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Developmental Signaling: Does It Bridge the Gap Between Cilia Dysfunction and Renal Cystogenesis?

Pamela V. Tran^{1,2,*}, Madhulika Sharma^{2,3}, Xiaogang Li^{2,3,4}, and James P. Calvet^{2,4}

¹Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas

²The Kidney Institute, University of Kansas Medical Center, Kansas City, Kansas

³Department of Internal Medicine, University of Kansas Medical Center, Kansas City, Kansas

⁴Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas

Abstract

For more than a decade, evidence has accumulated linking dysfunction of primary cilia to renal cystogenesis, yet molecular mechanisms remain undefined. The pathogenesis of renal cysts is complex, involving multiple cellular aberrations and signaling pathways. Adding to this complexity, primary cilia exhibit multiple roles in a context-dependent manner. On renal epithelial cells, primary cilia act as mechanosensors and trigger extracellular Ca^{2+} influx in response to laminar fluid flow. During mammalian development, primary cilia mediate the Hedgehog (Hh), Wnt, and Notch pathways, which control cell proliferation and differentiation, and tissue morphogenesis. Further, experimental evidence suggests the developmental state of the kidney strongly influences renal cystic disease. Thus, we review evidence for regulation of Ca^{2+} and cAMP, key molecules in renal cystogenesis, at the primary cilium, the role of Hh, Wnt, and Notch signaling in renal cystic disease, and the interplay between these developmental pathways and Ca^{2+} signaling. Indeed if these developmental pathways influence renal cystogenesis, these may represent novel therapeutic targets that can be integrated into a combination therapy for renal cystic disease.

Keywords

cilia; cystogenesis; signaling; renal cysts

Introduction: Linking Primary Cilia to Renal Cystogenesis

Renal cystic diseases are characterized by fluid-filled cysts, which compress surrounding renal parenchyma and compromise kidney function (Grantham et al., 2011). Most renal cystic diseases are genetic, classified as polycystic kidney disease (PKD) or non-PKD,

depending on the genes that are mutated. Autosomal dominant PKD (ADPKD) is among the most common, life-threatening, genetic diseases, affecting 1:500 adults worldwide. ADPKD is caused by mutations in *PKD1* or *PKD2*, which encode Polycystin 1 (PC1) and Polycystin 2 (PC2) proteins, respectively (Torres and Harris, 2006). Renal cysts can initiate as early as during fetal development and progress to cause end-stage renal disease during the midlife years of an individual. Autosomal recessive PKD (ARPKD) affects 1:20,000 children and is caused by mutations in *PKHD1*, encoding fibrocystin/polyductin (FPC). PC1, PC2, and FPC are transmembrane proteins, which localize to multiple subcellular compartments, including the primary cilium, an antenna-like sensory organelle. For a review on primary cilia, see Satir et al. (2010).

Mutations of ciliary genes, which encode proteins that synthesize and maintain primary cilia result in ciliopathies, a collective term for pediatric genetic disease syndromes, including nephronophthisis, Bardet–Biedl syndrome, Meckel syndrome (MKS), and Joubert syndrome (JS). Ciliopathies often affect multiple organ systems, but the most prevalent clinical feature across ciliopathies is renal cysts (Quinlan et al., 2008). These genetic diseases suggest a prominent role for primary cilia dysfunction in the etiology of renal cystic diseases, although molecular mechanisms remain unclear.

The pathogenesis of renal cystogenesis is complex. Multiple cellular aberrations are implicated, including increased cell proliferation, fluid secretion, dedifferentiation, and altered polarity (Calvet and Grantham, 2001; Menezes and Germino, 2009; Zhou, 2009). Further, studies in mice show that the developmental state of the kidney significantly influences severity of cystic disease; loss of cystoproteins in developing kidneys causes severe disease, while loss of cystoproteins in mature kidneys results in mild, late onset disease (Piontek et al., 2007). The undefined role of primary cilia in establishing and maintaining renal tubular integrity adds to the molecular complexity of renal cystogenesis. Cilia play multiple roles that are context-dependent. In the embryonic node, cilia generate leftward fluid flow to establish left–right asymmetry (Komatsu and Mishina, 2013). Cilia act as chemosensors in olfaction (McEwen et al., 2008). Ciliary proteins can also regulate the cell cycle (Pan et al., 2013). On renal epithelial cells, primary cilia project from the apical surface and function as mechanosensors, deflecting in response to urine flow and causing extracellular Ca^{2+} entry (Zhou, 2009). During mammalian development, primary cilia are required to transduce the Hedgehog (Hh) signal (Eggenchwiler and Anderson, 2007) and can also regulate Wnt and Notch signaling pathways (Corbit et al., 2008; Ezratty et al., 2011). Thus, what is the main task of this multitasking organelle that when perturbed causes renal cysts? We begin by reviewing the regulation of Ca^{2+} and cAMP at the primary cilium. We then review experimental evidence suggesting Hh, Wnt, and Notch signaling influence renal cystogenesis and interconnect with PKD biology, such as Ca^{2+} signaling.

Calcium and cAMP Regulation at the Primary Cilium

The second messenger cAMP is elevated in human ADPKD renal epithelial cells (Belibi et al., 2004) and in affected kidneys of orthologous PKD rodent models, *Pkd1* (Hopp et al., 2012) and *Pkd2* (Torres et al., 2004) mutant mice, and the *Pkhd1*-deficient Polycystic Kidney (PCK) rat (Wang et al., 2008). Cyclic AMP is also elevated in cystic kidneys of

ciliary mouse models of PKD, *pcy* (Yamaguchi et al., 1997), *cpk* (R. Rao, personal communication), *jck* (Smith et al., 2006), *Kif3a* (Choi et al., 2011), and *Thml/Ttc21b* (Tran et al., 2014), suggesting increased cAMP occurs universally in renal cystogenesis, independent of the genetic mutation. Primary cilia seem to regulate cAMP in multiple contexts. Loss of primary cilia on renal epithelial cells induced either by genetic deletion of *Kif3a*, a component of the anterograde kinesin motor required to build the ciliary axoneme, or by pharmacological treatment with dibucaine, elevated cAMP levels (Choi et al., 2011). Similarly, in experiments using brain choroid plexus epithelial cells to understand molecular mechanisms underlying hydrocephalus, deciliation with chloral hydrate led to increased cAMP levels and fluid secretion. In contrast, chloral hydrate treatment of Caco-2 gut epithelial cells, which inherently lack primary cilia, did not alter cAMP levels (Narita et al., 2010). The renal collecting duct is a prominent site for cyst development in ADPKD and is composed of ciliated principal cells and interspersed, unciliated intercalated cells. This raises questions regarding the molecular mechanisms by which intercalated cells regulate cAMP and contribute to renal cystogenesis, which must differ from those of principal cells.

Notably, increased cAMP levels are pathogenic in ADPKD because Ca^{2+} homeostasis is disrupted (Calvet, 2008; Wallace, 2011). *PKD1* or *PKD2* mutations of human ADPKD renal epithelial cells lower intracellular Ca^{2+} , allowing elevated cAMP to cause aberrant activation of ERK signaling and activation of the cystic fibrosis transmembrane conductance regulator, inducing cell proliferation, and transepithelial fluid secretion, respectively, resulting in cyst growth and expansion (Yamaguchi et al., 2004). Addition of a Ca^{2+} channel activator or of a Ca^{2+} ionophore to cultured ADPKD epithelial cells rescued the cAMP-induced proliferative phenotype, substantiating the crucial role of Ca^{2+} in determining whether or not cAMP functions as a mitogen (Yamaguchi et al., 2006).

Upon fluid flow, primary cilia of cultured Madin–Darby Canine Kidney (MDCK) epithelial cells bend and induce an increase in intracellular Ca^{2+} (Praetorius and Spring, 2001). Primary cilia of MDCK cells are essential for this fluid flow–induced Ca^{2+} response, which was obliterated in MDCK cells deciliated with chloral hydrate (Praetorius and Spring, 2003). This response was also disrupted in renal epithelial cells either derived from *Pkd1^{del34/del34}* mutant mice or incubated with a PC2 antibody, suggesting that PC1 and PC2 at the primary cilium mediate this mechanotransduction (Nauli et al., 2003). It has been proposed that the large extracellular domain of the transmembrane protein, PC1, mediates this mechanosensation in response to urine flow, which in turn, activates the Ca^{2+} channel activity of PC2, causing extracellular Ca^{2+} entry and subsequent release of intracellular Ca^{2+} stores. Other proteins, including FPC, TRPV4, a member of the transient receptor potential (TRP) family, which also includes PC1 (TRPP1) and PC2 (TRPP2), and the ciliary kinase NEK8, bind to PC2 and may function as mechanosensors (Wang et al., 2007; Kottgen et al., 2008; Manning et al., 2013). However, *Trpv4* knockout mice do not develop renal cysts, which led to questioning of the contribution of an impaired fluid flow–induced Ca^{2+} response to renal cystogenesis (Kottgen et al., 2008). A recent study showed that TRPV4 mechanosensory function is diminished in cyst-lining epithelial cells of PCK rats with ARPKD and that TRPV4 activation attenuated renal cystogenesis in this rat ARPKD model, suggesting fluid flow–induced Ca^{2+} responses may not initiate, but can certainly modulate

renal cystogenesis (Zaika et al., 2013). Importantly, *Pkhd1* knockout mice do not develop renal cysts, yet mutations in *PKHD1* cause ARPKD in humans and in the PCK rat. It is possible, therefore, that these mechanosensors have varying degrees of influence on fluid flow–induced Ca^{2+} responses, which may differ further among mouse, rat, and human kidneys. Interestingly, *Nek8*-null mice do not develop renal cysts (Manning et al., 2013), yet the *jck* mutant allele, which harbors a 2 bp mutation in *Nek8*, causes PKD in mice (Liu et al., 2002). To further evaluate the role of impaired fluid flow–induced Ca^{2+} responses in renal cystogenesis, the effect of the *jck* mutation on *Nek8* mechanosensory function could be examined. Measurements of fluid flow–induced Ca^{2+} responses of cell monolayers created by split-opening mouse renal tubules have been achieved, and these may provide closer representations of responses occurring in vivo (Zaika et al., 2013). Using this approach, Zaika et al. (2013) observed fluid flow–induced Ca^{2+} responses also in unciliated intercalated cells, suggesting that a cilia-independent mechanism of sensing fluid flow occurs as well.

Studies by Choi et al. (2011) suggest that PC2 Ca^{2+} channel activity regulates cAMP levels in a compartmentalized manner at the primary cilium of renal epithelial cells. These authors identified a protein complex composed of scaffolding A-kinase anchoring protein 150 (AKAP150), protein kinase A (PKA), adenylyl cyclases 5 and 6 (AC5/6), which synthesize cAMP, phosphodiesterase 4C (PDE4C), which catabolizes cAMP, and PC2. Each of these components localized to the primary cilium and ciliary localization of AC5/6 and PDE4C was dependent on the presence of PC2. Further, elevated cAMP levels in renal epithelial cells derived from *Pkd2*^{−/−} mice were decreased by expression of wild type PC2 but not of a PC2 mutant lacking Ca^{2+} channel activity, suggesting a specific role for the PC2 Ca^{2+} channel in modulating cAMP levels.

Until recently, flow-induced Ca^{2+} entry into primary cilia has been inferred, since use of commercially available Ca^{2+} indicators cannot reliably distinguish between Ca^{2+} signaling in the primary cilium and that in the cytoplasm. Three groups have successfully realized the highly technical challenge of visualizing Ca^{2+} signaling specifically in the primary cilium, in part by fusing a ciliary targeting sequence derived from cilia-localized proteins (e.g., FPC, serotonin receptor isoform 6, or smoothened) to a Ca^{2+} indicator (Delling et al., 2013; Jin et al., 2013; Su et al., 2013). When renal epithelial cells were subjected to fluid flow, an increase in Ca^{2+} was visualized first in the primary cilium, then in the cytoplasm (Jin et al., 2013; Su et al., 2013). While *Pkd2* siRNA knock-down abolished this fluid-flow Ca^{2+} response entirely, caffeine inhibition of the ryanodine receptor inhibited only the increase in cytosolic Ca^{2+} (Jin et al., 2013). Converse to the effect of fluid flow, addition of ATP, which activates release of intracellular Ca^{2+} stores, caused an increase in Ca^{2+} first in the cytoplasm, then in the primary cilium (Su et al., 2013). These novel live-cell imaging tools allow for molecular dissection of Ca^{2+} signaling and the possibility to extend these methods to visualize dynamics of other signaling pathways in primary cilia. These cilia-targeted Ca^{2+} indicators could also be combined with analysis of fluid-flow Ca^{2+} responses of epithelial cell monolayers of split-open renal tubules of different *Pkd* and ciliary mouse mutants. For instance, increased expression of *Pkd1* or of the PC1 extracellular domain also causes PKD in mice (Kurbegovic et al., 2010; Kurbegovic and Trudel, 2013), and determining the effect

of different levels of PC1 expression on fluid flow–induced Ca^{2+} response could be informative.

In summary, while the importance of Ca^{2+} and cAMP has been clearly established in renal cyst growth, the ciliary mechanisms regulating Ca^{2+} and cAMP are not as clear, and more experiments will be required to determine the degree to which fluid-flow ciliary bending is required for cyst initiation and enlargement.

Developmental Signaling Pathways in ADPKD

A genome-wide expression analysis of affected human ADPKD kidneys revealed increased expression of Hh signaling components and upregulation of Wnt and Notch signaling pathways (Song et al., 2009). Over the past 6 years, a large focus has been to distinguish between molecular processes that initiate renal cystogenesis and those that result from the cystic disease process, since identifying initiating molecular mechanisms will enable development of therapeutic strategies that will slow or prevent the disease. In Table 1, we list whether Hh, canonical Wnt, planar cell polarity (PCP), or Notch signaling was misregulated before or after renal cysts formed in experimental models of renal cystic disease.

Hedgehog Signaling

REGULATION AT THE PRIMARY CILIUM

The Hh signaling pathway is essential for proper development and tissue homeostasis, controlling cell proliferation and differentiation, and determining cell fate (Ingham et al., 2011). In the core mammalian pathway, Hh ligands—Sonic Hh (SHH), Indian Hh (IHH), or Desert Hh—bind Patched1 (PTCH1) receptor to activate the smoothened (SMO) signal transducer, and ultimately, the Glioblastoma transcription factors, GLI1, GLI2, and GLI3. For a detailed review on Hh signaling, see Nozawa et al. (2013). The Hh pathway is intricately regulated by cellular extensions, including both microtubule-based primary cilia and actin-based filopodia. Live imaging of limb mesenchymal cells revealed a special class of filopodia, which transport SHH ligand from a SHH-producing cell to PTCH1 at the primary cilium and to alternate receptors, Cell adhesion molecule downregulated by oncogenes (CDO) and Brother of CDO, distributed on filopodia of a Hh-receiving cell (Sanders et al., 2013). Once bound by Hh ligand, PTCH1 diminishes from the cilium, and concomitantly, SMO is enriched in the primary cilium and is activated (Corbit et al., 2005; Rohatgi et al., 2007). Full-length GLI2 and GLI3 are enriched at the distal tip of the cilium (Haycraft et al., 2005; Kim et al., 2009b; Wen et al., 2010). A series of dephosphorylation and phosphorylation events at multiple sites on full-length GLI2 and GLI3 results in their activation (Niewiadomski et al., 2014). In the absence of Hh signal, full-length GLI3 is cleaved to form GLI3 repressor (GLI3R), a process that is also dependent on primary cilia (Wang et al., 2000; Huangfu and Anderson, 2005; Humke et al., 2010). Different levels of Hh signaling are achieved by the balance of Gli activator (GLIA) and GLI3R activities in the nucleus.

In mice, loss of all intraflagellar transport (IFT) proteins, which are required to build and maintain ciliary structure and function, causes misregulation of Hh signaling (Nozawa et al., 2013). Loss of most complex B proteins, which mediate anterograde IFT, the trafficking of proteins from the ciliary base to the distal tip, results in loss of cilia and inability to respond to the Hh signal (Huangfu et al., 2003; Huangfu and Anderson, 2005; Liu et al., 2005). In contrast, loss of two IFT complex A proteins, THM1 and IFT122, which largely mediate retrograde IFT, the trafficking of proteins from the ciliary distal tip back to the base, causes shortened cilia with accumulation of proteins in bulb-like structures at the distal tip and enhanced activation of the Hh pathway, due to increased activities of GLI2A and GLI3A (Tran et al., 2008; Qin et al., 2011). In addition to cilia providing a milieu for the Hh signaling cascade, IFT is integral to Hh signaling by trafficking Hh signaling components within cilia. Loss of IFT25 does not affect ciliary structure, but impairs ciliary export of PTCH1 and SMO, ciliary import of GLI2, and activation of the Hh pathway (Keady et al., 2012). Similarly, hypomorphic loss of IFT80 does not disrupt ciliary structure but perturbs pathway activation (Rix et al., 2011).

INCREASED HH SIGNALING IN RENAL CYSTOGENESIS

Several studies suggest a role for Hh signaling in renal cystogenesis. SHH is essential for kidney development and 50% of *Shh*-null mouse embryos formed a single, ectopic, dysplastic kidney (Hu et al., 2006). For a detailed review on the role of Hh signaling in kidney development, see Cain and Rosenblum (2011). Mutation of the GLIS2 transcription factor, which belongs to the Kruppel-like C2H2 zinc finger protein subfamily, including the GLI proteins, causes NPHP in humans and mice. In *Glis2*-null kidneys, *Gli1*, a direct target of the Hh pathway, was upregulated, suggesting increased Hh signaling (Attanasio et al., 2007). Additionally, in a study examining the effects of corticosteroid overexposure on kidney development, hydrocortisone treatment of cultured mouse embryonic kidneys unexpectedly caused cysts and elevated *Ihh* transcripts (Chan et al., 2010). Addition of the SMO inhibitor, cyclopamine, reduced hydrocortisone-induced cysts, implicating enhanced Hh activity in this mechanism of cystogenesis. Deletion of complex A *IFT140* in the mouse kidney during embryogenesis resulted in renal cystic disease postnatally with increased expression of *Gli* transcripts in cystic kidneys (Jonassen et al., 2012). Similarly, ubiquitous deletion of *Thm1* during late embryogenesis in mice caused cystic kidney disease in adult mice and increased *Gli* expression in affected kidneys (Tran et al., 2014). Loss of *Gli2* in *Thm1*;*Gli2* double conditional knockout mice resulted in an attenuated renal cystic disease, supporting a causative role for increased Hh signaling in *Thm1* renal cystogenesis. *Gli* transcripts were also upregulated in cystic kidneys of *jck* mutants and *Pkd1* conditional knockout mice, and SMO and GLI inhibitors, Sant2 and Gant61, respectively, reduced cystogenic potential of *jck* and *Pkd1*-mutant embryonic cultured kidneys, suggesting Hh signaling may have a general role in renal cystogenesis.

CONNECTION OF HH PATHWAY TO PKD BIOLOGY AND CA²⁺SIGNALING

Molecular mechanisms by which Hh signaling may contribute to renal cystogenesis are emerging. Studies by Li et al. (2011) suggest low levels of Hh activity in mature kidneys maintain renal tubular epithelial cells in a differentiated state. In a *Glis2*-null mouse, renal tubular epithelial cells acquired a mesenchymal phenotype. In vitro, GLIS2 repressed Hh

signaling and treatment with GLI inhibitor, Gant58, sustained differentiation of *Glis2*-deficient renal epithelial cells (Li et al., 2011).

Several studies also suggest Hh signaling modulates Ca^{2+} levels. In two lung cancer cell lines, SMO inhibitor, GDC-0449, increased steady state levels of Ca^{2+} (Tian et al., 2012). Conversely, Ca^{2+} imaging of *Xenopus* embryonic spinal cells showed SHH ligand acutely increased Ca^{2+} spike activity through activation of SMO, which acts as a G-protein coupled receptor (Belgacem and Borodinsky, 2011). Recent studies further demonstrate an interconnection between the Hh pathway and Ca^{2+} signaling at the primary cilium (DeCaen et al., 2013; Delling et al., 2013). By patch-clamping primary cilia and subsequently rupturing the ciliary membrane at the distal tip, electrical currents and Ca^{2+} signals were recorded in primary cilia of retinal pigment epithelial (RPE) cells, and heteromers of TRP members, PKD1L1 and PKD2L1, were identified as the Ca^{2+} channels. In contrast to a fluid flow-induced Ca^{2+} response, in which increases in ciliary Ca^{2+} are followed by increases in intracellular Ca^{2+} , Delling et al. (2013) observed that rupture of the distal tip ciliary membrane triggered Ca^{2+} activity from the ciliary distal tip to the base, with very little subsequent effect on cytosolic Ca^{2+} . Approximately 50% of *Pkd2L1* knockout mice exhibited intestinal malrotation, which is associated with perturbed Hh signaling. In the presence of the SMO agonist, SAG, *Pkd2L1*-null mouse embryonic fibroblasts (MEF) showed reduced GLI1 protein levels and GLI2 localization at the ciliary distal tips, reflecting impaired activation of the Hh pathway. In wild type MEF, ciliary Ca^{2+} increased after 24 hr of SAG treatment, suggesting that SAG does not directly activate the Ca^{2+} channels, but recruits *Pkd2L1* to primary cilia. IFT25, which is proposed to traffic Hh components into and of cilia (Keady et al., 2012), has a Ca^{2+} binding site. Delling et al. (2013) suggest that *Pkd2L1* may “tune” Ca^{2+} levels for proper IFT25 function, and that function of other Hh signaling components should be examined for Ca^{2+} dependence.

Results of Tian et al. (2012) suggest effects of Hh activity on intracellular Ca^{2+} are opposite to those of Belgacem and Borodinsky (2011) and of Delling et al. (2013). Inhibition of SMO increased intracellular Ca^{2+} of lung cancer cells (Tian et al., 2012), yet activation of SMO also increased intracellular Ca^{2+} of *Xenopus* spinal cells (Belgacem and Borodinsky, 2011) and ciliary Ca^{2+} of wild type RPE cells (Delling et al., 2013). To further study the interdependence between Hh and Ca^{2+} signaling, comparison of effects of GDC-0449 between normal and cancerous cells and of SAG between wild type and *Pkd2L1*-mutant cells should be investigated. If too little or too much activation of the Hh pathway similarly affect Ca^{2+} signaling, this could account in part for the renal cystogenesis of IFT complex B mutants that cannot respond to the Hh signal. Finally, the interplay between Hh and Ca^{2+} signaling merits investigation in wild type and PKD renal epithelial cells because of the primary role of Ca^{2+} misregulation resulting from *PKD1* and *PKD2* mutations in ADPKD.

Wnt Signaling

REGULATION AT THE PRIMARY CILIUM

In contrast to Hh signaling, the Wnt pathway has been extensively studied in renal cystic disease, although regulation of Wnt signaling by the primary cilium is less understood. Wnt signaling is divided into the canonical branch, which governs cell proliferation,

differentiation and cell fate, and the noncanonical branch, which controls tissue organization and morphogenesis. Both canonical and noncanonical Wnt signaling initiates on binding of Wnt ligand to a Frizzled receptor (Fz) and recruitment of a scaffolding protein, Disheveled (Dvl), to Fz. While certain Wnt ligands and Fz receptors have been suggested to have predetermined roles in either canonical or noncanonical signaling, context has also been proposed to dictate whether a particular Wnt ligand or Fz receptor will participate in a particular Wnt branch. Downstream of Dvl, signaling proteins differ between the canonical and noncanonical branches. For a detailed review on Wnt signaling, see Oh and Katsanis (2013).

In the canonical Wnt pathway, in the absence of ligand, a destruction complex composed of scaffolding protein, axin2, adenomatous polyposis coli (APC), glycogen synthase kinase 3 β (GSK3 β), and casein kinase 1 (CK1), phosphorylates and targets β -catenin for degradation. In the presence of ligand, Dvl is recruited to a Wnt-Fz-low-density lipoprotein-related protein receptor complex, recruiting axin2 and GSK3 β of the destruction complex, which is concomitantly dissolved. β -catenin is stabilized in the cytoplasm, travels to the nucleus, and together with the T-cell factor/lymphoid enhancer factor (TCF) family of transcription factors, activates transcription of target genes, such as *c-myc*, *axin2*, and *Lef1*.

The noncanonical Wnt pathway is further partitioned into PCP signaling and Ca²⁺ signaling. The PCP pathway has attracted the most recent attention among the Wnt pathways in renal cystogenesis. PCP signaling regulates convergent extension movements and oriented cell division, which are essential for renal tubular elongation and morphogenesis (Karner et al., 2009; Lienkamp et al., 2012). Convergent extension refers to the directed intercalation of cells within an epithelium that results in lengthening and narrowing of the epithelial layer. Oriented cell division describes the angle of a cell during mitosis, relative to the longitudinal tubular axis. During late embryogenesis in the mouse (E13.5–E18.5), renal tubules undergo convergent extension processes, which lengthen and thin the tubules to establish tubular diameter. From postnatal day one (P1) to at least P5 in the mouse, oriented cell division maintains the diameter of postnatal renal tubules.

The PCP pathway still remains to be fully characterized, but two seemingly parallel pathways are implicated in establishing polarity. The Fz/Celsr pathway requires the atypical cadherin Celsr, which functions with Fz to regulate polarity. Diversin, Vangl2, and Prickle form a multiprotein complex that connects Fz and Celsr. Dvl interacts with Fz and the diversin/vangl2/prickle complex. The Dachous (Dchs1)/Fat (Ft) pathway appears to function in parallel. Ft acts as a receptor for Dchs1, and this binding represses transcription of *Four-jointed* (*Fjx1*; Saburi et al., 2008).

Experiments in *Xenopus laevis* embryos and mammalian renal epithelial cells suggested that *inversin*, which is mutated in patients with NPHP2, regulates the balance between canonical and noncanonical Wnt/PCP signaling, by promoting PCP signaling, and antagonizing canonical Wnt signaling (Simons et al., 2005). In gastrulating *X. laevis* embryos, antisense morpholinos against *inversin* impaired convergent extension movements of the dorsal mesoderm and neural tissues, demonstrating that *inversin* is required for PCP signaling. In HEK293T cells, *inversin* reduced levels of cytoplasmic β -catenin, and fluid flow across

IMCD cells increased expression of *inversin* and downregulated β -catenin. Thus it was postulated that early kidney development requires canonical Wnt signaling, and a switch to noncanonical Wnt signaling occurs once glomeruli are able to filter and urine is produced. However, contrary to this hypothesis, kidneys of *inv/inv* mouse mutants, devoid of *inversin*, did not show misregulation of canonical Wnt signaling as assessed by a TCF-LacZ reporter allele and by immunoblot analysis of β -catenin (Sugiyama et al., 2011). This may suggest that in vivo regulation of the balance between canonical and noncanonical Wnt signaling in mammals is more complex than what could be previously elucidated in vitro.

Wnt signaling components have been localized to the primary cilium or to the basal body. β -catenin and APC localized to the ciliary axoneme, while phosphorylated β -catenin targeted for degradation localized to the basal body (Corbit et al., 2008). *Inversin* localizes to the proximal part of the primary cilium, a region termed “the *inversin* compartment” (Shiba et al., 2009). PCP components *Fat4*, *Vangl2*, and *Fz3* have also been localized to the base of primary cilia (Saburi et al., 2008; Luyten et al., 2010).

A role for the primary cilium in regulating canonical Wnt signaling has been demonstrated in *Kif3a*^{−/−} mouse embryos and in cells deficient in *Kif3a*, *IFT88*, or *Odf1* (Corbit et al., 2008). These mutations result in the lack of primary cilia and in upregulated Wnt reporter activity in the presence of Wnt3a ligand. Thus, while primary cilia are essential to transduce the mammalian Hh signal, primary cilia have been proposed to act as a “brake” in canonical Wnt signal transduction (Corbit et al., 2008). Increased Wnt signaling caused by the absence of primary cilia may be explained by the role of Joubertin, which is encoded by *Abelson Helper Integration Site 1 (AH1)* and is mutated in JS (Lancaster et al., 2009). In vitro studies suggested that Joubertin facilitates nuclear translocation of β -catenin, and that the primary cilium sequesters Joubertin and β -catenin away from the nucleus (Lancaster et al., 2011).

Yet the functionality of primary cilia in regulating Wnt signaling remains unclear. IFT mouse mutant embryos, including *Kif3a*^{−/−} mutants, show Hh mutant phenotypes, rather than phenotypes characteristic of misregulated Wnt signaling (Eggenchwil and Anderson, 2007). Similar to IFT Complex B mutant cells (e.g., *Kif3a* and *IFT88*), MEF derived from E14.5 *Thm1*-null embryos harboring a BAT-gal Wnt reporter allele exhibited upregulated canonical Wnt signaling in response to Wnt3a ligand (Tran et al., 2014). Yet E8.5-E10.5 *Thm1*-null;BAT-gal⁺ embryos showed normal Wnt activity (Stottmann et al., 2009). *Ahi1*^{−/−};BAT-gal⁺ embryos also exhibited normal Wnt reporter activity (Lancaster et al., 2011). In cultured cells, differing levels of knock-down of *Dnchc2*, which encodes a subunit of the IFT retrograde dynein motor, resulted in opposing Wnt signaling effects. Moderate silencing of *Dnchc2*, which did not affect cilia structure, dampened the Wnt response, while greater silencing of *Dnchc2*, which shortened cilia, elevated the Wnt response (Lancaster et al., 2011). Thus, while in vitro studies demonstrate an effect of IFT/primary cilia on canonical Wnt signaling, more in vivo studies are required to substantiate functionality of primary cilia in transducing the canonical Wnt signal.

ALTERED CANONICAL WNT SIGNALING IN RENAL CYSTOGENESIS

A role for increased canonical Wnt signaling in renal cystogenesis was shown by renal-specific overexpression of active β -catenin or loss of APC, a negative regulator of canonical Wnt signaling, which resulted in renal cysts in mice (Saadi-Kheddouci et al., 2001; Qian et al., 2005). Further studies have supported a role for increased canonical Wnt signaling in renal cystogenesis. *Pkd1* conditional knockout mice showed increased canonical Wnt signaling before cysts were formed (Luyten et al., 2010). Canonical Wnt was also upregulated in a *Pkd2*-null renal cell line, although Wnt signaling has not been examined in *in vivo* *Pkd2* mutant mice (Kim et al., 2009a). In the postnatal kidneys of conditional knockout mice of ciliary genes, *Kif3a*, *IFT20*, *IFT140*, and of the *TMEM67* knockout mouse, a model for MKS and JS, canonical Wnt signaling was shown to be upregulated after renal cysts had formed (Lin et al., 2003; Jonassen et al., 2008, 2012; Leightner et al., 2013). Finally, in the *Mks1* knockout mouse, canonical Wnt signaling was upregulated before cysts were formed in mutant embryonic kidneys (Wheway et al., 2013).

Yet controversy regarding the role of canonical Wnt signaling in renal cystogenesis has also emerged. In kidneys of P1 *Wnt9b* knockout mice when cysts were prominent, changes in active β -catenin were not observed (Karner et al., 2009). Also, *inv/inv;TCF-lacZ*⁺ mice did not show misregulation of Wnt reporter activity nor of β -catenin levels in nuclear or cytoplasmic lysates of early postnatal affected kidneys (Sugiyama et al., 2011). Similarly, *Pkd1*^{-/-};TCF-lacZ⁺ embryonic kidneys and *Pkd2*/WS25;TCF-lacZ⁺ postnatal kidneys showed normal canonical Wnt reporter activity in cyst-lining epithelia and normal levels of β -catenin in whole kidney lysates, even after cysts had formed (Miller et al., 2011). In direct contrast, Qin et al. (2012) reported upregulated canonical Wnt reporter activity in E17.5 *Pkd1*^{-/-};TCF-lacZ⁺ renal cystlining epithelia, and increased levels of total and active β -catenin in *Pkd1*^{-/-} whole kidney lysates (Qin et al., 2012). Different Wnt reporter lines have been reported to yield differences in expression levels and patterns. The discrepancy in β -catenin levels is more difficult to account for, but perhaps mouse strain differences may have resulted in these differences.

While inappropriate activation of canonical Wnt signaling has been implicated in renal cystogenesis, too little canonical Wnt signaling can also result in renal cysts, as demonstrated in the *Ahi1*^{-/-} mouse, a model of NPHP and JS. *Ahi1*^{-/-} mice displayed decreased canonical Wnt signaling in the kidney at 5 months of age, before developing microcysts and tubular dilations at 1 year of age (Lancaster et al., 2009). Thus, the appropriate level of canonical Wnt signaling is essential for maintaining renal tubular integrity.

DISRUPTED PCP SIGNALING IN RENAL CYSTOGENESIS

In renal cystogenesis, misoriented cell division was first shown in renal tubules of conditional knockout mice of *Hnf1 β* , which encodes a transcriptional regulator of *Pkhd1*, and in the PCK rat before cyst formation (Fischer et al., 2006). Following this, loss of noncanonical Wnt signaling components, Fat4, Wnt9b, and inversin, were also shown to cause misoriented cell division in early postnatal kidneys accompanied by cysts, demonstrating a functional role for PCP signaling in establishing renal tubular integrity

(Saburi et al., 2008; Karner et al., 2009; Sugiyama et al., 2011). Kidney-specific loss of IFT proteins, Kif3a and IFT20, in mice also resulted in misoriented cell division in precystic tubules (Jonassen et al., 2008; Patel et al., 2008). Misoriented cell division was further demonstrated in *Pkd1*-mediated cyst development, occurring in precystic tubules of *Pkd1* conditional knockout mice in which *Pkd1* was knocked out either in the early postnatal period or in adulthood followed by kidney injury, induced either by S-1,2-dichlorovinyl-L-cysteine (DCVC) administration or by ischemia-reperfusion injury (IRI; Happe et al., 2009; Luyten et al., 2010). Further supporting misregulated PCP signaling, protein levels of CDC42, a downstream PCP component, were increased in precystic and cystic kidneys of *Pkd1* conditional knockout mice and Fz3, Fz6, and CDC42 protein levels were increased in human ADPKD kidney lysates (Luyten et al., 2010).

Controversy regarding the role of misoriented cell division in initiating renal cystogenesis has also arisen. Nishio et al. (2010) reported that oriented cell division was only disrupted in *Pkd1* and *Pkd2* conditional knockout renal tubules that were already dilated. Additionally, misoriented cell division was also present in *Pkhd1^{del4/del4}* renal tubules, which do not develop cysts (Nishio et al., 2010). Thus, while misoriented cell division may contribute to cyst progression, these data argue against its role in the initiation process.

Two mouse models show that cyst formation and progression can occur, even in the absence of misoriented cell division. Renal tubules of *IFT140* conditional knockout mice and of *TMEM67* knockout mice displayed normal oriented cell division, even after cysts had formed (Jonassen et al., 2012; Leightner et al., 2013).

Recently, convergent extension defects were reported in E15.5 cystic renal tubules of *Pkd1^{C/C}* mice that lack the C-terminal tail of PC1 (Castelli et al., 2013). PC1 was demonstrated to interact with the aPKC/Par3 complex. In *Par3* conditional knockout mice, renal tubules showed altered convergent extension, although cysts occurred in only 40% of *Par3* conditional knockout mice. Thus defects in convergent extension alone seem insufficient to initiate cyst formation, and may require an additional trigger, such as misoriented cell division.

The vast amount of experimental data on Wnt signaling and renal cystogenesis yield different results regarding the role of canonical Wnt and PCP signaling in initiating and/or progressing renal cystic disease. This suggests heterogeneity of molecular mechanisms underlying renal cystogenesis likely arising from different genetic mutations, different background strains, time at which genetic mutation was induced and time period in development or adulthood that renal cystic disease was investigated.

Notch Signaling

REGULATION AT THE PRIMARY CILIUM

While Notch signaling is essential for kidney development and commonly implicated in renal diseases, such as diabetic nephropathy (Bonegio and Susztak, 2012), HIV-associated nephropathy (Sharma et al., 2010), and glomerulosclerosis (Niranjan et al., 2008), it remains understudied in PKD. In the Notch pathway, Delta-like 1, 3, 4 (DL1, 3, and 4), and Jagged 1

and 2 (Jag1 and 2) ligands bind to one of four receptors, Notch 1–4. On ligand binding, the Notch receptors undergo a series of proteolytic cleavages mediated by γ -secretase, which results in the release of the Notch intracellular domain (NICD). NICD translocates to the nucleus, where it forms a complex with RBP-J and Mastermind (Mam1) to activate target genes (e.g., *Hes* and *Hey*). For a review on Notch signaling, see Hori et al. (2013).

Notch signaling has been shown to play a role in multiciliated cells (MCC) in vertebrates. In zebrafish, Notch signaling regulates ciliary formation in Kupffer's vesicle (Lopes et al., 2010) and in MCC of the pronephros (Liu et al., 2007). More recently, primary cilia were shown to mediate Notch signaling in skin cells of the spinous layer of mice during embryonic development (Ezratty et al., 2011). In these cells, Notch3 and presenilin-2, the catalytic subunit of γ -secretase, localized along the ciliary axoneme and to the basal body, respectively, and importantly, NICD3 localized to the nucleus. In *Kif3a*-null skin cells of the spinous layer of E16.5 embryos mosaic for loss of *Kif3a*, nuclear localization of NICD3 was abolished, demonstrating that primary cilia are essential in mediating Notch signal transduction. In vitro, a shift from low to high Ca^{2+} medium is required to induce Notch signaling and differentiation of primary mouse epidermal keratinocytes (MK). Loss of primary cilia on MK induced by shRNA knockdown of *IFT74*, *IFT88*, or *IFT172* resulted in significantly reduced Notch activity and failure to differentiate upon a shift to high Ca^{2+} medium. Whether Notch signaling in mammalian renal epithelial cells is similarly mediated by primary cilia and regulated by extracellular Ca^{2+} are yet to be determined.

Notch receptors are composed of an N-terminal extracellular domain containing Ca^{2+} binding Lin12-Notch (LN) modules, a protein motif unique to Notch receptors, and a C-terminal transmembrane domain. Previously, it was reported that deletion of Ca^{2+} -binding LN modules abolishes stable association of the extracellular domain with the transmembrane domain and leads to rapid shedding of the extracellular domain and subsequent upregulation of NICD and RBP-J in the nucleus (Rand et al., 2000). Thus, contrary to high Ca^{2+} being required to activate the Notch pathway, this earlier study suggests absence of Ca^{2+} binding of Notch receptors induces pathway activation. Similar to the Hh- Ca^{2+} signaling interplay, more experiments are required to understand the interconnection between Notch and Ca^{2+} .

DISRUPTED NOTCH SIGNALING IN RENAL CYSTOGENESIS

Clinical and experimental data suggest a role for Notch signaling in renal cystogenesis. Mutations in *JAG1* and *NOTCH2* cause Alagille syndrome (AGS), which affects children and is characterized by a deficiency in intrahepatic bile ducts, facial and skeletal anomalies, and renal manifestations (McDaniell et al., 2006). While renal dysplasia accounts for most of the renal manifestations, bilateral renal cysts have also been identified in patients with AGS (Pombo et al., 1995; McDaniell et al., 2006).

In mice, downregulation of Notch signaling in the kidney mesenchyme caused proximal tubular cysts, with severity corresponding to degree of inhibition of the Notch pathway (Surendran et al., 2010). Genetic deletion of *Notch1* or *Notch2* resulted in renal cysts by 3 weeks of age, but ablation of *Notch1* and *Notch2* simultaneously, or of *RBP-J*, resulted in renal cysts at birth. In mice lacking *Notch2* and one allele of *Notch1*, mitotic spindle orientation of epithelial cells of precystic proximal tubules was disrupted relative to the

basement membrane. This was proposed to result in epithelial stratification, which was observed as protrusions into the cystic lumen in some of the renal cysts. This cellular defect does not commonly occur in PKD rodent models. Still these data suggest that mitotic spindle orientation defects (whether they are relative to the tubular axis or to the basement membrane) may be a plausible mechanism in the pathogenesis of renal cystogenesis.

While these data suggest that Notch signaling is required to establish renal tubular integrity and prevent renal cyst formation, studies of the zebrafish *double bubble* (*dbb*) ciliary pronephros mutant indicate that inhibition of Jag2 or of Notch3 reduces cyst formation (Drummond et al., 1998; Liu et al., 2007). In the zebrafish pronephros, MCC, designed to propel fluid, and transporting epithelial cells (TEC), which recover filtered blood solutes, are intermingled, similar to principal and intercalated cells of the collecting ducts of mammalian kidneys. MCC and TEC express *Jag2* and *Notch3*, respectively. Knock-down of *Jag2* or *Notch3* expanded expression of the ciliary gene, *rfx*. Inhibition of Notch signaling, either by administering γ -secretase inhibitor, *N*-[*N*-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), or by knocking down *Jag2*, rescued cyst formation in *dbb* mutants. These studies indicate that increased Notch signaling causes ciliary dysfunction, leading to renal cysts in vertebrate kidneys. In contrast, Notch1 upregulation in proximal tubules of humans and mice leads to tubular cell proliferation and interstitial fibrosis, but not to cysts (Bielez et al., 2010).

Thus the role of the Notch pathway in cyst formation is unclear, and these data may indicate that different molecular mechanisms may be at play in developmental and adult models of renal cystogenesis.

Perspectives

The molecular mechanisms initiating renal cystogenesis remain in question. While PKD is considered to be monogenic, renal cystogenesis is sensitive to both environmental factors (e.g., infection and renal injury) and genetic factors (e.g., ciliary mutations and epigenetics), suggesting PKD and other renal cystic diseases may be more complex disorders etiologically. The success of cAMP modulators in attenuating PKD in animal models and in recent clinical trials, and the experiments that point to the proximal role of Ca^{2+} argue that the Ca^{2+} and cAMP mechanism is central to renal cystogenesis. Ciliary regulation of developmental signaling may feed into the Ca^{2+} and cAMP mechanism, and together these pathways may potentiate or mollify renal cyst growth. However, we are far from understanding convergence of these mechanisms.

Genetic ablation of *Pkd1* or *Pkd2* together with *Kif3a* in mice was shown to reduce severity of *Pkd* renal cystogenesis (Ma et al., 2013). These experiments are reminiscent of experiments performed in the cancer field. Tissue-specific expression of constitutively active SMO resulted in medulloblastoma and basal cell carcinoma in wild type mice, but not in *Kif3a* conditional knockout mice, presumably because activation of the Hh signaling cascade from SMO is dependent on primary cilia (Han et al., 2009; Wong et al., 2009). Similarly, loss of Hh signaling may have contributed to the attenuation of renal cystic disease in *Pkd;Kif3a* double knockouts.

In contrast to the improved *Pkd1*;*Kif3a* double mutant cystic phenotype, combining a *Pkd1*-null allele and a *jck* allele caused renal cysts in adult *Pkd1*^{+/-};*Nek8*^{jck/+} double heterozygous mice, although *Pkd1*^{+/-} and *Nek8*^{jck/+} single heterozygotes did not form renal cysts, suggesting interaction between PC1 deficiency and the Nek8 mutant protein (Natoli et al., 2008). It is generally observed that ADPKD is dominant in humans but recessive in the mouse, suggesting that allele combinations that differ in the human and mouse are modifying disease expression. These studies highlight the importance of fully characterizing the functions of individual ciliary proteins to understand why certain interactions exacerbate or ameliorate cystic disease. IFT proteins affect cell function differently. For instance, in primary MK, *Kif3a* knock-down caused hypoproliferation, while knock-down of *IFT88*, *IFT74*, or *IFT172* resulted in hyperproliferation. Additionally, knockdown of *Kif3a*, *IFT88*, or *IFT172* affected cytoplasmic microtubules, but knock-down of *IFT74* did not (Ezratty et al., 2011). Combining ciliary alleles with those of *Pkd1* and/or *Pkd2* should contribute to uncovering the ciliary mechanisms involved in renal cystogenesis. Human PKD may indeed be modified by alleles of any of these ciliopathy genes that modify the growth and development of renal tubules. Conversely, combining non-PKD alleles experimentally requires caution, as certain alleles may phenocopy *Pkd1*/*Pkd2* renal cystic disease, which may be thought-provoking, but potentially off-target.

Epigenetic modulation of gene expression and protein function in PKD has recently become a focus of scientific investigation (Fan et al., 2012; Zhou et al., 2013). Evidence suggests that one class of epigenetic regulators, histone deacetylases (HDACs), may be a factor in ciliopathies. HDAC6 (class II HDAC) and SIRT2 (class III HDAC) regulate cilia disassembly through deacetylating α -tubulin (Zhou et al., 2014). Fluid flow stimulation of polarized epithelial monolayers induced phosphorylation and nuclear export of HDAC5 (Xia et al., 2010). Targeting class I and/or II HDACs with trichostatin A or the class III HDAC, SIRT1, with nicotinamide (Vitamin B3) delayed cyst growth in *Pkd1* and *Pkd2* knockout mice (Xia et al., 2010; Fan et al., 2012; Zhou et al., 2013), suggesting that epigenetic regulators could become an essential component in the clinical management of ADPKD. The involvement of other epigenetic regulators, such as histone methyltransferases, and the interplay between epigenetic regulators and Hh, Wnt, and Notch signaling should also be actively investigated.

Several therapies are being evaluated in clinical trials (Chang and Ong, 2012). Tolvaptan, an antagonist of the Vasopressin 2 receptor, which positively regulates cAMP levels, has proven effective at slowing renal cystic disease progression in ADPKD patients (Torres et al., 2012), and thus far, is the only therapy that has been effective in patients. The success of Tolvaptan demonstrates that the molecular pathways and targets discovered in cells and mice are indeed the correct ones. The challenge now is to identify other targets that can be used in combination with Tolvaptan. Hh, Wnt, and Notch signaling have been shown to be misregulated in kidneys of experimental models of renal cystic disease, and appear to be a link between ciliary dysfunction and renal cystogenesis. Key studies will be to modulate these pathways in orthologous PKD models to attenuate renal cystic disease. If these developmental pathways contribute to renal cystogenesis, either in concert with or parallel to

cAMP, then a combination therapy, which modulates several pathways simultaneously, may be most effective.

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TABLE 1
Misregulation of Hh, Wnt, PCP and Notch Signaling Pathways in Experimental Models of Renal Cystic Disease

Experimental model	Renal phenotype	Hedgehog	Canonical Wnt	Oriented cell division	Convergent extension	Notch	Reference
ADPKD renal cystic tissue	Cystic	Increased <i>GLI2</i> , <i>PTCH1</i> and <i>GAS1</i>	Increased			Increased	Song et al., 2011
<i>Pkd1</i> cko; <i>Mx1</i> -Cre-deletion at 1 week	Severe cysts at 5 weeks postinjection		Increased	Disrupted 2 weeks before cysts have formed			Luyten et al., 2010
<i>Pkd1</i> cko; <i>Mx1</i> -Cre-deletion at 5 week; IRI at 8 weeks	Cysts at 4 weeks post-IRI			Disrupted 48 hours after IRI			Luyten et al., 2010
<i>Pkd1</i> cko; <i>Ksp</i> -Cre ^{ERT1} ; deletion at 11 weeks; DCVC 6 days later	Mild dilations begin 2 weeks after injury		Increased 1 week following injury	Disrupted 1–2 weeks following injury			Happe et al., 2009
<i>Pkd1</i> cko; <i>Ksp</i> -Cre	Cysts appear at P3			Disrupted only in dilated tubules at P3			Nishio et al., 2010
<i>Pkd1</i> ko	Cysts by E16.5		Normal regulation even after cysts have formed				Miller et al., 2011
<i>Pkd1</i> ko	Cysts by E16.5		Increased at E17.5 after cysts have formed				Qin et al., 2012
<i>Pkd1</i> ^{Ci} c	Cysts by E15.5				Disrupted before cysts formed		Castelli et al., 2013
<i>Pkd2</i> cko; <i>Pkhd1</i> -Cre	Cysts appear at P8–P10			Disrupted only in dilated tubules at P8–P10			Nishio et al., 2010
<i>Pkd2</i> /WS25	Cysts by 2 weeks of age		Normal regulation even after cysts have formed				Miller et al., 2011
<i>Pkd2</i> -null collecting duct cell line			Increased				Kim et al., 2009
PCK rat (<i>Pkhd1</i> -deficient)	Cysts at 3 weeks of age			Disrupted at P8–P12			Fischer et al., 2006
<i>Pkhd1</i> ^{del4/del4}	Does not develop cysts			Disrupted at P7–P10			Nishio et al., 2010
<i>Hnf1β</i> cko; <i>Ksp</i> -Cre	Cysts at P8			Disrupted at P3–P4			Fischer et al., 2006
<i>Kif3a</i> cko; <i>Ksp</i> -cre	Cysts by P5		Increased at P21				Lin et al., 2003
<i>Kif3a</i> cko; <i>Pkhd1</i> -Cre	Cysts evident at P14		Not examined, but proliferation not affected	Disrupted by P7–P10			Patel et al., 2008

Experimental model	Renal phenotype	Hedgehog	Canonical Wnt	Oriented cell division	Convergent extension	Notch	Reference
<i>IFT20</i> cko; <i>HoxB7</i> -Cre	Cysts by P5		Increased at P15	Disrupted at P5			Jonassen et al., 2008
<i>IFT140</i> cko; <i>HoxB7</i> -Cre	Cysts by P5	increased <i>Gli</i> by P20	Increased by P20	Not affected			Jonassen et al., 2012
<i>Thm1</i> cko; <i>R26R-Cre^{ERT}</i> - deletion at E17.5	Dilations begin by P15	increased <i>Gli</i> at 6 weeks					Tran et al., in press
<i>TMEM67</i> ko (bpck)	Tubular dilations by E16.5		Increased at P1 in cystlining epithelial cells	Not affected			Leightner et al., 2013
<i>Mks1</i> ko	Small cysts by E16.5		Increased in E14.5 kidneys				Wheway et al., 2013
<i>Ahl1</i> ko	NPHP microcysts and tubular dilations at 1 year		Decreased at 5 months				Lancaster et al., 2009
<i>APC</i> cko; <i>Ksp</i> -Cre	Severe renal cystic disease by P2		Increased				Qian et al., 2005
active β -catenin over-expression in kidney	Renal cysts in all tubules by P18		Increased				Saadi-Kheddouci et al., 2001
<i>Fat4</i> ko	Small cysts by E16.5			Disrupted			Saburi et al., 2008
<i>Wnt9b</i> cko; <i>Ksp</i> -Cre	Cysts by P10, few cysts at P1		Not affected in cystic tubules	Disrupted, evident at P5			Karner et al., 2009
<i>inv/inv</i>	Die by first week of age		Not affected	Disrupted, evident before cysts have formed and before increased cell proliferation			Sugiyama et al., 2011
<i>Notch1</i> cko; <i>Six2</i> -GFP::Cre	Proximal tubular cysts in of 3–5-week-old cko mice					Decreased	Surendran et al., 2010
<i>Notch2</i> cko; <i>Six2</i> -GFP::Cre	Proximal tubular cysts in 3-week-old mice					Decreased	Surendran et al., 2010
<i>Notch1</i> cko; <i>Notch2</i> cko; <i>Six2</i> -GFP::Cre	Proximal tubular cysts at birth					Decreased-disrupted mitotic spindle orientation relative to the basement membrane before cysts have formed	Surendran et al., 2010

Experimental model	Renal phenotype	Hedgehog	Canonical Wnt	Oriented cell division	Convergent extension	Notch	Reference
<i>RBP-J</i> cko; <i>Slx2</i> -GFP::Cre	Proximal tubular cysts at birth					Decreased	Surendran et al., 2010
<i>Gli3</i> ^{lacZ/lacZ}	NPHP renal tubular dilations begin at 8 weeks	Increased <i>Gli1</i> at 4 weeks					Attanasio et al., 2009
<i>Par3</i> cko; <i>HoxB7</i> -Cre	40% Develop renal cysts				Disrupted		Castelli et al., 2013
<i>Notch1</i> cko; <i>Notch2</i> cko; <i>Slx2</i> -GFP::Cre	Proximal tubular cysts at birth					Decreased-disrupted mitotic spindle orientation relative to the basement membrane before cysts have formed	Surendran et al., 2010
<i>RBP-J</i> cko; <i>Slx2</i> -GFP::Cre	Proximal tubular cysts at birth					Decreased	Surendran et al., 2010
<i>Gli3</i> ^{lacZ/lacZ}	NPHP renal tubular dilations begin at 8 weeks	Increased <i>Gli1</i> at 4 weeks					Attanasio et al., 2009
<i>Par3</i> cko; <i>HoxB7</i> -Cre	40% Develop renal cysts				Disrupted		Castelli et al., 2013

An empty cell indicates analysis of a signaling pathway has not been reported for a particular mutant.
ko, knockout; cko, conditional knockout.