



Published in final edited form as:

Kidney Int. 2015 October ; 88(4): 699–710. doi:10.1038/ki.2015.207.

A polycystin-centric view of cyst formation and disease: the polycystins revisited

Albert CM Ong¹ and Peter C Harris²

¹Kidney Genetics Group, Academic Nephrology Unit, Department of Infection and Immunity, University of Sheffield Medical School, Sheffield, UK

²Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA

Abstract

It is 20 years since the identification of *PKD1*, the major gene mutated in autosomal dominant polycystic kidney disease (ADPKD), followed closely by the cloning of *PKD2*. These major breakthroughs have led in turn to a period of intense investigation into the function of the two proteins encoded, polycystin-1 and polycystin-2 and how defects in either protein lead to cyst formation and non-renal phenotypes. In this review, we summarise the major findings in this area and present a current model of how the polycystin proteins function in health and disease.

Keywords

ADPKD; PKD1; PKD2; polycystin-1; polycystin-2; TRPP2; primary cilia; cysts

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the fourth ranked cause of kidney failure and the most common inherited nephropathy. Beyond the kidney, the major organs affected include the liver, pancreas, heart and intracerebral arteries. Although long considered an untreatable disease, the availability of new treatments that could alter its natural history are becoming a reality^{1,2}.

Mutations in two genes, *PKD1* and *PKD2*, cause ADPKD, with the existence of '*PKD3*' in doubt following reanalysis of the reported unlinked cases³. In typical renal clinic populations, PKD1 accounts for 80–85% of cases with PKD2 accounting for the remainder. Although PKD1 and PKD2 patients have overlapping renal and extra-renal features, truncating *PKD1* mutations are associated with the poorest renal survival whereas *PKD2* mutations have the best outcomes; non-truncating *PKD1* mutations display an intermediate spectrum⁴.

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:http://www.nature.com/authors/editorial_policies/license.html#terms

Correspondence: Albert CM Ong (a.ong@sheffield.ac.uk) or Peter C. Harris (harris.peter@mayo.edu).

Disclosure: None declared

PKD1 and *PKD2* encode the proteins, polycystin-1 (PC1) and polycystin-2 (PC2), respectively⁵. Early studies showed that tissue expression of both proteins was largely overlapping although not identical^{6, 7}. In addition, *Pkd2* expression appeared more constant while that of *Pkd1* was developmentally regulated⁸. Biochemical studies indicate that both proteins interact to form a polycystin (PC) complex^{9–11}. However this needs to be reconciled with the non-overlapping subcellular location/s of both proteins, especially the predominant localisation of PC2 in the ER¹² where it may function as a Ca²⁺ release channel^{13–15}.

ADPKD disease mechanism

There is agreement that ADPKD is associated with loss of function; null *Pkd1* or *Pkd2* mouse models are lethal and develop cysts by ~E13.5^{16, 17}. Disagreement has centred on whether a complete loss of the normal allele is required (two hit hypothesis) or whether cysts can develop once the level of function protein falls below a specific level (threshold hypothesis)¹⁸. Consistent with the need for two hits, inactivating and base pair somatic mutations have been identified in ADPKD renal and liver cystic epithelium, and cysts are clonal and so may be derived from a single cell^{19–21}. The hypermutable *Pkd2* allele (WS25), develops cysts following spontaneous loss of the normal allele²² and induced mutation in conditional models shows that loss of the second allele causes cystogenesis. However, the timing of this event significantly influences disease severity; before P13, cysts rapidly develop, while later loss results in slowly progressive disease^{23, 24}. The timing of somatic mutation thus significantly influences growth rates, possibly due to the basal level of proliferation or a critical developmental window. Somatic mutation could explain the focal nature of cyst development, found in a limited number of nephrons. It has also been suggested that PKD1 is a more severe disease than PKD2 due to *PKD1* being a larger mutational target²⁵.

On the other hand, hypomorphic models indicate that cysts can develop even if low levels of normal PC1 (15–20%) are present^{26, 27} and cysts in *Pkd1*^{+/+}; *Pkd1*^{-/-} chimeric mice initially comprise cells of both genotypes, only later (and associated with histological changes) being all *Pkd1*^{-/-}²⁸. *PKD2* somatic mutations in PKD1 cysts and vice versa^{29, 30}, plus other karyotypic changes in developing cysts³¹ also indicate complexity in cyst development, rather than due simply to the loss of both alleles¹⁸.

A threshold model of cystogenesis in ADPKD

The data presented above and recent evidence that different types of *PKD1* mutation (truncating and non-truncating) are associated with different disease severity⁴, that humans and mice with two incompletely penetrant alleles can be viable (severe to typical disease), and that *in utero* onset ADPKD can be due to biallelic *PKD1* or *PKD2* mutations (inactivating plus hypomorphic or two hypomorphic)^{32–36} suggests that a threshold or dosage model best explains cystogenesis in ADPKD (Figure 1). In patients with a 50% reduction of functional PC1 or PC2 (typical patients with an inactivating mutation), cysts can occur if the level of functional PC falls below the cystogenic threshold. This may occur by somatic mutation to the other allele, but stochastic cellular variability of expression of the remaining ADPKD allele^{37, 38} and other factors, such as renal damage (which has been

shown to promote cystogenesis in mice), may determine if a cyst develops^{39, 40}. It is possible that this minimum threshold itself could vary between animals, by nephron segment, developmental stage, tissue and even cell type^{23, 41, 42}. In mice, slowly progressive disease occurs if the level of functional PC1 is ~40%, but rapidly progressive disease occurs with ~20% functional PC1, highlighting the influence of dosage³⁶ (Figure 1). Since the level of functional PC seems linked to renal disease severity, treatments that can promote that level may help to slow disease progression.

Once initiated, further genetic events at the disease locus and elsewhere, similar to tumour development in cancer, plus other environmental influences, may favour growth and survival of a cyst. Of note, even in hypomorphic models, cyst development is focal³⁶ suggesting that factors in addition to the level of the functional protein are important but that once tipped into a cystogenic cascade, possibly irreversible. In conditional knockouts with low levels of induced deletion of the normal allele during adulthood, cyst development is slow but later cysts cluster around early ones, indicating that paracrine cystogenic factors may diffuse from developed cysts, although the non-randomness of Cre-inactivation should also be considered⁴³.

Mutation to the transcription factor HNF1B leads to a diverse range of kidney phenotypes including renal agenesis, dysplasia and renal malformations but most commonly cysts, occasionally phenocopying ADPKD as the Renal Cystic and Diabetes Syndrome (RCAD)⁴⁴. Both *PKD2* and *PKHD1*, the autosomal recessive PKD (ARPKD) gene, are under the transcriptional control of HNF1B⁴⁵, and a combination of *PKD1* and *HNF1B* pathogenic alleles may cause *in utero* onset PKD³⁴. This may be the result of the combination of altered regulation of HNF1B targets and the *PKD1* mutation.

The microRNA-17 (miRNA17) binds to the 3' UTR of *PKD2* and regulates the stability of *PKD2* mRNA and its translation. Transgenic overexpression of miRNA17 leads to a cystic phenotype in *Xenopus* and mice, through down-regulating *Pkd2* mRNA, confirming the importance of gene dosage⁴⁶. A transgenic model with two miRs targeting *Pkd1* also resulted in a slowly progressive PKD model⁴⁷. The RNA-binding protein, Bicaudal C (*Bicc1*), is mutated in several mouse models of PKD (*bpc*, *jcpk*) and competes with miRNA17 for binding to *Pkd2* mRNA: *Pkd2* levels are reduced with *Bicc1* mutation⁴⁸.

Disease mechanisms in non-renal tissues

A striking finding from *Pkd1* and *Pkd2* null animals is the uniform lack of liver cysts, common pancreatic cysts and variable expression of vascular abnormalities^{16, 17, 49}. These findings indicate that each tissue may have a different threshold for disease onset and expression. An unusual feature of the liver phenotype is the strong female predisposition to develop polycystic liver disease⁵⁰. This is the converse of the modest male predisposition to develop larger polycystic kidneys²⁵ and earlier ESRD⁴, which implies different modifiers of this phenotype.

The prevalence of intracranial aneurysms (ICA) in ADPKD varies between 6–16% with evidence of familial clustering^{51, 52}. ICA rupture can occur in younger individuals and before the onset of hypertension, suggesting a primary link between ICA formation and

polycystin function^{53, 54}. ICAs have been reported in only a few *Pkd1* mice and these have tended to be hypomorphic, transgenic or challenged heterozygous animals^{55–57} rather than vascular-specific knockouts⁵⁸. These findings suggest that gene dosage is important in the genesis of ICAs but that disease penetrance is determined by other factors such as genetic background and environmental ‘stress’. An early predisposing factor could be endothelial dysfunction, detected in resistance arteries of normotensive non-cystic heterozygous mice⁵⁹.

A common polycystin pathway for cystogenesis

Genetic interaction between cystogenes

Consistent with a dosage model of ADPKD cystogenesis, human and mouse studies have shown genetic interaction between cystogenes (Figure 2). Contiguous deletion of *PKD1* and the adjacent tuberous sclerosis gene (*TSC2*) results in much more severe PKD than a *PKD1* or *TSC2* mutation alone^{60, 61}. The mechanism may be related to overlap between their downstream signalling pathways involving mTOR and possibly others^{62, 63}, but a direct interaction between PC1 and the TSC2 protein, tuberin, may also be relevant⁶⁴.

Early-onset PKD foetuses with co-inheritance of mutations in *PKD2* and *PKHD1* have also been reported, suggesting another potential genetic interaction³⁴ (Figure 2). The ARPKD protein, fibrocystin (FPC), may bind to PC2 and regulate its Ca²⁺ permeability⁶⁵. Genetic studies in mice have also shown a genetic interaction between *Pkd1* and *Pkhd1*⁴¹. In one family, patients with digenic *PKD1* and *PKD2* mutations had ESRD ~20 years earlier than single mutations to either gene^{66, 67} and digenic mouse studies with hypermutable (*Pkd2*) and hypomorphic (*Pkd1*) alleles showed a similar dose dependent, synergistic phenotypic enhancement⁶⁸.

Studies of conditional kidney inactivation of polycystic liver disease (PCLD) genes (*Prkcsb*, *Sec63*) or *Pkhd1* with the ADPKD genes provide further evidence of cystogenic interaction and a dosage dependence of *Pkd1*, where the level of PC1 plays a central role⁴¹ (Figure 2). In this model, *Pkd2* cannot substitute for *Pkd1* but is required for *Pkd1* function suggesting that the PC1–PC2 complex is essential. In PCLD, the disease proteins are involved in glycosylation or translocation of membrane proteins into the ER, plus ER quality control^{69, 70} and hence, involved with processing the large and complex PC1 or PC2. It is not clear if PCLD is restricted to the liver because this organ is more dosage sensitive for these proteins or if somatic mutations occur more frequently in the liver⁴¹.

Polycystin structure and function

Polycystin-1

The 3D structure of PC1 remains unresolved and continues to prove challenging in view of the size, and complexity of the protein. Nonetheless, the modular domain structure of the protein has stimulated study of isolated domains. Figure 3 depicts our current view of the structure of the protein.

The GAIN domain—A major advance has been the crystallisation of the GPCR-Autoproteolysis Inducing (GAIN) regulatory domain⁷¹, a 320 residue region which includes

the 50 residue G protein-coupled receptor proteolysis site (GPS) motif which mediates *cis*-autoproteolysis of PC1 to generate a 320kDa (unglycosylated) N-terminal product (NTP) and a 140kDa C-terminal product (CTP) which remain non-covalently bound⁷². The GAIN domain is evolutionarily conserved and found in all 33 cell-adhesion GPCRs, orphan receptors with no known ligands, as well as all 5 PC1-like proteins⁷¹ (Figure 3). The functional role of this cleavage is not clear but may be a mechanism of stabilising protein folding and hence trafficking. An unanswered question is whether the uncleaved form of PC1 is functional since the extent of cleavage of the endogenous protein varies in different cells. A *Pkd1* GPS cleavage mouse mutant (*Pkd1*^{v/v}, T3041V) escapes embryonic lethality but develops rapidly progressive post-natal PKD⁷², suggesting a role for the uncleaved form during development. The interaction of PC1 with PC2 has been suggested as required for efficient GPS cleavage⁷³. However, recent data indicates that PC1 is efficiently cleaved even in *Pkd2* null cells and in mutant PC1 with the C-tail deleted^{68, 74}. An abundant, surface expressed cleaved NTP, detached from the CTP, of unknown function has been reported⁷⁵.

The PKD domain—An extracellular region that comprises 30% of PC1 (16 copies) is the Polycystic Kidney Disease (PKD) domain, which has similar topology to Ig-like and fibronectin type III domains⁷⁶. The first PKD domain is separated from the other 15, which exist in tandem (Figure 3). This arrangement of tandem Ig-like domains is reminiscent of proteins with structural and mechanical roles such as titin, fibronectin and NCAM. PKD domains have been identified in surface layer proteins of some archaeobacteria where they may mediate the formation of multicellular structures. The crystal structure of a *Methanosarcina* PKD domain shows the highest similarity to the NMR structure of PKD domain 1, revealing its possible role in evolution from unicellular to multicellular organisms⁷⁷.

Biophysical studies have shown that the PC1 extracellular domain is highly extensible through the folding and unfolding of the PKD domains^{78, 79}. In addition, the PKD domains (human and archae) appear to be stabilised by force-induced formation of a stable intermediate state⁸⁰. These studies are consistent with a role for PC1 in mechanical coupling between cells, e.g. in maintaining normal tubular lumen diameter under flow. Indeed, a functional role for the PC1 extracellular domains (including the PKD domains) in mediating cell adhesion and/or cell junction formation has been demonstrated in mammalian cells from several species^{81–83}, likely via *trans*-homophilic interactions of the PKD domains and *cis*-heterophilic interactions with E-cadherin⁸¹.

The PLAT domain—The Polycystin-1 Lipoygenase Alpha-Toxin (PLAT) domain is considered a signature domain of the PC1-like proteins^{84, 85} (Figure 3), and is identified in over 1000 different proteins. The PLAT domain crystal structure determined from murine Rab6-interacting protein 1 (Rab6IP1) and white sea coral 11R-lipooxygenase^{86, 87} show a β sandwich with two sheets of four strands each. Sequence homology to PC1 PLAT is however low (20%). Genetic studies of LOV-1, the *C.elegans* PC1 homologue, have implicated PLAT in mediating male mating behaviour in worms⁸⁸, but the domain structure and function of worm and human PC1 are clearly divergent. Recent NMR studies of human PC1 PLAT show discrete binding motifs to acidic phospholipids and Ca²⁺ and that it is

phosphorylated (Ong ACM, submitted). It is likely that PC1 PLAT functions as a lipid/protein binding scaffold to integrate cell signalling and PC1 trafficking.

The C-terminal tail—The best studied region of PC1 is the ~200aa C-terminal tail although its 3D structure is still unknown. This contains several functional motifs including a coiled-coil domain (aa4220–4251) which mediates binding to PC2 and a G-protein binding and activation sequence (aa4111–4184) for heterotrimeric G proteins. It can be phosphorylated *in vitro* and has been shown to bind protein phosphatases which regulate PC1 and PC2 phosphorylation^{89, 90}. Other key signalling proteins or effectors (eg. mTOR, Wnt, Jak/Stat) may be similarly regulated^{91–93} (see below).

Polycystin-2

A strong body of evidence indicates that PC2 functions primarily as a high conductance non-selective Ca²⁺ permeable channel^{13, 94, 95} and specific missense mutations (eg D511V) abolish Ca²⁺ permeability in the mutant protein^{13, 14}. Sequence homology to other TRP channels supports this hypothesis: PC2 is also known as TRPP2⁹⁶. What is less clear is where its primary site of action resides (or whether there are multiple sites of action) and whether channel opening is directly regulated by PC1, Ca²⁺ binding (via its EF-hand) and/or phosphorylation (see below). However it is predicted that mutation of either *PKD1* or *PKD2* leads to lower cytoplasmic Ca²⁺ concentrations (reduced entry and/or release) resulting in dysregulated cAMP levels and downstream signalling⁹⁷.

A second coiled coil domain (CC2)—A second coiled coil domain (CC2) distal to the original, CC1, mediates oligomerisation of the PC2 C-terminus (CT2) (to form dimers or trimers), an event essential for recognition and binding of the PC1 C-terminus (CT1) to form a functional PC1/PC2 protein complex^{14, 98} (Figure 3). The binding region in CT1 involves the coiled coil domain, although the precise stoichiometry of the complex is still debated.

Other oligomerisation motifs—Native PC2 forms higher order oligomers (tetramers, others) consistent with the likely tetrameric structure of other TRP channels^{11, 99}. An N-terminal dimerization motif and a single cysteine residue (C632), which mediates disulphide bonding, likely contribute to the formation of PC2 tetramers^{100, 101}.

EF-hand—Invertebrate PC2 homologues have two canonical EF hands (EF1, EF2), whereas vertebrate homologues have a single functional Ca²⁺ binding motif (EF2) due to a 4aa deletion in the Ca²⁺ binding loop of EF1¹⁰². The functional effect of Ca²⁺ binding may favour monomer over dimer formation¹⁰³ and/or regulate certain Ca²⁺ dependent interactions, rather than directly impact channel open probability¹⁰⁴.

Membrane targeting motifs—The detection of discrete ciliary and plasma membrane (PM) pools of PC2 has led to a search for distinct targeting signals for each compartment. Conserved ciliary targeting (⁶RVxP⁹) and PM (⁵⁷²KxxxF⁵⁷⁶) export motifs have been reported^{105, 106}. Mutation of the RVxP motif does not abolish localisation of PC2 to nodal cilia or its function in left-right determination¹⁰⁷ but the functionality of the KxxxF motif has not been tested *in vivo*. It has been postulated that PC2 could be directed from the *cis*-

Golgi without transversing the *trans*-Golgi to the cilia or PM, independently of PC1¹⁰⁶. Two different PC1 sequences (aa4296–4302; aa4100–4204) have been proposed as required for ciliary localisation by heterologous expression^{108, 109}. Several other pathways have been reported to regulate the surface expression of mammalian PC2. These include GSK3, PKA and CAMK-dependent pathways^{90, 110, 111}. However, it is not known if these signals are independent of PC1 or the targeting motifs reported.

Post-translational modification

Phosphorylation and Dephosphorylation

Although multiple phosphorylated residues have been identified from MS analysis, functional evidence for a regulatory role is limited. The best evidence comes from studies of five serine residues (S76, S80, S801, S812, S829) in PC2, each phosphorylated by a different kinase (GSK3, PrKD1, CK2, PKA)^{90, 110, 112, 113}. These appear to regulate a number of key steps such as forward and retrograde trafficking, surface localisation, Ca²⁺ permeability and Ca²⁺ regulation of PC2^{90, 110, 112–114}. An important link to PC1 was the striking finding that S829, a PKA activated site in PC2, remains constitutively phosphorylated in the absence of PC1⁹⁰. The mechanism appears due to loss of protein phosphatase-1 action (PP1 binds to CT1)^{89, 90}. PC1 is reported to bind to several receptor tyrosine phosphatases but it is unclear whether endogenous PC1 or PC2 are substrates for tyrosine kinases¹¹⁵.

The NEK8 kinase is mutated in patients with nephronophthisis (NPHP9), in the spontaneous *jck* cystic mouse and the Lewis polycystic kidney (*lpk*) rat^{116–118}. In *jck* kidneys, PC2 appears to be hyperphosphorylated¹¹⁹. NEK8 is known to act on other substrates such as ANKS1 but there is no evidence yet that PC2 is a substrate¹²⁰.

Ubiquitination and proteosomal degradation

Both PC1 and PC2 are subject to ubiquitin-dependent proteosomal degradation through the binding of different E3 ubiquitin ligases, Siah-1 and TAZ^{121, 122}. *Taz* mutant mice develop predominant glomerular cysts and are associated with lower PC2 levels¹²³. Nek1 in turn has been shown to phosphorylate TAZ leading to its degradation and increased PC2 levels, possibly leading to cystogenesis¹²⁴. TAZ is known to mediate both Hippo signalling and canonical Wnt responses through regulating the degradation of β -catenin¹²⁵. ER associated degradation (ERAD) of PC2 can be regulated by PRKCSH^{126, 127}. These pathways may be especially important during nephrogenesis where the dosage of both proteins is critical for tubular elongation or in the adult kidney for appropriate repair following injury^{128, 129}.

PC1 proteolytic cleavage

Additional CT1 cleavage (following GPS cleavage) generating two CT1 cleavage products (CCP) of differing length (17, 34 kDa) may occur^{130, 131} with these products acting as co-activators of several transcriptional pathways (Stat, Wnt, CHOP), independent of the NTP^{130, 131}. A third reported cleavage event generating a P100 CCP might regulate store-operated Ca²⁺ entry independently of PC2 through inhibiting translocation of the ER Ca sensor STIM-1¹³².

Is ADPKD a ciliopathy?

Accumulated data over the past 15 years has implicated functional defects in primary cilia in ADPKD pathogenesis but there is also conflicting information¹³³.

Primary cilia involved in PKD

Most cell types have a primary cilium whose role is sensory/mechanosensory. The cilium is rooted in the basal body and has a specific mechanism, intraflagellar transport (IFT), for distributing proteins anterograde and retrograde, which is essential for cilia formation and resorption¹³⁴. The cilium is a partially separate compartment with the transition zone and transition fibers acting as a ciliary gate. In epithelial cells, the cilium extends from the apical surface into the tubule lumen. Cilia are essential for certain developmental signaling pathways, most notably sonic hedgehog (Shh), and mechanosensory functions, like detecting flow; functional cilia are essential for normal development¹³⁵.

The initial evidence that PKD (ADPKD) is a ciliopathy came from the determination that homologous proteins to PC1 (*lov1*) and PC2 (*pkd2*) in *C. elegans* are localized to sensor cilia and loss of either results in defective mating behaviour^{136, 137}. Next, a mouse model defective for the IFT protein, IFT88, was found to develop PKD^{138, 139}. Subsequently, both PC1 and PC2 were localized to cilia in mammalian cells^{140, 141}; recent data has emphasized this localization and interaction of these proteins in cilia^{68, 74}. Another study showed that knocking out *Kif3A* (an IFT motor subunit) in the collecting duct resulted in PKD in this segment¹⁴². While structural cilia abnormalities are found in some ciliopathies, including ARPKD¹⁴³, cilia from *Pkd1* or *Pkd2* mutations were considered normal. Recently, study of the *Pkd1*^{RC/RC} and *Pkd1*^{RC/null} hypomorphic models has revealed longer cilia, with cilia length associated with the level of functional PC1³⁶. It is unclear if the length difference is directly associated with the reduced level of PC1 or compensation for reduced cilia signalling¹⁴⁴.

Recessively inherited developmental disorders with renal defects from PKD to nephronophthisis (NPHP), Meckel syndrome (MKS), Joubert syndrome (JBTS) and Bardet Biedl syndrome (BBS) are ciliopathies¹³⁵. The cilia link includes localization of the proteins to the transition zone, other regions of the cilia, the basal body or to a protein complex termed the BBSome shown to be important for localizing membrane associated proteins to the cilium^{145, 146}. Cilia in these mutants have altered protein composition. Many of the extra-renal phenotypes observed in these disorders are also consistent with ciliary defects: retinal defects (connecting cilium are required to form the outer segment), polydactyly (cilia dependent Shh is involved in limb bud development), *situs inversus* (defects in cilia in the embryonic node), anosmia (defective olfactory cilia) and infertility (due to sperm flagella defects)¹³⁵. It seems likely but not proven that the cystic defects in these pleiotropic disorders are due to mislocalization/function of the PC-complex and FPC.

Role of the PC complex on cilia—Although there is good evidence that the PC complex is found on cilia and that cilia are associated with cyst development, the precise role of the PC complex on the cilium is more controversial. It is known that cilia can act as a flow sensor and that changes in flow or mechanical movement of the cilium induces an

influx of Ca^{2+} ¹⁴⁷. Cells null for *Pkd1*, or blocked for PC2, have been shown to lose the Ca^{2+} influx in response to flow, suggesting that the PC complex is a ciliary flow receptor^{148, 149}. Additional evidence suggests that PC2 may be involved in flow detection in the cilia, but in combination with a different TRP channel, TRPV4¹⁵⁰. In the embryonic node, PC2 also plays a role in flow detection, but this time in combination with the PC1-like protein, PC1L1, with the PC2/ PC1L1 complex sensing nodal flow^{151–153} (Figure 3). This is consistent with *Pkd2* but not *Pkd1* mice having *situs inversus*^{154, 155}. A non-dimerizing PC2 mutant (PC2-4M) that cannot bind to PC1 but still has the ability to function as an ER-located Ca^{2+} release channel and can partially rescue LR asymmetry in a *pkd2* zebrafish model, may be associated with this role for PC2¹⁴. However, inactivation of *Trpv4* or *Pkd11l1* is not associated with cyst development, questioning the link between ciliary flow sensing and PKD.

Polycystins and ciliary calcium regulation—Recently, the concept that the PC1/2 complex regulates the level of ciliary Ca^{2+} has also been thrown into doubt with the PC1L1/PC1L2 complex implicated in that task^{156, 157} (Figure 3). Furthermore, the authors argue that a change in Ca^{2+} concentration in the cilium is insufficient to trigger a global cytoplasmic Ca^{2+} response. Nevertheless, given the likely involvement of the PC1/2 complex in Ca^{2+} regulation and the links to cilia and PKD, this issue is incompletely resolved. It is possible that a stimulus other than flow (ligand/s) may be important to activate the PC complex. In addition, localized differences in Ca^{2+} concentration, such as around the ciliary base and/or additional signal amplification, e.g. through cAMP^{158, 159}, should be considered as ways that a cilia cue could be perpetuated to the cell.

Cilia, polycystins and other signalling—PC1 and PC2 are secreted at high levels in exosomes and may be involved in mediating distal nephron signalling possibly by interacting with cilia^{160–162}. Other signalling pathways likely involving cilia have also been implicated in PKD. Changes in oriented cell division (OCD) in elongating tubules were found in the PCK rat model of ADPKD¹⁶³. Planar cell polarity (PCP) which is linked to functional cilia, regulates this process, suggesting a role for PCP in controlling tubule dilatation¹⁶⁴. PC1 deficient cells also appear to lack directional movement in relation to a stimulus possibly reflecting a PCP defect¹⁶⁵. However, evidence of an essential role of abnormal OCD in cyst formation in ADPKD is conflicting^{166, 167}. A role for PCP more generally in ciliopathies is also questioned by findings of normal classical PCP processes, such as orientation of motile cilia and asymmetric expression of Vangl1 during inner ear development, in an MKS animal model, *bpck*¹⁶⁸. An alternative explanation involves the related process, convergent-extension, which controls cell movements during tubular elongation¹⁶⁹; which is dysregulated in *Pkd1* mice¹⁷⁰.

Recently, a role for Shh signaling in PKD was suggested with mutation to an IFT component IFT139 that results in abnormal Shh signaling causing cyst development, as well as other ciliopathy phenotypes¹⁷¹. Interestingly, inhibition of Shh in kidney organ culture resulted in reduced cystogenesis in a *Pkd1* model¹⁷². Mutations in the Kruppel-like zinc finger transcription factor, Glisimilar 3 (*Glis3*), result in neonatal diabetes, hypothyroidism and PKD in humans and mice^{173, 174}. GLIS3 has been localised to primary cilia and

interacts with the transcriptional co-regulator protein, WWtr1/Taz, and *Glis3* mutant kidneys have lower *Pkd1* mRNA levels, indicating that GLIS3 may regulate *Pkd1* transcription¹⁷⁴.

Cell cycle regulation and centrosome duplication—PC1 and PC2 appear to regulate the transcription of the cyclin-dependent kinase inhibitor p21, via JAK2-STAT3 signalling⁹³. This could explain in part, the increased proliferation rate observed in non-cystic tubules and the exaggerated proliferative response following renal injury^{128, 175}. In addition, PC2 may further regulate the nuclear translocation of p21 through its cytoplasmic retention by binding to the Id2 protein¹⁷⁶. PC1 and PC2 deficient cells also have an abnormal increase in centrosome number, which could lead to abnormalities in proliferation control or result in apoptosis¹⁷⁷. It is not clear whether this represents a primary effect of centrosomal duplication or is secondary to abnormal cell cycle regulation.

Cilium signalling promotes cyst growth—A surprising study in conditional knockout models of *Pkd1* and *Pkd2*, and genes essential for cilia formation, *Ift88* and *Ift20*, showed that elimination of cilia as well as loss of the PC resulted in much milder disease than loss of the PC alone¹⁷⁸. This has been interpreted as the presence of a growth-promoting stimulus from cilia that is normally suppressed by the PC complex. However, while it is known that ciliary signalling can have a growth stimulating effect through regulation of the cell/centrosome cycles, direct linkage with the PC complex seems less certain.

Non-cilia functions of polycystins

Regulation of stretch-activated channels

In mesenteric arteries, a mechanism involving PC2 in suppressing stretch activated currents (SAC) has been proposed which does not involve its channel activity¹⁷⁹. The role of PC1 is to titrate the amounts of PC2. PC1 and PC2 also regulate the opening of stretch-activated K (2P) channels in kidney cells conferring protection against apoptosis induced by mechanical stress: a mechanism that may be operational during cyst growth¹⁸⁰.

Basolateral localisation function and polarity

The proposed basolateral location of PC1 fits well with evidence that PC1 regulates or mediates cell-cell or cell-matrix adhesion⁸². Results from a recent study supports this idea showing a remarkable suppression of the *Pkd1* cystic phenotype when combined with deletion of $\beta 1$ integrin¹⁸¹.

Abnormalities in apicobasal polarity are an inconsistent finding in PC1 and PC2 deficient cells but abnormalities in cell-cell junction formation are commonly found^{182, 183}. Alterations in the basolateral trafficking of E-cadherin have been linked to misregulation of components of the exocyst complex (sec6, sec8), which are important in the establishment of the basolateral domain of epithelial cells¹⁸⁴. PC2 has also been shown to bind to Sec10, although this has been linked to cilia rather than basolateral trafficking of PC2¹⁸⁵. It is conceivable that the role of PC1 in mediating cell-cell adhesion, through *trans*-homophilic interactions, and to recruit E-cadherin through *cis*-heterophilic interactions, could regulate this process⁸¹. Changes in intercellular adhesion are paralleled by changes in cell-matrix adhesion especially to collagen I¹⁸⁶.

Functions of other polycystins

We have discussed the role of PC1L1 with PC2 in the embryonic node and PC1L1 and PC2L1 in regulating ciliary calcium, but other members also have diverse functions (Figure 3). PC1REJ has been shown to mediate the sperm acrosome reaction¹⁸⁷ and PC1L3 to be the sour taste (H⁺) detector¹⁸⁸. The role of PC1L2 is less clear but up-regulation in mice results in chronic neuromuscular impairment¹⁸⁹ while PC2L2 has been implicated in spermatogenesis¹⁹⁰. In addition to what is presently known, other combinations between PC1 and PC2 paralogs likely mediate other cilia related and unrelated functions.

Polycystin complex formation, maturation and trafficking

The localisation of both PC1 and PC2 to different subcellular compartments (cilia, PM, ER and exosomes) has led to uncertainty whether the trafficking of both proteins is co-dependent, the nature of the targeting, export or retrieval motifs, and the functional site/s of action to prevent PKD.

Ciliary and PM complexes

PC2 is most abundantly found in the ER, consistent with the normal appearance of only an immature (EndoH sensitive) glycoform¹². PC1 generates mature (EndoH resistant; NTR, CTR) and immature (EndoH sensitive; NTS, CTS) glycoforms¹¹ with NTR found on the PM, cilia and exported via the multivesicular pathway in exosomes^{36, 68, 74, 75, 161} (Figure 4). In contrast, NTS likely remains in the ER along with any unGPS-cleaved PC1. PC1 binding to PC2 is absolutely dependent on PC2 oligomerisation via its C-terminus¹⁴ and PC1 maturation and localization requires PC2 in a dose-dependent manner^{68, 74}. PC1 cleavage at the GPS/GAIN domain is a requirement for PC1 maturation and the generation of PC1 NTR^{68, 74}. A PC1 NTP unlinked to the PC1 CTP has been reported at the plasma membrane⁷⁵ but transgenic expression an NTP-like protein (F3043X) could not rescue the phenotype of GPS cleavage-deficient *Pkd1*^{v/v} (T3041V) mice⁷⁵. It is unclear if the isolated PC1-CTP is functional but one study has shown that expression of the transgene is stabilised by PC2 binding¹⁰⁹.

Both PC1 and PC2 are present in the *cis*-Golgi, but at this point it is uncertain whether they continue to be co-transported through the Golgi for maturation and export to cilia. It has been suggested that they become uncoupled at the *cis*-Golgi and undergo independent trafficking at this point and recouple in a post-Golgi compartment¹⁰⁶. However, recent evidence of a cilia localized EndoH-resistant (mature) form of PC2 suggests that they may traffic together to that organelle⁷⁴. Transport of PC2 and PC1 to PM membranes may be similarly co-dependent^{73, 74, 191}. The low level of surface labelling of PC2 compared to PC1, questions whether PM PC1 may interact with ER PC2 in membrane subdomains^{14, 68}, possibly in cooperation with IP3 receptors¹⁵.

In conclusion

In 20 years, extensive information about the mechanism of disease in ADPKD, the structure of the polycystins and where they are localized has been determined. The challenges now are to determine exactly what the PC1/2 complex does in the cilium, if PC1/2 localization

elsewhere is significant to pathogenesis and whether PC1/2 levels are rate-limiting for the onset of PKD with other cystoproteins. Answers to these questions would stimulate new therapeutic options to target cyst initiation in ADPKD and other cystic diseases.

Acknowledgements

Work in the author's laboratories was supported by grants from the Wellcome Trust, Medical Research Council, Research Councils UK, Kidney Research UK, PKD Foundation, European Union (EUF7/2007–2013, grant agreement no. 317246, TranCYST) and NIH/NIDDK (DK058816 and DK090728). We thank past and present members of the Ong and Harris laboratories for significant contributions to this work and for many stimulating discussions.

References

1. Ong AC, Devuyst O, Knebelmann B, et al. Autosomal dominant polycystic kidney disease: the changing face of clinical management. *Lancet*. 2015 in press.
2. Chang MY, Ong AC. New treatments for autosomal dominant polycystic kidney disease. *Br J Clin Pharmacol*. 2013; 76:524–535. [PubMed: 23594398]
3. Paul BM, Consugar MB, Ryan Lee M, et al. Evidence of a third ADPKD locus is not supported by re-analysis of designated PKD3 families. *Kidney Int*. 2014; 85:383–392. [PubMed: 23760289]
4. Cornec-Le Gall E, Audrezet MP, Chen JM, et al. Type of PKD1 mutation influences renal outcome in ADPKD. *J Am Soc Nephrol*. 2013; 24:1006–1013. [PubMed: 23431072]
5. Ong AC, Harris PC. Molecular pathogenesis of ADPKD: The polycystin complex gets complex. *Kidney Int*. 2005; 67:1234–1247. [PubMed: 15780076]
6. Ong AC, Ward CJ, Butler RJ, et al. Coordinate expression of the autosomal dominant polycystic kidney disease proteins, polycystin-2 and polycystin-1, in normal and cystic tissue. *Am J Pathol*. 1999; 154:1721–1729. [PubMed: 10362797]
7. Foggensteiner L, Bevan AP, Thomas R, et al. Cellular and subcellular distribution of polycystin-2, the protein product of the PKD2 gene. *J Am Soc Nephrol*. 2000; 11:814–827. [PubMed: 10770959]
8. Guillaume R, Trudel M. Distinct and common developmental expression patterns of the murine Pkd2 and Pkd1 genes. *Mech Dev*. 2000; 93:179–183. [PubMed: 10781953]
9. Qian F, Germino FJ, Cai Y, et al. PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nature genetics*. 1997; 16:179–183. [PubMed: 9171830]
10. Tsiokas L, Kim E, Arnould T, et al. Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. *Proc Natl Acad Sci U S A*. 1997; 94:6965–6970. [PubMed: 9192675]
11. Newby LJ, Streets AJ, Zhao Y, et al. Identification, characterization, and localization of a novel kidney polycystin-1-polycystin-2 complex. *J Biol Chem*. 2002; 277:20763–20773. [PubMed: 11901144]
12. Cai Y, Maeda Y, Cedzich A, et al. Identification and characterization of polycystin-2, the PKD2 gene product. *J Biol Chem*. 1999; 274:28557–28565. [PubMed: 10497221]
13. Koulen P, Cai Y, Geng L, et al. Polycystin-2 is an intracellular calcium release channel. *Nat Cell Biol*. 2002; 4:191–197. [PubMed: 11854751]
14. Giamarchi A, Feng S, Rodat-Despoix L, et al. A polycystin-2 (TRPP2) dimerization domain essential for the function of heteromeric polycystin complexes. *EMBO J*. 2010; 29:1176–1191. [PubMed: 20168298]
15. Mekahli D, Sammels E, Luyten T, et al. Polycystin-1 and polycystin-2 are both required to amplify inositol-trisphosphate-induced Ca(2+) release. *Cell calcium*. 2012
16. Lu W, Peissel B, Babakhanlou H, et al. Perinatal lethality with kidney and pancreas defects in mice with a targeted Pkd1 mutation. *Nat Genet*. 1997; 17:179–181. [PubMed: 9326937]
17. Wu G, Markowitz GS, Li L, et al. Cardiac defects and renal failure in mice with targeted mutations in Pkd2. *Nat Genet*. 2000; 24:75–78. [PubMed: 10615132]

18. Ong AC, Harris PC. Molecular basis of renal cyst formation--one hit or two? *Lancet*. 1997; 349:1039–1040. [PubMed: 9107237]
19. Qian F, Watnick TJ, Onuchic LF, et al. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell*. 1996; 87:979–987. [PubMed: 8978603]
20. Watnick TJ, Torres VE, Gandolph MA, et al. Somatic mutation in individual liver cysts supports a two-hit model of cystogenesis in autosomal dominant polycystic kidney disease. *Mol Cell*. 1998; 2:247–251. [PubMed: 9734362]
21. Pei Y, Watnick T, He N, et al. Somatic PKD2 mutations in individual kidney and liver cysts support a "two-hit" model of cystogenesis in type 2 autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 1999; 10:1524–1529. [PubMed: 10405208]
22. Wu G, D'Agati V, Cai Y, et al. Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell*. 1998; 93:177–188. [PubMed: 9568711]
23. Piontek K, Menezes LF, Garcia-Gonzalez MA, 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]
24. Lantinga-van Leeuwen IS, Leonhard WN, van der Wal A, et al. Kidney-specific inactivation of the Pkd1 gene induces rapid cyst formation in developing kidneys and a slow onset of disease in adult mice. *Hum Mol Genet*. 2007; 16:3188–3196. [PubMed: 17932118]
25. Harris PC, Bae KT, Rossetti S, et al. Cyst number but not the rate of cystic growth is associated with the mutated gene in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2006; 17:3013–3019. [PubMed: 17035604]
26. Lantinga-van Leeuwen IS, Dauwese JG, Baelde HJ, et al. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. *Hum Mol Genet*. 2004; 13:3069–3077. [PubMed: 15496422]
27. Jiang ST, Chiou YY, Wang E, et al. Defining a link with autosomal-dominant polycystic kidney disease in mice with congenitally low expression of Pkd1. *Am J Pathol*. 2006; 168:205–220. [PubMed: 16400024]
28. Nishio S, Hatano M, Nagata M, et al. Pkd1 regulates immortalized proliferation of renal tubular epithelial cells through p53 induction and JNK activation. *J Clin Invest*. 2005; 115:910–918. [PubMed: 15761494]
29. Watnick T, He N, Wang K, et al. Mutations of PKD1 in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. *Nat Genet*. 2000; 25:143–144. [PubMed: 10835625]
30. Koptides M, Mean R, Demetriou K, et al. Genetic evidence for a trans-heterozygous model for cystogenesis in autosomal dominant polycystic kidney disease. *Hum Mol Genet*. 2000; 9:447–452. [PubMed: 10655555]
31. Gogusev J, Murakami I, Doussau M, et al. Molecular cytogenetic aberrations in autosomal dominant polycystic kidney disease tissue. *J Am Soc Nephrol*. 2003; 14:359–366. [PubMed: 12538736]
32. Rossetti S, Kubly VJ, Consugar MB, et al. Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. *Kidney Int*. 2009; 75:848–855. [PubMed: 19165178]
33. Vujic M, Heyer CM, Ars E, et al. Incompletely penetrant PKD1 alleles mimic the renal manifestations of ARPKD. *J Am Soc Nephrol*. 2010; 21:1097–1102. [PubMed: 20558538]
34. Bergmann C, von Bothmer J, Ortiz Bruchle N, et al. Mutations in multiple PKD genes may explain early and severe polycystic kidney disease. *Journal of the American Society of Nephrology : JASN*. 2011; 22:2047–2056. [PubMed: 22034641]
35. Losekoot M, Ruivenkamp CA, Tholens AP, et al. Neonatal onset autosomal dominant polycystic kidney disease (ADPKD) in a patient homozygous for a PKD2 missense mutation due to uniparental disomy. *Journal of medical genetics*. 2012; 49:37–40. [PubMed: 22114106]
36. Hopp K, Ward CJ, Hommerding CJ, et al. Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *The Journal of clinical investigation*. 2012; 122:4257–4273. [PubMed: 23064367]

37. Raj A, Rifkin SA, Andersen E, et al. Variability in gene expression underlies incomplete penetrance. *Nature*. 2010; 463:913–918. [PubMed: 20164922]
38. Ong AC, Harris PC, Davies DR, et al. Polycystin-1 expression in PKD1, early-onset PKD1, and TSC2/PKD1 cystic tissue. *Kidney Int*. 1999; 56:1324–1333. [PubMed: 10504485]
39. Bastos AP, Piontek K, Silva AM, et al. Pkd1 haploinsufficiency increases renal damage and induces microcyst formation following ischemia/reperfusion. *Journal of the American Society of Nephrology : JASN*. 2009; 20:2389–2402. [PubMed: 19833899]
40. Happe H, Leonhard WN, van der Wal A, et al. Toxic tubular injury in kidneys from Pkd1-deletion mice accelerates cystogenesis accompanied by dysregulated planar cell polarity and canonical Wnt signaling pathways. *Human molecular genetics*. 2009; 18:2532–2542. [PubMed: 19401297]
41. Fedeles SV, Tian X, Gallagher AR, et al. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. *Nature genetics*. 2011; 43:639–647. [PubMed: 21685914]
42. Raphael KL, Strait KA, Stricklett PK, et al. Inactivation of Pkd1 in principal cells causes a more severe cystic kidney disease than in intercalated cells. *Kidney Int*. 2009; 75:626–633. [PubMed: 19145237]
43. Leonhard WN, Zandbergen M, Veraar K, et al. Scattered Deletion of PKD1 in Kidneys Causes a Cystic Snowball Effect and Recapitulates Polycystic Kidney Disease. *J Am Soc Nephrol*. 2014
44. Faguer S, Chassaing N, Bandin F, et al. The HNF1B score is a simple tool to select patients for HNF1B gene analysis. *Kidney Int*. 2014; 86:1007–1015. [PubMed: 24897035]
45. Gresh L, Fischer E, Reimann A, et al. A transcriptional network in polycystic kidney disease. *EMBO J*. 2004; 23:1657–1668. [PubMed: 15029248]
46. Patel V, Williams D, Hajarnis S, et al. miR-17~92 miRNA cluster promotes kidney cyst growth in polycystic kidney disease. *Proc Natl Acad Sci U S A*. 2013; 110:10765–10770. [PubMed: 23759744]
47. Wang E, Hsieh-Li HM, Chiou YY, et al. Progressive renal distortion by multiple cysts in transgenic mice expressing artificial microRNAs against Pkd1. *J Pathol*. 2010; 222:238–248. [PubMed: 20814903]
48. Tran U, Zakin L, Schweickert A, et al. The RNA-binding protein bicaudal C regulates polycystin 2 in the kidney by antagonizing miR-17 activity. *Development*. 2010; 137:1107–1116. [PubMed: 20215348]
49. Kim K, Drummond I, Ibraghimov-Beskrovnaya O, et al. Polycystin 1 is required for the structural integrity of blood vessels. *Proc Natl Acad Sci U S A*. 2000; 97:1731–1736. [PubMed: 10677526]
50. Gevers TJ, Drenth JP. Diagnosis and management of polycystic liver disease. *Nature reviews Gastroenterology & hepatology*. 2013; 10:101–108. [PubMed: 23296249]
51. Rossetti S, Chauveau D, Kubly V, et al. Association of mutation position in polycystic kidney disease 1 (PKD1) gene and development of a vascular phenotype. *Lancet*. 2003; 361:2196–2201. [PubMed: 12842373]
52. Ring T, Spiegelhalter D. Risk of intracranial aneurysm bleeding in autosomal-dominant polycystic kidney disease. *Kidney Int*. 2007; 72:1400–1402. [PubMed: 17882153]
53. Ong AC. Screening for intracranial aneurysms in ADPKD. *Bmj*. 2009; 339:b3763. [PubMed: 19770180]
54. Thong KM, Ong AC. Sudden death due to subarachnoid haemorrhage in an infant with autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant*. 2014; 29(Suppl 4):iv121–iv123. [PubMed: 25165178]
55. Kurbegovic A, Cote O, Couillard M, 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]
56. Qian Q, Hunter LW, Li M, et al. Pkd2 haploinsufficiency alters intracellular calcium regulation in vascular smooth muscle cells. *Hum Mol Genet*. 2003; 12:1875–1880. [PubMed: 12874107]
57. Hassane S, Claij N, Lantinga-van Leeuwen IS, et al. Pathogenic Sequence for Dissecting Aneurysm Formation in a Hypomorphic Polycystic Kidney Disease 1 Mouse Model. *Arterioscler Thromb Vasc Biol*. 2007

58. Hassane S, Claij N, Jodar M, et al. Pkd1-inactivation in vascular smooth muscle cells and adaptation to hypertension. *Lab Invest.* 2011; 91:24–32. [PubMed: 20856231]
59. Brookes ZL, Ruff L, Upadhyay VS, et al. Pkd2 mesenteric vessels exhibit a primary defect in endothelium-dependent vasodilatation restored by rosiglitazone. *American journal of physiology Heart and circulatory physiology.* 2013; 304:H33–H41. [PubMed: 23103499]
60. Brook-Carter PT, Peral B, Ward CJ, et al. Deletion of the TSC2 and PKD1 genes associated with severe infantile polycystic kidney disease—a contiguous gene syndrome. *Nat Genet.* 1994; 8:328–332. [PubMed: 7894481]
61. Sampson JR, Maheshwar MM, Aspinwall R, et al. Renal cystic disease in tuberous sclerosis: role of the polycystic kidney disease 1 gene. *Am J Hum Genet.* 1997; 61:843–851. [PubMed: 9382094]
62. Hartman TR, Liu D, Zilfou JT, et al. The Tuberous Sclerosis Proteins Regulate Formation of the Primary Cilium via a Rapamycin-Insensitive and Polycystin 1-Independent Pathway. *Hum Mol Genet.* 2008
63. Bonnet CS, Aldred M, von Ruhland C, et al. Defects in cell polarity underlie TSC and ADPKD-associated cystogenesis. *Hum Mol Genet.* 2009; 18:2166–2176. [PubMed: 19321600]
64. Kleymenova E, Ibraghimov-Beskrovnaya O, Kugoh H, et al. Tuberin-dependent membrane localization of polycystin-1: a functional link between polycystic kidney disease and the TSC2 tumor suppressor gene. *Mol Cell.* 2001; 7:823–832. [PubMed: 11336705]
65. Kim I, Fu Y, Hui K, et al. Fibrocystin/polyductin modulates renal tubular formation by regulating polycystin-2 expression and function. *J Am Soc Nephrol.* 2008; 19:455–468. [PubMed: 18235088]
66. Pei Y, Paterson AD, Wang KR, et al. Bilineal disease and trans-heterozygotes in autosomal dominant polycystic kidney disease. *Am J Hum Genet.* 2001; 68:355–363. [PubMed: 11156533]
67. Pei Y, Lan Z, Wang K, et al. A missense mutation in PKD1 attenuates the severity of renal disease. *Kidney international.* 2012; 81:412–417. [PubMed: 22031115]
68. Gainullin VG, Hopp K, Ward CJ, et al. Polycystin-1 maturation requires polycystin-2 in a dose-dependent manner. *J Clin Invest.* 2015
69. Hofherr A, Wagner C, Fedeles S, et al. N-glycosylation determines the abundance of the transient receptor potential channel TRPP2. *J Biol Chem.* 2014; 289:14854–14867. [PubMed: 24719335]
70. Li A, Davila S, Furu L, et al. Mutations in PRKCSH Cause Isolated Autosomal Dominant Polycystic Liver Disease. *Am J Hum Genet.* 2003; 72:691–703. [PubMed: 12529853]
71. Arac D, Boucard AA, Bolliger MF, et al. A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *The EMBO journal.* 2012; 31:1364–1378. [PubMed: 22333914]
72. Yu S, Hackmann K, Gao J, et al. Essential role of cleavage of Polycystin-1 at G protein-coupled receptor proteolytic site for kidney tubular structure. *Proc Natl Acad Sci U S A.* 2007; 104:18688–18693. [PubMed: 18003909]
73. Chapin HC, Rajendran V, Caplan MJ. Polycystin-1 surface localization is stimulated by polycystin-2 and cleavage at the G protein-coupled receptor proteolytic site. *Molecular biology of the cell.* 2010; 21:4338–4348. [PubMed: 20980620]
74. Kim H, Xu H, Yao Q, et al. Ciliary membrane proteins traffic through the Golgi via a Rabep1/GGA1/Arl3-dependent mechanism. *Nat Commun.* 2014; 5:5482. [PubMed: 25405894]
75. Kurbegovic A, Kim H, Xu H, et al. Novel functional complexity of polycystin-1 by GPS cleavage in vivo: role in polycystic kidney disease. *Mol Cell Biol.* 2014; 34:3341–3353. [PubMed: 24958103]
76. Bycroft M, Bateman A, Clarke J, et al. The structure of a PKD domain from polycystin-1: implications for polycystic kidney disease. *EMBO J.* 1999; 18:297–305. [PubMed: 9889186]
77. Jing H, Takagi J, Liu JH, et al. Archaeal surface layer proteins contain beta propeller, PKD, and beta helix domains and are related to metazoan cell surface proteins. *Structure.* 2002; 10:1453–1464. [PubMed: 12377130]
78. Qian F, Wei W, Germino G, et al. The nanomechanics of polycystin-1 extracellular region. *J Biol Chem.* 2005; 280:40723–40730. [PubMed: 16219758]
79. Forman JR, Qamar S, Paci E, et al. The remarkable mechanical strength of polycystin-1 supports a direct role in mechanotransduction. *J Mol Biol.* 2005; 349:861–871. [PubMed: 15894330]

80. Forman JR, Yew ZT, Qamar S, et al. Non-native interactions are critical for mechanical strength in PKD domains. *Structure*. 2009; 17:1582–1590. [PubMed: 20004162]
81. Streets AJ, Wagner BE, Harris PC, et al. Homophilic and heterophilic polycystin 1 interactions regulate E-cadherin recruitment and junction assembly in MDCK cells. *J Cell Sci*. 2009; 122:1410–1417. [PubMed: 19351715]
82. Streets AJ, Newby LJ, O'Hare MJ, et al. Functional analysis of PKD1 transgenic lines reveals a direct role for polycystin-1 in mediating cell-cell adhesion. *J Am Soc Nephrol*. 2003; 14:1804–1815. [PubMed: 12819240]
83. Ibraghimov-Beskrovnaya O, Bukanov NO, Donohue LC, et al. Strong homophilic interactions of the Ig-like domains of polycystin-1, the protein product of an autosomal dominant polycystic kidney disease gene, PKD1. *Hum Mol Genet*. 2000; 9:1641–1649. [PubMed: 10861291]
84. Bateman A, Sandford R. The PLAT domain: a new piece in the PKD1 puzzle. *Curr Biol*. 1999; 9:R588–R590. [PubMed: 10469604]
85. Ponting CP, Hofmann K, Bork P. A latrophilin/CL-1-like GPS domain in polycystin-1. *Curr Biol*. 1999; 9:R585–R588. [PubMed: 10469603]
86. Recacha R, Boulet A, Jollivet F, et al. Structural basis for recruitment of Rab6-interacting protein 1 to Golgi via a RUN domain. *Structure*. 2009; 17:21–30. [PubMed: 19141279]
87. Eek P, Jarving R, Jarving I, et al. Structure of a calcium-dependent 11R-lipoxygenase suggests a mechanism for Ca²⁺ regulation. *J Biol Chem*. 2012; 287:22377–22386. [PubMed: 22573333]
88. Hu J, Barr MM. ATP-2 interacts with the PLAT domain of LOV-1 and is involved in *Caenorhabditis elegans* polycystin signaling. *Mol Biol Cell*. 2005; 16:458–469. [PubMed: 15563610]
89. Parnell SC, Puri S, Wallace DP, et al. Protein phosphatase-1alpha interacts with and dephosphorylates polycystin-1. *PLoS one*. 2012; 7:e36798. [PubMed: 22675472]
90. Streets AJ, Wessely O, Peters DJ, et al. Hyperphosphorylation of polycystin-2 at a critical residue in disease reveals an essential role for polycystin-1-regulated dephosphorylation. *Human molecular genetics*. 2013; 22:1924–1939. [PubMed: 23390129]
91. Shillingford JM, Murcia NS, Larson CH, et al. The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc Natl Acad Sci U S A*. 2006; 103:5466–5471. [PubMed: 16567633]
92. Lal M, Song X, Pluznick JL, 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]
93. Bhunia AK, Piontek K, Boletta A, et al. PKD1 Induces p21(waf1) and Regulation of the Cell Cycle via Direct Activation of the JAK-STAT Signaling Pathway in a Process Requiring PKD2. *Cell*. 2002; 109:157–168. [PubMed: 12007403]
94. Gonzalez-Perret S, Kim K, Ibarra C, et al. Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca²⁺-permeable nonselective cation channel. *Proc Natl Acad Sci U S A*. 2001; 98:1182–1187. [PubMed: 11252306]
95. Ma R, Li WP, Rundle D, et al. PKD2 functions as an epidermal growth factor-activated plasma membrane channel. *Mol Cell Biol*. 2005; 25:8285–8298. [PubMed: 16135816]
96. Montell C, Birnbaumer L, Flockerzi V, et al. A unified nomenclature for the superfamily of TRP cation channels. *Mol Cell*. 2002; 9:229–231. [PubMed: 11864597]
97. Torres VE, Harris PC. Strategies targeting cAMP signaling in the treatment of polycystic kidney disease. *J Am Soc Nephrol*. 2014; 25:18–32. [PubMed: 24335972]
98. Yu Y, Ulbrich MH, Li MH, et al. Structural and molecular basis of the assembly of the TRPP2/PKD1 complex. *Proc Natl Acad Sci U S A*. 2009; 106:11558–11563. [PubMed: 19556541]
99. Zhang P, Luo Y, Chasan B, et al. The multimeric structure of polycystin-2 (TRPP2): structural-functional correlates of homo- and hetero-multimers with TRPC1. *Hum Mol Genet*. 2009; 18:1238–1251. [PubMed: 19193631]
100. Feng S, Okenka GM, Bai CX, et al. Identification and Functional Characterization of an N-terminal Oligomerization Domain for Polycystin-2. *J Biol Chem*. 2008; 283:28471–28479. [PubMed: 18701462]
101. Feng S, Rodat-Despoix L, Delmas P, et al. A Single Amino Acid Residue Constitutes the Third Dimerization Domain Essential for the Assembly and Function of the Tetrameric Polycystin-2

- (TRPP2) Channel. *The Journal of biological chemistry*. 2011; 286:18994–19000. [PubMed: 21474446]
102. Petri ET, Celic A, Kennedy SD, et al. Structure of the EF-hand domain of polycystin-2 suggests a mechanism for Ca²⁺-dependent regulation of polycystin-2 channel activity. *Proc Natl Acad Sci U S A*. 2010; 107:9176–9181. [PubMed: 20439752]
 103. Schumann F, Hoffmeister H, Bader R, et al. Ca²⁺-dependent conformational changes in a C-terminal cytosolic domain of polycystin-2. *J Biol Chem*. 2009; 284:24372–24383. [PubMed: 19546223]
 104. Cantero Mdel R, Cantiello HF. Calcium Transport and Local Pool Regulate Polycystin-2 (TRPP2) Function in Human Syncytiotrophoblast. *Biophysical journal*. 2013; 105:365–375. [PubMed: 23870258]
 105. Geng L, Okuhara D, Yu Z, et al. Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *J Cell Sci*. 2006; 119:1383–1395. [PubMed: 16537653]
 106. Hoffmeister H, Babinger K, Gurster S, et al. Polycystin-2 takes different routes to the somatic and ciliary plasma membrane. *J Cell Biol*. 2011; 192:631–645. [PubMed: 21321097]
 107. Yoshiba S, Shiratori H, Kuo IY, et al. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. *Science*. 2012; 338:226–231. [PubMed: 22983710]
 108. Ward HH, Brown-Glaberman U, Wang J, et al. A conserved signal and GTPase complex are required for the ciliary transport of polycystin-1. *Mol Biol Cell*. 2011; 22:3289–3305. [PubMed: 21775626]
 109. Cai Y, Fedeles SV, Dong K, et al. Altered trafficking and stability of polycystins underlie polycystic kidney disease. *J Clin Invest*. 2014; 124:5129–5144. [PubMed: 25365220]
 110. Streets AJ, Moon DJ, Kane ME, et al. Identification of an N-terminal glycogen synthase kinase 3 phosphorylation site which regulates the functional localization of polycystin-2 in vivo and in vitro. *Hum Mol Genet*. 2006; 15:1465–1473. [PubMed: 16551655]
 111. Miyakawa A, Ibarra C, Malmersjo S, et al. Intracellular calcium release modulates polycystin-2 trafficking. *BMC Nephrol*. 2013; 14:34. [PubMed: 23398808]
 112. Cai Y, Anyatonwu G, Okuhara D, et al. Calcium dependence of polycystin-2 channel activity is modulated by phosphorylation at Ser812. *J Biol Chem*. 2004; 279:19987–19995. [PubMed: 14742446]
 113. Streets AJ, Needham AJ, Gill SK, et al. Protein kinase D-mediated phosphorylation of polycystin-2 (TRPP2) is essential for its effects on cell growth and calcium channel activity. *Molecular biology of the cell*. 2010; 21:3853–3865. [PubMed: 20881056]
 114. Kottgen M, Benzing T, Simmen T, et al. Trafficking of TRPP2 by PACS proteins represents a novel mechanism of ion channel regulation. *EMBO J*. 2005; 24:705–716. [PubMed: 15692563]
 115. Boucher CA, Ward HH, Case RL, et al. Receptor protein tyrosine phosphatases are novel components of a polycystin complex. *Biochimica et biophysica acta*. 2011; 1812:1225–1238. [PubMed: 21126580]
 116. Otto EA, Trapp ML, Schultheiss UT, et al. NEK8 mutations affect ciliary and centrosomal localization and may cause nephronophthisis. *J Am Soc Nephrol*. 2008; 19:587–592. [PubMed: 18199800]
 117. Liu S, Lu W, Obara T, et al. A defect in a novel Nek-family kinase causes cystic kidney disease in the mouse and in zebrafish. *Development*. 2002; 129:5839–5846. [PubMed: 12421721]
 118. McCooke JK, Appels R, Barrero RA, et al. A novel mutation causing nephronophthisis in the Lewis polycystic kidney rat localises to a conserved RCC1 domain in Nek8. *BMC Genomics*. 2012; 13:393. [PubMed: 22899815]
 119. Sahara E, Luo Y, Zhang J, et al. Nek8 regulates the expression and localization of polycystin-1 and polycystin-2. *J Am Soc Nephrol*. 2008; 19:469–476. [PubMed: 18235101]
 120. Hoff S, Halbritter J, Epting D, et al. ANKS6 is a central component of a nephronophthisis module linking NEK8 to INVS and NPHP3. *Nature genetics*. 2013
 121. Kim H, Jeong W, Ahn K, et al. Siah-1 interacts with the intracellular region of polycystin-1 and affects its stability via the ubiquitin-proteasome pathway. *J Am Soc Nephrol*. 2004; 15:2042–2049. [PubMed: 15284290]

122. Tian Y, Kolb R, Hong JH, et al. TAZ promotes PC2 degradation through a SCFbeta-Trcp E3 ligase complex. *Mol Cell Biol*. 2007; 27:6383–6395. [PubMed: 17636028]
123. Makita R, Uchijima Y, Nishiyama K, et al. Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ. *American journal of physiology Renal physiology*. 2008; 294:F542–F553. [PubMed: 18172001]
124. Yim H, Sung CK, You J, et al. Nek1 and TAZ interact to maintain normal levels of polycystin 2. *Journal of the American Society of Nephrology : JASN*. 2011; 22:832–837. [PubMed: 21474562]
125. Azzolin L, Panciera T, Soligo S, et al. YAP/TAZ incorporation in the beta-catenin destruction complex orchestrates the Wnt response. *Cell*. 2014; 158:157–170. [PubMed: 24976009]
126. Liang G, Li Q, Tang Y, et al. Polycystin-2 is regulated by endoplasmic reticulum-associated degradation. *Hum Mol Genet*. 2008; 17:1109–1119. [PubMed: 18178578]
127. Gao H, Wang Y, Wegierski T, et al. PRKCSH/80K-H, the protein mutated in polycystic liver disease, protects polycystin-2/TRPP2 against HERP-mediated degradation. *Hum Mol Genet*. 2010; 19:16–24. [PubMed: 19801576]
128. Prasad S, McDaid JP, Tam FW, et al. Pkd2 dosage influences cellular repair responses following ischemia-reperfusion injury. *Am J Pathol*. 2009; 175:1493–1503. [PubMed: 19729489]
129. Zhao Y, Haylor JL, Ong AC. Polycystin-2 expression is increased following experimental ischaemic renal injury. *Nephrol Dial Transplant*. 2002; 17:2138–2144. [PubMed: 12454224]
130. Merrick D, Chapin H, Baggs JE, et al. The gamma-secretase cleavage product of polycystin-1 regulates TCF and CHOP-mediated transcriptional activation through a p300-dependent mechanism. *Developmental cell*. 2012; 22:197–210. [PubMed: 22178500]
131. Low SH, Vasanth S, Larson CH, et al. Polycystin-1, STAT6, and P100 Function in a Pathway that Transduces Ciliary Mechanosensation and Is Activated in Polycystic Kidney Disease. *Dev Cell*. 2006; 10:57–69. [PubMed: 16399078]
132. Woodward OM, Li Y, Yu S, et al. Identification of a polycystin-1 cleavage product, P100, that regulates store operated Ca entry through interactions with STIM1. *PloS one*. 2010; 5:e12305. [PubMed: 20808796]
133. Ong AC, Wheatley DN. Polycystic kidney disease--the ciliary connection. *Lancet*. 2003; 361:774–776. [PubMed: 12620752]
134. Sung CH, Leroux MR. The roles of evolutionarily conserved functional modules in cilia-related trafficking. *Nat Cell Biol*. 2013; 15:1387–1397. [PubMed: 24296415]
135. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. *The New England journal of medicine*. 2011; 364:1533–1543. [PubMed: 21506742]
136. Barr MM, DeModena J, Braun D, et al. The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs *lov-1* and *pkd-2* act in the same pathway. *Curr Biol*. 2001; 11:1341–1346. [PubMed: 11553327]
137. Barr MM, Sternberg PW. A polycystic kidney-disease gene homologue required for male mating behaviour in *C. elegans*. *Nature*. 1999; 401:386–389. [PubMed: 10517638]
138. Pazour GJ, Dickert BL, Vucica Y, 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]
139. Haycraft CJ, Swoboda P, Taulman PD, et al. The *C. elegans* homolog of the murine cystic kidney disease gene *Tg737* functions in a ciliogenic pathway and is disrupted in *osm-5* mutant worms. *Development*. 2001; 128:1493–1505. [PubMed: 11290289]
140. Yoder BK, Hou X, Guay-Woodford LM. The polycystic kidney disease proteins, polycystin-1, polycystin-2, *polaris*, and *cystin*, are co-localized in renal cilia. *J Am Soc Nephrol*. 2002; 13:2508–2516. [PubMed: 12239239]
141. Pazour GJ, San Agustin JT, Follit JA, et al. Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in *orpk* mice with polycystic kidney disease. *Curr Biol*. 2002; 12:R378–R380. [PubMed: 12062067]
142. Lin F, Hiesberger T, Cordes K, et al. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc Natl Acad Sci U S A*. 2003; 100:5286–5291. [PubMed: 12672950]

143. Masyuk TV, Huang BQ, Ward CJ, et al. Defects in cholangiocyte fibrocystin expression and ciliary structure in the PCK rat. *Gastroenterology*. 2003; 125:1303–1310. [PubMed: 14598246]
144. Ong AC. Primary cilia and renal cysts: does length matter? *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2013; 28:2661–2663.
145. Nachury MV, Loktev AV, Zhang Q, et al. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell*. 2007; 129:1201–1213. [PubMed: 17574030]
146. Garcia-Gonzalo FR, Reiter JF. Scoring a backstage pass: mechanisms of ciliogenesis and ciliary access. *J Cell Biol*. 2012; 197:697–709. [PubMed: 22689651]
147. Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol*. 2001; 184:71–79. [PubMed: 11687880]
148. Nauli SM, Alenghat FJ, Luo Y, et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet*. 2003; 33:129–137. [PubMed: 12514735]
149. Xu C, Rossetti S, Jiang L, et al. Human ADPKD primary cyst epithelial cells with a novel, single codon deletion in the PKD1 gene exhibit defective ciliary polycystin localization and loss of flow-induced Ca²⁺ signaling. *Am J Physiol Renal Physiol*. 2007; 292:F930–F945. [PubMed: 17090781]
150. Kottgen M, Buchholz B, Garcia-Gonzalez MA, et al. TRPP2 and TRPV4 form a polymodal sensory channel complex. *J Cell Biol*. 2008; 182:437–447. [PubMed: 18695040]
151. McGrath J, Somlo S, Makova S, et al. Two populations of node monocilia initiate left-right asymmetry in the mouse. *Cell*. 2003; 114:61–73. [PubMed: 12859898]
152. Field S, Riley KL, Grimes DT, et al. Pkd111 establishes left-right asymmetry and physically interacts with Pkd2. *Development*. 2011; 138:1131–1142. [PubMed: 21307093]
153. Kamura K, Kobayashi D, Uehara Y, et al. Pkd111 complexes with Pkd2 on motile cilia and functions to establish the left-right axis. *Development*. 2011; 138:1121–1129. [PubMed: 21307098]
154. Pennekamp P, Karcher C, Fischer A, et al. The ion channel polycystin-2 is required for left-right axis determination in mice. *Curr Biol*. 2002; 12:938–943. [PubMed: 12062060]
155. Karcher C, Fischer A, Schweickert A, et al. Lack of a laterality phenotype in Pkd1 knock-out embryos correlates with absence of polycystin-1 in nodal cilia. *Differentiation; research in biological diversity*. 2005; 73:425–432.
156. Delling M, DeCaen PG, Doerner JF, et al. Primary cilia are specialized calcium signalling organelles. *Nature*. 2013; 504:311–314. [PubMed: 24336288]
157. DeCaen PG, Delling M, Vien TN, et al. Direct recording and molecular identification of the calcium channel of primary cilia. *Nature*. 2013; 504:315–318. [PubMed: 24336289]
158. Choi YH, Suzuki A, Hajarnis S, et al. Polycystin-2 and phosphodiesterase 4C are components of a ciliary A-kinase anchoring protein complex that is disrupted in cystic kidney diseases. *Proc Natl Acad Sci U S A*. 2011; 108:10679–10684. [PubMed: 21670265]
159. Raychowdhury MK, Ramos AJ, Zhang P, et al. Vasopressin receptor-mediated functional signaling pathway in primary cilia of renal epithelial cells. *Am J Physiol Renal Physiol*. 2009; 296:F87–F97. [PubMed: 18945824]
160. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U S A*. 2004; 101:13368–13373. [PubMed: 15326289]
161. Hogan MC, Manganelli L, Woollard JR, et al. Characterization of PKD protein-positive exosome-like vesicles. *J Am Soc Nephrol*. 2009; 20:278–288. [PubMed: 19158352]
162. Bakeberg JL, Tammachote R, Woollard JR, et al. Epitope-tagged Pkhd1 tracks the processing, secretion, and localization of fibrocystin. *Journal of the American Society of Nephrology : JASN*. 2011; 22:2266–2277. [PubMed: 22021705]
163. Fischer E, Legue E, Doyen A, et al. Defective planar cell polarity in polycystic kidney disease. *Nat Genet*. 2006; 38:21–23. [PubMed: 16341222]
164. Simons M, Gloy J, Ganner A, 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]

165. Boca M, D'Amato L, Distefano G, et al. Polycystin-1 induces cell migration by regulating phosphatidylinositol 3-kinase-dependent cytoskeletal rearrangements and GSK3beta-dependent cell cell mechanical adhesion. *Mol Biol Cell*. 2007; 18:4050–4061. [PubMed: 17671167]
166. Luyten A, Su X, Gondela S, et al. Aberrant regulation of planar cell polarity in polycystic kidney disease. *J Am Soc Nephrol*. 2010; 21:1521–1532. [PubMed: 20705705]
167. Nishio S, Tian X, Gallagher AR, et al. Loss of oriented cell division does not initiate cyst formation. *J Am Soc Nephrol*. 2010; 21:295–302. [PubMed: 19959710]
168. Leightner AC, Hommerding CJ, Peng Y, et al. The Meckel syndrome protein meckelin (TMEM67) is a key regulator of cilia function but is not required for tissue planar polarity. *Hum Mol Genet*. 2013; 22:2024–2040. [PubMed: 23393159]
169. Karner CM, Chirumamilla R, Aoki S, et al. Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. *Nat Genet*. 2009; 41:793–799. [PubMed: 19543268]
170. Castelli M, Boca M, Chiaravalli M, et al. Polycystin-1 binds Par3/aPKC and controls convergent extension during renal tubular morphogenesis. *Nat Commun*. 2013; 4:2658. [PubMed: 24153433]
171. Tran PV, Haycraft CJ, Besschetnova TY, et al. THM1 negatively modulates mouse sonic hedgehog signal transduction and affects retrograde intraflagellar transport in cilia. *Nat Genet*. 2008; 40:403–410. [PubMed: 18327258]
172. Tran PV, Talbott GC, Turbe-Doan A, et al. Downregulating hedgehog signaling reduces renal cystogenic potential of mouse models. *J Am Soc Nephrol*. 2014; 25:2201–2212. [PubMed: 24700869]
173. Senee V, Chelala C, Duchatelet S, et al. Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. *Nat Genet*. 2006; 38:682–687. [PubMed: 16715098]
174. Kang HS, Beak JY, Kim YS, et al. Glis3 is associated with primary cilia and Wwtr1/TAZ and implicated in polycystic kidney disease. *Mol Cell Biol*. 2009; 29:2556–2569. [PubMed: 19273592]
175. Chang MY, Parker E, Ibrahim S, et al. Haploinsufficiency of Pkd2 is associated with increased tubular cell proliferation and interstitial fibrosis in two murine Pkd2 models. *Nephrol Dial Transplant*. 2006; 21:2078–2084. [PubMed: 16720597]
176. Li X, Luo Y, Starremans PG, et al. Polycystin-1 and polycystin-2 regulate the cell cycle through the helix-loop-helix inhibitor Id2. *Nat Cell Biol*. 2005; 7:1202–1212. [PubMed: 16311606]
177. Battini L, Macip S, Fedorova E, et al. Loss of polycystin-1 causes centrosome amplification and genomic instability. *Hum Mol Genet*. 2008; 17:2819–2833. [PubMed: 18566106]
178. Ma M, Tian X, Igarashi P, et al. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. *Nature genetics*. 2013; 45:1004–1012. [PubMed: 23892607]
179. Sharif-Naeini R, Folgering JH, Bichet D, et al. Polycystin-1 and -2 dosage regulates pressure sensing. *Cell*. 2009; 139:587–596. [PubMed: 19879844]
180. Peyronnet R, Sharif-Naeini R, Folgering JH, et al. Mechanoprotection by polycystins against apoptosis is mediated through the opening of stretch-activated K(2P) channels. *Cell Rep*. 2012; 1:241–250. [PubMed: 22832196]
181. Lee K, Boctor S, Barisoni LM, et al. Inactivation of Integrin-beta1 Prevents the Development of Polycystic Kidney Disease after the Loss of Polycystin-1. *J Am Soc Nephrol*. 2014
182. Silberberg M, Charron AJ, Bacallao R, et al. Mispolarization of Desmosomal Proteins and Altered Intercellular Adhesion in Autosomal Dominant Polycystic Kidney Disease. *Am J Physiol Renal Physiol*. 2005
183. Natoli TA, Gareski TC, Dackowski WR, et al. Pkd1 and Nek8 mutations affect cell-cell adhesion and cilia in cysts formed in kidney organ cultures. *Am J Physiol Renal Physiol*. 2008; 294:F73–F83. [PubMed: 17928412]
184. Charron AJ, Nakamura S, Bacallao R, et al. Compromised cytoarchitecture and polarized trafficking in autosomal dominant polycystic kidney disease cells. *J Cell Biol*. 2000; 149:111–124. [PubMed: 10747091]

185. Fogelgren B, Lin SY, Zuo X, et al. The exocyst protein Sec10 interacts with Polycystin-2 and knockdown causes PKD-phenotypes. *PLoS Genet.* 2011; 7:e1001361. [PubMed: 21490950]
186. Battini L, Fedorova E, Macip S, et al. Stable knockdown of polycystin-1 confers integrin- α 2 β 1-mediated anoikis resistance. *J Am Soc Nephrol.* 2006; 17:3049–3058. [PubMed: 17005934]
187. Sutton KA, Jungnickel MK, Ward CJ, et al. Functional characterization of PKDREJ, a male germ cell-restricted polycystin. *J Cell Physiol.* 2006; 209:493–500. [PubMed: 16883570]
188. Ishimaru Y, Inada H, Kubota M, et al. Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. *Proc Natl Acad Sci U S A.* 2006; 103:12569–12574. [PubMed: 16891422]
189. Mackenzie FE, Romero R, Williams D, et al. Upregulation of PKD1L2 provokes a complex neuromuscular disease in the mouse. *Human molecular genetics.* 2009; 18:3553–3566. [PubMed: 19578180]
190. Chen Y, Zhang Z, Lv XY, et al. Expression of Pkd2l2 in testis is implicated in spermatogenesis. *Biol Pharm Bull.* 2008; 31:1496–1500. [PubMed: 18670078]
191. Hanaoka K, Qian F, Boletta A, et al. Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature.* 2000; 408:990–994. [PubMed: 11140688]
192. Inoue Y, Sohara E, Kobayashi K, et al. Aberrant glycosylation and localization of polycystin-1 cause polycystic kidney in an AQP11 knockout model. *J Am Soc Nephrol.* 2014; 25:2789–2799. [PubMed: 24854278]
193. Cogswell C, Price SJ, Hou X, et al. Positional cloning of jcpk/bpk locus of the mouse. *Mamm Genome.* 2003; 14:242–249. [PubMed: 12682776]
194. Wodarczyk C, Distefano G, Rowe I, et al. Nephrocystin-1 forms a complex with polycystin-1 via a polyproline motif/SH3 domain interaction and regulates the apoptotic response in mammals. *PLoS one.* 2010; 5:e12719. [PubMed: 20856870]
195. Su X, Driscoll K, Yao G, et al. Bardet-Biedl syndrome proteins 1 and 3 regulate the ciliary trafficking of polycystic kidney disease 1 protein. *Hum Mol Genet.* 2014; 23:5441–5451. [PubMed: 24939912]

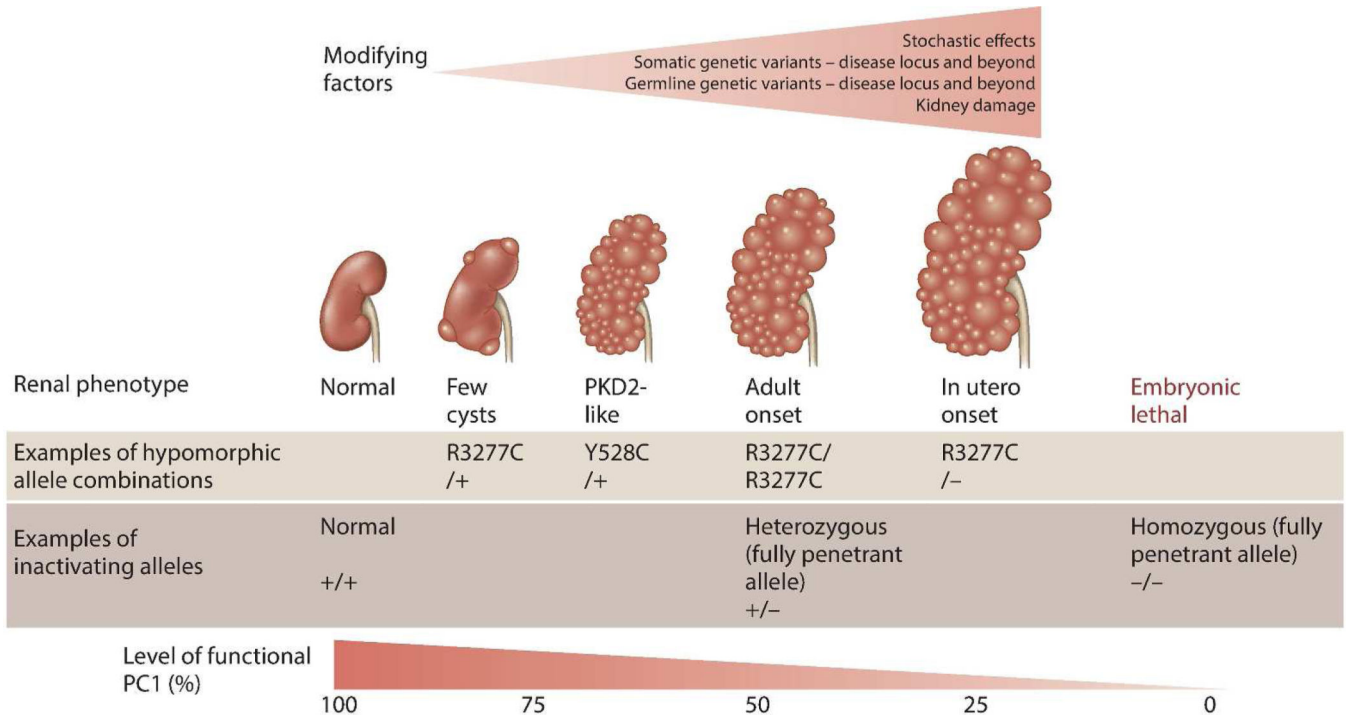


Figure 1. Dosage dependent disease mechanism in PKD1

The level of functional PC1 (bottom) directly influences the renal phenotype with a ~50% reduction (haploinsufficiency due to a single inactivating allele) associated with adult onset disease and no PC1 incompatible with life. Incompletely penetrant (hypomorphic) PC1 alleles of different strengths and combinations can significantly influence the renal phenotype. The PC1 allele p.Y528C has a phenotype similar to PKD2⁶⁷ while p.R3277C can result in a few cysts, adult onset disease or early onset disease depending on the *in trans* combination^{32, 36}. Additional mutations/variants at the disease locus and elsewhere (somatic and germline), along with chance and environmental factors influence the disease course by determining the frequency of cyst development.

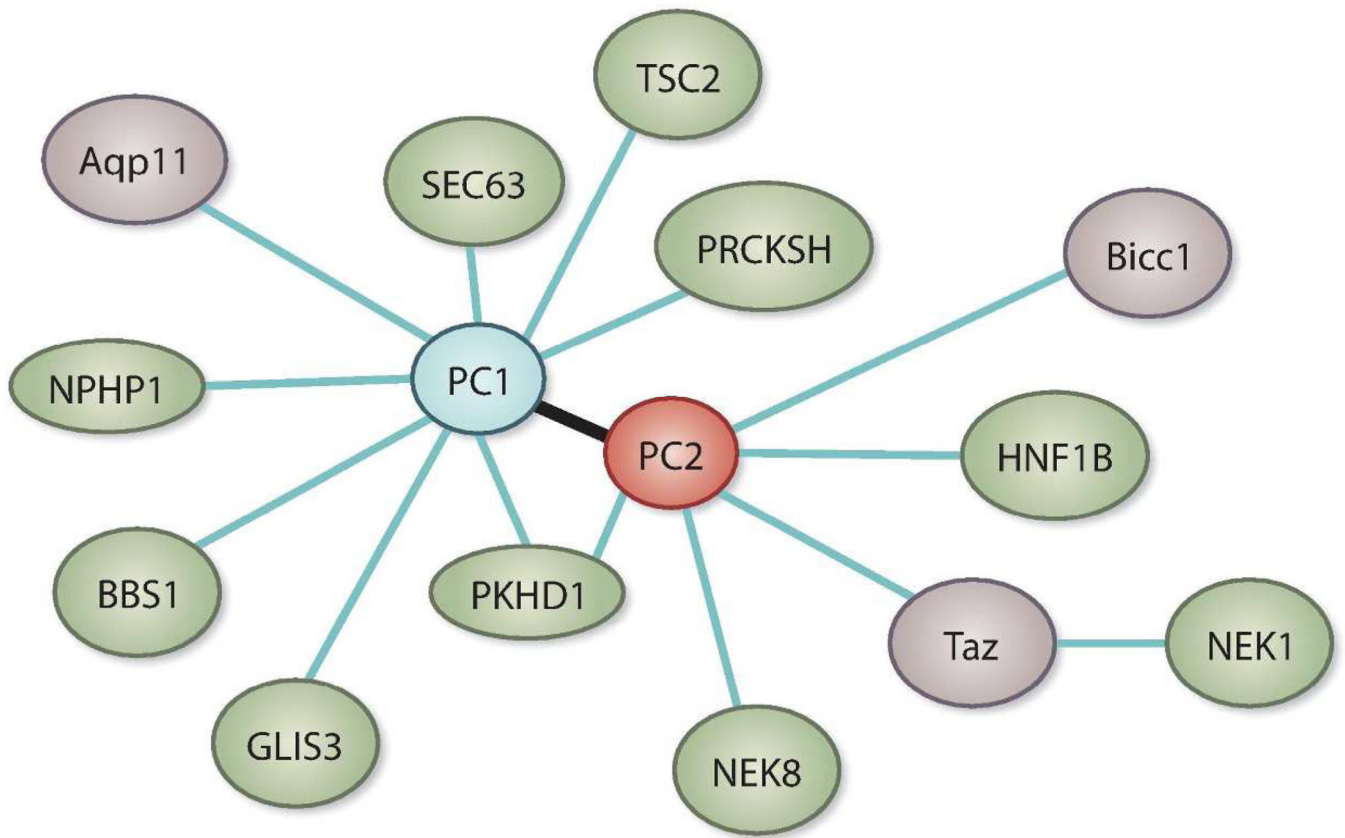


Figure 2. Genetic and biochemical interactions between known cystoproteins

Genetic and/or biochemical interactions are shown as solid lines. The murine cystoproteins shaded in grey (Aqp11, Bicc1, Taz) represent those where a human PKD phenotype has not yet been reported^{123, 192, 193}. Biochemical interactions between PC1 and NPHP1 and BBS1 have been reported^{194, 195}.

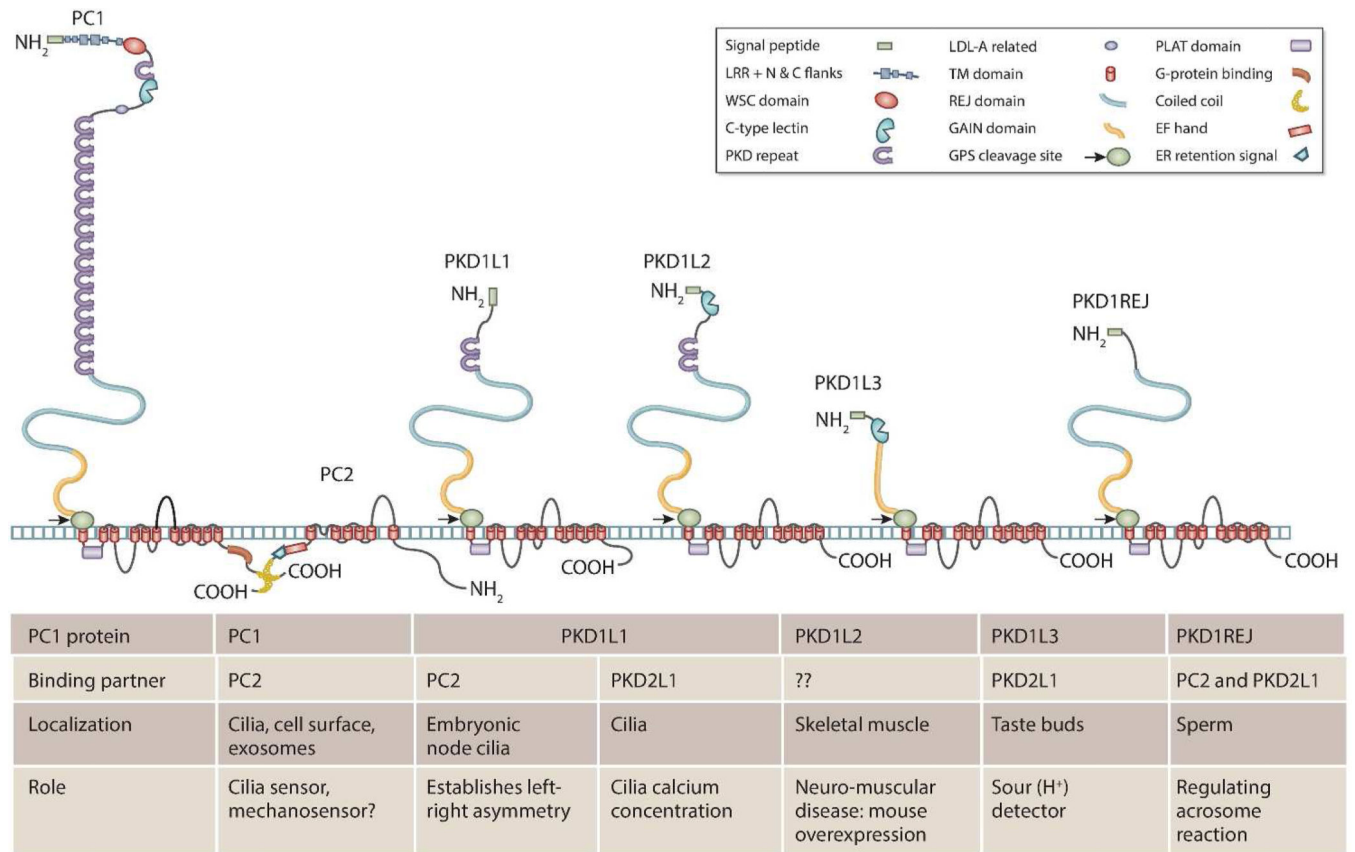


Figure 3. Structure and functions of PC1 and related polycystin-like proteins, plus PC2
 The Key shows the different domains and other motifs found in these proteins. PC2 is shown complexed with PC1 via PC2 CC2 and the PC1 coiled coil region. A high degree of similarity between PC1 paralogs is found in the transmembrane regions but with more variation in the N-terminal ectodomains and the cytoplasmic tails. The structure of PC2L1 and PC2L2 are similar to PC2 but PC2L2 does not have an EF-hand.

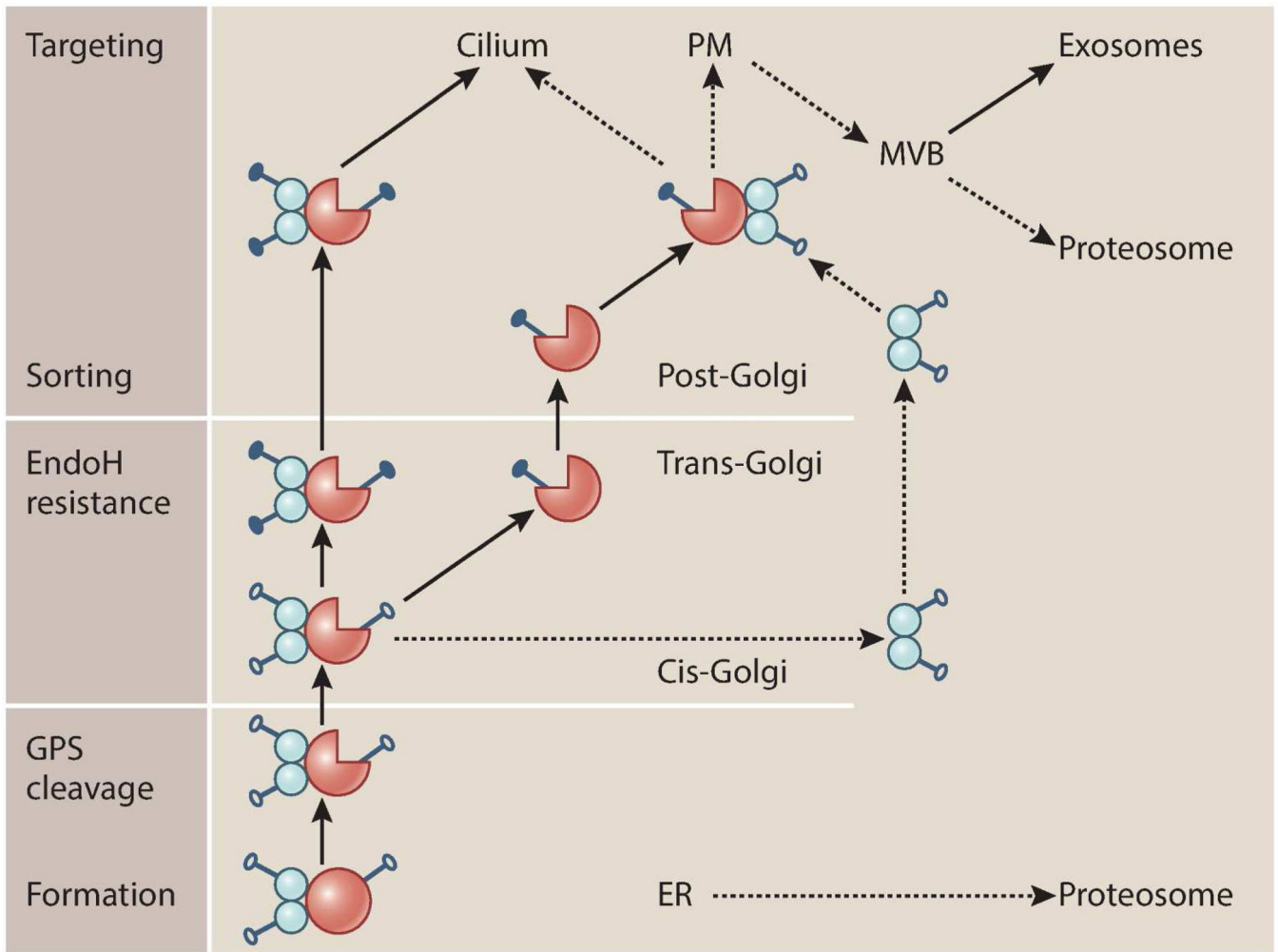


Figure 4. Models of the biosynthesis, maturation and trafficking of the polycystin complex
 PC1 (red) binds to PC2 (blue) shown as a putative dimer in the ER prior to undergoing GPS cleavage. At this stage, both proteins are expressed as EndoH-sensitive glycoforms (empty circles). EndoH-resistance (filled circles) is acquired with passage through the *trans*-Golgi with a small pool of an EndoH-resistant complex detectable in primary cilia. An alternative model proposes that EndoH-sensitive PC2 exits the *cis*-Golgi and traffics independently of PC1 which undergoes normal Golgi maturation, acquiring Endo H-resistance. The broken lines indicate other regulatory pathways which could determine the levels of both proteins, complex formation and subcellular localisation.