Chapter 1

Normal Human Kidney Development and Congenital Anomalies of the Kidneys and Urinary Tract

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1.1 Normal Human Kidney Development

1.1.1 Introduction

Our kidneys are essential for normal postnatal life, controlling diverse processes including excretion of nitrogenous waste products, homeostasis of water, electrolyte and acid base balance, and hormone secretion. The paired kidneys are located in the retroperitoneum lateral to, and extending between, the 12th thoracic and 3rd lumbar vertebrae. An average mature human male kidney measures around 12 × 6 × 2.5 cm in size and weighs up to 170 g; females have slightly smaller organs, although kidney size tends to correlate with body surface area in both genders.

The basic functional unit reiterated throughout the kidney is the nephron, comprising the glomerulus, which filters the blood, and then specialised epithelia from the proximal tubule to loop of Henle to distal tubule. These are connected to a tree-like collecting duct system that conveys the urine into the renal pelvis, from which it passes into the ureter and then the bladder. Glomeruli are located in the renal cortex, a 1 cm thick strip that forms the outermost part of the kidney; other nephron components extend from there into the medulla. The cortex is continuous in humans, whereas the medulla consists of around 14 discrete pyramids. This arrangement is termed 'multipapillary' and contrasts with the 'unipapillary' kidneys found in rodents, rabbits and many other species.

Nephron number or 'endowment' is a key determinant of renal function, and historical reports often state that humans have around 1 million nephrons in each kidney. This is inaccurate, however, because nephron number varies considerably between individuals and different populations, with estimates based on serial sectioning and counting glomeruli using stereology ranging from 0.6 to 1.3 million per kidney [1]. Techniques to gauge live nephron number are being developed [2] but are still a long way from routine use.

1.1.2 Timetable of Nephrogenesis

Human kidney formation, usually termed 'nephrogenesis', begins around 22 days after fertilisation and completes around the 34th to 36th week of gestation. There are three pairs of 'kidneys' in human development (and other mammals): the pronephros, mesonephros and metanephros, which arise sequentially from intermediate mesoderm on the dorsal body wall. The human pronephros and mesonephros are transient structures that degenerate and are resorbed during fetal life, but they are essential precursors to the metanephros; normal adult kidneys do not develop if they are disrupted. Timing of the stages seen in human kidney development is outlined in Table 1.1 as days post fertilisation. Other texts may cite days post ovulation, which is 12–24 hours earlier, or Carnegie Staging (CS), which relates to size and gross development of the embryo (for reference, CS13 is around 28–32 days, CS18 44–51 and CS23 56–60). Times are contrasted with that of the mouse because this is the main experimental animal used to

Table 1.1. Summary of important stages during nephrogenesis, comparing human and murine milestones. One essential difference is that formation of new nephrons is complete before birth in humans but continues in the first postnatal week in mice, which makes it easier for experimental studies.

Structure		Human	Mouse
Pronephros	Appears	22 days	9 days
	Regresses	25 days	10 days
Mesonephros	Appears	24 days	10 days
	Regresses	16 weeks	14 days
Metanephros		32 days	11.5 days
Renal pelvis		33 days	12.5 days
Collecting tubules/nephrons		44 days	13 days
Glomeruli (from)		9 weeks	14 days
Nephrogenesis ceases		34–36 weeks	3–7 days after birth
Length of gestation		40 weeks	20 days

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further molecular understanding of nephrogenesis, and there is a frequently updated mouse database at GUDMAP (www.gudmap.org), with an evolving human counterpart in the Edinburgh Human Anatomy Project Atlas [3]. Most major genes are expressed similarly between man and mouse, although there is an increasing understanding of the differences too [4]. It is noteworthy that the pronephros is the functioning kidney of adult hagfish and some amphibians, as is the mesonephros in adult lampreys, some fishes and amphibians. Zebrafish are another good model for some aspects of nephrogenesis [5].

1.1.3 Anatomy

Despite unchanging anatomy, our understanding of nephrogenesis is constantly evolving. Anatomical descriptions have been published over many centuries with the Italian Marcello Malpighi highlighting many microscopical features in the seventeenth century. These were updated several times through the twentieth century by authors such as Kampmeier [6], who described how early structures are transient (and remodelled by a process that we now know to be apoptosis [7]), and seminal work by Edith Potter and colleagues based on microdissection of thousands of fetal autopsies (see below) [8–10]. These concepts are now being refined by histological analysis combined with powerful 3D imaging allied to molecular reports that identify the important gene pathways in different lineages [11–14].

1.1.4 Early Kidneys: The Pronephros and Mesonephros

The human pronephros develops around 22 days after fertilisation from the intermediate mesoderm lateral to the notochord adjacent to the ninth somite (Figure 1.1). It comprises simple tubules that develop sequentially alongside the pronephric duct, which elongates caudally to fuse with the cloaca on day 26. At this point, the duct is renamed as the mesonephric or Wolffian duct. Pronephric tubules and the cranial part of the duct involute by day 25.

The long, sausage-shaped mesonephros appears similar to the pronephros initially, with simple tubules adjacent to a duct, but glomerulus-like structures also become identifiable from around 24 days of gestation as the duct grows towards the cloaca (see Figure 1.1). The connection between these primitive nephron-like structures and filtration function is uncertain in humans because the mesonephric duct is initially thought to be a solid rod of cells that only develops a lumen after fusion with the cloaca. Mesonephric tubules develop from intermediate mesoderm medial to the duct by 'mesenchymal to epithelial' transformation, a process that is subsequently reiterated during nephron formation in metanephric development. In humans, a total of around 40 mesonephric tubules are produced (several per somite), but the cranial tubules regress at the same time as caudal ones are forming, hence there are never more than 30 pairs at any time.

Each human mesonephric tubule consists of a medial cup-shaped sac encasing a knot of capillaries, analogous to the Bowman's capsule and glomerulus of the mature kidney, and a lateral portion attached to the mesonephric duct. Other segments of the tubule resemble mature proximal and distal tubules histologically but there is no loop of Henle. Small quantities of urine are thought to be made after duct canalisation in the human mesonephros, whereas the murine organ is much more rudimentary and does not contain well-differentiated glomeruli. Most mesonephric structures involute during the third month of gestation, except in males, where some tubules contribute to the efferent ducts of the epididymis and the mesonephric duct is incorporated into ductular parts of the epididymis, the seminal vesicle and the ejaculatory duct.

1.1.5 The Definitive Kidney: The Metanephros

Osathanondh and Potter split development of the metanephros into four phases: 1 (5–15 weeks), 2 (14–15 to 20–22 weeks), 3 (20–22 to 32–36 weeks) and 4 (32–36 weeks to adulthood) [10]. During phase 1, metanephrogenesis begins in the caudal part of the embryo (Figures 1.1 and 1.2). The developing organ comprises two main cell lineages at inception: the epithelial cells of the ureteric bud, and the mesenchymal cells of the metanephric blastema. Reciprocal interactions promote ureteric bud branching to form collecting tubules, calyces and the renal pelvis, with proximal parts forming the ureter. In contrast, the mesenchyme has a more varied fate with some undergoing epithelial conversion to form the nephrons from glomerulus to distal tubule, while some contribute to vascular development or the interstitial cells/stroma in the mature kidney.

Human metanephric kidney development begins around day 28 post conception when the ureteric bud arises as an outpouching of the distal mesonephric duct / Wolffian duct. Perturbation of these processes can lead to a range of phenotypes including renal agenesis, dysplasia and duplex kidneys, as well as malpositioning defects such as pelvic or horseshoe kidneys if the kidneys fail to ascend to their normal 12th thoracic to 3rd lumbar vertebral site during development (see Section 1.2 and Table 1.2).

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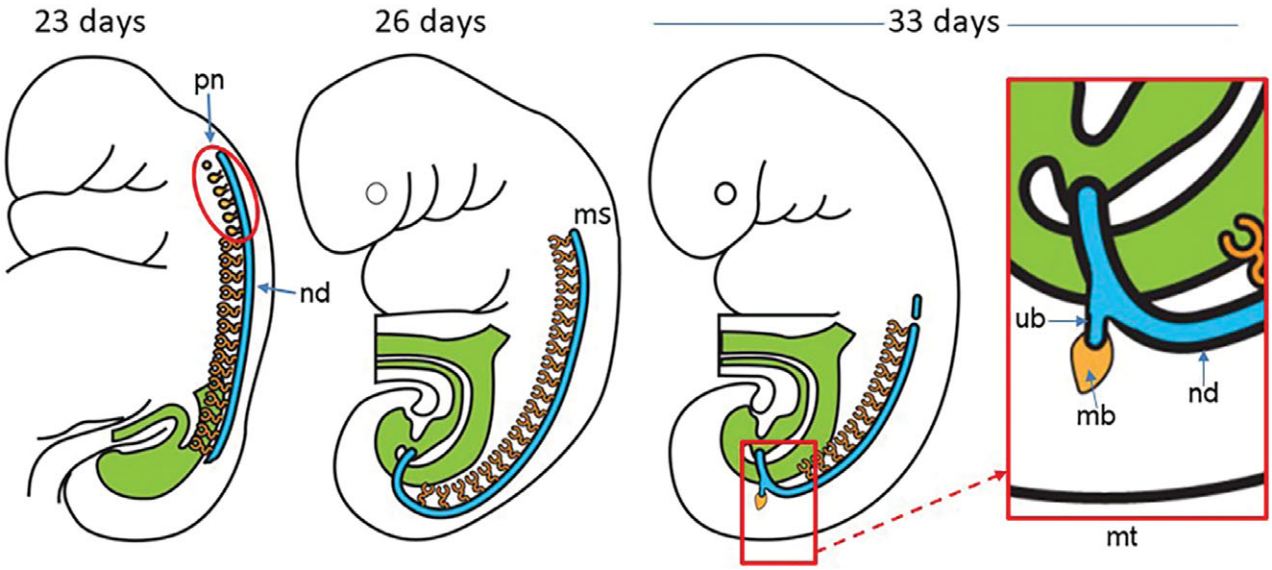

Figure 1.1 Early development. Initiation of human kidney development from 23 to 33 days post fertilisation. The three stages are demonstrated sequentially: pronephros (pn), mesonephros (ms) and metanephros (mt). These are mirrored on the right and left of the developing embryo. The nephric duct (nd) originates in the thoracic region, along with the tubules of the pronephros. It migrates caudally, stimulating mesonephric glomeruli and tubules, and is then renamed the mesonephric or Wolffian duct as it fuses with the cloaca. The ureteric bud (ub) branches from the duct close to the cloaca and grows into the pre-programmed mesenchyme of the metanephric blastema (mb) to form the metanephros, which develops into the definitive adult kidney.

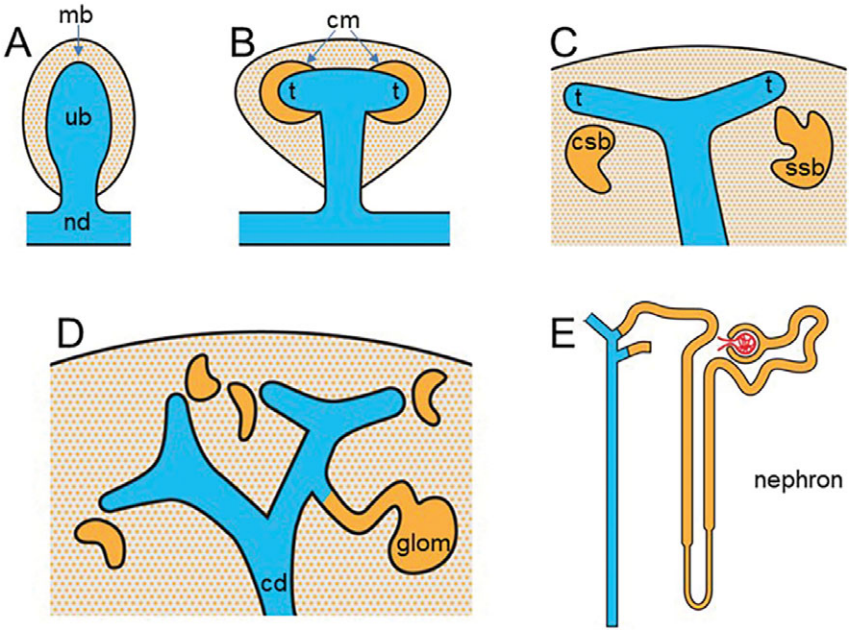

Figure 1.2 Metanephric development. (A) The first step in metanephrogenesis is outpouching of the epithelial ureteric bud (ub) from the nephric duct (nd, also termed the Wolffian duct) and extension into the metanephric mesenchyme (mb). (B) Mutual induction between the bud and mesenchyme causes the bud to branch serially. Bud tips (t) stimulate the mesenchyme to condense (cm, sometimes termed compact mesenchyme) and undergo epithelial conversion. (C, D) The newly formed epithelia go through renal vesicle, comma and S-shaped (S) stages as the nephron is formed from glomerulus (glom) through to distal tubule. csb, Comma-shaped body; ssb, S-shaped body; cd, collecting duct. (E) The eventual structure consists of ureteric bud–derived collecting ducts (in blue) connected to mesenchyme-derived epithelia (in orange) from glomerulus, through proximal tubule and loop of Henle, to distal tubule. This image depicts individual attachment points, which is correct in phase 1 of nephrogenesis up to about 15 weeks' gestation, whereas later connections have multiple nephrons arranged in arcades attached to a single point.

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Table 1.2. Genetic causes of congenital anomalies of the kidneys and urinary tract (CAKUT).

Gene/gene family	CAKUT type
Angiotensin-related (*ACE, AGT, AGTR1*)	Renal tubular dysgenesis (can also be induced by angiotensin-related medications)
BBS genes	Multiple Bardet–Biedl syndrome mutations causing tubulointerstitial and glomerular changes with or without cysts and dysplasia
BICC1	Multicystic dysplastic kidney
BMP4 or *7*	Renal hypodysplasia/agenesis; flow impairment/obstruction
BNC2	CAKUT, dominant lower urinary tract obstruction
CDC5L	Renal agenesis
CDKN1C	Renal medullary dysplasia
CHD1L	CAKUT
CHD7	VACTERL
CHRM3	Functional bladder outlet obstruction/prune-belly syndrome
DNAJB11	ADPKD
DSTYK	CAKUT
EYA1	Branchio-oto-renal syndrome; renal hypoplasia
FGFR1 and *2*	Renal agenesis
FOXC1 or *2*	CAKUT
FRAS1, FREM1 or *2, GRIP1*	Fraser syndrome–associated genes, CAKUT
GANAB	ADPKD
GATA3	VUR
GDF11	Renal agenesis
GDNF	Hirschsprung disease; VUR, renal agenesis, CAKUT
GFRA1	CAKUT (GFRA1 is the coreceptor for GDNF)
GLI3	Pallister–Hall syndrome
GPC3	Cystic and dysplastic kidneys
GREB1L	Renal agenesis, genital tract malformation/agenesis
GREM1	Renal agenesis
HNF1β	Renal hypodysplasia, frequently with renal cysts; renal cysts and diabetes syndrome (RCAD), maturity onset diabetes of the young
HPSE2	Urofacial syndrome/Ochoa syndrome with dysmorphic, poorly emptying bladder
JAG1	Alagille syndrome
KAL1	Kallmann syndrome, renal agenesis and hypodysplasia
NOTCH2	Alagille syndrome
NPHP1	Renal hypodysplasia, nephronophthisis
OFD1	Oral-facial-digital syndrome with glomerulo-cystic kidneys
PAX2	Renal coloboma syndrome, renal hypodysplasia, VUR
PAX8	Hypothyroidism and renal agenesis
PBX1	Renal hypoplasia
PKD1/2	ADPKD; a few large cysts arising from all nephron segments
PKHD1	ARPKD; many cysts arising from collecting ducts only
REN	Renal tubular dysgenesis

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Table 1.2. (*cont.*)

Gene/gene family	CAKUT type
RET	Hirschsprung disease/CAKUT (RET is the main receptor for GDNF)
ROBO2	VUR/CAKUT
SALL1	Townes–Brocks syndrome, renal hypoplasia
SIX1; 2 or 5	Branchio-oto-renal syndrome, renal hypodysplasia, CAKUT
SOX9	CAKUT
SOX17	Pelviureteric junction obstruction/VUR
TBX18	CAKUT – dominantly inherited
UMOD	Renal hypodysplasia, occasional cysts
UPIIIA	Renal hypodysplasia
WNT4	Renal hypoplasia
WT1	Denys–Drash, WAGR and Frasier syndromes; mesangial sclerosis, focal glomerular sclerosis or Wilms tumours with other urogenital abnormalities; renal agenesis
XPNPEP3	Nephronophthisis, tremor, sensorineural hearing loss, mitochondriopathy

ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; VACTERL, vertebral defects, anal atresia, cardiac defects, tracheo-oesophageal fistula, renal anomalies and limb abnormalities; VUR, vesicoureteric reflux; WAGR, Wilms tumour, aniridia, genitourinary anomalies and mental retardation.

By day 32, the tip (ampulla) of the bud has extended into a specific area of sacral intermediate mesoderm, the metanephric blastema. This mesoderm is pre-programmed to respond to signals from the ureteric bud, and many of the current experimental efforts to grow kidneys in vitro depend on conditioning the cells into intermediate mesoderm then exposing them to sequential inductive signals normally secreted by the ureteric bud [15, 16]. Glomeruli form from 8 to 9 weeks, and nephrogenesis continues in the outer rim of the cortex until 34 weeks (Figure 1.3) [10]. Nephrons elongate and continue to differentiate postnatally, but new nephrons are not formed. In mice, the ureteric bud enters the metanephric mesenchyme by embryonic day 10.5, the first glomeruli form by embryonic day 14 and nephrogenesis continues until the end of the first week after birth (although many older texts incorrectly state until 14 days).

1.1.6 Nephrons from Glomerulus to Distal Tubule

Every early nephron develops from mesenchyme adjacent to an ampullary tip of the ureteric bud. The mesenchyme is initially loosely arranged but the cells destined to become nephrons grow closely together and compact/condense around the bud tips before undergoing phenotypic transformation into epithelial renal vesicles. Each vesicle elongates to form an initial comma shape that then folds back on itself to become S-shaped [17]. The proximal part of the S-shape develops into the glomerulus whilst the distal portion elongates and differentiates into nephron segments from proximal convoluted tubule to distal convoluted tubule. Other mesenchymal cells give rise to renal interstitial cells and contribute to vessel development. Many mesenchymal cells destined to become different final lineages can be identified at early stages by their molecular markers, with the transcription factors Six2 and Cited1 marking nephron progenitors whereas Foxd1 marks stromal precursors [18–20]. Lack of these factors was identified originally in mice with kidney defects but is now implicated in human renal disease too [21].

There has been some debate over the likely function of early glomeruli, particularly because they are eventually lost as the original outer part of the metanephros becomes incorporated into the developing medulla. Recent work using 31 human embryos between 46 and 60 days' gestation (CS 19 to 23), from the Kyoto Collection in Japan, defined five different maturity stages of nascent glomeruli and proposed that it is only after the fifth generation of ureteric bud branching that these connect (and presumably function) [13]. Similarly, another study from around 37–41 days (CS16) reported

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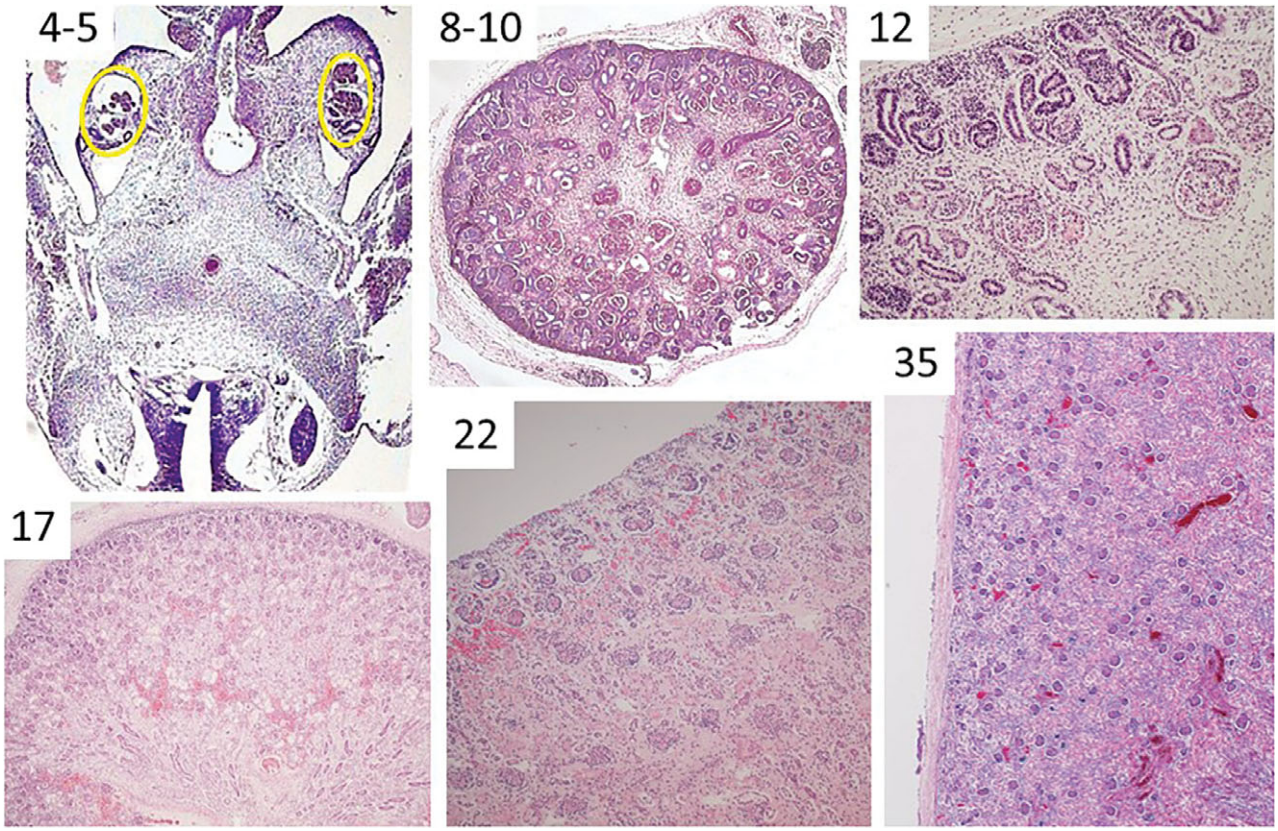

Figure 1.3 Histology. Human developing kidneys labelled with gestation in weeks. The mesonephros is visible at 4–5 weeks (outlined in yellow) and contains recognisable glomeruli and tubules, which are believed to have some excretory function in humans (but not mice). Metanephric glomeruli are visible by 9–10 weeks, deep to the active nephrogenic layer, which contains ureteric bud tips adjacent to condensing mesenchyme (see higher power at 12 weeks). Later sections demonstrate increasing layers of glomeruli with gestation. The nephrogenic cortex has disappeared by 35 weeks because new nephrons are no longer forming in the majority of fetuses by this stage.

that it takes between 3 and 14 days from the first condensates (pretubular aggregates) until s-shaped bodies connect fully, then a further 1–10 days before vascularised glomeruli are observed at around 56–60 days (CS23) [12].

1.1.7 Collecting Duct Lineage

The solitary ureteric bud grows into the metanephric blastema (see Figures 1.1 and 1.2) and signalling from this mesenchyme stimulates the epithelial ampulla to divide into a T-shape with two tips. Tips have two functions: (1) to induce adjacent mesenchyme to condense and start epithelial transition (see Section 1.1.5), and (2) to extend and divide so that further tips can repeatedly go through this cycle to generate a tree-like collecting duct system draining the nephrons. Serial branching of the bud changes it from a simple 180 degree T pattern, through a Y orientation to a V-shaped morphology with a prominent lumen by around 60 days [12]. Potter reported that the degree of branching differs in different parts of the kidney in humans [10], with a recent study confirming a faster rate at the upper and lower poles versus the interpolar regions deeper within the organ [14]. Mathematical modelling can explain some of the changes in branching pattern [22], but Davies and colleagues from Edinburgh proposed an extra layer of complexity after observing that tips actively avoid branching towards other tips [23]. They also demonstrated (in mice) how remodelling makes Y-shaped branches retract, shortening the stalk of the Y into a V; computer simulation shows how this can transform the spreading tree arrangement of the early kidney into a system with long, almost-parallel medullary rays, as seen in the mature organ [24].

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Twenty cycles of binary division (e.g., 2 to the power of 20) would generate just over a million nephrons if each tip induced a single condensate. This calculation oversimplifies kidney development, however, because the one tip to one condensate/nephron relationship persists only during the first phase of nephrogenesis up to 15 weeks according to Potter [10, 25]. More recent publications bear this out with Ishiyama and colleagues [14] calculating that fifteen generations of ureteric bud branching have already been reached after 60 days (end of CS23). It is intriguing that there were nearly five times as many bud tips as nephrons at the early stages (0.21 ± 0.14 at CS19) suggesting that nephron maturation lags branching. This is unlikely to affect eventual nephron number because we have known for nearly a century that early nephrons (and branching generations) are transient and destined for remodelling by a process that we now know is apoptosis [6, 7]. It is estimated that the first three to five generations contribute to the renal pelvis and the next three to five give rise to the minor calyces and papillae [10, 14].

Bifid branching morphogenesis does not feature in Potter's second phase after 15 weeks' gestation; instead nephrons form arcades with several draining via the same ureteric bud attachment point [10, 14]. It becomes technically difficult to identify or visualise the number and pattern of ureteric bud branching histologically as the human kidney grows, and the focus switches to the number of glomerular generations as an indicator of the stage of nephrogenesis. A recent study investigated mid- to late-gestation human development and demonstrated a linear relationship between age (or body weight) and glomerular generations between 20 and 40 weeks [4], with an eventual complete number of 9.9 ± 0.23 generations in females and 10.4 ± 0.29 in males. This gender difference was not significant and may only reflect the slightly higher weight of males. Interestingly, these authors also noted that nephrogenesis was complete by 36 weeks in most cases, but one sample had a persistent nephrogenic zone at 37 weeks suggesting wider variability than previously suspected [4].

1.1.8 Stromal and Vascular Tissues

Most early work on nephrogenesis ignored non-nephron-forming mesenchyme, regarding it as a source of unremarkable, undifferentiated stroma. This heterogeneous tissue gives rises to several specialised cell types in the renal capsule, vasculature and interstitium, however, and it is also critical for normal nephron and collecting duct patterning [26, 27]. Moreover, aberrant stroma has been linked to both dysplastic and fused horseshoe kidneys [28] and expansion of the renal stroma with fibrotic changes is a key factor in many progressive renal diseases. Hence, further study of both normal and abnormal stromal development is warranted in any search to find new renal treatment strategies.

Up to a fifth of cardiac output is directed to the kidneys in humans, and there is specialised vascular patterning in different areas including glomerular capillaries adapted for filtration, the juxtaglomerular apparatus and vasa rectae, which pass alongside loops of Henle into the medulla. Anatomists have debated the source of renal vessels for many years, championing either vasculogenesis where mesenchyme differentiates *in situ* to form capillary endothelia, or angiogenesis in which vessels grow in from outside. We now believe that both processes are important, as endothelial precursors are present within early mesenchyme and there is also some sprouting from existing vessels [29, 30]. Developmental vascular patterning is not well studied in human kidneys, but a recent report in mice demonstrated endothelial plexuses from the very earliest stages, encircling the ureteric bud and then organising around condensing mesenchymal cells and bud branches in a repeated, predictable pattern [31]. It is not just arteriovenous patterning that is important, though, with an increasing focus on renal lymphatics demonstrating important roles in both normal development and disease [32, 33].

1.2 Congenital Anomalies of the Kidneys and Urinary Tract (CAKUT)

The acronym CAKUT encompasses a wide spectrum of kidney and urinary tract abnormalities and pathologies that are diagnosed in up to 1% of pregnancies, although many of these are mild abnormalities that do not require further treatment. We will briefly consider kidney abnormalities here, although there is often concomitant maldevelopment of the kidney and urinary tract which may impact renal function further [34, 35]. CAKUT are routinely diagnosed by antenatal screening before any pathological defect is defined, so representative ultrasound images are given in Figure 1.4 to demonstrate the possible presentation of these abnormalities [36, 37]. Based on the stages of normal development outlined in earlier sections, there are several key phases that should be considered in CAKUT including:

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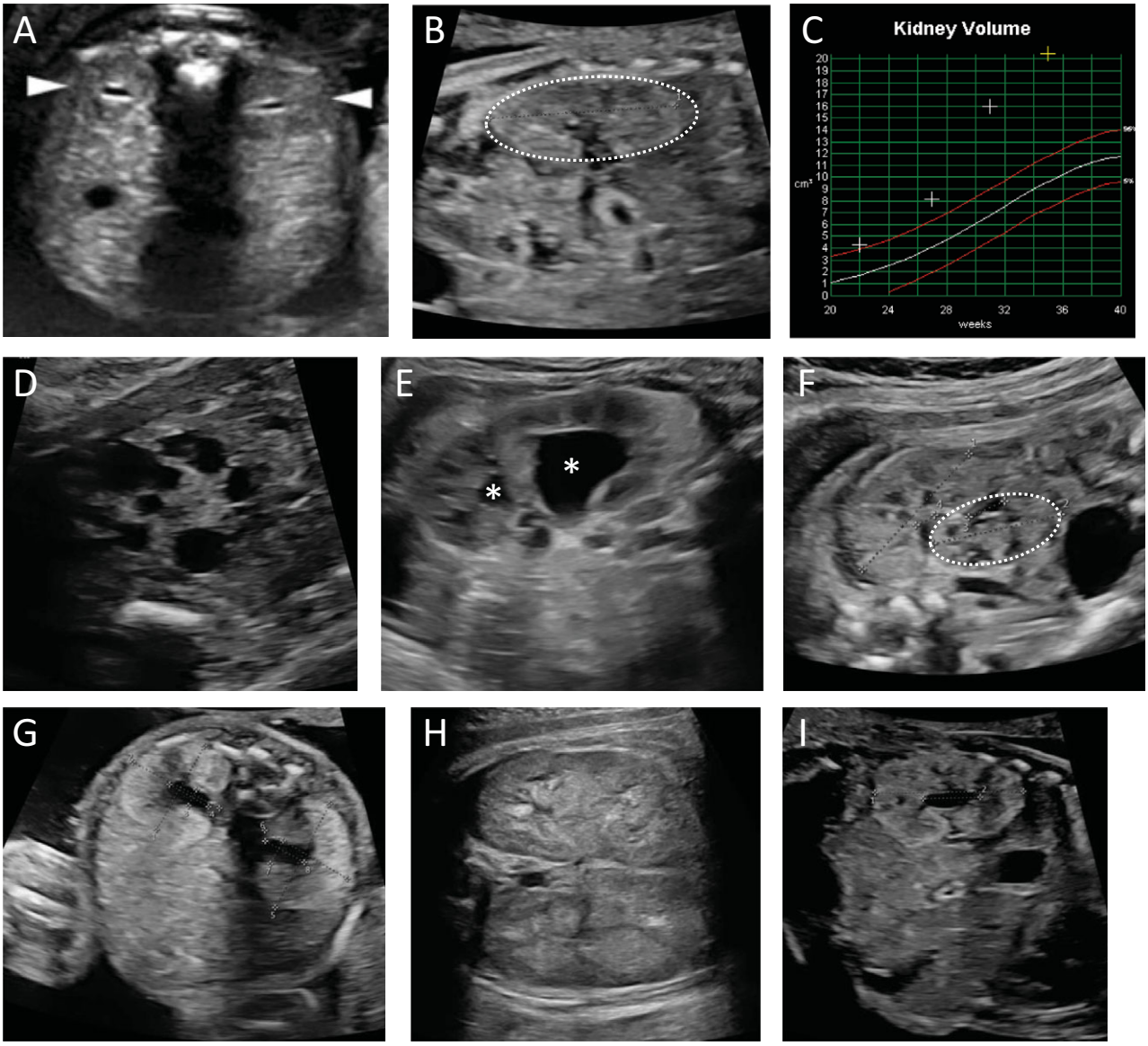

Figure 1.4 Antenatal ultrasound. (A) Normal kidney with bilateral symmetrical appearance antero-lateral to the spine. (B) A normal single kidney (outlined) is shown in the upper part of the scan but the contralateral kidney is absent (note, both kidneys are not always visible in the same field, but this kidney was absent on multiple views). (C) With normal single kidneys, compensatory growth as seen on this centile chart is usually a positive sign that this kidney is likely to have reasonable function, particularly if amniotic fluid levels are also normal. (D) Multicystic dysplastic kidney with irregular cysts and echo-bright (i.e., abnormal) parenchyma. (E) Duplex kidney with the separate renal pelves indicated by asterisks. (F) Cross-fused ectopia with the crossed moiety outlined. (G) Bilateral bright kidneys with loss of normal corticomedullary differentiation. Note how the echogenicity is brighter than that of surrounding soft tissues and approaching that of the spine. (H) Massively enlarged bilateral bright kidneys occupying almost all of the abdomen. This was associated with anhydramnios caused by autosomal recessive polycystic kidney disease; this often presents with large bright kidneys rather than frank cysts because the cysts are below the level of resolution for detection on conventional ultrasound. (I) Unilateral bright cystic dysplastic kidney.

- Initial transient pronephric and mesonephric stages needed to establish the developmental renal field.
- Co-ordinated outgrowth of the ureteric bud at the correct time in the right orientation to make contact with pre-specified metanephric mesenchyme.
- Multiple inductive interactions between epithelial cells in the bud and mesenchymal cells destined to become nephrons, stroma or vessels.

Most diagnoses can only be presumed from ultrasound and need histology for definitive confirmation, but this rarely changes management in live infants so it is almost never

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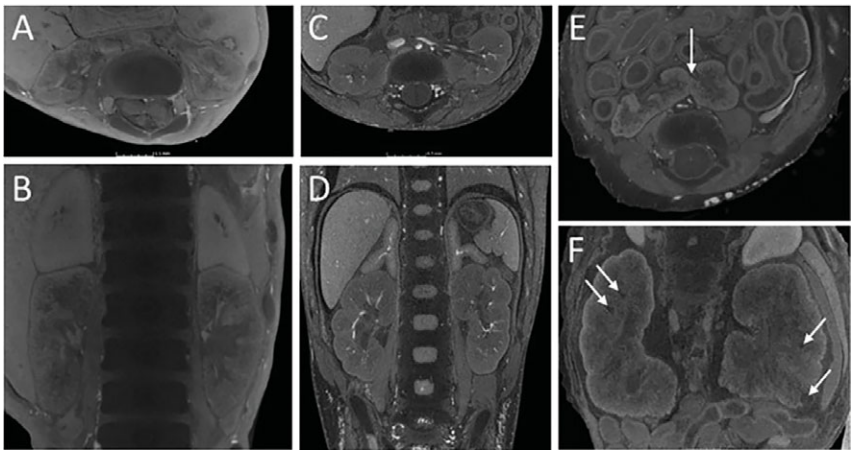

Figure 1.5 MicroCT. Postmortem microCT imaging and other less invasive techniques can provide useful information on both normal and abnormal kidney development. (A–D) Normal kidney images at 12 (A and B) and 20 (C and D) weeks' gestation. Note how large the adrenals are at 12 weeks, almost the same size as the kidneys. This can confound the diagnosis of renal agenesis when the adrenal sits in the renal bed and mimics the kidney on early ultrasound. (E) 15 weeks; crossed fused ectopia (arrowed). (F) 17 weeks; bilateral cysts (arrowed) in dysplastic kidneys (in a fetus with trisomy 13). Images courtesy of Dr Susie Shelmerdine, Great Ormond Street Hospital for Sick Children NHS Foundation Trust.

obtained. Postmortem should always be offered if the infant dies, because making a secure diagnosis can help planning and counselling for future pregnancies. Many parents are understandably reluctant to consent to full postmortem, however, since the loss of a baby is so traumatic. Hence newer, less invasive assessment is being developed, including microCT, which is illustrated in Figure 1.5.

1.2.1 Absent Kidneys

Renal agenesis or aplasia results in the same phenotype, a missing kidney. However the aetiology is different: agenesis is a primary defect with initiation of the pronephros-mesonephros sequence and/or failure of ureteric bud outgrowth; in contrast, renal aplasia usually represents a kidney that formed initially but later regressed, suggesting potential failure in epithelial-mesenchymal induction. A common cause of aplasia is multicystic dysplastic kidneys (MCDK), since at least two thirds of these involute eventually, but it is difficult to give a precise incidence because few older people had fetal ultrasound to ascertain the presence of MCDK along with later scans to document disappearance [38]. Absence of both kidneys is rare at 1:4 000 to 1:10 000 births with a slight male bias. These babies usually die at birth with the Potter sequence of anhydramnios–defective lung maturation–respiratory insufficiency, along with limb and face deformities. Lack of one kidney is more common, affecting 1:1 000 individuals with an equal gender ratio. The left kidney is more likely to be affected; this may reflect the slightly different timing of nephrogenesis on each side. Uncomplicated unilateral agenesis may be clinically silent if not detected on antenatal ultrasound because there will be minimal early symptoms with a normal contralateral kidney that hypertrophies and compensates. Up to 50% may have associated urogenital anomalies, particularly vesicoureteric reflux but also contralateral renal dysplasia, absence of vas deferens, absent adrenals and malpositioning (usually in the pelvis, likely reflecting failure to ascend during development). Bilateral or unilateral renal agenesis accompanies many syndromes such as the branchio-oto-renal syndrome, renal cysts and diabetes (RCAD) syndrome and the hypoparathyroidism, sensorineural deafness and renal anomalies (HDR) syndrome, Fanconi anaemia, and Fraser, Kallmann, Di George and Smith–Lemli–Opitz syndromes (see Table 1.2 and references 21, 39–41).

1.2.2 Dysplastic Kidneys

Dysplastic kidneys reflect a failure of normal differentiation, most likely via perturbed inductive interactions after the metanephros has formed. They can be either larger or smaller than normal and diffusely or partly cystic [42]. Diagnosis is routinely made by characteristic renal ultrasound appearances ranging from subtle changes in echogenicity resembling a 'bright kidney', through loss of corticomedullary differentiation to frank cystic changes (see Figure 1.4D–I) [36, 37]. Pathological features include disrupted renal architecture with a paucity of nephrons, abundance of undifferentiated cells, tortuous vessels,

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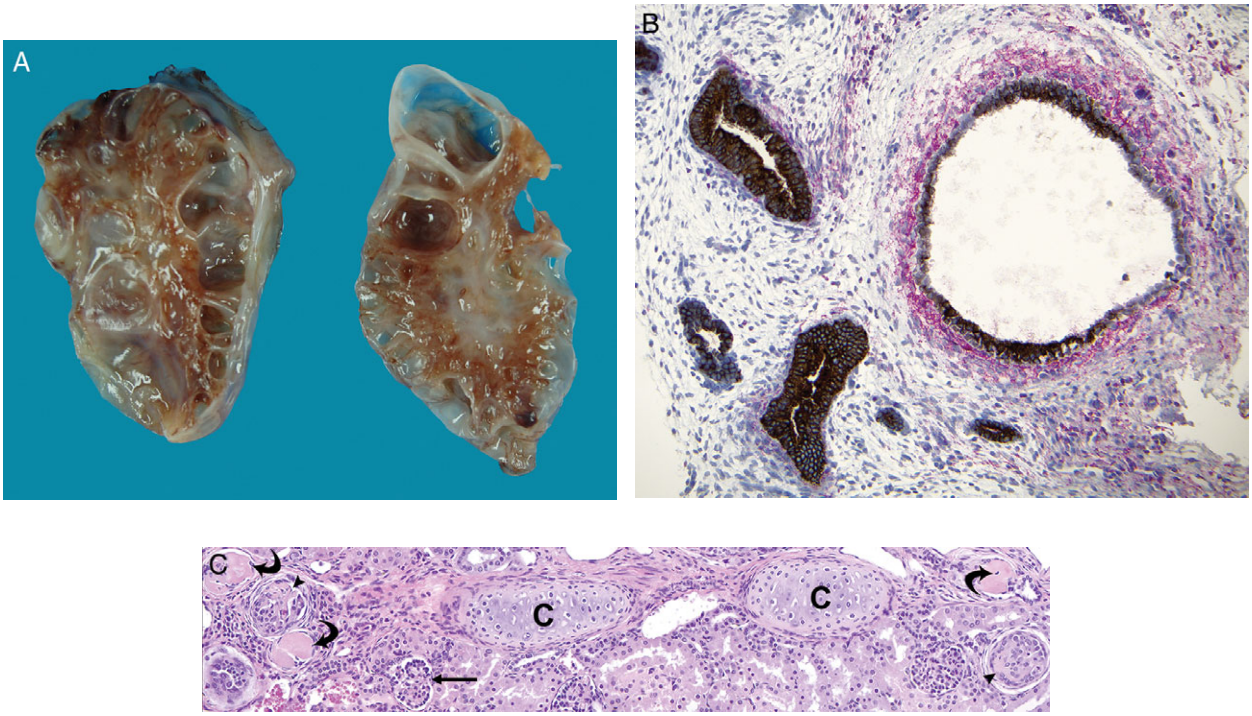

Figure 1.6 Dysplasia. (A) Bilateral cystic dysplasia in a stillborn male infant, approximately 20–21 weeks' gestational age (body weight ~390 g). The kidneys were grossly enlarged and had numerous cysts as seen in the cross sections; their combined weight was 24 g (expected weight for age is 4.2 g). (B) Microscopically, dysplastic kidneys show epithelial-lined tubules and cysts (CAM 5.2 antibody, brown) surrounded by undifferentiated mesenchyme. A collar of smooth muscle (smooth muscle actin antibody, red) encircles a cyst (immunohistochemistry × 20). (C) Glomeruli are small and fetal looking (arrow) or obsolete (curved arrows). Islands of cartilage (C) are present (haematoxylin and eosin × 20). (A) Courtesy Dr. Philip Faught, Indiana University School of Medicine, Indianapolis, IN, USA. (B and C) Courtesy Dr. Carrie Phillips, Indiana University, Indianapolis, IN.

metaplastic cartilage and primitive, poorly branched ducts with smooth muscle collars (Figure 1.6A, B). Cartilage is pathognomonic but only present in around a third of cases (Figure 1.6C). MCDK represents one end of the range, with no functioning renal tissue and all of the parenchyma replaced by cysts and poorly differentiated interstitial tissues [38]. Such kidneys are often attached to an atretic ureter, raising the possibility of early obstructive pathogenesis from the ureteric bud outgrowth stage.

Unilateral dysplasia is more common than bilateral, and in 50–75% of patients co-existing abnormalities of the urinary tract occur, particularly renal ectopias such as horseshoe kidney (Figure 1.7), ureteral duplication, hydroureters, ureteropelvic and ureterovesical junction obstruction and vesicoureteric reflux [43]. The prognosis for renal function can be poor for children with bilateral dysplasia because of their intrinsic nephron deficit, with severity ranging from the lethal Potter sequence, through neonatal renal failure to later chronic kidney disease. Conversely, children with unilateral dysplasia generally

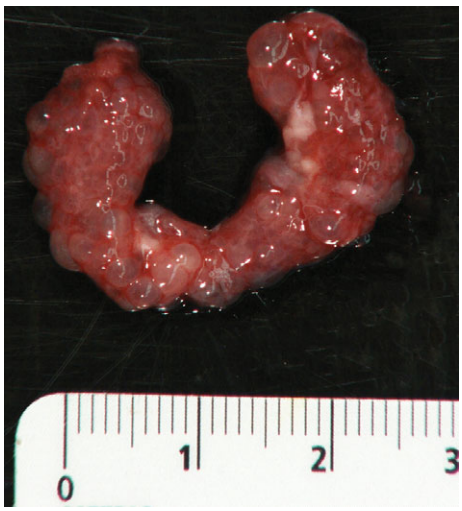

Figure 1.7 Horseshoe kidney. Autopsy of a baby boy shows a horseshoe kidney fused in the middle with multiple cysts throughout indicating concurrent dysplasia. Courtesy Helen Liapis.

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do well if the contralateral kidney is normal and hypertrophies during development, although co-existing anomalies may also affect prognosis. Many older texts suggest removing dysplastic kidneys because of the increased risk of hypertension and Wilms tumours. However, these risks appear overstated: over 70% of MCDK involute and disappear without intervention, as do a significant proportion of non-cystic dysplastic kidneys [44, 45].

The most common genetic cause of dysplastic kidneys is thought to be mutations of the *TCF2* gene in the RCAD syndrome [46]. *TCF2* encodes the transcription factor hepatocyte nuclear factor 1β, which is expressed in the mesonephric duct, ureteric bud lineage and early nephron epithelia as well as the adjacent paramesonephric ducts that should differentiate into the uterus and fallopian tubes. Renal malformations in RCAD are highly variable, ranging from grossly cystic dysplastic kidneys through hypoplasia with oligomeganephronia to apparent unilateral agenesis, along with diverse uterine abnormalities in females and familial gout [47].

1.2.3 Hypoplastic Kidneys

Hypoplastic kidneys weigh less than 50% of the expected mean for age but do not display any primary histological abnormality, for example there may be significantly fewer nephrons but those present are normal initially (this may change with hyperfiltration and nephron dropout later). There are three main hypoplasia variants: simple hypoplasia, oligomeganephronic hypoplasia (oligomeganephronia) and segmental hypoplasia (Ask-Upmark kidney) [48].

Oligomeganephronia is defined as bilateral reduction in kidney size, reduction in estimated glomerular filtration rate and no evidence of vesicoureteric reflux. Histologically, the number of glomeruli in the cortex is reduced and there is a marked increase in the number of peripheral capillary loops, glomerular diameter and volume. Tubules are also enlarged compared to age-matched controls. Children that present with progressive renal dysfunction may show focal segmental glomerulosclerosis or global glomerulosclerosis, interstitial fibrosis and tubular atrophy. Typically, end stage kidney disease ensues from 6 months to 17 years.

Hypoplasia may be isolated or part of a wider malformation syndrome such as the renal coloboma syndrome that is caused by mutations in the *PAX2* gene which is critical for epithelial-mesenchymal transition in early nephron formation [48, 49]. In theory, detailed histologic examination of the kidney is required to exclude evidence of dysplasia before labelling as hypoplasia, but this is often not done if the renal appearance is normal (albeit small) on ultrasound because it is risky to biopsy small kidneys and confirmation would rarely alter conservative 'watch and wait' treatment. Bilateral small kidneys are occasionally seen in children with multiple congenital malformations, Down syndrome, or longstanding disease or anomalies of the central nervous system. These may represent a failure of renal growth rather than an intrinsic defect in early nephrogenesis.

1.2.4 Cystic Kidney Diseases and Ciliopathies

Renal cysts can develop at any stage of life, from *in utero* through to late adulthood. A key distinction between different conditions relating to CAKUT, however, is whether the cysts arise during kidney development or after the full nephron complement has been reached. Multicystic dysplastic kidneys clearly combine cysts and severe perturbation of early nephrogenesis. In contrast, most polycystic kidney diseases and the new group of diseases called ciliopathies (including Bardet–Biedl syndromes and nephronophthisis, which have molecular defects in components of the primary cilium [50]) demonstrate relatively normal early development, then cysts develop later (i.e., there is a secondary, induced nephron defect). Hence, we will not consider them further in this kidney development chapter.

1.2.5 Duplex Kidneys

Duplication of the renal pelvis and ureter (duplex kidney) is common, occurring in about 5% of unselected autopsies. The spectrum ranges from simple bifurcation of the extrarenal renal pelvis to complete duplication with two distinct (but contiguous) kidneys, separate ureters and two uretero-vesical openings [51, 52]. Many cases will be asymptomatic, but up to a half may develop complications requiring treatment. Mackie and Stevens proposed a mechanism for the degree of renal abnormality almost 50 years ago [53] based on the displacement of the ureteric orifice from its normal site, with more cranial or caudal location generating more severe hypoplasia or dysplasia. In practice, the upper pole in which the ureter usually connects distally to the bladder is more likely to have obstruction and dysplasia, whilst the lower renal moiety has a higher risk of reflux.

The most logical aetiology of a duplex system is a double outgrowth of the ureteric bud from the mesonephric duct around 28 days post conception. To ensure

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that the bud emerges at the right site in the correct orientation this process is tightly controlled, particularly by the GDNF/RET/GFRa system whereby GDNF secreted by mesenchyme acts on its receptors RET and GFRa in the epithelial mesonephric duct (and in ureteric tip branching later). This process has multiple negative regulator pathways including Spry1, Slit2/Robo and semaphorins to restrict branching to the correct position; mutations in some of these have been implicated in cystic dysplastic kidneys, unilateral renal agenesis and duplicated collecting systems [52, 54–56].

1.2.6 Ectopic Kidneys and Malrotation

The kidneys should ascend from their initiation position in the sacral region towards the upper lumbar region during development, accompanied by rotation of the renal pelvis from an anterior- to a medial-facing orientation. Failure to ascend completely is relatively common at 1:800 on routine renal imaging, and usually associated with retention of a more anterior-facing renal pelvis [57]. Isolated ectopic kidneys are often asymptomatic but there is a risk of reduced nephron number and dysplasia because of perturbed development. Reflux or hydronephrosis/obstruction secondary to the abnormal orientation and ureteric positioning increases the risk of urinary tract infection and kidney stones. Ectopic kidneys can also be part of a multisystem disorder such as CHARGE syndrome (coloboma, heart disease, atresia choanae, retarded growth, genital hypoplasia and ear abnormalities) and VACTERL malformations (vertebral, anal, cardiac, tracheal, oesophageal, renal and limb anomalies) [57].

Occasionally, in crossed ectopia, the kidney is on the wrong side as well as in the wrong position and it may also be fused to the contralateral kidney in cross-fused ectopia. Kidneys can also be fused in a horseshoe kidney (1 in 400–600); these are usually situated lower than normal (see Figure 1.7) and, again, have an increased risk of vesicoureteric reflux or hydronephrosis [58]. Pancake or lump kidneys are rare variants. The vascular supply to ectopic kidneys can be complex, including direct from the aorta, iliac or hypogastric arteries, or from mid-sacral vessels. This is important when surgery is required, particularly in emergency situations with traumatic injury [59].

1.2.7 Underlying Pathogenesis of CAKUT

1.2.7.1 Genetic

There have been major advances in understanding the genetic and molecular pathogenesis of CAKUT over the last two decades, with new studies and approaches being reported almost weekly. Many gene mutations have been implicated across the spectrum and a selection of these are listed in Table 1.2 [21, 34, 60–62]. It is striking, however, that even the best studies find a genetic cause in only 20–25% of cases. This could be because we lack the technology to unravel complex, yet subtle interactions between multiple genes across different developmental stages but could also mean that extrarenal, environmental and stochastic (simple chance) factors may be as important as genetics in CAKUT.

1.2.7.2 Obstruction

Urinary flow impairment, such as posterior urethral valves, is commonly found distal to dysplastic kidneys [63, 64] and there is strong experimental evidence that obstruction reproduces many of the histological and molecular changes seen in renal dysplasia [65, 66]. Moreover, the most severe part of the dysplasia spectrum, MCDK, is associated with 'atretic' (e.g., obstructed ureters). Hence, obstruction must always be considered in the aetiology of CAKUT, but the pathogenesis is complex because obstruction may induce both primary and secondary effects, with early damage causing a reduction in the number of nephrons formed, then later bladder dysfunction potentiating renal injury [67]. One must not forget, however, that some obstructive lesions may also have a genetic underpinning although only rare genes have been implicated thus far [68].

1.2.7.3 Teratogens and Maternal Factors

Teratogens and maternal factors are also implicated in CAKUT [34, 69–71]. Some therapies perturb nephrogenesis and should be avoided around pregnancy. The most widely used are modulators of the renin-angiotensin system, which mimic the effects of gene mutations in this pathway and can lead to renal tubular dysgenesis (see Chapter 9) and skeletal malformations [72]. Natural factors may also play a role; one example is retinoic acid, a natural metabolite of vitamin A, excess levels of which perturb experimental nephrogenesis [73] whilst deficiency may have more subtle roles in determining nephron mass [74], making it one of the many prenatal factors that can programme adult diseases such as hypertension [75]. Maternal diabetes is also associated with an increased incidence of kidney and lower urinary tract malformations [76, 77]. Some of these cases may represent the first presentation of a family with *HNF1β* mutations and the RCAD syndrome (see Section 1.2.2) but there is also a significant positive correlation between the odds of a fetus having CAKUT and the severity of maternal obesity [78].

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Key Points/Summary

- There are three paired kidneys at different developmental stages in humans – the pronephros, mesonephros and metanephros – with only the latter persisting beyond fetal life.
- Mature human kidneys contain around a million nephrons as a population average, but this figure conceals a wide variation between 600,000 and 1,300,000 in normal individuals, which may predispose to earlier renal disease in those with lower numbers.
- New nephron formation finishes by 34 to 36 weeks gestation in most fetuses, and further nephrons do not develop after that time, even when there is significant renal injury.
- Congenital anomalies of the kidneys and urinary tract (CAKUT) occur in up to 1% of fetuses, encompassing a wide spectrum of positional and structural defects.
- CAKUT causes can be genetic, secondary to obstruction or teratogens/maternal environment, or a combination of these. At present, however, most cases do not have a clear aetiology.

Knowledge Gaps

- Nephron number varies between individuals and different populations, but we do not know how nephron number is controlled during development. It would be helpful in designing novel regenerative therapies to understand which factors instruct the kidney to stop making nephrons.
- Studies have identified many of the molecular processes in normal mouse kidney development but there is much less data in humans and there is some evidence of divergence. More comparative studies are required to identify and understand both common processes and differences.
- Much research is now focused on human kidney organoids, as a precursor to making transplantable neo-kidneys. These currently replicate some parts of the nephron but they are small and lack connections with a single ureter and vessels. More work is needed to understand scalability and gross organisation.

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