—— REVIEW ARTICLE ——

Genetics of Renal Hypoplasia: Insights Into the Mechanisms Controlling Nephron Endowment

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ABSTRACT: Renal hypoplasia, defined as abnormally small kidneys with normal morphology and reduced nephron number, is a common cause of pediatric renal failure and adult-onset disease. Genetic studies performed in humans and mutant mice have implicated a number of critical genes, *in utero* environmental factors and molecular mechanisms that regulate nephron endowment and kidney size. Here, we review current knowledge regarding the genetic contributions to renal hypoplasia with particular emphasis on the mechanisms that control nephron endowment in humans and mice. (*Pediatr Res* **68:** 91–98, 2010)

Renal hypoplasia is a common, yet poorly understood and misused term describing congenital renal anomalies. Renal hypoplasia is defined as abnormally small kidneys (<2 SD below the expected mean when correlated with age or parameters of somatic growth) with normal morphology and reduced nephron number. This definition predicts that $\sim2.2\%$ of the population exhibit renal hypoplasia, whereas epidemiologic studies suggest an estimated incidence of 1 in 400 births (1). Much confusion and the misapplication of this definition have arisen because the majority of congenitally small kidneys also exhibit evidence of tissue maldifferentiation, defined as renal dysplasia. The exact incidence of pure renal hypoplasia (without dysplasia) is difficult to define as renal dysplasia has often been incorrectly described as hypoplasia. This has predominantly been due to the lack of noninvasive diagnostic tools (*i.e.* Ultrasound) with resolution power adequate enough to discriminate dysplasia from hypoplasia in such settings. Severe reductions in nephron number that are characteristic of renal hypoplasia/dysplasia are the leading cause of childhood end stage renal disease. Indeed, if severe enough, these conditions can lead to significant impairment of intrauterine renal function which can in turn lead to the oligohydramnios sequence, a condition not compatible with extrauterine life. This includes severe and modest bilateral renal hypoplasia. More subtle defects in nephron number, such as those at the lower end of the normal range caused by mild bilateral renal hypoplasia, have been associated with the development of adult-onset hypertension and chronic renal failure (2–6). Here, we focus on knowledge derived from the study of human syndromic forms of renal hypoplasia and mouse mutants that provide insights into the molecular mechanisms that underlie renal hypoplasia and control nephron endowment. Congenital renal abnormalities characterized by nephron number and other renal pathologies including renal dysplasia, hydroureter, cystic dysplasia, and agenesis are reviewed in detail elsewhere (7–9).

OVERVIEW OF KIDNEY DEVELOPMENT

Development of the mammalian metanephric kidney is dependent on reciprocal inductive interactions between two distinct cell lineages, the ureteric cell lineage and the metanephric mesenchyme (MM) cell lineage (Fig. 1). At the onset of metanephric development, signals emanating from the mass of uninduced MM initiate the formation of an epithelial bud (the ureteric bud) from the adjacent Wolffian duct (Fig. 1*A*). Reciprocal inductive interactions between the ureteric bud (UB) tip and adjacent MM result in continued/successive branching of the UB, ultimately forming the mature collecting duct system of the kidney (Fig. 1*B*). Simultaneously, the UB tips signal to the adjacent MM cells causing them to aggregate, undergo a mesenchymal-epithelial conversion, and progress through a series of maturation steps to form a fully functional nephron (Fig. 1*C* and *D*). This iterative cycle of ureteric branching morphogenesis and nephron induction results in the formation of $\sim60,000$ collecting ducts and an average of $\sim785,000$ (range: 210,332–1,825,380) nephrons in humans (10). Human metanephric development commences at ~5–6 wk after fertilization, and nephrogenesis is completed by 36-wk gestation. Therefore, final nephron endowment is

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Abbreviations: FGF(R), fibroblast growth factor (receptor); **HH,** Hedgehog; **MM,** metanephric mesenchyme; **Rar,** retinoic acid receptor; **UB,** ureteric bud; **UBM,** ureteric branching morphogenesis
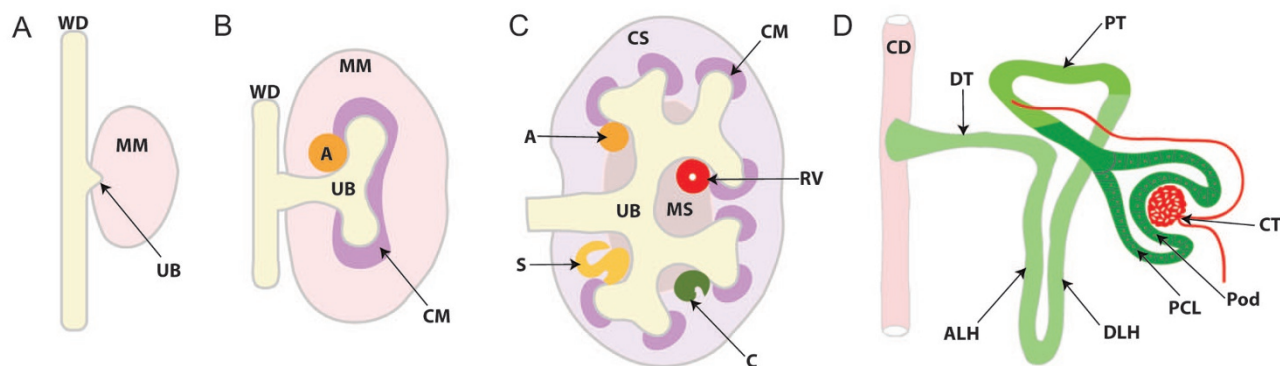
Figure 1. Morphogenesis of the metanephric kidney. *A*, Metanephric kidney development commences with metanephric mesenchyme (MM) induced formation of the ureteric bud (UB) from the caudal aspect of the Wolffian duct (WD). *B*, The UB grows toward and invades the MM and reciprocal inductive interactions between the two result in reiterative branching of the UB, ultimately forming the mature collecting duct system of the kidney. *C*, Simultaneously, the UB tips signal adjacent MM cells to condense (CM) and undergo a mesenchyme-epithelial transformation forming the aggregate (A), renal vesicle (RV), comma-shaped body (C), and S-shaped body (S). *D*, The S-shaped body undergoes further differentiation to form the functional nephron. Ascending loop of Henle (ALH), cortical stroma (CS), capillary tuft (CT), distal loop of Henle (DLH), distal tubules (DT), medullary stroma (MS), parietal cell layer (PCL), podocyte cell layer (Pod), proximal tubule (PT).

Table 1. *Human gene mutations associated with renal hypoplasia*

Gene	Primary disease	Kidney phenotype	OMIM no.	Reference(s)
BMP4	Isolated renal hypoplasia	Hypoplasia, VUR		50
CREBBP	Rubinstein-Taybi syndrome	Agenesis, hypoplasia,	180849	98
DHCR7	Smith-Lemli-Optiz syndrome	Agenesis, cystic dysplasia, hypoplasia	270400	37
EYA1, SIX1, SIX5	Branchio-Oto-Renal syndrome	Agenesis, ectopia, cystic/dysplasia, duplication, hypoplasia, hydronephrosis/ureter	113650, 610896	48, 49, 99
FRAS1, FREM2	Fraser syndrome	Agenesis, cystic/dysplasia, hypoplasia	219000	100
GLI3	Pallister-Hall syndrome	Agenesis, cystic/dysplasia, hypoplasia, hydronephrosis/ureter	146510	35
HNF1β	Renal cysts and diabetes syndrome	Cystic/dysplasia, hypoplasia	137920	32
PAX2	Renal-Coloboma syndrome	Hypoplasia, VUR	120330	22–24
PAX2	Isolated renal hypoplasia	Hypoplasia		25, 26
RET, UPK3a	Potter syndrome (oligohydramnios) Renal adysplasia	Agenesis, cystic/dysplasia, hypoplasia	191830	13
RET	Isolated renal hypoplasia	Hypoplasia		14
SALL1	Townes-Brocks syndrome	Agenesis, cystic dysplasia, hypoplasia	107480	54
SALL4	Acrorenalocular syndrome (Okihiro syndrome)	Agenesis, ectopia, hypoplasia, hydronephrosis/ureter, VUR	607323	53
SIX2	Isolated renal hypoplasia	Hypoplasia, VUR		50
7q 11.23	Williams syndrome	Ectopia, hypoplasia, hydronephrosis/ureter	194050	101

completed during embryogenesis, after which no new nephrons can be formed, although the kidney tubules continue to mature into the postnatal period. The fetal metanephric kidney begins to function at ~10-wk gestation when the first signs of urinary output become evident.

Much of the insight into the genetics of human renal hypoplasia has been obtained *via* studies into rare syndromic conditions (Table 1). These important genetic associations identified in human renal hypoplasia have provided a platform for many researchers to investigate the molecular mechanisms by which these genes function in both normal and abnormal kidney development. Animal model systems (predominantly murine) have provided significant insights into the molecular mechanisms underlying the development of renal hypoplasia. In turn, many of these same mechanisms have been corroborated in the human condition. Here, we discuss the most significant findings generated and our current understanding of how these genes interact with each other and within different cell lineages.

CONTRIBUTION OF THE URETERIC CELL LINEAGE TO RENAL HYPOPLASIA

Nephron formation in the MM requires reciprocal interaction with the UB tips. The proper elaboration of the UB tree and the secretion of tip-derived signals are essential for adequate nephrogenesis. Multiple processes are required within the UB for development: induction, growth and branching, survival, and differentiation. These processes are primarily regulated by receptor tyrosine kinase signaling pathways and transcription factors expressed in the UB (Table 2).

Growth and branching. The interactions between the UB tip-specific receptor RET and its ligand GDNF are crucial for the initial UB induction from the Wolffian duct. Loss of these interactions leads to a failure of kidney development as $Ret^{-/-}$ mice present with renal agenesis (11). However, recent work exploring the participation of the *Ret* signaling axis throughout kidney development has begun to tease out a role for this

Table 2. *Targeted gene disruption leading to renal hypoplasia in mice*

Gene	Genetic model	Mechanism	Reference(s)
UB			
Egfr	Egfr$^{neo/neo}$, Hoxb7Cre;Egfr$^{loxP/loxP}$	Growth and branching, elongation	20
Fgfr2	Hoxb7Cre;Fgfr2$^{loxp/-}$	Growth and branching	17
Frs2α	Hoxb7Cre;Frs2α$^{loxP/loxP}$	Growth and branching	16
Gli3R	Hoxb7Cre;Ptc1$^{-/loxP}$, Hoxb7Cre;Ptc1$^{-/loxP}$; Gli3$^{Δ699/+}$, Gli3$^{-/-}$	Differentiation	34
Lim1	Hoxb7Cre;Lim1$^{loxP/loxP}$	Growth and branching, differentiation	21
Met	Hoxb7Cre;Met$^{loxP/loxP}$	Growth and branching	19
p53	p53 MMTV	Differentiation	33
Pax2	Pax2$^{1Neu/+}$	Growth and branching, survival	27
Ret	RetY1062F	Growth and branching	12
Hnf1β	vHnf1$^{-/-}$ (tetraploid)	Growth and branching, induction	31
Wnt11	Wnt11$^{-/-}$	Growth and branching	15
MM/stroma			
Bcl2	Bcl2$^{-/-}$	Survival	66, 67
C-myc	Bmp7Cre;C-myc$^{loxP/loxP}$	Survival, progenitor pool	64
Eya1	Eya1$^{+/-}$	UBM	47
Fgf7	Fgf7$^{-/-}$	UBM	60
Fgf10	Fgf10$^{-/-}$	UBM	62
Gdf	Gdf11$^{-/-}$	UBM	45
Gdnf	Gdnf$^{+/-}$	UBM	41, 42
Hoxa11/Hoxd11	Hoxa11$^{-/-}$;Hoxd11$^{-/-}$	UBM	46
N-myc	N-myc$^{-/-}$	Survival	63
Raldh2	Raldh2$^{-/-}$	UBM	56
RARαβ2	Rarα$^{-/-}$; Rarβ2$^{-/-}$	UBM	55
Sall1	Sall1$^{-/-}$	UBM	51
Sall4	Sall4$^{+/-}$	UBM	52
Six2	Six2$^{-/-}$, Br$^{+/-}$	Progenitor pool	69, 70

pathway during all phases of UB development. Three tyrosine residues on the intracellular portion of RET, Tyr1015, Tyr1096, and Tyr1062, act as P-Tyr binding sites for multiple adaptors and effectors, with Y1062 activating the Ras/Erk, PI3 K/Akt, and JNK pathways. Mutation of the Y1062 residue in *RetY1062F* knockin mice abolishes this binding site. In contrast to *Ret*$^{-/-}$ mice which lack kidneys due to deficient UB induction, *RetY1062F* knockin mice demonstrate renal hypoplasia, characterized by normal UB induction and initial branching but decreased branching from E13.5 onwards, suggesting an additional role for *Ret* in the regulation of branching in late embryogenesis (12). Moreover, the observation that these knockin mice do not phenocopy *Ret*$^{-/-}$ mice indicates a role for multiple P-Tyr binding sites downstream of *Ret* in guiding proper kidney development throughout embryogenesis. In humans, mutations in the *RET* gene have been identified in some patients presenting with features of Potter Syndrome and renal anomalies, including renal hypoplasia (13). Furthermore, a common single-nucleotide polymorphism within the exon splicing enhancer of exon 7 of the human *RET* gene, predicting diminished function, has been identified in newborns with subtle renal hypoplasia (Table 1; 14).

Maintenance of *Gdnf* and *Ret* expression is controlled by an autoregulatory feedback loop with *Wnt11*. WNT11 secretion from the UB tip is responsible for the maintenance of GDNF expression in the MM. *Wnt11*$^{-/-}$ mice develop defective UB branching and failure to maintain *Gdnf* expression in the MM (15). Newborn mutant mice are characterized by a 64% decrease in glomerular number. Synergistic genetic interactions between *Ret* and *Wnt11* have been demonstrated by a more severe hypoplastic phenotype in *Wnt11*$^{+/-}$;*Ret*$^{+/-}$ mice, as well

as the loss of *Wnt11* in the few *Ret*$^{-/-}$ mice that develop hypoplastic kidney tissue (15).

FGF signaling *via* the FGFR2 receptor, expressed on UB cells, has also been shown to play a crucial role in ureteric development, with multiple knockout models resulting in renal hypoplasia. The docking protein *Frs2α* is classically thought of as a major intracellular docking protein for FGFR2 signaling. Conditional inactivation of *Frs2α* in the UB leads to mild renal hypoplasia with a reduced but normal pattern of UB growth and branching after the T-stage (16). This is in contrast to the *Fgfr2*$^{-/-}$ mice that exhibit renal hypoplasia but an abnormally thin and elongated ureteric branching pattern resulting in an abnormal mature kidney shape (17). This phenotypic difference and the lack of renal phenotype in mice that express point mutations of the *Frs2α* docking site of FGFR2 suggest that FGFR2 signals through other adaptor molecules in the UB and that *Frs2α* may transmit signals downstream of other receptor tyrosine kinases (RTKs). The finding of decreased expression of *Ret* and *Wnt11* in *Frs2α* mutants suggests that this docking protein may function downstream of RET in the RET/GDNF autoregulatory pathway. Furthermore, the *Ret* and *Fgfr2β* RTK pathways converge on common transcription factors *Etv4* and *Etv5* (18).

Signaling from two additional RTKs, *Met* and *Egfr*, have also been shown to cooperate in the regulation of late UB branching. *Met* is expressed in both the UB and MM and is the receptor for *Hgf*. Targeted deletion of *Met* to the ureteric cell lineage results in a 35% decrease in nephron number by 12 wk of age (19). The *Egfr* was shown to be up-regulated in the collecting ducts of these mice. Elimination of *Egfr* on the *Met* deficient background causes a more severe deficit in ureteric

branching suggesting that these two RTK pathways act cooperatively in directing late ureteric branching (19). *Egfr* null mutants alone also present with renal hypoplasia, characterized by atrophy of the renal papilla and decreased ureteric branching (20). Interestingly, conditional inactivation of *Egfr* in the UB lineage results in normal ureteric branching but a marked reduction in collecting duct elongation (20).

Ureteric branching and growth is also regulated at the transcriptional level. The transcription factor *Lim1* is essential in the UB for growth and branching as well as *Ret* expression in the tip domain. Targeted deletion of *Lim1* in the UB lineage also demonstrated a requirement for *Lim1* in the timing of UB induction and nephric duct maintenance (21). Analysis of chimeric embryos consisting of a mix of wild type and *Lim1*$^{-/-}$ cells demonstrated a cell autonomous requirement for *Lim1* in the nephric duct and UB tip domain.

Ureteric cell survival. Renal-Coloboma syndrome (RCS) is an autosomal dominant disorder that is caused by heterozygous loss of function mutations in the human *PAX2* gene (Table 1; 22). Kidneys of affected individuals are characterized by normal nephron structure but a substantial reduction in total nephron number often leading to chronic renal failure (23,24). Supporting the importance of *PAX2* in human renal hypoplasia, mutations have also been detected in cases of isolated renal hypoplasia and a common variant in the *PAX2* gene is associated with reduced kidney size (subtle renal hypoplasia) in newborns who lack any other phenotypic characteristic of *PAX2* deficiency (25,26). Insights into the underlying mechanisms governing renal hypoplasia in RCS have been obtained *via* studies in mice and suggest that *PAX2* in the UB lineage is crucial for ureteric growth, branching, and survival. A frameshift mutation of *Pax2*, identical to the G619 insertion mutation identified in some humans with RCS (*Pax2*^{1Neu}) in mice results in renal agenesis in homozygotes and renal hypoplasia in heterozygotes (27). Renal hypoplasia in this model is associated with a 40% reduction in nephron number, elevated apoptosis in the UB epithelium, and reduced number of ureteric branches (27,28). Constitutive expression of the proapoptotic gene, *Bax*α, in the ureteric cell lineage driven by the *Pax2* promoter results in renal hypoplasia, elevated UB apoptosis and reduced branching, identical to the phenotype observed in the *Pax2*$^{1Neu/+}$ mice (29). Further supporting a role of ureteric cell apoptosis in renal hypoplasia, transgenic mice expressing the antiapoptotic factor, *Bcl2*, in ureteric cells in *Pax2*$^{1Neu/+}$ mice, prevents apoptosis and normalizes ureteric branching, nephron number, and renal function (28). The ability of *Pax2* to directly activate transcription of *Ret* and its ligand, *Gdnf*, in mice, provides further evidence of the importance of these genes in renal development and the pathogenesis of human renal hypoplasia (30).

Induction of nephrogenesis. Recently, the transcription factor *Hnf1*β was demonstrated to participate in a regulatory network that maintains the expression of *Lim1*, *Pax2*, and *Wnt9b* expression in the UB (31). *Hnf1*β deficient embryos develop severe renal hypoplasia with a delay in UB induction, loss of Wolffian duct maintenance, and reduced UB growth and branching. *Hnf1*β-regulated gene transcription is critical to the UB and MM interplay as it exhibits *in vivo* binding to

noncoding regulatory DNA sequences of *Pax2*, *Lim1*, and *Wnt9b* (31). This is particularly clear given the essential requirement for WNT9b secretion from the UB stalk domain and its effect on MM nephrogenic program induction. Disruption of these events likely provides insight into the rare association of renal hypoplasia with Renal Cysts and Diabetes syndrome (RCAD) and mutations in human *HNF1*β (Table 1) (32).

Ureteric cell differentiation. The transcriptional control of UB differentiation, especially of the tip domain, is essential for UB development and proper nephron formation. Ectopic overexpression of nuclear phospho protein p53 in the UB results in defective differentiation of the UB and a secondary survival defect in the MM leading to a 50% decrease in both kidney size and nephron number in adult mice (33). Normally expressed at low levels in the UB, excessive amounts of wild-type p53 in the UB resulted in defective expression and localization of *Ret*, a marker of the ureteric tip, as well as decreased expression of tubular differentiation markers DBA lectin and Aquaporin-2.

Hedgehog signaling effectors also regulate the differentiation of the UB tip domain. HH activity is normally restricted to the medullary domain of the kidney. Ectopic pathway activation in the cortical UB domain *via* targeted deletion of *Ptc1* in the ureteric cell lineage results in mild renal hypoplasia characterized by abnormal UB tip morphology, decreased UB branching and glomerular number, and a severe reduction in *Ret* and *Wnt11* expression in ureteric tip cells (34). Constitutive expression of the truncated repressor form of *Gli3* in the *Ptc1* deficient background rescued the expression of *Ret* and *Wnt11* in tip cells and normalized kidney size and glomerular number. Thus, *Gli3* repressor activity is required in UB tip cells for *Wnt11* and *Ret* expression and subsequent control of ureteric growth and branching. The requirement for tight regulation of HH signaling during metanephric development is also evident in humans with Pallister-Hall syndrome (PHS) and renal abnormalities (Table 1; 35). PHS is an autosomal dominant disorder caused by frameshift/nonsense and splicing mutations that exclusively affects the second third of the *GLI3* gene (nucleotides 1998–3481) predicting a truncated functional repressor form of the GLI3 protein (36). Further implicating a role for HH signaling in human renal hypoplasia is Smith-Lemli-Opitz syndrome (SLOS), an autosomal recessive disorder caused by heterozygous mutations in the *DHCR7* gene (Table 1; 37). The *DHCR7* locus encodes sterol delta-7-reductase that is required in mammalian sterol biosynthesis to convert 7-dehydrocholesterol into cholesterol. As HH proteins undergo cholesterol modifications, it is plausible that disruption in genes responsible for these posttranslational modifications (*DHCR7*) may lead to HH functional abnormalities and therefore developmental malformations associated with SLOS.

CONTRIBUTION OF THE MESENCHYMAL/ STROMAL CELL LINEAGE TO RENAL HYPOPLASIA

Mesenchymal/stromal-dependent ureteric branching morphogenesis. Because nephron formation is dependent on inductive signals from the ureteric tip, the number of ureteric

tips is a considered a strong determinant of nephron endowment. However, it is the inductive signals emanating from the MM that are critical for inducing the UB tip to divide. This process of ureteric branching is primarily mediated by the GDNF/RET signaling axis. GDNF, the ligand for RET (and GFRα1), is exclusively expressed in the MM and is critical for ureteric branching morphogenesis. Although *Gdnf* null mice lack kidneys and ureters due to deficient UB induction (38–40), *Gdnf* heterozygous mice exhibit reduced ureteric branching, significantly reduced kidney size and a 30% nephron number deficit (41). Moreover, *Gdnf* heterozygous mice demonstrate increased mean arterial blood pressure and glomerular hypertrophy at 14 mo of age (42). The absence of known human pathogenic mutations in *GDNF* suggests an importance of gene dosage, as is evident from the mouse models (43). Consistent with the importance of the GDNF/RET signaling axis in ureteric branching and subsequent nephrogenesis, perturbations in mesenchymal factors that regulate *Gdnf* and/or modify its signaling are also associated with renal hypoplasia. Members of the *Hox11* gene cluster and *Gdf11* are required for *Gdnf* expression in the MM. *Gdf11* null mice and compound *Hoxa11/Hoxd11* null mice exhibit smaller kidneys, reduced branching, and a loss of *Gdnf* expression (44–46). Similarly, although *Eya1* null mice fail to form kidneys due to absent *Gdnf* expression in the uninduced MM population, *Eya1* heterozygote mice demonstrate a low incidence of renal hypoplasia (47). Genetic studies in mice have shown that EYA1 acts in an EYA1-SIX-PAX gene complex to regulate gene transcription. Interestingly, *Eya1* heterozygous mice also display other developmental anomalies in common with human Branchio-Oto-Renal syndrome that is caused by mutations in the *EYA1* gene (Table 1). Mutations in *SIX1, SIX2,* and *SIX5* have also been identified in patients with Branchio-Oto-Renal syndrome and are predicted to disrupt formation of the EYA1-SIX complex and/or SIX-DNA binding and subsequent gene transcription (48–50). *Sall1* null mice and *Sall4* heterozygous mice also exhibit renal agenesis and hypoplasia associated with reduced mesenchymal *Gdnf* expression (51,52). Mutations in the human *SALL1* and *SALL4* genes result in human Townes-Brocks syndrome and Okihiro syndrome, respectively, which both demonstrate a range of renal abnormalities including hypoplasia (Table 1) (53,54).

The renal stroma is also critical for nephron endowment, primarily *via* regulation of ureteric branching morphogenesis by the retinoic acid-signaling axis. Retinoic acid is the active form of dietary Vitamin A that is synthesized by enzymes, including *Raldh2*, and signals through retinoic acid receptors (Rar). Genetic elimination of *Raldh2* and compound null mutants for *Rarα* and *Rarβ2* exhibit renal hypoplasia and reduced expression of *Ret* in ureteric tip cells (55,56). Interestingly, constitutive *Ret* expression in *Rarα;Rarβ2* compound null mice normalizes kidney development, suggesting a critical role for retinoic acid in the maintenance *Ret* expression (57). A similar pattern of defects is observed in offspring of Vitamin A deficient mothers (58,59), discussed in more detail below. *Fgf7*, expressed in stromal mesenchyme, has also been implicated in renal hypoplasia. Genetic elimination of *Fgf7* results in reduced ureteric branching and a 30% reduction in

nephron number (60). *Fgf10* null mutants also exhibit smaller kidneys with reduced branching morphogenesis (61,62). Recently, genetic analyses have demonstrated a critical role for *Fgf10* in UB induction and branching morphogenesis in cooperation with *Gdnf* (61).

Mesenchyme survival and maintenance of nephrogenic progenitors. The population of MM and nephrogenic precursors provides a potential limiting factor for nephron endowment. Defects in cell proliferation, survival and progenitor cell self-renewal, and commitment can result in fewer cells able to contribute to nephron formation.

Members of the *Myc* family of genes largely mediate cell growth, proliferation, and apoptosis. During metanephric development, expression of *c-myc* is restricted to early uninduced mesenchyme and *n-myc* to early mesenchymal aggregates. Targeted deletion of *c-myc* to the MM lineage or *n-myc* deficiency results in renal hypoplasia due to a significant decrease in mesenchymal proliferation, independent of changes in apoptosis (63,64). Interestingly, a progressive loss of nephrogenic progenitor cell marker expression, *Six2* and *Cited1*, was also observed in the *c-myc* deficient kidneys suggesting that *c-myc* functions to modulate the proliferation, and likely self-renewal, of the nephrogenic progenitor cell population (64).

Bcl-2 is an oncogene that inhibits apoptotic cell death and is expressed in both the UB and MM (65). *Bcl-2* null mutant mice develop renal hypoplasia and severe renal failure (66,67). Interestingly, these mutants exhibit a significant increase in apoptosis, predominantly in the MM, resulting in reduced ureteric branching and nephrogenesis.

Six2 is exclusively expressed in the cap mesenchyme, marking the nephron progenitor population (68,69). Genetic elimination of *Six2* results in severe renal hypoplasia, characterized by premature and ectopic differentiation of nephrogenic tubules and a rapid depletion of the nephrogenic progenitor population (69). Furthermore, the *Brachyrrine* mouse model (*Br*) demonstrates a marked decrease in the embryonic expression of *Six2* (70). *Br* heterozygous mice exhibit a severe reduction in kidney size, 88% decrease in nephron number, elevated mean arterial pressure, and declined renal function. Further implicating a requirement for progenitor cell maintenance in normal nephron endowment, *Six2* mutations have recently been identified in humans with isolated renal hypoplasia (Table 1; 50).

IN UTERO ENVIRONMENT AND RENAL HYPOPLASIA

There is an increasing amount of evidence, derived from human epidemiologic studies and animal models, demonstrating an important role for the intrauterine environment and fetal programming in the pathogenesis of renal hypoplasia and predisposition to later kidney disease (Table 3) (71,72).

Low birth weight or IUGR is generally considered a clinical outcome of suboptimal *in utero* environment. In these instances, the fetal kidney is particularly susceptible leading to reduced nephron number. Among the most common causes of human IUGR, animal models of uteroplacental insufficiency

Table 3. *Factors influencing* in utero *environment that are associated with renal hypoplasia*

Fetal exposure to	Renal phenotype	Reference(s)
Uteroplacental insufficiency	Hypoplasia	73
Vitamin A deficiency	Hypoplasia, hydronephrosis/ureter	58, 59
Low protein diet	Hypoplasia	75–80
Hyperglycemia	Agenesis, ectopic/horseshoe, cystic/dysplasia, hypoplasia, hydronephrosis/ureter	83, 102
Cocaine	Agenesis, hypoplasia, hydronephrosis/ureter	84–86
Alcohol	Agenesis, ectopia/horseshoe, cystic dysplasia, hypoplasia, hydronephrosis/ureter	87–90
Dexamethasone/ corticosterone	Hypoplasia	91–95
Thalidomide	Agenesis, ectopic/horseshoe, cystic dysplasia, hypoplasia, hydronephrosis/ureter	96, 97

and maternal undernutrition are known to exhibit significant reductions in nephron endowment (73,74). In particular, the association between maternal nutrition and nephron endowment is striking. Above, we have already discussed the mechanisms by which perturbations of the retinoic acid signaling axis cause renal hypoplasia. Retinoids are active metabolites of Vitamin A. Vitamin A deficiency is a global health problem, particularly in developing countries where poor nutrition commonly results in developmental anomalies of the genitourinary tract. Interestingly, these anomalies can be prevented by early maternal administration of vitamin A during the period of kidney development (59). Furthermore, vitamin A deficiency in rats results in a significant decrease in glomerular number and *Ret* expression, consistent with the genetic studies described above (58). In addition, maternal dietary protein restriction results in decreased nephron number, reduced renal function, and hypertension in a variety of species including rodents and sheep (75–80). Although the precise mechanisms governing this are not well defined, there is some evidence suggesting that the maternal diet programs the expression of critical genes required for embryonic kidney development, cell survival, and renal function (76,81,82). Interestingly, a single midgestation retinoic acid administration is able to normalize kidney size and nephron number in rat offspring exposed to maternal protein restriction raising the possibility of preventative approaches in humans (78).

Reduced nephron number and IUGR are not always synonymous. Maternal diabetes and *in utero* exposure to drugs and alcohol have all been linked to renal hypoplasia in the absence of reduced birth weight. In animal experiments, offspring of hyperglycemic or diabetic mothers demonstrate a significant nephron deficit (83). Human studies have demonstrated that infants exposed to cocaine *in utero* had an increased risk of renal tract anomalies including renal hypoplasia (84–86). Similarly, infants with Fetal Alcohol Syndrome also have a higher incidence of renal malformations, including small kidneys (87–89), and a recent study in sheep demonstrated that repeated alcohol exposure in late gestation leads to a mild nephron deficiency (90). Several clinical medications have

also proven detrimental to fetal nephron endowment. Dexamethasone, a synthetic glucocorticoid that readily crosses the placental barrier, is commonly used in obstetrics to promote fetal lung maturation and in general therapeutic use as an anti-inflammatory. However, sheep and rodent investigations have demonstrated that the presence of maternal corticosterone elevations due to the natural stress response or exogenous fetal dexamethasone exposure are associated with a reduction in nephron endowment and subsequent development of hypertension in offspring (91–95). Thalidomide is another clinical drug associated with renal abnormalities and hypoplasia (96,97). A powerful tranquilizer and painkiller, it was widely prescribed in the late 1950s before the finding that it too could readily cross the placental barrier, resulting in severe birth defects.

Importantly, the underlying genetic and molecular mechanisms that cause renal hypoplasia in response to suboptimal *in utero* environment are largely unknown. A greater understanding in these cases will provide a significant opportunity for future preventative interventions.

CONCLUSION

Genetic studies in humans and mice have provided valuable insights into the genetic contribution and molecular mechanisms leading to normal nephron endowment and renal hypoplasia. Specifically, the roles of several critical parallel and interacting signaling pathways, including GDNF/Ret, FGF, PAX2, and HH, have been strongly implicated in the pathogenesis of renal hypoplasia. Furthermore, factors influencing the *in utero* environment are also critical in establishing sufficient nephron number, albeit by mostly unknown mechanisms. With the continual advancements in human and mouse genetic analyses, the identification of other known and novel genes in nephron endowment is sure to follow. Understanding of the precise mechanisms governing nephron endowment is required to advance the prediction, diagnosis, prevention, and treatment of renal hypoplasia and the health risks associated with low nephron endowment.

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