Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common human monogenic diseases, with a prevalence of about 1 in 1,000 [refs 1,2]. This multisystem inherited disorder is characterized by the progressive development of fluid-filled cysts in the kidney, liver and pancreas and is associated with hypertension, kidney failure and brain aneurysms3,4. Mutations that cause ADPKD occur on \(PKD1\) and \(PKD2\), which encode polycystin 1 (PC1; also known as \(PKD1\)) and polycystin 2 (PC2; also known as \(PKD2\) or \(TRPP2\)), respectively5,6. Mutations in \(PKHD1\), which encodes fibrocystin (also known as polyductin), cause the autosomal recessive form of the disease (ARPKD)7. One study proposed that the carboxy-terminal domain of fibrocystin binds to the intracellular amino terminus of PC2 and that loss of fibrocystin results in reduced PC2 expression8; however, another study did not confirm this interaction between fibrocystin and PC2 (refs 8,9). Additional reported partners for PC2 include other ion channel subunits such as the transient receptor potential (TRP) channels TrpV4 and TrpC1 and the PIEZO1 mechanosensitive ion channel10–15.

Genetically, ADPKD is autosomal dominant as the mutations associated with the disease are heterozygous. However, at the cellular level, cyst formation requires a second somatic mutation in the normal allele that results in either reduced expression or inactivation of the wild-type gene16. These pathogenic mutations affect intracellular calcium homeostasis and numerous additional signalling pathways19. Notably, in ADPKD, low cytosolic calcium concentration is associated with enhanced cAMP levels, owing to calcium-dependent regulation of adenyl cyclase and phosphodiesterases, which are responsible for the synthesis and degradation of cAMP, respectively. The rise in intracellular cAMP promotes the proliferation of tubular epithelial cell and cystic fibrosis transmembrane conductance regulator (CFTR)-mediated fluid secretion20–22.

The genetic basis of ADPKD is well known, including the identity of the mutated genes and mutational mechanisms, but the biological function of polycystins remains poorly understood. Initial reports indicated that PC1 and PC2 combine to form a calcium-permeable channel at the plasma membrane23,24. However, subsequent studies in which PC1 expression was targeted with small interfering RNAs (siRNAs) or through a conditional knockout of \(PKD1\) challenged this assertion25,26. Instead, those data suggested that PC2 can function independently of PC1 in the primary cilium of renal collecting duct epithelial cells to form a channel that is mainly permeant to monovalent cations and is activated by both membrane depolarization and an increase in intraciliary calcium. In addition, PC2 functions as a calcium-activated calcium release channel at the endoplasmic reticulum membrane. Structural studies indicate that the heteromeric PC1–PC2 complex comprises one PC1 and three PC2 channel subunits. Surprisingly, several positively charged residues from PC1 occlude the ionic pore of the PC1–PC2 complex, suggesting that pathogenic polycystin mutations might cause ADPKD independently of an effect on channel permeation. Emerging reports of novel structural and functional findings on polycystins will continue to elucidate the molecular basis of ADPKD.
The pore of the ion channel.

Channel that attract cations from the extracellular side of the membrane is known as a cation sink. This confers voltage sensitivity. Channel (segment 4 (s4)) that is involved in the voltage sensor of the protein.

Charged domain of an ion channel is involved in ionic permeability. Ion channel that controls its permeability.

Permeation

Permeability of ions through channels

Gating mechanism

Molecular mechanism of channel opening and closing

Coiled-coil domains

Structural motifs in proteins in which 2–7 α-helices are coiled together and mediate protein–protein interaction.

Lipid nanodiscs

Lipid bilayer mimetics that function as synthetic model membranes in which purified proteins can be reconstituted for structural determination with cryo-electron microscopy.

Selectivity filter

Segment within the pore of an ion channel that controls its ionic permeability.

Voltage sensor

Charged domain of an ion channel (segment 4 (S4)) that confers voltage sensitivity.

Cation sink

Negative charges present at the extracellular side of the channel that attract cations towards the selectivity filter.

Conduction pathway

The pore of the ion channel.

and is not observed in other areas of the plasma membrane. Moreover, some reports indicated that PC1–PC2 complexes in the primary cilium of both renal tubular epithelial cells and endothelial cells are responsible for sensing shear stress27–29, whereas subsequent findings showed that primary cilia are not calcium-responsive mechanosensors30.

The resolution of the atomic structure of both PC1 and PC2 is important for understanding the permeation and gating mechanism of the PC2 cationic channel. Surprisingly, it also revealed a pore-blocking function for PC1 (REFS 11–15). In this Review, we describe the most recent structural and functional findings about polycystins and tentatively reconcile some of the earlier controversies in the field.

Pre-structural studies of polycystins

Mutations in PKD1 or PKD2 produce a similar cystic phenotype, suggesting that both proteins might function in the same pathway and/or complex. Indeed, PC1 interacts with PC2 through coiled-coil domains within the cytosolic carboxy-terminal domains. It was initially reported that co-assembly of PC1 and PC2 resulted in a non-selective cation-permeable current at the plasma membrane when overexpressed in transfected Chinese hamster ovary (CHO) cells, whereas neither PC1 nor PC2 alone could produce a current. In the absence of PC1, PC2 was retained in the endoplasmic reticulum (ER) and translocated to the plasma membrane only when co-expressed with PC1 (REF 21). PC2 contains a carboxy-terminal ER retention motif. One of the disease-causing mutated forms of PKD1, R4227X, encodes a truncated PC1 protein that lacks the last 76 amino acids, which include the coiled-coil domain. Notably, co-expression of this mutated isoform of PC1, which cannot interact with PC2, did not result in a measurable current. Similarly, co-expression of full-length PC1 with PC2 742X, a pathogenic mutated isoform of PC2-like 1 protein is active at the plasma membrane when overexpressed in CHO cells, whereas neither PC1 nor PC2 was detected when co-expressed with the mutated PC2 (Glu631–Pro658) with the core domain of PC2-like 1 protein is active at the plasma membrane, chimeric constructs that combined the pore domain of PC2 (Glu631–Pro658) with the core domain of PC2-like 1 protein have been used to evaluate the ionic selectivity of PC2 (REF 36). The data obtained from these constructs indicated that PC2 is mainly a sodium and potassium conducting channel (PNa⁺:Pe− permeability ratio = 2.2 K⁺:1 Na⁺; 0.5 Ca²⁺)31.

Calcium binding sites

The structural analysis of full-length PC2 in complex with calcium and lipids revealed two distinct channel states. The structure with a larger pore radius of 1.7 Å contained five cation binding sites along the binding sites of extracellular polycystin domains in PC1 and PC2 are hot spots for pathogenic mutations.

Atomic structure of homomeric PC2

PC2, which is found at the membrane of the primary cilium and at the ER, belongs to the TRP ion channel family. Its structure was first solved by cryogenic electron microscopy at a resolution of 3 Å in lipid nanodiscs (BOX 1). PC2 forms a homotetramer and each subunit contains a pore domain, composed of two pore helices (PH1 and PH2) and a selectivity filter, as well as a voltage sensor-like domain (VSD) that is formed by S1–S4. The external part of the pore is enriched in negative charges and probably acts as a cation sink. In addition, the pore is constricted within the selectivity filter between amino acids Leu641-Gly642-Asp643 (fig. 1e), which narrows the ionic pathway so that only dehydrated cations can move through the pore. Within the homotetramer, the negatively charged Asp643 residues point to the centre of the pore, presumably interacting with cations while repelling anions (fig. 1e). The Leu-Gly-Asp sequence found in the PC2 selectivity filter is also present in PC2-like 1 protein, a TRP homologue (71% sequence similarity) encoded by PKD2L1 that is responsible for sour taste sensing and other pH-dependent processes that occur at the primary cilium. Because, unlike PC2, transfected PC2-like 1 protein is active at the plasma membrane, chimeric constructs that combined the pore domain of PC2 (Glu631–Pro658) with the core domain of PC2-like 1 protein have been used to evaluate the ionic selectivity of PC2 (REF 36). The data obtained from these constructs indicated that PC2 is mainly a sodium and potassium conducting channel (Ps:PsNa⁺:Pe− permeability ratio = 2.2 K⁺:1 Na⁺; 0.5 Ca²⁺)31.

These seminal reports put forward the notion that PC1 controls both the trafficking and the gating of PC2 and that pathogenic mutations in either subunit impair calcium-dependent signalling in renal tubular epithelial cells, as well as in other cell types affected by ADPKD, which include arterial smooth muscle cells. Consequently, before the structural determination of polycystins, the dogmatic view was that PC1 acts as an obligatory positive regulator of PC2 (REFS 11,12,14). Surprisingly, structural studies suggest an intriguing alternative hypothesis in which PC1 blocks cation permeation through the heteromeric PC1–PC2 complex.
The PC1–PC2 heteromeric complex. a | Topological model of polycystin 1 (PC1) and PC2. PC1 comprises an amino-terminal domain (NTD), which includes an intracellular PC1 lipoxygenase and α-toxin (PLAT) domain between transmembrane helix 1 (TM1) and TM2, followed by a voltage sensor-like domain (VSD) that contains an extracellular polycystin domain (PCD) between segment 1 (S1) and S2. The region indicated by the dashed line between S5 and S6 was not resolved in the PC1 structure. b | Topology of a PC2 subunit, including a VSD and a pore domain between S5 and S6, which includes two pore helices (PH1 and PH2) that surround the selectivity filter (SF). c | Structure of a PC1 subunit. The dashed line represents a 29-amino-acid-long segment (4,051–4,080), upstream of S6, which was not resolved in the PC1 structure. In PC2 and in other transient receptor potential (TRP) subunits, this region corresponds to the pore domain. Pathogenic substitution mutations, as described by the ADPKD mutation database, are indicated by grey spheres. d | Structure of PC2. Asp643 in the SF is responsible for the binding of extracellular calcium and inhibition of PC2 permeation. Leu677 at the intracellular side of S6 acts as a hydrophobic gate. e | Structure of the heteromeric polycystin complex, including one PC1 (in blue and red) and three PC2 subunits (in green). For easier visualization, the PCDs are not represented. Magnification of the pore region shows that positively charged residues Arg4100, Arg4107 and His4111 from PC1 occlude the permeation pathway in the PC1–PC2 heteromeric complex. PDB accession number: 6A70; PyMOL 1.3 software. EF, EF-hand motif.
Cryo-electron microscopy

Crystallization of transmembrane proteins in well-ordered crystals suitable for X-ray crystallography remains a difficult task. As an alternative approach, cryogenic electron microscopy (cryo-EM) is a combination of methods that enable the creation of 3D protein models using focused beams of electrons and super-cold temperatures. The protein is frozen in a thin, single-molecule-thick layer of glass-like ice that is then bombarded with electrons. An advantage of the freezing process is that it preserves proteins and complexes in their native state. In addition, for the study of membrane proteins in their nearly native lipid bilayer environment, the transmembrane domain can be stabilized with lipid nanodiscs. The irradiation of these nanodiscs with low-energy electrons produces 2D images of individual protein particles in many orientations. Cryo-EM methods then sort and average hundreds of thousands of these images to build a 3D map and computationally construct a 3D model of the protein. Cryo-EM is an expanding structural biology technique that allows high-resolution (~3–5 Å) structural determination of large biomolecules and thus complements other structural biology techniques such as X-ray crystallography and NMR.

The polycystin domain

PC2 contains a large extracellular domain (~200 amino acids long), termed the polycystin domain (PCD), which is also present in PC1 and PC2-like 1 protein (fig. 1b,d). The PCD, found between segment 1 (S1) and S2 and extending 35 Å above the membrane, is involved in the assembly of the ion channel subunits and might have a role in the regulation of channel gating (fig. 1b,d,3). The importance of PCDs is highlighted by the clinical mechanisms of PC2 (refs 35,36). Leu677 at the cytoplasmic end of S6 forms a single-residue hydrophobic barrier that is predicted to prevent ion permeation by repulsing water molecules at the cytoplasmic side of the channel (fig. 2a). Accordingly, the non-pathogenic Leu677Asn or Leu677Gly mutants of PC2, which were created by systematic site-directed mutagenesis and in which Leu677 is replaced with polar residues, greatly enhanced PC2 currents (fig. 2b,3). Similarly, the Phe604Pro mutation, another experimental mutation that is not reported in patients, results in constitutive activation of PC2, probably by bending S5 and pulling on the Leu677 hydrophobic gate (fig. 3b). This model suggests that changes in the conformation of the PCD might contribute to channel activation (fig. 3b), in which case the PCD might act as a lid on top of the channel and regulate channel activation in response to physical and/or chemical stimuli (fig. 3b).

The voltage sensor-like domain

The S4 of the PC2 VSD contains only two of the four positive charges that are usually found in voltage-gated channels (fig. 3). Of note, these positive charges are facing Asp511 in S3, which is a frequent site of missense pathogenic mutations in patients with ADPKD. An alternative model to the aforementioned model of PCD-regulated activation of PC2 (ref. 35) is that PC2 gating is controlled by conformational changes within the VSD (that is, segments S1–S4) (fig. 3d). In this model, the PCD would remain mostly static upon membrane depolarization, whereas the positively charged voltage-sensing domain S4 would be predicted to undergo a clockwise twist of about 4° together with an outward movement of about 4 Å (fig. 3d). Such a conformational change in the VSD is anticipated to result in an iris-like opening of the lower end of S6, where the hydrophobic gate (Leu677) that keeps the ionic pore closed is located (fig. 3a,d). The last nine residues of S4 (Ile571–Phe579) form a 3_10 helical region (that is, three residues per turn) that might be involved in gating movements of the S4–S5 linker, which is coupled to S6 via a hydrogen bond between Glu585 and Lys688 (ref. 35).

The inner activation gate

Before the publication of the structural studies, very limited information was available about the gating mechanisms of PC2 (refs 35,36). Leu677 at the cytoplasmic end of S6 forms a single-residue hydrophobic barrier that is predicted to prevent ion permeation by repulsing water molecules at the cytoplasmic side of the channel (fig. 2a). The PCD interacts with the pore of the constitutively open channel composed of Phe604Pro mutant subunits, indicating that the inner hydrophobic gate Leu677 alone might control the opening of PC2 currents (fig. 2b,3). The S6 of PC2 is characterized by the presence of α helix at amino acids Met668–Phe–Phe–Ile–Leu672, which possibly acts as a point of flexibility that causes the rotation of the Leu677 hydrophobic gate so that it points away from the centre of the pore. In the Phe604Pro mutant, the inner pore diameter increases from 5 Å to 7.6 Å, allowing cation permeation (fig. 2b,3). No major difference was found in the diameter of the selectivity filter between the closed channel and the constitutively open channel composed of Phe604Pro mutant subunits, indicating that the inner hydrophobic gate Leu677 alone might control the opening of PC2 (refs 35,36). (fig. 2). However, the selectivity filter might still possibly act as a secondary gate (fig. 2). Interestingly, a fifth binding site at the channel exit involving Asn681 and Ser685 (ref. 34). These structural data support electrophysiological studies indicating that PC2 permeation to monovalent cations is blocked by extracellular calcium, presumably through binding of calcium to Asp643 with a helix–loop–helix EF-hand motif (fig. 1b). The high-resolution structure of the EF-hand domain of human PC2 was determined using NMR spectroscopy (refs 37,38). The PC2 region 717–792 was shown to bind a single calcium ion with low affinity (122 μM), and this binding was abolished when Glu774 was mutated into Gln. Therefore, whereas calcium binding to the cytosolic carboxy-terminal EF-hand motif results in the activation of PC2, extracellular calcium binding to Asp643 blocks permeation of monovalent cations (refs 39,40,41,42).
the gain-of-function mutants Phe604Pro and Leu677Gly rescued the cystic phenotypes induced by *pkd2* knockdown in zebrafish more efficiently than wild-type PC2; this rescue effect was even more pronounced in the double mutant (Phe604Pro and Leu677Gly) 35. These in vivo findings provide further functional evidence that both Phe604Pro and Leu677Gly are gain-of-function mutations that open the PC2 channel.

The structural studies of PC2 provide novel insights into the permeation and the gating mechanisms of the PC2 ion channel. Currently, no structure for PC1 alone is available, and whether PC1 might form a biologically relevant multimer in the absence of PC2 is unknown. However, the reported structure of the PC1–PC2 heteromeric complex suggests an unexpected function for PC1 33.

The heteromeric PC1–PC2 complex
The structure of the PC1–PC2 complex was solved by cryo-EM at a 3.6 Å resolution 33 (Box 1). The amino-terminal domain (NTD) of PC1, formed by transmembrane helix 1 (TM1)–TM5, lies on the side of the PC1 VSD and contains a PC1 lipoxygenase and α-toxin (PLAT) domain that is located between TM1 and TM2 and faces the cytosol33 (fig. 1a,c). The PLAT domain can be found in a variety of membrane-associated or lipid-associated proteins, where it is involved in lipid binding and/or trafficking. Of note, the PC1 protein used for the structural studies of PC1–PC2 lacked the large cleavable extracellular NTD53.

The carboxy-terminal domain of the PC1 subunit is similar to that of PC2 and includes a VSD formed by S1–S4 as well as a PCD between S1 and S2 (fig. 1a,b). Importantly, sequences corresponding to the selectivity filter and the supporting pore helices (PH1 and PH2), as found in PC2, are absent in PC1 (fig. 1c,d). However, a 29-amino-acid-long segment (4,051–4,080), within the predicted pore region, was not resolved in the PC1 structure 53. The heteromeric PC1–PC2 complex is composed of one PC1 and three PC2 subunits, as previously suggested by studies of single molecules 54 (fig. 1e). Early studies indicated that PC1 and PC2 interact through their carboxy-terminal coiled-coil domains 38. However, the PC1 construct used for the subsequent structural determination of the complex showed that heteromultimerization with PC2 was possible, even in the absence of the cytosolic coiled-coil domains 33. Similarly to the homotetrameric PC2 channel, the channel core of the PC1–PC2 heteromeric complex is formed by 24 transmembrane segments, but it also contains an additional 5 transmembrane segments from the NTD of PC1, which renders the complex asymmetric (fig. 1e). The four VSDs in the heteromeric complex show a pseudo-fourfold symmetry, although the S6 of PC1 is bent in the middle of the helix (fig. 1a,e). Strikingly, three positively charged residues from the S6 — Arg4100, Arg4107 and His4111 — face the conduction pathway and are predicted to prevent cation permeation through the pore (fig. 1e). These findings suggest that the heteromeric PC1–PC2 complex may be non-conductive and that PC1 could act as a dominant-negative subunit of the PC2 channel 33.

**Fig. 2** | Closed and open states of PC2. a Wild-type polycystin 2 (PC2) channel in a closed state. The ionic pore is constricted at the external side of the channel within the selectivity filter between amino acids Leu641-Gly-Asp643. Leu677 forms an internal hydrophobic gate. b The Phe604Pro gain-of-function mutation, located within segment 5 (S5), constitutively opens PC2. The hydrophobic gate Leu677 swings away from the ionic path in Phe604Pro, but the external constriction at the selectivity filter is not altered. The solvent-accessible pathway is shown as a blue cloud (calculated with the HOLE program). PDB accession numbers: 5T4D and 6D1W; PyMOL 1.3 software.

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**References**
33. The amino-terminal domain (NTD) of PC1, formed by transmembrane helix 1 (TM1)–TM5, lies on the side of the PC1 VSD and contains a PC1 lipoxygenase and α-toxin (PLAT) domain that is located between TM1 and TM2 and faces the cytosol. The PLAT domain can be found in a variety of membrane-associated or lipid-associated proteins, where it is involved in lipid binding and/or trafficking. Of note, the PC1 protein used for the structural studies of PC1–PC2 lacked the large cleavable extracellular NTD. The carboxy-terminal domain of the PC1 subunit is similar to that of PC2 and includes a VSD formed by S1–S4 as well as a PCD between S1 and S2. Importantly, sequences corresponding to the selectivity filter and the supporting pore helices (PH1 and PH2), as found in PC2, are absent in PC1. However, a 29-amino-acid-long segment (4,051–4,080), within the predicted pore region, was not resolved in the PC1 structure. The heteromeric PC1–PC2 complex is composed of one PC1 and three PC2 subunits, as previously suggested by studies of single molecules. Early studies indicated that PC1 and PC2 interact through their carboxy-terminal coiled-coil domains. However, the PC1 construct used for the subsequent structural determination of the complex showed that heteromultimerization with PC2 was possible, even in the absence of the cytosolic coiled-coil domains. Similarly to the homotetrameric PC2 channel, the channel core of the PC1–PC2 heteromeric complex is formed by 24 transmembrane segments, but it also contains an additional 5 transmembrane segments from the NTD of PC1, which renders the complex asymmetric. The four VSDs in the heteromeric complex show a pseudo-fourfold symmetry, although the S6 of PC1 is bent in the middle of the helix. Strikingly, three positively charged residues from the S6 — Arg4100, Arg4107 and His4111 — face the conduction pathway and are predicted to prevent cation permeation through the pore. These findings suggest that the heteromeric PC1–PC2 complex may be non-conductive and that PC1 could act as a dominant-negative subunit of the PC2 channel.
Modelling PC1 in an ‘activated’ state (based on the Phe604Pro PC2 structure) shows that at least one positively charged residue remains in the conduction pathway (D.D., unpublished observations), suggesting that permeation through the complex might be impaired independently of PC1 activation. It will be interesting to study the co-expression of PC1 with PC2 Phe604Pro and/or Leu677Asn (constitutively open mutant forms of PC2) to functionally determine whether PC1 still inhibits opening of the activated PC2 channel.

These findings contrast with previous functional data that suggested that PC1 is required for the channel activity of PC2 at the plasma membrane 24,55,56. However, it remains possible that either direct or indirect binding of a specific ligand to the PC1–PC2 complex alters the conformation of PC1 and opens the channel 24,55,56. Alternatively, auxiliary subunits that can complex with PC1 or PC2, such as other TRP channel subunits, might modify the structural properties of the complex and allow ion permeation through the heteromeric PC1–PC2 channel. Another possibility is that the absence of the coiled-coil domain in the construct used for structural determination might have favoured a heteromeric assembly between truncated PC1 and the PC2 subunits that is not physiologically relevant.

These structural studies support the notion that PC1 might prevent cation permeation through the PC1–PC2 ion channel complex (Fig. 1e), which is rather unexpected as both PKD1 and PKD2 loss-of-function mutations cause ADPKD. The only difference is that the phenotype associated with PKD2 mutations is milder than that associated with PKD1 mutations and is characterized by a lower number of cysts in renal parenchyma, although the rates of cyst growth are identical 67. The difference in phenotype might be due to the difference in the size of both genes — the larger size of PKD1 might increase its probability of acquiring the required second hit mutation on the somatic allele 67.

In addition to ion permeation, the PC1–PC2 complex is also involved in regulation of the cell cycle, cell
adhesion and intracellular signalling. Disruption of any of these functions due to loss-of-function mutations in PKD1 or PKD2 could potentially contribute to polycystic disease. Notably, and similarly to PC2, the PCD of PC1 (REF.33) (Fig. 1a,b) is a hot spot for pathogenic mutations33 (Fig. 1c); the PC1 PLAT domain is another common site for pathogenic mutations33 (Fig. 1c). Strikingly, pathogenic mutations in the pore domain of PC1 have not been described, further suggesting that the pathogenic mechanisms of polycystin mutations might be independent of channel permeation (Fig. 1c). Clearly, further investigation will be needed to confirm and elucidate the significance of the reported structural findings.

**Ion channel functions of polycystins**

Polycystins are located at the membrane of the primary cilium and of the ER. The ion channel function of PC2 was demonstrated at both locations, activated by an increase in cytosolic calcium25,26,39 (Fig. 4).

**Cationic channels at the primary cilium**

Single-channel currents at the primary cilium of renal epithelial cells were first described in 2005 (REF.58), and electrophysiological findings reported in 2017 and 2018 suggest that these currents are PC2-dependent25,26. The electrophysiological activity of native ciliary PC2 was recorded in a murine cell line derived from inner

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**Fig. 4 | Ion channel function of PC2 at the primary cilium and the ER.**

(a) Patch clamp recording (inside-out configuration) of polycystin 2 (PC2) at the primary cilium of renal collecting duct cells. Once the electrode is attached to the primary cilium, the patch is excised in the inside-out configuration, depolarized and exposed to free intracellular calcium ([free-Ca$^{2+}$]$_{in}$) concentrations. (b) Inside-out cillum patch record from an inner medullary collecting duct epithelial cell cilium. The patch contained at least three active channels; O1, O2 and O3 indicate open states. Cytosolic calcium was progressively elevated from 0.09 µM to 0.3 µM, then to 3 µM and finally washed out. (c) Relationship between open-state probability of ciliary PC2 and membrane voltage with increasing internal calcium concentrations. (d) PC2 opening amplifies the release of calcium through the inositol-1,4,5-trisphosphate receptor (InsP3R) by a calcium-induced calcium release mechanism39,44,75; the endoplasmic reticulum (ER) PC2 open-state probability over cytosolic calcium concentration is normally distributed44. DIC, differential interference contrast. Parts a and b are adapted from REF.25, CC-BY-4.0 (https://creativecommons.org/licenses/by/4.0/). Part c is adapted with permission from REF.26, American Physiological Society.
medullary tubules, mIMCD-3, as well as in primary cultures of mouse collecting duct cells (FIG. 4a–c); of note, cysts in ADPKD are mainly found in the renal medulla. The researchers recorded a large-conductance non-selective cationic channel of 97 pS in physiological solution that had a PK⁺:PNa⁺ permeability ratio of 2.4, indicating that potassium is more permeant than sodium (FIG. 4b,c). Importantly, knockout of PKD2 with CRISPR–Cas9 technology suppressed this cationic current in mIMCD-3 cells26. Transfection of collecting duct cells with siRNAs against PKD2 or genetic deletion of this gene also eliminated cationic currents at the primary cilium35. Provocatively, PC2 channel activity at the primary cilium was still present in cells that lacked PC1 (REF. 25), a finding that is again in sharp contrast with previous observations that suggest an obligatory role for PC1 in the regulation of PC2 trafficking to the plasma membrane and channel gating23,24. In line with this finding, the amino-terminal domain of PC2 contains a ciliary trafficking motif (R6VxP)59. However, the possibility that the conditional Pkd1 mice used to test the activity of PC2 in the absence of PC1 might have some residual PC1 expression in tubular cells cannot be disregarded35. In addition, these findings do not support an inhibitory function of PC1 on PC2 permeation, as suggested by the structural data25. One possibility for this discrepancy is that the trafficking of heteromeric PC1–PC2 and homomeric PC2 complexes to the primary cilium occurs through independent pathways. In this scenario, inactivation of PKD1 might not influence the electrophysiological function of homotetrameric PC2 at the primary cilium35. However, even if the basal activity of PC2, which is presumably driven by the homotetramer of PC2 complex, occurs independently of PC1, it is still possible that PC1 regulates the PC2 channel. For example, PC1 activation in response to the binding of a ligand such as Wnt might result in the activation of PKD1–PC2 heteromeric complex35. Binding of Wnt to the extracellular domain of PC1 has been shown to result in PC2-dependent calcium influx27, although subsequent findings from experiments that used HEK and CHO cells co-transfected with both PC1 and PC2 have challenged the potential role of Wnt in PC2 activation27. Further experiments will be required to explain whether or not additional subunits and/or receptors are involved in the possible regulation of PC1–PC2 by Wnt.

Calcium permeation. The selectivity of PC2 for calcium is controversial, as one group reported a substantial PCa²⁺:PK⁻ permeability ratio of 0.55 (REF. 29) whereas another study reported a negligible PCa²⁺:PK⁻ ratio of 0.025, which suggested instead that PC2 is poorly permeant to calcium5,28. However, such low calcium permeability through PC2 (REF. 23) is at odds with previous findings from functional studies3,5,24,27,29,49. Moreover, ion channel chimaeras in which the selectivity filter of PC2 was inserted into the core of PC2-like 1 protein also resulted in substantial calcium permeability (PCa²⁺:PNa⁺ permeability ratio of 0.5)41. However, both the pore dilation and electrostatic fields of PC2-like protein 1 might influence calcium permeability of the chimeric channel25,42. Whether native PC2 permeates calcium therefore remains an open question.

Activation of PC2 at the primary cilium. The latest electrophysiological findings indicate that PC2 functions as a cationic channel at the primary cilium but not at the plasma membrane25,26. In the Xenopus oocyte, despite obvious targeting of PC2 to the plasma membrane, no significant channel activity could be detected35. Similarly, no current was detected at the plasma membrane outside the primary cilium in HEK or COS cells that overexpressed PC2 (REFS 55,60). These findings suggest that wild-type PC2 is in a closed conformation when expressed at the plasma membrane and becomes active at the primary cilium25,26.

In addition, the open-state probability of PC2 is dramatically enhanced by membrane depolarization, and by elevated intracellular calcium, possibly detected by the intracellular EF-hand motif5,26 (FIG. 1b,c). Under physiological conditions, cation influx (mostly potassium within the collecting duct) through PC2 is predicted to depolarize the primary cilium, although with a limited impact on the whole cell potential as the membrane resistance of collecting duct cells is low25.

Extracellular calcium blocks permeation of monovalent cations through PC2, presumably by interacting with Asp643 within the selectivity filter (half-maximal inhibitory concentration (IC₅₀) = 17 mM)25,34 (FIG. 1d,e). However, because urinary concentration of calcium is highly variable, ranging from 5 to 20 mM, PC2 is likely to be mostly active when urinary calcium concentration is in the lower range.

By contrast, intracellular calcium binding to the EF-hand motif within the cytosolic carboxy-terminal domain of PC2 is thought to promote channel activation30,40,49 (FIG. 1f). Because the resting membrane potential of primary cilia is –18 mV and intracellular calcium concentration is estimated to be in the range of 700 nM30, PC2 is predicted to be in the closed conformation under basal conditions as the intracellular calcium is too low and the membrane is not sufficiently depolarized to mediate channel opening26 (FIG. 4c). Thus, additional stimuli such as the stimulation of a membrane receptor and/or the presence of an intracellular second messenger or specific lipids might be required for the activation of PC2 and channel opening within the primary cilium. For example, PC2 might function as an epidermal growth factor (EGF)-activated ion channel40. EGF activates PC2 by releasing a protein diaphanous homologue 1 (mDia1) block at negative membrane potentials41. Of note, in mIMCD3 cells, the number of active channels per cilium was relatively low; 66% of cilia did not show PC2 activity and in the remaining cilia, only 1–6 channels were active at the primary cilium25.

Mechanosensing by the primary cilium

Initial reports claimed that PC1 and PC2 mediate mechanosensation at the primary cilium of kidney epithelial cells37. In response to shear stress (that is, elevated fluid flow), an increase in intracellular calcium was detected in cells that naturally express PC1 and PC2. Remarkably, this was not observed in cells that lacked PC1 (REF. 25).
Review

**Ratiometric calcium indicator**
A calcium-imaging method based on the use of a ratio between two fluorescent intensities (for example, the Fura-2 calcium probe).

**Mechanoprotection**
Inhibition of mechanosensitive ion channels by the cytoskeleton.

Following inhibition of PC2 with antibodies. The conclusion was that the PC1–PC2 complex was required for fluid flow sensing by the primary cilium in renal tubular epithelial cells\(^{25,26}\). In addition, it was suggested that loss of mechanosensation by the primary cilium upon pathogenic mutation of PC1 or PC2 might contribute to cystogenesis\(^{25,26}\); subsequent findings challenged this interesting hypothesis. One study showed that TrpV4, another TRP family channel subunit, interacts with PC2 at the primary cilium and is critically required for flow sensing, as visualized by calcium imaging\(^{25}\). However, because zebrafish or mice that are deficient in TrpV4 do not develop renal cysts, it seems unlikely that defective flow sensing by the primary cilium alone has a causative role in ADPKD\(^{25}\).

The proposed role of primary cilia as calcium-responsive mechanosensors was challenged by one study that used cultured cells derived from a transgenic mouse line that selectively expresses a genetically encoded ratiometric calcium indicator in all primary cilia\(^{40}\). The researchers reported that cilia-specific calcium influx did not occur in response to fluid flow, even when supraphysiological fluid flows were applied\(^{40}\). These results suggest that mechanosensation by the primary cilium might be independent of ciliary calcium signalling\(^{25}\). However, potential technical issues such as oversaturation of the calcium reporter due to a relatively high calcium concentration in the primary cilium might complicate the interpretation of these data\(^{1}\). In fact, several other reports support the increase in ciliary calcium in response to mechanical stimuli in cultured renal cells\(^{35,40}\).

Another hypothesis that warrants further investigation suggests that potassium and sodium permeability through PC2, independently of calcium influx\(^{25}\), could contribute to flow sensing by the primary cilium and that disruption of this mechanism might have a role in ADPKD. PC2 activation might also be secondary to the opening of a neighbouring depolarising and/or calcium-permeable ciliary channel or plasma membrane channel, such as PIEZO1 (REFS\(^{38,39}\)), in response to shear stress.

**Box 2 | PC2 and the determination of left–right asymmetry**

Although vertebrates show a bilaterally symmetrical body plan, visceral organs show left–right asymmetry in their structure and position, which is best exemplified by the heart. Symmetries are broken during early development, in a process that involves a unidirectional fluid flow, termed nodal flow, within an embryonic cavity at the ventral midline\(^{14,29}\). Polycystin 2 (PC2) (PC2) is exclusively expressed in the crown cells at the edge of the ventral node\(^{29}\). Nodal flow generated by the rotational movement of motile node cell monocilia is detected by the non-motile perinodal sensory monociliated cells\(^{30–40}\). Leftward nodal flow is responsible for breaking left–right symmetry in the embryo through activation of PC2 in crown cells\(^{30–40}\). Accordingly, homozygous PC2 mutant embryos show laterality defects\(^{30}\). Notably, PC1 is not expressed in nodal crown cells\(^{30}\), further indicating that PC2 can operate independently of PC1 (REF\(^{30}\)); moreover, left–right asymmetry is unaffected in Pak1-knockout mice\(^{30}\). By contrast, a laterality defect is also observed in a PC1-like 1 protein loss-of-function mutant and in Pak1D111–/– mice models as well as in humans carrying mutations in this gene\(^{20–24}\). Accordingly, PC1-like 1 protein interacts with PC2, and this interaction has a role in flow detection by nodal cells\(^{20–24}\). When nodal flow occurs, an asymmetric calcium increase is detected at the left margin of the node, upstream of the asymmetrical expression of nodal genes\(^{20–24}\).

Notably, ciliary localization of PC2 is essential for the control of left–right asymmetry\(^{20–24}\), but whether or not PC2 directly senses nodal flow or molecules transported by unidirectional nodal flow remains an open question\(^{20–24}\).

In the context of vertebrate left–right asymmetry determination during embryonic development, PC2 might also have a role in cilia mechanosensation, independent of PC1 (BOX 2).

Interestingly, in comparison to wild-type cells, endothelial cells that lack PC1 release less nitric oxide (NO), a vasorelaxing factor, in response to shear stress\(^{45}\). Of note, patients with ADPKD suffer from hypertension, a vascular dysfunction that might precede kidney failure\(^{46}\). However, shear-stress-induced activation of the endothelium seems to depend on the activation of the mechanosensitive ion channel PIEZO1 at the plasma membrane\(^{46,47}\). Deletion of Piezo1 not only prevents activation of the endothelium in response to shear stress but also causes vascular and valvular developmental defects as well as impaired NO release and associated hypertension in adult mice\(^{49,76,77,78}\). Interestingly, polycystins might regulate the function of PIEZO1. One study showed that Pkd1 deletion in arterial myocytes impairs activation of PIEZO1-dependent stretch-activated cationic channels at the plasma membrane\(^{46,47}\). Moreover, in both vascular and renal cells, the PC1:PC2 ratio influences PIEZO1 activity, at least partly through a mechanoprotection mechanism mediated by the filamin A–actin cytoskeleton network\(^{44,46}\). In the absence of PC1 (or when PC2 is elevated), the cortical skeleton becomes stiffer, which inhibits opening of PIEZO1 induced by membrane stretch. Thus, the impaired shear stress response of renal and endothelial cells in Pkd1-knockout mice might be explained by the resulting blunted activation of PIEZO1 rather than the loss of a direct contribution of polycystins to the primary cilium mechanosensation\(^{46,47}\). Of note, kidney-specific Piezo1-knockout mice do not have a cystic phenotype\(^{45}\).

**Calcium release through PC2 at the ER**

The carboxy-terminal domain of PC2 includes EF hands, as well as an ER retention signal. Accordingly, the majority of PC2 is found at the ER membrane\(^{46}\), where it forms a calcium-activated channel that is permeable to divalent cations, including Ca\(^{2+}\) and Ba\(^{2+}\) (REF\(^{46}\) (FIG. 4d)). When PC2 is overexpressed in the LLC-PK1 kidney proximal tubule cell line, vasopressin-induced calcium responses are greatly enhanced, whereas the pathogenic loss-of-function mutant PC2-D511V showed no effect\(^{46}\). These findings indicate that PC2 at the ER membrane is permissive to divalent cations\(^{4,43,44,46}\) (FIG. 4d). The open-state probability of PC2 in response to cytosolic calcium concentrations follows a normal distribution pattern that peaks at 0.3 μM (REF\(^{44–46}\)), meaning that channel activity is potentiated at calcium concentrations below 0.3 μM but becomes inhibited at higher concentrations\(^{44,46}\) (FIG. 4d). Interestingly, the calcium sensitivity of ER PC2 is modulated by casein kinase phosphorylation of Ser812 within its cytosolic carboxy-terminal domain\(^{46}\); the Ser812Ala mutant is approximately tenfold less sensitive to calcium activation than the wild-type PC2. In line with these findings, the Ser812Ala mutant greatly reduces potentiation of the vasopressin response by PC2 (REF\(^{46}\)). One study demonstrated that at the ER membrane, inositol-1,4,5-trisphosphate receptor (InsP\(_3\),R)-mediated release of Ca\(^{2+}\) from the ER induces
openings of the PC2 channel (calcium-induced calcium release), which in turn amplifies the Ca\(^{2+}\) release induced by InsP\(_3\), \textit{REF\(^{17}\)} \textit{FIG. 4d}). When calcium is released, the ER lumen becomes negatively charged and a potassium influx is required to balance the positive charge deficit. Whether PC2 also has a role in counterion conductance at the ER membrane and indirectly influences calcium release through InsP\(_3\)Rs has not yet been explored. Collectively, these findings suggest that the selectivity, and possibly the gating, of PC2 might differ depending on its subcellular localization (that is, primary cilium, plasma membrane or ER), as location might determine, for example, which lipids are available for binding to polycystins\(^{11}\), as well as other potential binding partners, such as other TRP channels, including TrpV4 and TrpC1 \textit{REF\(^{11,11}\)}). The potential modulation of polycystin functions by subcellular localization needs to be further evaluated. At this stage, it is also unclear whether PC2 at the ER requires PC1, or another related molecule, to function. Moreover, whether PC2 at the ER is relevant to ADPKD remains to be examined.

**Conclusions and perspectives**

PC2 is a non-selective cationic channel that is activated by both membrane depolarization and intracellular ciliary calcium and is inhibited by high extracellular calcium\(^{12,13}\). PC2 localizes to the primary cilium and ER membrane, and its ability to permeate calcium might be conditioned by its subcellular localization. This effect might be due to the specific membrane environment and/or the presence and contribution of auxiliary proteins such as PC1, fibrocystin, PC1-like proteins, PC2-like proteins or other TRP subunits. PC2 might also have a non-channel function, for example, in mitotic spindles, with potential effects on cell division, or even as a negative regulator of other types of ion channels, including the ryanodine receptor at the ER\(^{14}\), two-pore domain potassium channel TREK-2\(^{15}\) and PIEZO1 \textit{REF\(^{16}\)} at the plasma membrane. Breakthroughs in the structural characterization of PC1–PC2 revealed that PC1 might occlude the PC2 ion channel pore, suggesting that ion channel permeation might be unrelated to ADPKD. This hypothesis is reinforced by the observation that no pathogenic mutations are found in the putative pore region of PC1 \textit{REF\(^{17}\)} \textit{FIG. 1b}). However, these findings come from a single report and await confirmation; importantly, they need to be functionally validated by electrophysiological data. Nonetheless, these unexpected observations raise important novel questions. Is the solved PC1–PC2 structure physiologically relevant? What is the function of the homomeric PC2 channel in addition to its recognized role in nodal cells during embryonic development? What mediates PC2 calcium permeability in the ER? If channel activity is unrelated to ADPKD, what is the function of the PC1–PC2 heteromeric complex and how do pathogenic mutations cause the disease? Because numerous mutations are found in the PCD of both PC1 and PC2, what is the role of this extracellular ‘lid’ that sits on top of the channel complex? Is the PCD involved in either physical and/or chemical activation of the heteromeric PC1–PC2 complex? What function might the PC1 PLAT domain fulfill? Which lipids bind to PC1–PC2 and how does this affect its function or relate to ADPKD?

Despite being discovered more than 20 years ago, the biological role of polycystins and how mutations in \textit{PKD1} and \textit{PKD2} cause ADPKD are still obscure and require substantial further work to enable the identification of potential therapeutic options.

Published online 4 April 2019

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1. Harris, P. C. & Torres, V. E. Polycystic kidney disease. \textit{Annu. Rev. Med.} \textbf{60}, 321–337 (2009).
2. Arnaout, M. A. Molecular genetics and pathogenesis of autosomal dominant polycystic kidney disease. \textit{Annu. Rev. Med.} \textbf{52}, 93–123 (2001).
3. Torres, V. E. & Harris, P. C. Mechanisms of disease: autosomal dominant and recessive polycystic kidney diseases. \textit{Nat. Clin. Pract. Nephrol.} \textbf{6}, 530–538 (2010).
4. Patel, A. & Honore, E. Polycystins and renovascular mechanosensory transduction. \textit{Nat. Rev. Nephrol.} \textbf{2}, 40–55, quiz 55 (2006).
5. Hughes, J. et al. The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. \textit{Nat. Genet.} \textbf{10}, 151–160 (1995).
6. Mochizuki, T. et al. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. \textit{Science} \textbf{272}, 1359–1364 (1996).
7. Wilson, P. D. Polycystic kidney disease. \textit{N. Engl. J. Med.} \textbf{350}, 151–164 (2004).
8. Kim, I. et al. Polycystin-2 expression is regulated by a PC2-binding domain in the intracellular portion of fibrocystin. \textit{J. Biol. Chem.} \textbf{283}, 31559–31566 (2008).
9. Ousted, P. A. et al. A novel model of autosomal recessive polycystic kidney disease questions the role of the fibrocystin C-terminus in disease mechanism. \textit{Kidney Int.} \textbf{92}, 1130–1144 (2017).
10. Kotting, M. et al. TRPP2 and TRPV4 form a polyomodal sensory channel complex. \textit{J. Cell Biol.} \textbf{182}, 437–447 (2008).
11. Kobori, T., Smith, G. D., Sandford, R. & Edwardson, J. M. The transient receptor potential channels TRPP2 and TRPC1 form a heterotetramer with a 2-2 stoichiometry and an alternating subunit arrangement. \textit{J. Biol. Chem.} \textbf{284}, 35507–35513 (2009).
12. This work demonstrates a relationship between PC2 and TrpC1.
13. Anyanwu, I. N., Estrada, M., Tian, X., Somlo, S. & Ehrlich, B. E. Regulation of ryanodine receptor-dependent calcium signaling by polycystin-2. \textit{Proc. Natl Acad. Sci. USA} \textbf{104}, 6454–6459 (2007).
14. Li, Y. et al. Polycystin-1 interacts with inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca\(^{2+}\) signaling with implications for polycystic kidney disease. \textit{J. Biol. Chem.} \textbf{284}, 56431–56441 (2009).
15. This study indicates that PC2 allows the amplification of the InsP\(_3\)-dependent calcium release from the ER.
16. Li, Y. W., Johnston, J. M., Qian, F., Germain, G. G. & Guggino, W. B. Polycystin-2 interacts with type 1 inositol 1,4,5-trisphosphate receptor to modulate intracellular Ca\(^{2+}\) signaling. \textit{J. Biol. Chem.} \textbf{280}, 41298–41306 (2005).
17. Peyruron, R. et al. Piezo1-dependent stretch-activated channels are inhibited by Polycystin-2 in renal tubular epithelial cells. \textit{EMBO Rep.} \textbf{14}, 1143–1148 (2013).
18. This article presents evidence that PC2 inhibits PIEZO1 opening in renal epithelial cells through a cytoskeleton-mediated mechanoprotection mechanism.
19. Lantinga-van Leeuwen, I. S. et al. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. \textit{Hum. Mol. Genet.} \textbf{15}, 3069–3077 (2004).
20. This study demonstrates that a hypomorphic effect on the Pkd1 somatic allele is sufficient to cause ADPKD.
21. Piontek, K., Menezes, L. F., Garcia-Gonzalez, M. A., Huss, D. L. & Germain, G. C. A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1. \textit{Nat. Med.} \textbf{13}, 1490–1495 (2007).
22. \textit{This report shows that loss of Pkd1 before postnatal day 13 results in severely cystic kidneys.}
23. Wu, G. et al. Somatic inactivation of Pkd2 results in polycystic kidney disease. \textit{Cell} \textbf{93}, 177–188 (1998).
24. Delmas, P. Polycystins: from mechanosensation to gene regulation. \textit{Cell} \textbf{118}, 145–148 (2004).
25. Choi, Y. H. et al. Polycystin-2 and phosphorhadiesterase 4C are components of a cilary A-kinase anchoring protein complex that is disrupted in cystic kidney diseases. \textit{Proc. Natl Acad. Sci. USA} \textbf{108}, 10679–10684 (2011).
26. Yamaguchi, T., Hemison, S. J., Reif, G. A., Hedges, A. M. & Wallace, D. P. Calcium restores a normal proliferation phenotype in human polycystic kidney disease epithelial cells. \textit{J. Am. Soc. Nephrol.} \textbf{17}, 178–187 (2006).
27. Yamaguchi, T. et al. Calcium restriction allows cAMP activation of the B-Raf/ERK pathway, switching cells to a AMPD-dependent growth-stimulated phenotype. \textit{J. Biol. Chem.} \textbf{279}, 40419–40430 (2004).
28. Hanaoka, K. et al. Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. \textit{Nature} \textbf{408}, 990–994 (2000).
29. This study demonstrates that, in transfected CHO cells, PC1 interacts with PC2 at the plasma membrane to form a cationic channel.
30. Delmas, P. et al. Gating of the polycystin ion channel signaling complex in neurons and kidney cells. \textit{FASEB J.} \textbf{18}, 740–742 (2004).
31. This report demonstrates that, when overexpressed in sympathetic neurons, the PC1–PC2 complex can be activated at the plasma membrane by a PC1-targeting antibody.
Zheng, W. et al. Hydrophobic pore gates regulate ion channel activity by both depolarization and an increase in intracellular calcium.

Koulen, P. et al. Polycystin-2 is an intracellular calcium release channel. Proc. Natl Acad. Sci. USA 107, 9176–9181 (2010).

Petri, E. T. 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. USA 107, 24372–24385 (2010).

Ishii, T. et al. Polycystin-2 activation by inositol 1,4,5-trisphosphate-induced Ca2+-release requires its direct association with the inositol 1,4,5-trisphosphate receptor in a cilia-derived microdomain. J. Biol. Chem. 285, 18794–18800 (2010).

This study demonstrates that opening of PC2 at the ER membrane amplifies the release of calcium through the InsP3R.

Peyronnet, R. et al. Mechanosensation by polycystins against apoptosis is mediated through the opening of stretch-activated K(+/) channels. Cell 1, 241–250 (2012).

This study predicts that PC2 inhibits two-pore domain potassium channel TREK-2 opening by membrane stretch.

Shen, P. S. The 2017 Nobel Prize in Chemistry: cilia: the new organelle. Annu. Biomed. Chem. 410, 2053–2057 (2017).

McGrath, J., Somlo, S., Makova, S., Tian, X. & Bruckner, M. Two populations of node monocilia initiate left-right asymmetry in the mouse. Cell 114, 61–73 (2003).

Pennenkamp, P. et al. The ion channel polycystin-2 is required for left-right axis determination in mice. Curr. Biol. 12, 938–943 (2002).

This report shows that loss of PKD2 causes embryonic lethal defects.

Yoshida, S. et al. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. Science 338, 226–231 (2012).

This study demonstrates that flow-induced PC2 opening in nodal cilia is responsible for left–right asymmetry.

Karcher, C. et al. Lack of a latexin phenotype in Pkd1 knock-out embryos correlates with absence of polycystin-1 in nodal cilia. Differentiation 75, 425–432 (2005).

Field, S. et al. Pkd1 establishes left-right asymmetry and physically interacts with Pkd2. Development 138, 1131–1142 (2011).

Vogel, P. et al. Situs inversus in Dpck2−/−: Nme7−/−, and Pkd1−/− mice. Vet. Pathol. 47, 120–131 (2009).

Vetrie, F. et al. Bile-duct malformations in Pkd1−/− are associated with leaflet defects in humans. Am. J. Hum. Genet. 99, 866–895 (2016).

Kamura, K. et al. Pkd1 regulates motile cilia and functions to establish the left-right axis. Development 138, 1121–1129 (2011).

Grimes, D. T. et al. Cerebral analysis reveals a hierarchy of interactions between polycystin-encoding genes and genes controlling cilia function during left-right determination. PLOS Genet. 12, e1006070 (2016).

Acknowledgements
The authors thank the Human Frontier Science Program, the Fondation pour la Recherche Médicale and the Agence Nationale de la Recherche for support.

Author contributions
E.H. wrote the manuscript, D.D. performed the structural modelling and A.P. edited the manuscript.

Competing interests
The authors declare no competing interests.

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Reviewers
Nature Reviews Nephrology thanks S. Nauli and the other anonymous reviewers for their contribution to the peer review of this work.

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