Cystic Kidney Disease: the Role of Wnt Signaling

Madeline A. Lancaster and Joseph G. Gleeson

Biomedical Sciences Program Howard Hughes Medical Institutes Department of Neurosciences
University of California, San Diego

Abstract

Wnt signaling encompasses a variety of signaling cascades that can be activated by secreted Wnt ligands. Two such pathways, the canonical or β -catenin pathway and the planar cell polarity pathway, have recently received attention for their roles in multiple cellular processes within the kidney. Both of these pathways are important for kidney development as well as homeostasis and injury repair. Disruption of either pathway can lead to cystic kidney disease, a class of genetic diseases that includes the most common hereditary life-threatening syndrome polycystic kidney disease. Recent evidence implicates canonical and noncanonical Wnt pathways in cyst formation and points to a remarkable role for developmental processes in the adult kidney.

Keywords

cyst; Wnt; cilia; ciliopathy; signaling; planar cell polarity; kidney; injury; repair; development

Cystic kidney diseases

Cystic kidney disease is the most common genetic cause of end-stage renal failure¹, with polycystic kidney disease (PKD) having a prevalence rate of approximately 1 in 500 (Box 1). These diseases are characterized by the progressive development of cysts of the nephron and collecting ducts, and patients often require dialysis and kidney transplantation². Not only are these life-threatening illnesses, but current treatments are exceedingly costly. This class of disease, therefore, represents a major clinical priority, especially because there are currently no approved drug treatments and no preventive strategies.

There are two types of PKD classified by their mode of inheritance: autosomal dominant (ADPKD) and autosomal recessive PKD (ARPKD). In PKD, cyst formation can occur in all segments of the nephron (Figure 1), which leads to overall enlargement of the kidney and eventually to end-stage renal failure in approximately 50% of patients³. ADPKD typically arises during adulthood, whereas ARPKD arises during childhood and is much more severe. The stochastic spatial and temporal occurrence of cysts in ADPKD has led to the proposal of a “second-hit” hypothesis, which suggests the disease, though dominantly inherited, might occur recessively at the cellular level⁴. This model, similar to the two-hit hypothesis in

cancer progression, proposes that an inherited germline mutation combined with a second spontaneous somatic mutation triggers cyst formation.

Two genes have been identified for ADPKD: polycystic kidney disease gene 1 (*PKD1*) and *PKD2* that encode polycystin-1 (PC1) and polycystin-2 (PC2)¹, respectively. ARPKD is caused by mutations in polycystic kidney and hepatic disease 1 (*PKHD1*), which encodes the fibrocystin (or polyductin, FPC) protein. Although the precise mechanism of these proteins in PKD pathogenesis remains unclear, many recent papers have shed light on the probable subcellular roles for these proteins in renal development and homeostasis. For example, PC2 is a cation channel with selectivity to calcium⁵ that is influenced by mechanosensation of fluid flow in renal epithelial cells⁶, and PC1 and FPC, each containing transmembrane domains, may modulate the function of PC2^{6,8}.

A second class of cystic nephropathy is characterized as glomerulocystic kidney disease, which is a descriptive term for pathology associated with a variety of syndromic and nonsyndromic cystic renal diseases⁹. As the name suggests, kidneys from patients with these disorders exhibit an enlarged Bowman's space in the glomerulus in addition to cystic tubules of the nephron and collecting ducts; examples include medullary cystic kidney disease (MCKD) and nephronophthisis (NPHP), and the phenotypes are related to those observed in PKD (Box 2). Indeed, certain forms of PKD exhibit glomerulocystic disease pathology¹⁰.

NPHP is inherited in a recessive fashion and like ARPKD leads to kidney impairment much earlier in life than ADPKD. However, NPHP kidneys exhibit some unique features including marked tubular basement membrane abnormalities and interstitial fibrosis¹¹. Additionally, cysts are primarily confined to the corticomedullary junction. To date, 11 genes have been identified in the NPHP and NPHP-like disorders (Table 1) that overlap with a larger spectrum of syndromic forms of cystic kidney disease classified as “ciliopathies¹²” including PKD. If we take into account all of the known genes in both syndromic and nonsyndromic forms of cystic kidney disease, there are currently 57 genes identified in cystic nephropathies, including dominant, recessive and X-linked (Table 1). This poses quite a challenge in identifying precise mechanisms underlying the complex process of cyst formation for any given patient, but at the same time provides us with a multitude of molecular entry points to find commonalities and to establish treatment paradigms.

Canonical Wnt/ β -catenin signaling

Canonical Wnt signaling is a highly conserved developmental pathway involved in a variety of biological processes depending on the cellular context¹³. Wnt signaling regulates cell proliferation and differentiation from *Drosophila* to vertebrates and is even important in some models of regeneration¹⁴. PKD and NPHP proteins have roles in canonical Wnt signaling, suggesting this pathway affects cystogenesis.

The canonical Wnt or β -catenin pathway (Figure 2a) initiates when a canonical Wnt ligand binds to a cognate Frizzled receptor in the presence of the low-density lipoprotein (LDL) receptor-related protein-5 or -6 coreceptors (LRP5 or 6)¹⁵. This activates Dishevelled (Dvl), which in turn inhibits a complex of proteins termed the “destruction complex.” Included in this complex are glycogen synthase kinase- β (GSK3 β), adenomatous polyposis coli (APC), Axin and casein kinase 1 (CK1), all of which inhibit the major downstream mediator of the canonical Wnt pathway, β -catenin. When Wnt signaling is inactive, β -catenin is targeted (through phosphorylation by GSK3 β and CK1) for proteasome-mediated degradation. However, when the pathway is activated, this destruction is inhibited and β -catenin accumulates in the cytosol. β -catenin then enters the nucleus where it interacts with TCF transcription factors to activate transcription of Wnt target genes.

Several cystic renal disease genes are linked to canonical Wnt signaling. The first evidence pointing to a role for canonical Wnt signaling in cystic renal disease pathogenesis came from work that revealed a role for PC1 in the modulation of the downstream canonical Wnt response¹⁶. The carboxyl (C) terminus of PC1 augments canonical Wnt transcriptional response and overexpression of the C terminus of PC1 *in vivo* dorsalizes zebrafish embryos¹⁶, which is consistent with increased canonical Wnt signaling. These findings were subsequently supported by studies in cancer cells^{17, 18} and more recently in osteoblasts¹⁹ where PC1 is further implicated in positive modulation of canonical Wnt/ β -catenin signaling.

However, these findings are complicated by recent data indicating a potential inhibitory role for PC1 in the canonical pathway. For example, Lal and colleagues found that, although the C terminus of PC1 facilitates nuclear accumulation of β -catenin, downstream transcription is decreased with C terminus overexpression *in vitro*²⁰. Furthermore, this inhibition occurs with PC2 also in kidney cells *in vitro*²¹. Although these findings at first appear contradictory, one possible explanation is that there is a fine balance between PC1 expression and post-translational cleavage of the C terminus; disruption of this balance might lead to cyst development. This hypothesis is supported by the finding that cysts arise in animal models of PKD with complete or partial loss of protein (as in, *Pkd1*^{-/-}, *Pkd1*^{+/-} and hypomorphic mice)^{22, 23} and also with overexpression of the protein^{24, 25}, suggesting the kidney is especially sensitive to gene dosage. Indeed, increased PC1 expression in cystic epithelium of patients with PKD has been reported²⁶. Thus, PC1 expression seems to be tightly regulated in the kidney, and canonical Wnt signaling might also require similar fine tuning.

Balanced Wnt signaling is important in other models of cystic renal disease as well. For example, studies with conditional Wnt mutant mice such as β -catenin overexpression²⁷ or APC inactivation²⁸ have revealed that overactivation of the canonical Wnt pathway leads to cystic renal phenotypes, further supporting a role for canonical Wnt signaling in cystogenesis. However, these findings are complicated by data from canonical Wnt loss-of-function mutants that also display renal cysts^{29, 30}. Again, this suggests canonical Wnt/ β -catenin must be delicately balanced in the kidney.

Further evidence for a role of canonical Wnt signaling in renal cyst disease comes from work with NPHP animal models. Nphp2 (Inversin) negatively regulates the canonical Wnt cascade through regulation of Dvl, both *in vitro* and *in vivo* in *Xenopus laevis* embryos³¹. These findings were also replicated with Nphp3³². Canonical Wnt activity is also affected in a mammalian model of NPHP; knockout mice for *Ahi1*³³, a gene mutated in the ciliopathy Joubert syndrome (JS), have decreased canonical Wnt signaling in the kidney compared to wild-type mice³⁴. These results were initially surprising but are consistent with the hypothesis that disruption of the pathway in either direction can lead to cystogenesis. Furthermore, studies on other proteins involved in the cystic kidney ciliopathy spectrum, such as several of the proteins mutated in Bardet-Biedl syndrome (BBS)³⁵⁻³⁷, reveal similar dichotomies. Overall, the data suggest a unique mechanism of pathogenesis; unbalanced canonical Wnt signaling contributes to cystogenesis.

Noncanonical Wnt signaling (Planar cell polarity)

The work with Inversin not only shed light on the canonical Wnt pathway in cystic renal disease but also introduced a new concept: a switch between canonical and noncanonical branches of the Wnt pathway might be involved in disease pathogenesis³¹. There are several noncanonical forms of Wnt signaling; in cystic kidney disease, the planar cell polarity (PCP) is the branch most recently implicated². PCP is not nearly as well understood as canonical

Wnt signaling, but many key players have been identified³⁸. The pathway (Figure 2b) diverges from the canonical pathway at the level of Dvl, which, when localized to the membrane, can activate a cohort of downstream mediators involved in cell polarity and cytoskeletal rearrangements.

Inversin regulates the stability of Dvl; however, membrane-associated Dvl is resistant to Inversin-dependent degradation, suggesting the membrane-associated pool of Dvl is still active even when canonical Wnt signaling is inhibited by Inversin³¹. This suggests a switch between the two pathways and implicates PCP in the development of cystic renal diseases like NPHP. Indeed, Inversin is required for noncanonically mediated convergent extension movements *in vivo*³¹. Several other cystic disease proteins are also required for noncanonical Wnt signaling including Nphp^{32, 39}, BBS1, BBS4 and BBS6⁴⁰, PC1⁴¹, OFD1 (Orofacial digital syndrome-1; mutated in Orofaciodigital syndrome)⁴² and HNF1 β (hepatocyte nuclear factor 1 β ; mutated in renal cysts and diabetes syndrome/MODY5)⁴³. Additionally, a mutation of Fat4, a protein found to regulate upstream in the PCP pathway, leads to cystic kidney phenotypes⁴⁴ further supporting a role for PCP signaling in cystogenesis. However, given the role of Fat proteins in Hippo signaling⁴⁵, one cannot rule out the possibility that the phenotype is due to signaling other than the PCP pathway.

Exactly how PCP defects contribute to cyst development is unclear, but several studies have now shed light on two related processes involved in normal kidney tubule development: convergent extension and oriented cell division. Convergent extension is a process of intercalation of adjacent cells during development of various tissues⁴⁶, whereas oriented cell division refers to an alignment of the axis of cell division during mitosis⁴⁷. Both of these processes are at least partly dependent on PCP. Defective oriented cell division was proposed to cause cystogenesis by Fischer and colleagues based on the finding that two rodent models of cystic kidney disease, *Hnf1 β* mutant mice and *Pck* rats, have tubule epithelial cells with altered planes of division⁴³. *Wnt9b* mutant mice also have cystic kidneys with defects in both convergent extension and oriented cell division, although the two processes are not synchronized, suggesting partially independent mechanisms⁴⁸. Finally, a recent paper provides another side to the story; *PKD* mutant mice do not exhibit defects in oriented cell division at cyst initiation, though such defects are present in already dilated tubules⁴⁹. Thus, although the exact cause of cyst initiation is unclear, there is increasing evidence that PCP could be involved in both convergent extension and oriented cell division and that these processes are abnormal in cystic tubules. One possible explanation for these findings is that cystogenesis is triggered by defective elongation and the narrowing of kidney tubules; subsequently, these dilated tubules become further cystic through defective oriented cell division.

Crosstalk and the role of cilia in Wnt signaling

Many cystic renal disorders, including PKD and NPHP, are ciliopathies⁵⁰ (Box 1). Cilia are tiny extensions (usually 1-10 μ m in length⁵¹) relative to the cell body (average 50 μ m diameter). Primary cilia are distinguished from motile cilia by their lack of outer dynein arms required for movement as well as their presence on almost all vertebrate cell types⁵². Despite their abundance, however, primary cilia have been largely ignored, until now. Within the past ten years, there has been an explosion in research dedicated to uncovering the many diverse roles of these cellular protrusions. Cilia have now been linked to key cellular processes such as signaling, polarity, cell cycle/proliferation and cell fate/differentiation⁵³.

Cilia are implicated in several signaling pathways, including Hedgehog, platelet-derived growth factor (PDGF), calcium and Wnt⁵⁴. Research on the roles of cilia in both canonical

and noncanonical Wnt pathways is still in its infancy, but there is sufficient evidence to indicate that not only do cilia regulate the Wnt pathways but also Wnt signaling regulates ciliogenesis (Figure 3). Cilia were first implicated in the pathogenesis of the cystic kidney diseases when a mutation of the *Ift88* gene (intraflagellar transport gene 88) necessary for proper cilia formation⁵⁵ was found in a widely used model of cystic renal disease, the Oak ridge polycystic kidney (*Orpk*) mouse mutant⁵⁶. Subsequently, the role for Inversin, which affects left-right asymmetry and cilia function⁵⁷, in canonical and noncanonical Wnt regulation was identified and together, these findings stimulated more studies into the role of primary cilia in Wnt signaling and vice versa.

β -catenin signaling was first linked to primary cilia function through the finding that *Wnt3a* mutant mouse embryos exhibit left-right asymmetry defects and defective ciliary localization of PC1 in the node⁵⁸. Additionally, GSK3 β along with von Hippel-Landau protein (VHL) are required for primary cilium maintenance^{59, 60}. More recently, Chibby, an inhibitor of β -catenin signaling, was found to be necessary for the formation of motile cilia⁶¹. These findings suggest canonical Wnt signaling might be involved in the regulation of cilia formation; however, this connection is still tenuous since these proteins, particularly GSK3 β and VHL, have well-described roles in signaling pathways other than the Wnt pathway. Furthermore, other data suggest PC1 is not normally found in nodal cilia adding a perplexing complication to these findings⁶². Thus, whether canonical Wnt signaling is involved in regulation of cilia formation in general, and specifically within the context of the kidney, is a topic worthy of future scrutiny.

Conversely, regulation also seems to occur in the opposite direction with ciliary mediated inhibition of the canonical Wnt pathway. The presence of the primary cilium inhibits canonical Wnt signaling through regulation of Dvl⁶³ and the proteasome³⁶. Recent data supports this inhibitory role in mammary gland morphogenesis⁶⁴. However, there is also convincing data that suggests a lack of connection between cilia and canonical Wnt signaling^{65, 66}. Thus, although there is significant evidence suggesting a role for regulation of canonical Wnt signaling at the primary cilium, there are still many questions as to the extent of this regulation as well as the exact mechanism, which remain to be resolved.

Although there is evidence for ciliary regulation of PCP and vice versa, the exact connection is not well defined. Because PCP signaling progresses through multiple branches rather than through a clear linear path, it can be difficult to discern at which point the cilium plays a role. Many of the studies examining the relationship between cilia and PCP have used mutant animal models, and PCP or cilia phenotypes are used as read outs. Thus, the directionality of regulation and whether it is direct or indirect is often difficult to interpret. For example, the zebrafish cystic kidney *seahorse* mutant displays both cilia defects and convergent extension defects⁶⁷, suggesting a connection between cilia and PCP, although it is not clear which defect is primary. Similarly, both PCP and ciliary defects occur in mouse and zebrafish mutants of *Ofd1*⁴², *Tsc1* and *Tsc2* (mutated in Tuberous Sclerosis Complex)⁶⁸, *Pkd1*⁴¹ and *BBS4* and *BBS6*⁴⁰ as well as *duboraya* (*dub*) zebrafish mutants⁶⁹, supporting the hypothesis that there is crosstalk between cilia and PCP.

Strikingly, work with the PCP effectors Inturned and Fuzzy in *X. laevis* provides strong evidence that PCP signaling is required for ciliogenesis by establishing apical-basal polarity and affecting cytoskeleton organization⁷⁰. This is supported by studies showing that Dvl is required in the apical docking of basal bodies to form cilia in *X. laevis*⁷¹ and in determining cilia orientation in mice⁷². The dependence of cilia orientation on polarity is highly conserved and was identified as early as 1975 in *Paramecium*⁷³. Furthermore, several PCP proteins including Van Gogh-like 1 (Vangl1)⁷⁴ and Vangl2⁷⁵, Prickle2⁷⁴, Bicaudal C (BicC)⁷⁶, and Frizzled 3 (Fzd3)⁷⁵ are required for planar polarization and the orientation of

cilia in several cellular contexts. Thus, PCP plays a role in both cilia orientation and in another type of polarity along the apical-basal axis leading to basal body docking and cilia outgrowth.

Increasing data also indicate that cilia can regulate PCP. Jones and colleagues identified a striking defect in planar cell polarity of stereociliary bundles in cilia mutant mice⁷⁷. This defect, however, is associated with intact partitioning of core PCP components, suggesting the cilium might act downstream of PCP components or in a parallel pathway, which then interacts with PCP components in determining cell polarity. Further evidence for the role of cilia in PCP comes from the finding that loss of *Ift20*, which encodes intraflagellar transport protein-20 and leads to cilia loss, results in misorientation of cell division and cystic kidney disease in mice⁷⁸, suggesting the cilium is critical for oriented cell division.

Overall, increasing data indicate that cilia are important in both canonical and noncanonical Wnt signaling, though the exact mechanisms are still unclear. Because evidence also suggests that noncanonical and canonical Wnt signaling oppose one another and the cilium seems to play disparate roles in each, the cilium might act as a switch between the two pathways³¹, thereby controlling the cell's sensitivity or interpretation of the signal. Furthermore, ciliary regulation might represent a novel feedback mechanism. Support for this hypothesis comes from a study in which initial cell polarity determines cilia positioning followed by cilia-mediated refinement of cell polarity⁷⁹. Thus, given the strong evidence for a role of the cilium in cystic renal disease, ciliary regulation of Wnt signaling could be lost in the cystic kidney ciliopathies.

Cyst formation in the developing kidney

Although patients with the most common form of cystic kidney disease ADPKD do not typically display symptoms until adulthood, most other forms of cystic renal disease have a much earlier age of onset, usually during childhood or even infancy. Thus, there might be common pathogenic mechanisms for cysts that form both during adulthood and when renal development is still ongoing. Recently, a striking effect of developmental state on cyst formation was shown⁸⁰; conditional inactivation of *Pkd1* in mice before postnatal day 13 produces a severe and sudden cystic kidney phenotype, whereas mice with conditional inactivation of the gene after postnatal day 14 do not develop cysts until at least five months of age. This abrupt change in sensitivity at such a specific age in mice suggests a striking shift in renal developmental state at this time point. Indeed, dramatic expression pattern changes occur between P12 and P14 including many Wnt and cilia genes⁸⁰. This is the first indication that the developmental state of renal tubules plays an important role in cyst formation.

Wnt signaling is a crucial regulator of kidney morphogenesis. Wnt11 functions in ureteric bud branching, whereas both canonical and noncanonical Wnt signaling are required for nephron induction and tubule epithelial formation⁸¹⁻⁸³. Wnt9b, acting through the canonical β -catenin pathway, induces early nephron markers and is required for initial nephrogenesis, leading to the formation of pretubular aggregates⁸⁴. Wnt4, one of the early markers induced by Wnt9b, is then involved in the mesenchymal to epithelial transition (MET) to form renal tubular epithelium⁸³ at least partially through the β -catenin pathway⁸⁵. However, β -catenin overexpression reveals that the final step in this differentiation is actually inhibited by the canonical pathway⁸⁵. Thus, the activity of canonical Wnt signaling goes through a dramatic shift over the course of nephron development.

Although Wnt9b acts through the canonical pathway in early nephron induction, it is later required for convergent extension movements and oriented cell division⁴⁸, processes associated with PCP. Furthermore, Wnt7b acts through the canonical β -catenin pathway in

oriented cell division within the developing epithelium⁸⁶. Overall, there appears to be significant crosstalk between canonical and noncanonical Wnt pathways. Canonical Wnt signaling through Wnt9b and β -catenin is needed for the formation of condensed mesenchyme of the pretubular aggregate. A switch then deactivates canonical Wnt signaling and activates noncanonical Wnt pathways for MET and convergent extension movements that form properly elongated tubules. Thus, the two pathways are tightly controlled both spatially and temporally, and disruption of either can lead to numerous defects including early cystic disease.

The injury model in cystic kidney disease

Although many forms of cystic kidney disease exhibit an early age of onset, most patients with renal cysts do not exhibit signs or symptoms until adulthood after the kidney is completely developed. This presents a quandary because we hypothesize that the two forms have similar molecular mechanisms, yet cystogenesis has a clear dependence on the developmental state of renal tubules. One model, therefore, is that adult cysts form in tubules that revert to a state resembling a developing tubule and that then depend on some of the same developmental programs⁸⁷. One mechanism for this type of developmental reactivation could be through injury (Figure 4). Indeed, renal injury might be considered an alternative “second hit” or even a “third hit” in PKD, triggering rapid development of disease in *Pkd1* mutant mice⁸⁸. A major discovery was the finding that *Kif3a* mutant mice exhibit defective renal repair leading to kidney cysts⁸⁹, a finding also observed in *Ahi1*³³ and *Pkd1* mutant mice⁴¹, which suggests cyst formation could result from defective repair following renal injury.

There are two related hypotheses for why both developmental state and renal injury are relevant to cyst formation. One hypothesis is that both the developing kidney and the injured kidney exhibit high levels of cell proliferation so proliferation might be the common denominator^{89, 90}. The other hypothesis is that during both renal development and injury repair, developmental pathways are activated in order to form new renal tubule epithelium³³. These hypotheses are highly similar because proliferation is an important process in the formation of new tubule epithelium either during development or following injury. Therefore, one model, which reconciles these two possibilities, is that developmental signaling cascades such as the Wnt pathways are activated during development or following injury in order to stimulate proliferation and differentiation of new tubule epithelium. Defective cellular responses to these signals would then lead to cyst formation in either context.

In this latter model, we expect to see the reactivation of developmental signaling pathways following injury in a healthy adult kidney, whereas mice with cystic kidney gene mutations should exhibit defects in this cellular response. This hypothesis has now been tested using mouse models of cystic kidney disease. *Ahi1* mutant mice develop a slow onset cystic kidney disease with reduced basal canonical Wnt response³³. Injury in wild-type mice triggers an upregulation of canonical Wnt signaling, whereas in *Ahi1* mutant mice, canonical Wnt signaling is not responsive to injury and these mice develop cystic tubules. Similarly, *Pkd1* mutant mice⁴¹ and *Kif3a* conditional knockouts⁸⁹ display defective canonical Wnt and PCP following injury, suggesting that as seen in development, both Wnt pathways are necessary for renal injury repair.

We expect that similar regulatory mechanisms control Wnt signaling during injury repair and development. Indeed, the activation of canonical Wnt signaling following injury has been confirmed and at least one Wnt ligand, Wnt7b, is involved in injury repair⁹¹. This ligand is likely responsible for the injury response, which is abnormal in *Ahi1* mutant mice.

It remains to be determined whether the same delicate balance of Wnt signaling that is observed during development is also present during injury repair and whether a similar switching mechanism is present between the canonical and noncanonical branches. Nonetheless, evidence suggests that defective Wnt signaling in newly forming tubule epithelium, either during development or injury repair, is at least partly responsible for cystic kidney phenotypes.

Concluding remarks

Although we have focused on the roles of cystic kidney disease proteins in Wnt signaling, it is important to note that many of these proteins also have functions in other signaling cascades such as Hedgehog, Ras/MAPK (mitogen-activated protein kinase), and calcium signaling²³, which might or might not be independent of their roles in Wnt signaling. For example, in kidney cells *in vitro*, the primary cilium can bend in response to flow and elicit a calcium influx that depends upon PC2 as a calcium channel and PC1⁶. This observation led to the introduction of the flow hypothesis of cystogenesis that proposes cysts develop owing to a defect in homeostatic flow sensing by cilia⁴⁷. This hypothesis was further applied to Wnt signaling to suggest the cilium acts as a switch between canonical and noncanonical branches through a role in mechanotransduction³¹.

However, the finding that cystogenesis is highly dependent on renal tubule developmental state and can be triggered by injury has dramatically shifted the model of cystic kidney pathogenesis from this flow hypothesis to a more developmental cilia signaling-centric view. Findings from conditional cilia mutants such as *Ift88*⁹² and *Kif3a*^{89, 92} have recently turned the flow hypothesis on its head⁹³. Conditional ablation of the cilium during adulthood does not lead to immediate cyst formation despite the loss of cilia. Instead, injury is required to trigger cyst formation in these mice. Thus, although there is strong evidence that cilia detect flow *in vitro*⁶, whether this is actually involved in cyst formation is not clear. One possibility is that mechanotransduction is important only during development or repair rather than through constant homeostatic flow sensing.

Although there is convincing data that suggest both canonical and noncanonical Wnt signaling are involved in cyst formation, there are still many questions that remain to be answered (Box 3). For example, given the roles of cilia and cystic disease proteins in other signaling pathways, how might these pathways including the Wnt pathway influence each other during cyst formation? Studies examining signaling on a larger scale using bioinformatics will no doubt provide new insights. Additionally, increasing evidence suggests there is overlap between cystogenesis and cancer, because cysts exhibit similarities with renal neoplasms⁹⁴ and patients with TSC or VHL have a greatly increased risk of developing renal cell carcinomas⁹⁵. The nature of this connection and the role of cilia and Wnt signaling are not yet clear, but the evidence suggests cilia play a regulatory role in carcinogenesis at least in certain contexts^{96, 97}. Furthermore, although there is significant data suggesting the connection between cilia and Wnt signaling, exact mechanisms are still unclear. Finally, how defects in these processes lead to cyst formation and the exact cellular changes and movements that underlie cyst formation will need to be examined more in depth in order to begin to target these processes for therapeutic intervention. Several drugs are already being tested and some are even undergoing clinical trials, such as drugs which target cyclic AMP and mTOR (mammalian target of rapamycin) signaling¹, but none so far have been introduced that target the Wnt pathways. Whether the Wnt pathways represent good candidates for drug screening for the cystic kidney diseases remains to be determined.

Findings implicating abnormal injury repair suggest that slow onset cystic disease might result from a defective response to the slow accumulation of injury. The reactivation of

developmental pathways during repair might represent a broader response to injury in other organs as well. For example, canonical Wnt signaling is required during bone repair in a manner similar to its role in bone development⁹⁸, and in fact, a similar balance of canonical Wnt regulation to that seen in kidney development is maintained in bone⁹⁹. Thus, the Wnt pathway components might represent promising therapeutic targets not only for cystic renal disease, but also for kidney repair and the regeneration of other organs or tissues such as lung¹⁰⁰, nervous system¹⁰¹, liver¹⁰² and bone⁹⁸.

Box 1. Syndromic cystic kidney disease and the ciliopathies

Kidney cysts are quite common; they are present in approximately 10-20% of adults over the age of 50¹⁰³. Cysts are a feature of diverse syndromes from pseudothalidomide syndrome (a defect in sister chromatid cohesion) to multiple acyl-CoA dehydrogenase deficiency and to the ciliopathies⁹. The ciliopathy spectrum is so named because of the increasing evidence pointing to a role for cilia in pathogenesis of this class of disorders; most of the proteins involved localize to the cilium or the modified centrosome at its base, termed the basal body¹². This spectrum includes a variety of multisymptomatic disorders such as Bardet-Biedl syndrome (BBS), Joubert syndrome (JS), Meckel-Gruber syndrome (MKS) and orofacioidigital syndrome (OFD). Additionally, other syndromic glomerulocystic renal phenotypes can occur in association with diseases that have not (yet) been identified as ciliopathies, such as Marden-Walker's syndrome, Smith-Lemli-Opitz and even Down syndrome. The diversity of syndromes and the high prevalence of renal cysts suggest the kidney is highly susceptible to cyst formation. Defective Wnt signaling and cilia function are two of perhaps several possible causes.

Box 2. PKD and NPHP

ADPKD is primarily an adult-onset disorder, whereas NPHP mainly occurs in children and adolescents². Although the phenotypes are quite similar, there are notable differences. For example, ADPKD is marked by greatly enlarged kidneys, whereas kidneys of NPHP patients are typically smaller than normal. Additionally, NPHP is marked by extensive fibrosis and is, therefore, termed a fibrocystic kidney disease; ADPKD does not exhibit this fibrosis. These differences can provide clues as to the underlying pathogenic mechanisms. Although there have been only two studies on the roles of cystic kidney disease proteins in Wnt signaling following renal injury, these studies shed light on some of the pathogenic differences between PKD and NPHP. *Ahi1* is necessary in mice for the canonical Wnt response following injury and loss of this activity leads to NPHP³³. Injured *Pkd1* mutant mice have defects in PCP with an opposite effect on canonical Wnt activity⁴¹. These results suggest that in NPHP pathogenesis, early canonical Wnt-dependent differentiation is defective, which might lead to the overproduction of mesenchymal fibroblasts and fibrosis (Figure 3). Conversely, PKD might result from defective terminal differentiation of tubule epithelium, leading to tubule dilation without fibrosis. Although this hypothesis remains to be tested, the evidence suggests that potentially related but distinct molecular mechanisms underlie NPHP and PKD.

Box 3. Outstanding questions

- Does the primary cilium act as a switch between canonical and noncanonical Wnt pathways in the kidney?

- Does flow sensing play a role in Wnt signaling during development and injury repair?
- Are other signaling pathways that are implicated in cystic kidney disease, such as mTOR and calcium, also involved in renal injury repair?
- Is the reactivation of developmental signaling following injury a common paradigm?
- How is the delicate balance of canonical Wnt signaling in the kidney maintained?

Glossary

Cilia	small cellular protrusions composed of nine outer microtubule doublets and, in the case of most motile cilia, two center singlet microtubules. Intraflagellar transport (IFT), composed of IFT proteins and motor proteins, carries cargo up and down the cilium and is responsible for cilium assembly and disassembly
Ciliopathy	any of a number of syndromic genetic disorders that are characterized by a role for the cilium in their pathogenesis. Although the role(s) cilia play are not yet fully understood, all of the genes identified can be linked either to cilia structure or function. Phenotypes include brain malformation, polydactyly, retinal degeneration, facial dysmorphism, liver fibrosis and cysts, aneurysms, and many others in addition to kidney cysts
Mesenchymal epithelial transition (MET)	a programmed cell-fate transition that occurs during the development of many tissues and that is characterized by a loss of motility as well as an increase in cell polarization and cell adhesion
Nephron	the basic functional unit of the kidney; it filters blood to eliminate waste from the body. The nephron controls water and salt balance, and, therefore, regulates blood pressure and volume as well as electrolyte balance
Planar cell polarity (PCP)	a noncanonical Wnt pathway that regulates polarity of a plane of cells relative to each other. PCP also regulates cell movements relative to each other as in convergent extension
Wnts	a family of highly conserved secreted growth factors. These ligands regulate cell fate determination as well as tissue induction and proliferation during development and are also involved in tumorigenesis

References

1. Harris PC, Torres VE. Polycystic kidney disease. *Annu Rev Med.* 2009; 60:321–337. [PubMed: 18947299]
2. Bacallao RL, McNeill H. Cystic kidney diseases and planar cell polarity signaling. *Clin Genet.* 2009; 75:107–117. [PubMed: 19215242]
3. Walz G. Therapeutic approaches in autosomal dominant polycystic kidney disease (ADPKD): is there light at the end of the tunnel? *Nephrol Dial Transplant.* 2006; 21:1752–1757. [PubMed: 16705023]

4. Reeders ST. Multilocus polycystic disease. *Nat Genet.* 1992; 1:235–237. [PubMed: 1338768]
5. Gonzalez-Perrett S, et al. Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+} -permeable nonselective cation channel. *Proc Natl Acad Sci U S A.* 2001; 98:1182–1187. [PubMed: 11252306]
6. Nauli SM, et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet.* 2003; 33:129–137. [PubMed: 12514735]
7. Hanaoka K, et al. Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature.* 2000; 408:990–994. [PubMed: 11140688]
8. Wang S, et al. Fibrocystin/polyductin, found in the same protein complex with polycystin-2, regulates calcium responses in kidney epithelia. *Mol Cell Biol.* 2007; 27:3241–3252. [PubMed: 17283055]
9. Bissler JJ, et al. Glomerulocystic kidney disease. *Pediatr Nephrol.* 2010
10. Sharp CK, et al. Dominantly transmitted glomerulocystic kidney disease: a distinct genetic entity. *J Am Soc Nephrol.* 1997; 8:77–84. [PubMed: 9013451]
11. Hildebrandt F, et al. Nephronophthisis: disease mechanisms of a ciliopathy. *J Am Soc Nephrol.* 2009; 20:23–35. [PubMed: 19118152]
12. Lancaster MA, Gleeson JG. The primary cilium as a cellular signaling center: lessons from disease. *Curr Opin Genet Dev.* 2009; 19:220–229. [PubMed: 19477114]
13. Angers S, Moon RT. Proximal events in Wnt signal transduction. *Nat Rev Mol Cell Biol.* 2009; 10:468–477. [PubMed: 19536106]
14. Petersen CP, Reddien PW. Smed-betacatenin-1 is required for anteroposterior blastema polarity in planarian regeneration. *Science.* 2008; 319:327–330. [PubMed: 18063755]
15. MacDonald BT, et al. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell.* 2009; 17:9–26. [PubMed: 19619488]
16. Kim E, et al. The polycystic kidney disease 1 gene product modulates Wnt signaling. *J Biol Chem.* 1999; 274:4947–4953. [PubMed: 9988738]
17. Zhang K, et al. PKD1 inhibits cancer cells migration and invasion via Wnt signaling pathway in vitro. *Cell Biochem Funct.* 2007; 25:767–774. [PubMed: 17437318]
18. Zheng R, et al. Polycystin-1 induced apoptosis and cell cycle arrest in G0/G1 phase in cancer cells. *Cell Biol Int.* 2008; 32:427–435. [PubMed: 18289888]
19. Xiao Z, et al. Conditional disruption of Pkd1 in osteoblasts results in osteopenia due to direct impairment of bone formation. *J Biol Chem.* 2010; 285:1177–1187. [PubMed: 19887454]
20. Lal M, et al. Polycystin-1 C-terminal tail associates with beta-catenin and inhibits canonical Wnt signaling. *Hum Mol Genet.* 2008; 17:3105–3117. [PubMed: 18632682]
21. Kim I, et al. Conditional mutation of Pkd2 causes cystogenesis and upregulates beta-catenin. *J Am Soc Nephrol.* 2009; 20:2556–2569. [PubMed: 19939939]
22. Lantinga-van Leeuwen IS, et al. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. *Hum Mol Genet.* 2004; 13:3069–3077. [PubMed: 15496422]
23. Torres VE, Harris PC. Mechanisms of Disease: autosomal dominant and recessive polycystic kidney diseases. *Nat Clin Pract Nephrol.* 2006; 2:40–55. [PubMed: 16932388]
24. Thivierge C, et al. Overexpression of PKD1 causes polycystic kidney disease. *Mol Cell Biol.* 2006; 26:1538–1548. [PubMed: 16449663]
25. Kurbegovic A, et al. Pkd1 transgenic mice: adult model of polycystic kidney disease with extrarenal and renal phenotypes. *Hum Mol Genet.* 2010; 19:1174–1189. [PubMed: 20053665]
26. Ward CJ, et al. Polycystin, the polycystic kidney disease 1 protein, is expressed by epithelial cells in fetal, adult, and polycystic kidney. *Proc Natl Acad Sci U S A.* 1996; 93:1524–1528. [PubMed: 8643665]
27. Saadi-Kheddouci S, et al. Early development of polycystic kidney disease in transgenic mice expressing an activated mutant of the beta-catenin gene. *Oncogene.* 2001; 20:5972–5981. [PubMed: 11593404]
28. Qian CN, et al. Cystic renal neoplasia following conditional inactivation of apc in mouse renal tubular epithelium. *J Biol Chem.* 2005; 280:3938–3945. [PubMed: 15550389]

29. Marose TD, et al. Beta-catenin is necessary to keep cells of ureteric bud/Wolffian duct epithelium in a precursor state. *Dev Biol.* 2008; 314:112–126. [PubMed: 18177851]
30. Pinson KI, et al. An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature.* 2000; 407:535–538. [PubMed: 11029008]
31. Simons M, et al. Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. *Nat Genet.* 2005; 37:537–543. [PubMed: 15852005]
32. Bergmann C, et al. Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. *Am J Hum Genet.* 2008; 82:959–970. [PubMed: 18371931]
33. Lancaster MA, et al. Impaired Wnt-beta-catenin signaling disrupts adult renal homeostasis and leads to cystic kidney ciliopathy. *Nat Med.* 2009; 15:1046–1054. [PubMed: 19718039]
34. Louie CM, Gleeson JG. Genetic basis of Joubert syndrome and related disorders of cerebellar development. *Hum Mol Genet.* 2005; 14(2):R235–242. [PubMed: 16244321]
35. Wiens CJ, et al. Bardet-Biedl syndrome-associated small GTPase ARL6 (BBS3) functions at or near the ciliary gate and modulates Wnt signaling. *J Biol Chem.* 2010; 285:16218–16230. [PubMed: 20207729]
36. Gerdes JM, et al. Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. *Nat Genet.* 2007; 39:1350–1360. [PubMed: 17906624]
37. Marion V, et al. Transient ciliogenesis involving Bardet-Biedl syndrome proteins is a fundamental characteristic of adipogenic differentiation. *Proc Natl Acad Sci U S A.* 2009; 106:1820–1825. [PubMed: 19190184]
38. Axelrod JD. Progress and challenges in understanding planar cell polarity signaling. *Semin Cell Dev Biol.* 2009; 20:964–971. [PubMed: 19665570]
39. Zhou W, et al. Nephrocystin-3 is required for ciliary function in zebrafish embryos. *Am J Physiol Renal Physiol.* 2010
40. Ross AJ, et al. Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat Genet.* 2005; 37:1135–1140. [PubMed: 16170314]
41. Happe H, et al. Toxic tubular injury in kidneys from Pkd1-deletion mice accelerates cystogenesis accompanied by dysregulated planar cell polarity and canonical Wnt signaling pathways. *Hum Mol Genet.* 2009; 18:2532–2542. [PubMed: 19401297]
42. Ferrante MI, et al. Convergent extension movements and ciliary function are mediated by ofd1, a zebrafish orthologue of the human oral-facial-digital type 1 syndrome gene. *Hum Mol Genet.* 2009; 18:289–303. [PubMed: 18971206]
43. Fischer E, et al. Defective planar cell polarity in polycystic kidney disease. *Nat Genet.* 2006; 38:21–23. [PubMed: 16341222]
44. Saburi S, et al. Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease. *Nat Genet.* 2008; 40:1010–1015. [PubMed: 18604206]
45. Sopko R, McNeill H. The skinny on Fat: an enormous cadherin that regulates cell adhesion, tissue growth, and planar cell polarity. *Curr Opin Cell Biol.* 2009; 21:717–723. [PubMed: 19679459]
46. Nikolaidou KK, Barrett K. Getting to know your neighbours; a new mechanism for cell intercalation. *Trends Genet.* 2005; 21:70–73. [PubMed: 15661350]
47. Simons M, Walz G. Polycystic kidney disease: cell division without a clue? *Kidney Int.* 2006; 70:854–864. [PubMed: 16816842]
48. Karner CM, et al. Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. *Nat Genet.* 2009; 41:793–799. [PubMed: 19543268]
49. Nishio S, et al. Loss of oriented cell division does not initiate cyst formation. *J Am Soc Nephrol.* 2010; 21:295–302. [PubMed: 19959710]
50. Fliegauf M, et al. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol.* 2007; 8:880–893. [PubMed: 17955020]
51. Mokrzan EM, et al. Differences in renal tubule primary cilia length in a mouse model of Bardet-Biedl syndrome. *Nephron Exp Nephrol.* 2007; 106:e88–96. [PubMed: 17519557]

52. Cardenas-Rodriguez M, Badano JL. Ciliary biology: understanding the cellular and genetic basis of human ciliopathies. *Am J Med Genet C Semin Med Genet.* 2009; 151C:263–280. [PubMed: 19876935]
53. Nigg EA, Raff JW. Centrioles, centrosomes, and cilia in health and disease. *Cell.* 2009; 139:663–678. [PubMed: 19914163]
54. Berbari NF, et al. The primary cilium as a complex signaling center. *Curr Biol.* 2009; 19:R526–535. [PubMed: 19602418]
55. Pazour GJ, et al. Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol.* 2000; 151:709–718. [PubMed: 11062270]
56. Yoder BK, et al. Insertional mutagenesis and molecular analysis of a new gene associated with polycystic kidney disease. *Proc Assoc Am Physicians.* 1995; 107:314–323. [PubMed: 8608416]
57. Otto EA, et al. Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat Genet.* 2003; 34:413–420. [PubMed: 12872123]
58. Nakaya MA, et al. Wnt3a links left-right determination with segmentation and anteroposterior axis elongation. *Development.* 2005; 132:5425–5436. [PubMed: 16291790]
59. Thoma CR, et al. pVHL and GSK3beta are components of a primary cilium-maintenance signalling network. *Nat Cell Biol.* 2007; 9:588–595. [PubMed: 17450132]
60. Schermer B, et al. The von Hippel-Lindau tumor suppressor protein controls ciliogenesis by orienting microtubule growth. *J Cell Biol.* 2006; 175:547–554. [PubMed: 17101696]
61. Voronina VA, et al. Inactivation of Chibby affects function of motile airway cilia. *J Cell Biol.* 2009; 185:225–233. [PubMed: 19364920]
62. Karcher C, et al. Lack of a laterality phenotype in Pkd1 knock-out embryos correlates with absence of polycystin-1 in nodal cilia. *Differentiation.* 2005; 73:425–432. [PubMed: 16316413]
63. Corbit KC, et al. Kif3a constrains beta-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms. *Nat Cell Biol.* 2008; 10:70–76. [PubMed: 18084282]
64. McDermott KM, et al. Primary Cilia Regulate Branching Morphogenesis during Mammary Gland Development. *Curr Biol.* 2010
65. Ocbina PJ, et al. Primary cilia are not required for normal canonical Wnt signaling in the mouse embryo. *PLoS One.* 2009; 4:e6839. [PubMed: 19718259]
66. Huang P, Schier AF. Dampened Hedgehog signaling but normal Wnt signaling in zebrafish without cilia. *Development.* 2009; 136:3089–3098. [PubMed: 19700616]
67. Kishimoto N, et al. Cystic kidney gene seahorse regulates cilia-mediated processes and Wnt pathways. *Dev Cell.* 2008; 14:954–961. [PubMed: 18539122]
68. Bonnet CS, et al. Defects in cell polarity underlie TSC and ADPKD-associated cystogenesis. *Hum Mol Genet.* 2009; 18:2166–2176. [PubMed: 19321600]
69. Oishi I, et al. Regulation of primary cilia formation and left-right patterning in zebrafish by a noncanonical Wnt signaling mediator, duboraya. *Nat Genet.* 2006; 38:1316–1322. [PubMed: 17013396]
70. Park TJ, et al. Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat Genet.* 2006; 38:303–311. [PubMed: 16493421]
71. Park TJ, et al. Dishevelled controls apical docking and planar polarization of basal bodies in ciliated epithelial cells. *Nat Genet.* 2008; 40:871–879. [PubMed: 18552847]
72. Hashimoto M, et al. Planar polarization of node cells determines the rotational axis of node cilia. *Nat Cell Biol.* 2010; 12:170–176. [PubMed: 20098415]
73. Tamm SL, et al. The role of cortical orientation in the control of the direction of ciliary beat in Paramecium. *J Cell Biol.* 1975; 64:98–112. [PubMed: 45847]
74. Antic D, et al. Planar cell polarity enables posterior localization of nodal cilia and left-right axis determination during mouse and Xenopus embryogenesis. *PLoS One.* 2010; 5:e8999. [PubMed: 20126399]

75. Mitchell B, et al. The PCP pathway instructs the planar orientation of ciliated cells in the *Xenopus* larval skin. *Curr Biol*. 2009; 19:924–929. [PubMed: 19427216]
76. Maisonneuve C, et al. Bicaudal C, a novel regulator of Dvl signaling abutting RNA-processing bodies, controls cilia orientation and leftward flow. *Development*. 2009; 136:3019–3030. [PubMed: 19666828]
77. Jones C, et al. Ciliary proteins link basal body polarization to planar cell polarity regulation. *Nat Genet*. 2008; 40:69–77. [PubMed: 18066062]
78. Jonassen JA, et al. Deletion of IFT20 in the mouse kidney causes misorientation of the mitotic spindle and cystic kidney disease. *J Cell Biol*. 2008; 183:377–384. [PubMed: 18981227]
79. Mitchell B, et al. A positive feedback mechanism governs the polarity and motion of motile cilia. *Nature*. 2007; 447:97–101. [PubMed: 17450123]
80. Piontek K, et al. A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1. *Nat Med*. 2007; 13:1490–1495. [PubMed: 17965720]
81. Schmidt-Ott KM, et al. beta-catenin/TCF/Lef controls a differentiation-associated transcriptional program in renal epithelial progenitors. *Development*. 2007; 134:3177–3190. [PubMed: 17693601]
82. Schmidt-Ott KM, Barasch J. WNT/beta-catenin signaling in nephron progenitors and their epithelial progeny. *Kidney Int*. 2008; 74:1004–1008. [PubMed: 18633347]
83. Schedl A. Renal abnormalities and their developmental origin. *Nat Rev Genet*. 2007; 8:791–802. [PubMed: 17878895]
84. Carroll TJ, et al. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell*. 2005; 9:283–292. [PubMed: 16054034]
85. Park JS, et al. Wnt/beta-catenin signaling regulates nephron induction during mouse kidney development. *Development*. 2007; 134:2533–2539. [PubMed: 17537789]
86. Yu J, et al. A Wnt7b-dependent pathway regulates the orientation of epithelial cell division and establishes the cortico-medullary axis of the mammalian kidney. *Development*. 2009; 136:161–171. [PubMed: 19060336]
87. Calvet JP. Injury and development in polycystic kidney disease. *Curr Opin Nephrol Hypertens*. 1994; 3:340–348. [PubMed: 7922262]
88. Takakura A, et al. Renal injury is a third hit promoting rapid development of adult polycystic kidney disease. *Hum Mol Genet*. 2009; 18:2523–2531. [PubMed: 19342421]
89. Patel V, et al. Acute kidney injury and aberrant planar cell polarity induce cyst formation in mice lacking renal cilia. *Hum Mol Genet*. 2008; 17:1578–1590. [PubMed: 18263895]
90. Patel V, et al. Advances in the pathogenesis and treatment of polycystic kidney disease. *Curr Opin Nephrol Hypertens*. 2009; 18:99–106. [PubMed: 19430332]
91. Lin SL, et al. Macrophage Wnt7b is critical for kidney repair and regeneration. *Proc Natl Acad Sci U S A*. 2010; 107:4194–4199. [PubMed: 20160075]
92. Davenport JR, et al. Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. *Curr Biol*. 2007; 17:1586–1594. [PubMed: 17825558]
93. Kottgen M, et al. TRPP2 and TRPV4 form a polymodal sensory channel complex. *J Cell Biol*. 2008; 182:437–447. [PubMed: 18695040]
94. Moch H. Cystic renal tumors: new entities and novel concepts. *Adv Anat Pathol*. 2010; 17:209–214. [PubMed: 20418675]
95. Bonsib SM. Renal cystic diseases and renal neoplasms: a mini-review. *Clin J Am Soc Nephrol*. 2009; 4:1998–2007. [PubMed: 19875768]
96. Wong SY, et al. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med*. 2009; 15:1055–1061. [PubMed: 19701205]
97. Han YG, et al. Dual and opposing roles of primary cilia in medulloblastoma development. *Nat Med*. 2009; 15:1062–1065. [PubMed: 19701203]
98. Deschaseaux F, et al. Mechanisms of bone repair and regeneration. *Trends Mol Med*. 2009; 15:417–429. [PubMed: 19740701]

99. Chen Y, et al. Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. *PLoS Med.* 2007; 4:e249. [PubMed: 17676991]
100. Flozak AS, et al. Beta-catenin/T-cell factor signaling is activated during lung injury and promotes the survival and migration of alveolar epithelial cells. *J Biol Chem.* 2010; 285:3157–3167. [PubMed: 19933277]
101. Jagasia R, et al. New regulators in adult neurogenesis and their potential role for repair. *Trends Mol Med.* 2006; 12:400–405. [PubMed: 16890023]
102. Apte U, et al. Beta-catenin activation promotes liver regeneration after acetaminophen-induced injury. *Am J Pathol.* 2009; 175:1056–1065. [PubMed: 19679878]
103. Ravine D, et al. An ultrasound renal cyst prevalence survey: specificity data for inherited renal cystic diseases. *Am J Kidney Dis.* 1993; 22:803–807. [PubMed: 8250026]
104. Kim YS, et al. The Kruppel-like zinc finger protein Glis2 functions as a negative modulator of the Wnt/beta-catenin signaling pathway. *FEBS Lett.* 2007; 581:858–864. [PubMed: 17289029]
105. Chitalia VC, et al. Jade-1 inhibits Wnt signalling by ubiquitylating beta-catenin and mediates Wnt pathway inhibition by pVHL. *Nat Cell Biol.* 2008; 10:1208–1216. [PubMed: 18806787]
106. Lokmane L, et al. vHNF1 functions in distinct regulatory circuits to control ureteric bud branching and early nephrogenesis. *Development.* 2010; 137:347–357. [PubMed: 20040500]
107. Ma D, et al. Fidelity in planar cell polarity signalling. *Nature.* 2003; 421:543–547. [PubMed: 12540853]

Figure 1. The renal nephron

Drawing of the nephron segments within the mammalian kidney. Filtration begins at the glomerulus where water and solutes are transported from the blood into the renal tubules. The proximal tubule then performs the majority of reabsorption of salts and water that takes place. The thin descending segment of the Loop of Henle reabsorbs water, whereas the thin ascending segment and the distal tubule reabsorb salt. Finally, the connecting tubule and collecting duct can be triggered to absorb water from the urine in the presence of vasopressin, thus concentrating the urine. Cilia are present in almost all tubule segments. Patients with PKD exhibit cysts in all nephron segments, whereas patients with NPHP exhibit cysts preferentially in the proximal tubules and collecting ducts of the corticomedullary region (insets depict these regions of the nephron). NPHP kidneys also exhibit other abnormalities such as basement membrane thickening and interstitial fibrosis. Both types of cystic kidney disease are characterized by defects in urine concentrating ability.

a Canonical Wnt/ β -catenin

b Noncanonical/Planar Cell Polarity

Figure 2. The Wnt Pathways

(a) Drawing of the canonical Wnt/ β -catenin pathway. In the “off” state (right), β -catenin (β Cat) is targeted for degradation by phosphorylation by the destruction complex. When Wnt is present, the pathway is “on” (left), and the destruction complex is inhibited by Dvl, leading to cytosolic accumulation of β -catenin (β Cat) which is transported into the nucleus where it activates transcription through Tcf. Disease proteins involved in this process are shown; activators are on the left, whereas inhibitors are on the right: PC1, Jouberin (Jbn encoded by *Ahi1*), Gli-similar 2 (Glis2), BBS1, BBS3, BBS4, BBS6, BBS10, BBS12, Nph3, Inversin (Inv), Tsc1 and Tsc2 (Tsc1/2), VHL. Although not depicted, HNF1 β acts

upstream of the Wnt ligand¹⁰⁶. **(b)** Schematic of the noncanonical/planar cell polarity pathway. PCP might be activated by a Wnt ligand, followed by activation of Frizzled (Fzd) and membrane-associated Dvl. Dvl activates Ras homolog gene family member A (RhoA) or Ras-related C3 botulinum toxin substrate 1 (Rac1) to stimulate c-Jun N-terminal kinase (JNK) and Rho-associated kinase (Rock), which leads to downstream cytoskeletal rearrangements and transcriptional activation. Fuzzy (Fy) and Inturned (Int) are effectors that probably function downstream of Dvl in PCP signaling, while Fat and Dachshous (Ds) might regulate upstream of Fzd¹⁰⁷. Additional regulators and disease proteins involved include: Vangl, Prickle (Pk), c-Jun, Disheveled-associated activator of morphogenesis 1 (Daam1), Four-jointed (Fjx), Inv, Nphp3, PC1, Ofd1, BBS1, BBS4, and BBS6.

Figure 3. Crosstalk between Wnt pathways and cilia

Schematic showing the regulation of Wnt components by the cilium as well as the regulation of cilia formation by Wnt components. Canonical components are shown at the left and noncanonical at the right. The components involved in canonical Wnt signaling, which localize to or are regulated by the cilium, include Fzd1, phospho- β -catenin and the proteasome. Noncanonical components, which localize to the cilium, include Fat4, Vangl2, Fuzzy (Fy) and Inturned (In). Wnt components that regulate aspects of cilia formation include Chibby (Cby), Gsk3 β , Vangl, Prickle (Pk) and Fzd3. Disease genes involved in canonical Wnt-cilia crosstalk include PC1, Tsc1 and Tsc2 (Tsc1/2), BBS4, BBS6, BBS10, and BBS12, while Duboraya (Dub), Odf1, VHL, and Seahorse regulate noncanonical Wnt-cilia crosstalk.

Figure 4. Cyst formation during development and injury

The developing kidney tubule epithelium forms from the differentiation of mesenchymal precursor cells. This process is triggered by canonical Wnt signaling that induces pretubular aggregates, a cluster of cells which are epithelial progenitors. Subsequently, canonical Wnt signaling must be turned off and noncanonical Wnt signaling takes over to form the epithelium and the polarized tubule structure. When injury occurs, this process or a similar one is reactivated and both canonical and noncanonical Wnt signaling are again required for new tubule formation. Cyst formation results from abnormalities in any of these cellular processes during development or during injury repair. The inset depicts the model of convergent extension in tubule elongation during new renal tubule formation.

Table 1

Cystic renal diseases and their genetic causes

<u>Syndrome Name</u>	<u>Genes Identified</u>	<u>Syndrome Name</u>	<u>Genes Identified</u>
<u>Arthrogryposis-renal dysfunction cholestasis (ARC)</u>	<i>VPS33B</i>	<u>Congenital nephrotic syndrome, Finnish</u>	<i>NPHS1</i>
<u>Cornelia de Lange syndrome</u>	<i>NIPBL, SMC1L1, SMC3</i>	<u>Down syndrome</u>	Trisomy 21
<u>Multiple acyl-CoA dehydrogenase deficiency</u>	<i>ETFA, ETFB, ETFD</i>	<u>Phocomelia syndrome</u>	<i>ESCO2</i>
<u>Brachymesomelia-renal syndrome</u>	None identified	<u>Smith-Lemli-Opitz syndrome</u>	<i>DHCR7</i>
<u>Asplenia with cardiovascular abnormalities</u>	None identified	<u>Zellweger's cerebrohepatorenal syndrome</u>	<i>PEX1-6, 12, 14, 26</i>
<u>Marden-Walker syndrome</u>	None identified	<u>Maturity Onset Diabetes of the Young (MODY)/Renal cysts and diabetes syndrome</u>	<i>HNF1β</i> * ⁴³
Ciliopathies			
<u>Bardet-Biedl Syndrome (BBS)</u>	<i>BBS1</i> * ^{36, 40} , <i>BBS2</i> , <i>BBS3</i> / <i>ARL6</i> * ³⁵ , <i>BBS4</i> * ^{36, 40} , <i>BBS5</i> , <i>BBS6</i> / <i>MKKS</i> * ^{36, 40} , <i>BBS7</i> , <i>BBS8</i> / <i>TTC8</i> , <i>BBS9</i> / <i>PTHB1</i> , <i>BBS10</i> * ³⁷ , <i>BBS11</i> / <i>TRIM32</i> , <i>BBS12</i> * ³⁷	<u>Nephronophthisis (NPHP)</u>	<i>NPHP1</i> , <i>NPHP2</i> ^{Inv} * ³¹ , <i>NPHP3</i> * ³² , <i>NPHP4</i> , <i>NPHP5</i> / <i>IQCB1</i> , <i>NPHP6</i> / <i>CEP290</i> , <i>NPHP7</i> / <i>GLIS2</i> * ¹⁰⁴ , <i>NPHP8</i> / <i>RPGRIP1L</i> , <i>NPHP9</i> / <i>NEK8</i>
<u>Polycystic Kidney Disease (PKD)</u>	<i>PKD1</i> / <i>PC1</i> * ^{16, 20, 41} , <i>PKD2</i> / <i>PC2</i> * ²¹ , <i>PKHD1</i> / <i>FPC</i>	<u>NPHP-like</u>	<i>XPNPEP3</i>
<u>Joubert syndrome (JS)</u>	<i>AH11</i> / <i>Jbn</i> * ³³ , <i>NPHP1</i> , <i>CEP290</i> / <i>NPHP6</i> , <i>MKS3</i> / <i>TMEM67</i> , <i>RPGRIP1L</i> , <i>ARL13b</i> , <i>CC2D2A</i> , <i>INPP5E</i>	<u>Meckel-Gruber syndrome (MKS)</u>	<i>MKS1</i> , <i>MKS3</i> / <i>TMEM67</i> , <i>CEP290</i> / <i>NPHP6</i> , <i>CC2D2A</i>
<u>Alstrom syndrome</u>	<i>ALMS1</i>	<u>Jeune Asphyxiating Thoracic Dystrophy (JATD)</u>	<i>IFT80</i>
<u>Short-rib-polydactyly</u>	<i>DYNCH2H1</i>	<u>Von Hippel Lindau syndrome (VHL)</u>	<i>VHL</i> * ¹⁰⁵
<u>Oral-facial-digital syndrome (OFD)</u>	<i>OFD1</i> * ^{42, 63}	<u>Medullary Cystic Kidney Disease (MCKD)</u>	<i>UMOD</i>
<u>Tuberous Sclerosis Complex (TSC)</u>	<i>TSC1</i> * ⁶⁸ , <i>TSC2</i> * ⁶⁸		

Data available on OMIM.

* denotes genes with evidence for a role in canonical or noncanonical Wnt signaling (with references as indicated).