sonograms, and bilateral severe hydroureteronephrosis. In male infants, a distended bladder and bilateral hydroureteronephrosis may be secondary to posterior urethral valves, a condition which requires immediate renal imaging and clinical intervention. In general, unilateral anomalies do not require urgent investigation after birth. Renal ultrasound for unilateral hydronephrosis is not recommended within the first 72 h of life because urine output gradually increases over the first 24–48 h of life as renal plasma flow and glomerular filtration rate increase [161]. Thus, the degree of urinary tract dilatation can be underestimated during this period of transition.

A careful examination of the genitalia and the position of the anus are part of the initial assessment since CAKUT can occur in the context of cloacal malformations and with genital tract defects in females and males. The mesonephric duct gives rise to the developing kidneys, urinary tracts and the male genital tracts; therefore, a careful examination of the testes, the epididymis, and the ductus deferens is important. Congenital epididymal cysts are the most frequent anomaly noted in association with mesonephric duct anomalies and are usually asymptomatic. Other male genital duct anomalies that may occur in the context of CAKUT include an absent, ectopic or duplicated ductus deferens. Seminal vesicle cysts may also arise and typically present after puberty as pelvic pain or with urinary symptoms like dysuria, polyuria, or urinary retention [162].

Adjacent to the mesonephric ducts are the paired Müllerian or paramesonephric ducts that give rise to the fallopian tubes, the uterus, the cervix, and the upper two thirds of the vagina. Because Müllerian duct development is tightly linked to the growth and elongation of the mesonephric ducts, CAKUT is also observed with concurrent female Müllerian duct anomalies. Indeed, the Mayer-Rokitansky-Kuster-Hauser syndrome describes women with normal female external genitalia, but Müllerian duct anomalies that include aplasia of the uterus, the cervix, and the upper vagina. In a large cohort of 284 women with this syndrome, roughly 30% of them had associated CAKUT anomalies including renal agenesis, horseshoe kidney, ectopic kidney, and urinary tract defects including duplications [163]. Females with Müllerian duct anomalies are typically discovered because of primary amenorrhea, dyspareunia, infertility, and/or obstetric complications [164]. In females with CAKUT and a suspected Müllerian duct anomaly, MRI imaging may be indicated to define the anatomical defect with better precision.

Clinical Approach to Specific Malformations

Unilateral Renal Agenesis

A diagnosis of unilateral renal agenesis depends on the certainty that a second kidney does not exist in the pelvis or some other ectopic location. Since absence of one kidney induces compensatory hypertrophy in the existing kidney, the presence of a large kidney on one side suggests the possibility of unilateral renal agenesis. Interestingly, compensatory hypertrophy has been observed to begin as early as 20 weeks of gestation: van Vuuren et al. examined 67 fetuses with a diagnosis of MCDK or unilateral renal agenesis and noted that 87% of the cases of MCDK and 100% of the cases of unilateral renal agenesis exhibited compensatory hypertrophy of the contralateral kidney with kidney length greater than the 95th percentile for gestational age [165]. Since unilateral agenesis is associated with contralateral urinary tract abnormalities

including UPJO and VUR in 20–40% of the cases [166, 167], imaging of the contralateral side is suggested. Management of affected patients involves determining the functional status of the contralateral kidney. If the contralateral kidney is normal, the long-term renal functional outcome is usually excellent, although a recent study suggests that some patients may in fact have a poor long-term outcome and require dialysis [25]. It is therefore reasonable to propose that individuals with a single functioning kidney should have their blood pressure measured, urine tested for protein, and renal function measured periodically throughout life. While some have suggested that children with single kidneys should avoid contact/collision sports, at least one study suggests that kidney injuries occur much less frequently than other organ injuries, and thus sports restriction may not be indicated solely on the basis of having a single kidney [168].

Renal Hypoplasia

Unless associated with other malformations, renal hypoplasia can be asymptomatic. Unilateral hypoplasia is often discovered as an incidental finding during an abdominal sonogram or other imaging study. In contrast, patients with bilateral renal hypoplasia are at risk for decreased renal function and CKD.

Renal Dysplasia

The dysplastic kidney is generally smaller than normal. However, cystic elements can contribute to large kidney size, the most extreme example being the MCDK (see below). During the antenatal period, unilateral disease is likely to be discovered as an incidental finding. This may also be the case for bilateral renal dysplasia unless it is associated with oligohydramnios. After birth, bilateral renal dysplasia may limit GFR, causing renal failure that is usually progressive. Postnatal ultrasonography of the dysplastic kidney is characterized by small size, increased echogenicity, loss of corticomedullary differentiation and cortical cysts. Renal dysplasia is strongly associated with dilatation of the upper and lower urinary tract from VUR, posterior urethral valves, and/or other urinary tract obstruction [169]. Accordingly,

imaging of the lower urinary tract should be performed to determine whether these abnormalities are present.

Multicystic Dysplastic Kidney

The MCDK presents by ultrasonography as a large cystic non-reniform mass in the renal fossa and by palpation as a flank mass. The MCDK is nonfunctional, a condition that can be demonstrated by imaging with MAG3 or DTPA radio-nuclide scanning. The MCDK is usually unilateral. If bilateral, it is fatal. Complications of MCDK include hypertension (0.01–0.1%). Wilms tumour and renal cell carcinoma have also been described in MCDK, but the incidence of malignant complications is not significantly different from the general population [170]. In 25% of cases, the contralateral urinary tract is abnormal. Contralateral abnormalities can include rotational or positional anomalies, renal hypoplasia, VUR and UPJO [18]. Contralateral UPJO occurs in 5–10% of cases.

Gradual reduction in renal size and eventual resolution of the mass of the MCDK is common. At two years, an involution in size by ultrasound has been noted in up to 60% of the affected kidneys. Complete disappearance of the MCDK can occur in a minority of patients (3–4%) by the time of birth, and in 20–25% by two years. Increase in the size of MCDK can be seen in some cases. Several reports suggest that if the kidney length of the MCDK is less than 6.2 cm on the initial postnatal US, then complete resolution is likely to occur [171, 172]. The contralateral kidney usually shows compensatory hypertrophy by ultrasound evaluation. If the contralateral kidney does not show hypertrophy, it could be hypoplastic.

Management of patients with MCDK has shifted from routine nephrectomy in the past, to observation and medical therapy. Because of the risk of associated anomalies in the contralateral kidney, the possibility of VUR should be evaluated and blood pressure should be measured. For children with isolated MCDK and a contralateral kidney that is structurally normal with compensatory hypertrophy, the prognosis is excellent. While there exist no evidence-based guidelines

for long-term follow-up of these children, a review of published evidence and expert opinion supports serial investigation by ultrasound and urinalysis within the first two years of life to monitor MCDK involution and contralateral renal growth, and then very intermittent examination of renal growth, blood pressure and urine protein excretion through the end of puberty [173]. For the small number of patients with unilateral MCDK who develop hypertension, estimated to be 5.4 out of 1000 children [170], medical therapy is usually effective. Nephrectomy may be curative in resistant cases.

Renal Ectopia

Normally, the kidneys lie on either side of the spine in the lumbar region and are located in the retroperitoneal renal fossae. Rapid caudal growth during embryogenesis results in migration of the developing kidney from the pelvis to the retroperitoneal renal fossa. With ascension, comes a 90° rotation from a horizontal to a vertical position with the renal hilum finally directed medially. Migration and rotation are complete by 8 weeks of gestation.

Simple congenital ectopy refers to a low-lying kidney that failed to ascend normally. It most commonly lies over the pelvic brim or in the pelvis and is termed a pelvic kidney. Less commonly, the kidney may lie on the contralateral side of the body, a state that is termed crossed ectopy without fusion. Clinical presentation can be asymptomatic or symptomatic. Asymptomatic presentation is when the ectopic kidney has been diagnosed coincidentally such as might occur during routine antenatal sonography. Symptomatic presentation occurs with urinary tract infections. Symptoms such as abdominal pain or fever may occur. On examination, an abdominal mass may be palpable. Other presenting features include hematuria, incontinence, renal insufficiency and hypertension [21]. A high incidence of urological abnormalities has been associated with renal ectopia. VUR is the most common, occurring in 20% of crossed renal ectopia and 30% of simple renal ectopia. In bilateral simple renal ectopia, there is a higher incidence of VUR, occurring in 70% of cases. Other associ-

ated urological abnormalities include contralateral renal dysplasia (4%), cryptorchidism (5%) and hypospadias (5%) [21]. Reduced renal function is commonly observed by radionuclide scan in the ectopic kidney. Female genital anomalies such as agenesis of the uterus and vagina [174] or unicornuate uterus [175] have also been associated with ectopic kidneys. Other anomalies described include adrenal, cardiac and skeletal anomalies. Clinical assessment should therefore include a careful physical examination for other anomalies. Renal ultrasonography will help with diagnosis and defining the underlying anatomy. A VCUG should be undertaken, particularly if there is hydronephrosis, given the risk of VUR and obstruction. A DMSA scan is also recommended to assess for differential renal function.

Renal Fusion

Renal fusion is defined as the fusion of two kidneys. The most common fusion anomaly is the horseshoe kidney, in which fusion occurs at one pole of each kidney, usually the lower pole. The fused kidney may lie in the midline (symmetric horseshoe kidney) or the fused part may lie lateral to the midline (asymmetric horseshoe kidney). In a crossed fused ectopic kidney, the kidney from one side has crossed the midline to fuse with the kidney on the other side. Fusion is thought to occur before the kidneys ascend from the pelvis to their normal dorsolumbar position. This is usually between the fourth to ninth week of gestation. As a result, fusion anomalies seldom assume the high position of normal kidneys. The blood supply may therefore come from vessels such as the iliac arteries. Abnormal rotation is also associated with early fusion of the developing kidneys. The pelvis of each kidney lies anteriorly and the ureter, therefore, traverses over the isthmus of a horseshoe kidney or the anterior surface of the fused kidney. Ureteric compression may occur due to external compression by a traversing aberrant artery. The majority of patients are asymptomatic. Some, however, develop obstruction which presents with loin pain, hematuria and may be associated with urinary tract infections due to urinary stasis or VUR. Renal calculi may occur in up to 20% of cases [176].

Other associated urological anomalies include ureteral duplication, ectopic ureter and retrocaval ureter. Genital anomalies such as bicornuate and/or septate uterus, hypospadias, and undescended testis have also been described. Associated non-renal anomalies involve the gastrointestinal tract (anorectal malformations such as imperforate anus, malrotation, and Meckel diverticulum) the central nervous system (neural tube defects), and the skeleton (rib defects, clubfoot, or congenital hip dislocation). Investigations should include static imaging (renal ultrasound) and functional imaging (DMSA scan) and a VUCG.

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