Polycystins and renovascular mechanosensory transduction

Amanda Patel and Eric Honoré

Abstract | Autosomal dominant polycystic kidney disease is a common disorder, affecting approximately one in 1,000 individuals. This disease is characterized by the presence of renal and extrarenal cysts, as well as by cardiovascular abnormalities, including hypertension and intracranial aneurysms. Mutations in the *PKD1* gene account for 85% of cases, whereas mutations in *PKD2* account for the remaining 15% of cases. Findings from the past 10 years indicate that polycystins, the products of the *PKD* genes, have a key role in renal and vascular mechanosensory transduction. In the primary cilium of renal, nodal, and endothelial cells, polycystins are proposed to act as flow sensors. In addition, the ratio of polycystin-1 to polycystin-2 regulates pressure sensing in arterial myocytes. In this Review, we summarize the data indicating that polycystins are key molecules in mechanotransduction. Moreover, we discuss the role of nucleotide release and autocrine and/or paracrine purinergic signaling in both fluid flow and pressure responses. Finally, we discuss the possible role of altered mechanosensory transduction in the etiology of polycystic kidney disease.

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Introduction

Polycystin-1 is encoded by *PKD1*. This protein contains 4,302 amino acids and possesses a large extracellular domain of around 3,000 amino acids, in which several protein motifs are juxtaposed (Figure 1).[1–4] These motifs might be involved in protein–protein interactions, protein–sugar interactions, or the linking of polycystin-1 to one or several putative ligands; they are also capable of homophilic association, and therefore can behave as a membrane receptor and/or as a cell adhesion molecule.[1–4] Polycystin-1 has 11 transmembrane segments; the cytosolic carboxy terminal domain (which is 225 amino acids long) includes a coiled-coil domain that is implicated in protein–protein interactions. Polycystin-1 has an important role in cell–extracellular matrix interactions at focal adhesion sites, in cell–cell interactions at adherens junctions and/or at desmosomes, and in cellular signaling.[1–4] Several polycystin-1-like proteins have been identified, including PKDREJ, PKD1L1, PKD1L2 and PKD1L3.[5]

Polycystin-2 is encoded by *PKD2*. This protein contains 968 amino acids and includes six transmembrane segments, two cytosolic extremities with two EF-hands (helix–loop–helix domains that bind calcium), two coiled-coil domains, and an endoplasmic reticulum retention signal in the C terminus (Figure 1).[1–4] Polycystin-2 belongs to the transient receptor potential (TRP) family of cationic channels that are permeable to calcium.[6,7] Other members of the TRP polycystin (TRPP) subfamily include PKD2L1 and PKD2L2, which share structural homology with polycystin-2.[5]

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Competing interests
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Polycystin-1 and polycystin-2 interact with each other via their carboxy terminal coiled-coil domains (Figure 1).[1–4] Polycystin-1 is localized at the plasma membrane, whereas polycystin-2 is localized both at the plasma membrane and at the membrane of the endoplasmic reticulum. Importantly, the polycystin-1/polycystin-2 complex is present at the plasma membrane of primary cilium in a variety of cell types, including renal epithelial cells and endothelial cells.[3,8]

Over the past decade, the polycystin complex has been implicated in the regulation of both fluid flow and pressure sensing.[9–14] This Review describes evidence demonstrating that polycystins are key molecules in the regulation of mechanotransduction in various cells, including kidney epithelial cells, embryonic nodal cells, endothelial cells, and vascular smooth muscle cells.

Polycystins mediate flow sensing

Role of the primary cilium: *in vitro* evidence

Almost every cell type contains a primary cilium with a few exceptions, such as cells of the immune system.[3,15–17] The primary cilium is a nonmotile cilium with nine peripherally located microtubule pairs and the absence of a central doublet, which is only found in motile cilia (Figure 2a). In the kidney, each principal cell has one primary cilium during interphase and during the G0 phase of the cell cycle. This cilium subsequently disassembles during cell division. Multiple lines of evidence indicate that defects in ciliogenesis (for instance, caused by mutations in *IFT88*, which encodes the cilium cargo protein polaris) provoke polycystic kidney disease (PKD).[3,15–17] The primary cilium of Madin–Darby canine kidney (MDCK) cells, a renal epithelial cell line derived

from the canine distal tubule, bends and acts as a flow sensor in response to increased fluid flow rate (that is, in response to shear stress).[18,19] This response is thought to depend on the presence of the primary cilium, as cells treated with chloral hydrate or immature (that is, non-confluent) cells that lack a primary cilium do not respond to shear stress.[20] Of note, however, chloral hydrate not only induces ciliary loss but may also produce non-specific effects that are unrelated to the primary cilium, such as the inhibition of cell division and protein synthesis.[20] In addition, it should be noted that experiments supporting a role for the primary cilium in flow sensing in kidney tubules have to date been performed in cell lines and that *in vivo* evidence in support of this phenomenon is still lacking. Nevertheless, direct bending of the primary cilium in MDCK cells by use of a micropipette induces an increase in intracellular calcium level.[18] Thus, the primary cilium is proposed to act as a mechano-sensitive antenna that is able to sense fluid flow in the kidney tubule.[8,19] The initial part of the calcium response critically requires extracellular calcium and is inhibited by administration of the lanthanide ion Gd^{3+} or by amiloride—blockers of stretch-activated ion channels (SACs).[18] Flow-induced calcium entry is followed by the release of intracellular calcium from the endoplasmic reticulum, mediated either by inositol 1,4,5-trisphosphate or by activation of ryanodine receptors, depending on the cell type involved.[9,18] The initiation of this flow response is rather slow, occurring a maximum of about 30 s after shear stress is applied. The response also shows strong desensitization upon repetitive flow stimulation.[18] The physiological relevance of this adaptation has not yet been addressed.

Remarkably, renal cells isolated from transgenic mice that lack functional polycystin-1 do not show a calcium response to increased fluid flow (Figure 2b).[9] Moreover, blocking antibodies directed against polycystin-2 abolish the flow response of wildtype kidney epithelial cells.[9] The large extracellular domain of polycystin-1 is thought to serve as an extracellular sensor for the flow of urine in the kidney and the resulting activation (that is, a conformational switch) of polycystin-1 may result in opening of the ionic pore of polycystin-2, thereby allowing calcium entry into the primary cilium.[8,9] The unusual mechanical strength of this extracellular domain may be supportive of such a hypothesis.[21,22] The resulting local increase in cytosolic calcium concentration regulates various molecular pathways inside the cell that contribute to tissue development and morphogenesis.[3,19] The inability of cells to sense flow has been proposed to result in cyst formation.[9]

Polycystin-2 interacts with other TRP channels, including TRPC1 and TRPV4, which are also located in primary cilia.[23,24] Interestingly, TRPC1 has been proposed to form a calcium-permeable SAC, although direct mechanosensitivity of TRPC1 remains controversial.[25,26] Furthermore, knockdown of *TrpV4* in MDCK cells impairs the flow-induced increase in calcium concentration.[23] These findings suggest that heteromeric TRP channels, including TRPP2 and TRPV4, are probably involved in flow sensing by the primary cilum.

Key points

- The polycystin receptor–ion channel complex formed by polycystin-1 and polycystin-2 is localized in the primary cilium and is necessary for flow sensing in both renal epithelial and endothelial cells
- Bending of the primary cilium by fluid flow is anticipated to induce a conformational change in the polycystins, which results in calcium influx
- Fluid flow induces the cilium-dependent release of nucleotides and the autocrine and/or paracrine stimulation of purinergic receptors; pressure induces the cilium-independent release of ATP
- Activation of the endothelial primary cilium polycystin complex by fluid flow mediates nitric oxide release, causing vasodilation
- Polycystin-2 in nodal sensory cilia is involved in determination of left–right asymmetry
- The ratio of polycystin-1 to polycystin-2 regulates the opening of stretch-activated ion channels in arterial myocytes and affects myogenic tone

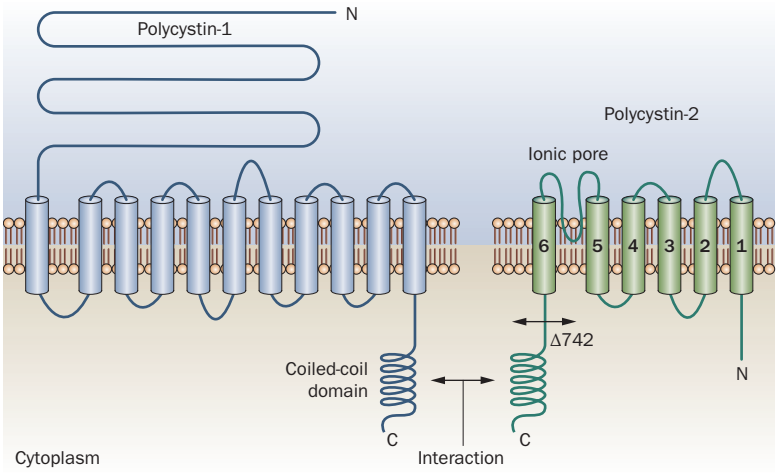

Figure 1 | Membrane topology of the receptor-ion channel complex polycystin-1/polycystin-2. The pore of the ion channel is located between transmembrane segments 5 and 6 of polycystin-2. Transient receptor potential channels, such as polycystin-2, have been shown to assemble as homotetramers or as heterotetramers,[73] although other data indicate that polycystin-2 may exist in the plasma membrane as a trimer, which can interact with polycystin-1 to form a heteromer with a 3:1 stoichiometry.[74] A truncated pathogenic polycystin-2 mutant (Δ742) lacks an endoplasmic reticulum retention signal. Polycystin-1 and polycystin-2 interact with each other via their carboxy terminal coiled-coil domains.

Surprisingly, in renal epithelial cells from patients with autosomal recessive PKD caused by mutations in the gene that encodes fibrocystin, an exaggerated flow-induced calcium response is seen compared with that seen in control cells.[27] Of note, expression of polycystin-2 tends to be higher in cells from these patients than in control cells, further suggesting a role for this channel in flow sensing.[27]

The primary cilium and cell polarity

Defects in planar cell polarity that lead to disoriented cell division have been associated with cystogenesis in autosomal dominant PKD.[28] The position of the primary cilium, which is determined by fluid flow, has been proposed to influence centrosome localization and therefore helps to define the plane of cell division within the renal tubule.[29] Whether flow-induced calcium signaling

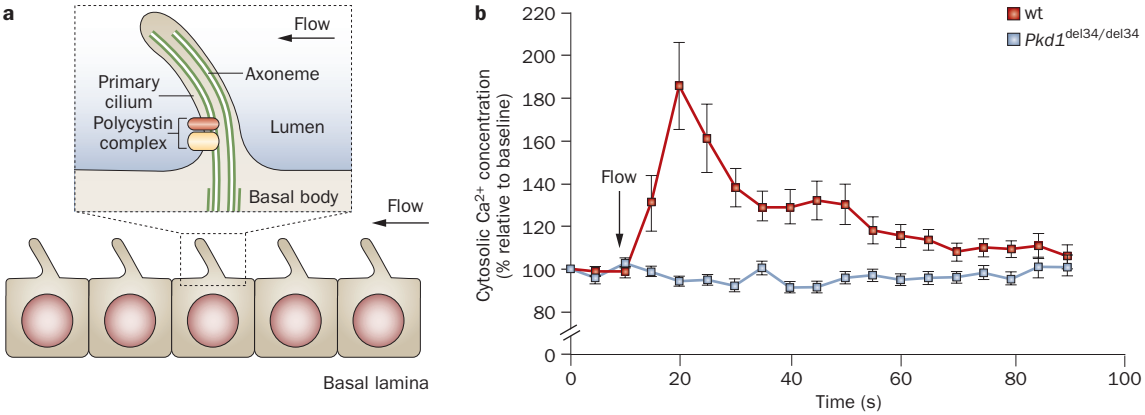

Figure 2 | Role of the primary cilium in fluid flow sensing. **a** | Fluid flow (that is, shear stress) in the lumen of the renal tubule is thought to bend the primary cilium of epithelial principal cells. The primary cilium contains tubulin in the form of an axoneme (shown in green). The polycystin complex polycystin-1/polycystin-2 (shown in yellow and red) is localized at the plasma membrane of the primary cilium. With kind permission from Springer © Delmas, P. *Pflugers Arch*. **451**, 264–276 (2005). **b** | Fluid flow is thought to induce cilium bending and induce a conformational change in the polycystin-1/polycystin-2 complex, which leads to channel opening and calcium influx. Epithelial cells from wildtype mice show an increase in intracellular calcium in response to fluid flow. This calcium signal is impaired in epithelial cells from mice with a targeted deletion of *Pkd1* exon 34 (Pkd1 $^{del34/del34}$ mice). Permission obtained from Nature Publishing Group © Nauli, S. M. *et al. Nat. Genet*. **33**, 129–137 (2003). Abbreviation: wt, wildtype.

in the primary cilium directly affects planar cell polarity remains to be defined.

Fluid flow and cystogenesis

Although strong evidence from *in vitro* studies are in favor of the polycystin-1/polycystin-2 complex acting as a flow sensor in the primary cilium of kidney epithelial cells,[9] the relevance of this mechanism to cystogenesis has been questioned over the past three years. Indeed, knockdown of *TrpV4* completely impairs the cilium-dependent flow-induced increase in calcium level both *in vitro* and *in vivo*, although interestingly, no cyst formation is observed in either *TrpV4* knockout mice or in knockdown zebrafish.[23] Furthermore, intrauterine kidney cysts occur in global *Pkd1*-knockout and in *Pkd2*-knockout mice, at a time when renal tubule flow is anticipated to be either absent or very low in the developing embryo.[30–33] In addition, inactivation of *Pkd1* before postnatal day 13 results in accelerated severe cyst formation whereas inactivation of *Pkd1* after postnatal day 13 results in delayed and mild cyst formation, revealing a restricted time window during which *Pkd1* inactivation leads to cyst formation and indicating that cystogenesis requires more than just the loss of cilia-mediated mechanosensation.[34] Similar results were reported for the conditional inactivation of either *Ift88* or *Kif3a*, which encode proteins that are involved in intracilium transport.[35] Loss of either of these genes results in a lack of cilium formation. As in the *Pkd1*-knockout model, cystogenesis in these conditional knockout models was dependent on the time of gene inactivation, and did not appear in adult mice until 6 months after gene deletion.

Polycystin-2 and left–right asymmetry

Polycystin-2 is also localized in primary cilia of the embryonic node.[12,13] During early embryonic development, the leftward flow of perinodal fluid (that is, nodal flow) generated by motile monocilia initiates left–right asymmetry of the embryo.[15] Nonmotile peripheral polycystin-2-containing cilia respond to nodal flow by inducing an asymmetric increase in calcium influx at the left margin of the node. This process activates molecular pathways that are responsible for inducing left–right asymmetry (Figure 3).[12,13]

Pkd2-knockout mice have abnormal left–right asymmetry and develop *situs inversus*.[13,30] The nonmotile sensory nodal cilia of *Pkd2*-knockout mice that would normally express polycystin-2 fail to respond to fluid flow generated by neighboring motile nodal cilia.[12,13] Of note, nodal cells that express *Pkd2* lack *Pkd1*, suggesting that polycystin-1 is not required for flow sensing in these cells.[36] Accordingly, no laterality defect is found in *Pkd1*-knockout mice.[36] Other subunits that interact with polycystin-2, including PKD1L1, may be involved in flow sensing in nodal cells.[37]

The endothelium and shear stress

Polycystin-1 and polycystin-2 are expressed in vascular smooth muscle and in the endothelium of most blood vessels, including the aorta and cerebral arteries (Figure 4).[38,39] The expression of polycystins in the vascular wall is complex and developmentally regulated.[40–42] A large number of patients affected by autosomal dominant PKD suffer from arterial lesions and arterial dysfunction.[38,39]

Arterial dysfunction associated with PKD

Intracranial aneurysms are associated with autosomal dominant PKD, occurring in around 10% of patients with this disease compared with just 1% of the general population.[40] Other cardiovascular anomalies are also common; prolapse of the mitral valve occurs in 20–30%

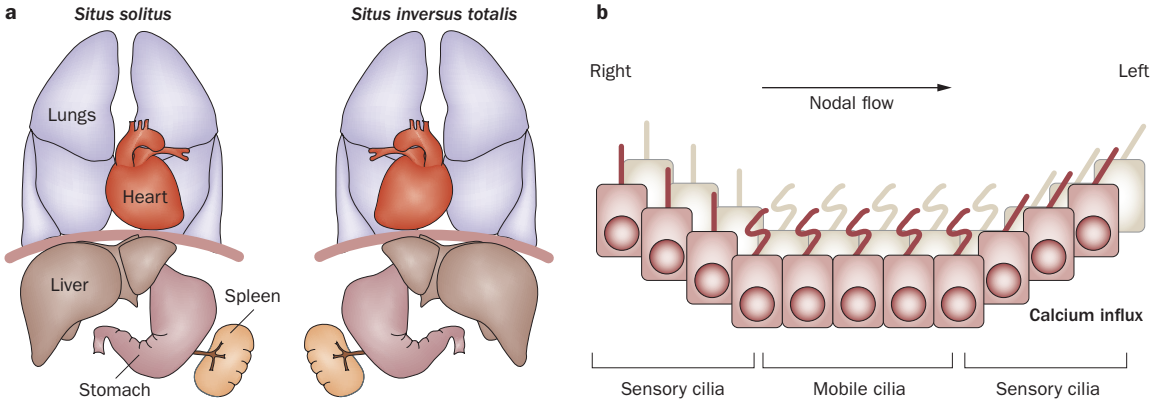

Figure 3 | Role of polycystins in left–right asymmetry. **a** | Normal left–right asymmetry (*situs solitus*) and *situs inversus* as is observed in *Pkd2*-knockout mice. **b** | Motile cilia in the center of the embryonic node generate a leftward nodal flow that induces bending of non-motile sensory primary cilia at the periphery of the node and triggers an asymmetric calcium distribution in the node. The bending of these cilia is thought to induce opening of polycystin-2, calcium influx, and an increase in the intracellular calcium concentration that initiates the development of left–right asymmetry. However, other signal transduction events including the ligand stimulation of membrane receptors are also likely to have a role. Permission obtained from Nature Publishing Group © Fliegauf, M. *et al. Nat. Rev. Mol. Cell Biol.* **8**, 880–893 (2007).

of patients with autosomal dominant PKD and aortic dissections have also been described.[40] Moreover, hypertension, which affects 50–70% of patients with autosomal dominant PKD, appears at early stages of the disease, well before the appearance of renal lesions. This finding is indicative of an endothelial defect as has been discussed in detail elsewhere.[38,39] The endothelial dysfunction that is associated with autosomal dominant PKD also potentially contributes to progression of renal disease.[43]

Response of the endothelium to shear stress

Similarly to renal epithelial cells, the endothelial cells that line the inside of blood vessels respond to shear stress (Figure 4).[44] Blood flow induces an increase in intracellular calcium concentration followed by the endothelial release of nitric oxide, which leads to vasodilation. The primary cilia of endothelial cells are also involved in blood flow sensing.[10] Indeed, endothelial cells isolated from *Ift88*-knockout mice that lack primary cilia fail to respond to fluid flow.[10] Moreover, endothelial cells isolated from aortas of *Pkd1*-knockout mice are not sensitive to fluid flow, although they are activated by acetylcholine (a natural vasodilator), demonstrating the selective impairment of flow sensing.[10] *Pkd1* haploinsufficiency in mice is associated with altered vascular reactivity and hypertension.[45] Interestingly, when endothelial cells are equilibrated for 30 min under conditions of high shear stress, they become desensitized to subsequent increases in fluid flow.[10] Under these conditions of chronic shear stress, polycystin-1 becomes truncated and almost the entire functional full-length polycystin-1 is lost, further indicative of a key role for polycystin-1 in flow sensing in the endothelium.[10] In an elegant study, the Nauli group showed that polycystin-2 is localized to the primary cilium of endothelial cells.[11] Furthermore, the flow-induced increase in intracellular calcium concentration and release of nitric oxide were impaired in endothelial cells from *Pkd2*-knockout mice.[11] Unlike endothelial cells from homozygous *Pkd2*-knockout mice, endothelial cells from heterozygous *Pkd2*-knockout mice respond to flow, which suggests that the vascular phenotype of autosomal dominant PKD may involve a two-hit mechanism, with one germline and one somatic mutation, as has previously been proposed for cystogenesis.[11,46] Although these results are indicative of a direct role for polycystin-2 in flow sensing in endothelial cells, its function as a calcium-release channel in the endoplasmic reticulum may also contribute to this hemodynamic

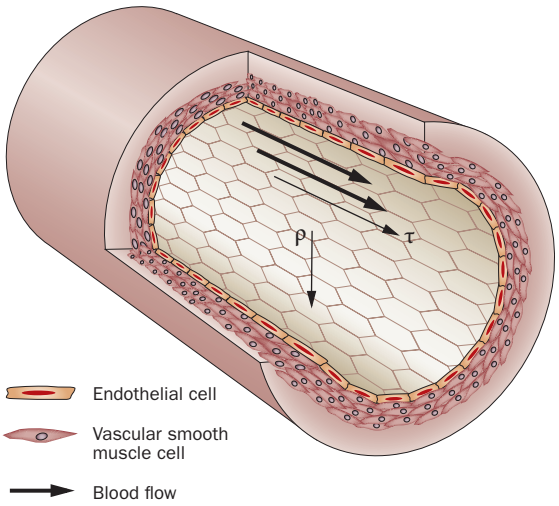

Figure 4 | Hemodynamic forces that act on blood vessels. Shear stress (τ) is a lateral force that activates the endothelium and induces nitric oxide release, which causes vasorelaxation. Pressure (ρ) is a force that acts perpendicular to the arterial wall, stretching myocytes and inducing contraction (also known as myogenic tone or the Bayliss response). Resistance arteries have a key role in the control of peripheral vascular resistance and tissue perfusion. They possess a basal myogenic tone, which is opposed by flow-mediated (that is, shear stress-mediated) dilation. Permission obtained from Nature Publishing Group © Hahn, C. & Schwartz, M. A. *Nat. Rev. Mol. Cell Biol.* **10**, 53–62 (2009).

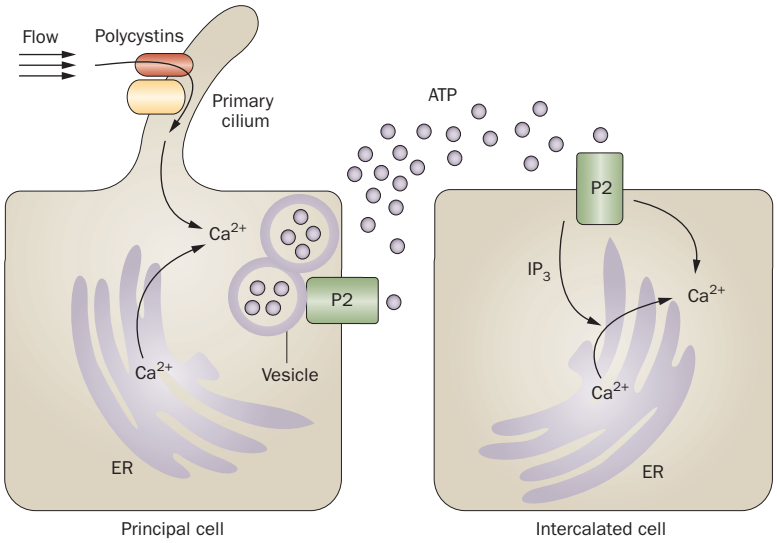

Figure 5 | Role of ATP and purinergic stimulation in the flow response. Renal epithelial ciliated cells (or endothelial cells) respond to fluid flow by increasing intracellular levels of calcium. This response is thought to depend on the activity of polycystins in the primary cilium, on ATP release (which is presumably vesicular although other mechanisms including connexins, anion channels or ABC transporters could also be involved[75]), and on the autocrine stimulation of P2 purinergic receptors. P2X and P2Y receptors are expressed by renal epithelial cells and by endothelial cells.[54] Intercalated renal epithelial cells probably lack a primary cilium, and are thought to respond to urine flow through the paracrine stimulation of purinergic receptors. Other flow sensors or autocrine purinergic signaling independent of the primary cilium may also be involved. Of note, polycystin-1 and polycystin-2 are not distributed evenly throughout the nephron; therefore, it may be questioned whether the presence of a polycystin-1/polycystin-2 complex is indeed critical for the flow response in all renal epithelial cells. Pressure also induces ATP release in renal epithelial and vascular cells, but does not involve the primary cilium. Abbreviations: ER, endoplasmic reticulum; IP3, inositol trisphosphate.

response.[47] Together, these findings indicate that flow stimulation may activate the polycystin-1/polycystin-2 complex in endothelial primary cilia to promote calcium influx that in turn triggers nitric oxide release and vasodilation. The failure to release nitric oxide in response to shear stress has been proposed to be clinically relevant to the development of hypertension in patients with autosomal dominant PKD.[11]

ATP release and purinergic stimulation

ATP is released in response to fluid flow

In the perfused rabbit collecting duct, the flow-induced elevation in calcium level occurs both in principal and intercalated cells. Although some controversy exists, intercalated cells are generally thought to lack a primary cilium.[8,19] Assuming that intercalated cells do not contain a primary cilium, these findings suggest that a diffusible factor may be released by flow-stimulated ciliated principal cells, which in turn may activate non-ciliated intercalated cells. Of note, a 2009 study demonstrated the flow-induced elevation of calcium level in MDCK cells to be mediated by ATP release and by stimulation of the P2Y purinergic receptor.[48] Stepwise increases in fluid flow in the absence of pressure changes in a semi-open flow chamber enhance the concentration of ATP in the superfusate of cultured ciliated MDCK cells.[48] Moreover, placing confluent ciliated MDCK cells upstream of the flow path of non-confluent cells (that is, immature cells that lack a primary cilium and that are normally non-responsive to flow, but are sensitive to extracellular ATP) induces the nonconfluent cells to respond to flow by increasing their intracellular calcium level.[48] This 'acquired' response is inhibited by the purinergic-receptor blocker suramin or by apyrase, which catalyzes the hydrolysis of ATP, further indicating that ciliated cells release ATP during flow stimulation, which stimulates the P2Y purinergic receptors of downstream non-ciliated cells.[48] Alternatively, it can be argued that other cilium-independent flow sensors may be present in the intercalated cells that may respond in an autonomous fashion to shear stress, although evidence in support of this hypothesis is lacking.

Role of ATP in the flow response

The role of ATP release in the shear-stress response has been further investigated using a mouse model of PKD called the Oak Ridge Polycystic Kidney (orpk) mouse. These mice have a mutation in *Ift88*, which encodes polaris. Collecting duct principal cells from orpk mice, which lack a primary cilium were grown as polarized cell monolayers and compared with collecting duct principal cells from orpk mice that had been genetically rescued.[49] Constitutive ATP release under basal conditions was found to be low and not different in mutant versus rescued monolayers.[49] However, the amount of ATP released in response to harsh pipetting of medium was threefold to fivefold higher in rescued cells than in mutant cells.[49] In addition, flow-induced calcium signaling was reduced by apyrase.[49] The authors of that study proposed a calcium-dependent vesicular mechanism of ATP release. Importantly, flow-induced ATP release from cyst cells from patients with autosomal dominant PKD is lower than that from normal kidney cells.[50] These findings link the sensory apical non-motile primary cilium to ATP secretion and flow-induced calcium signaling in renal cells (Figure 5). P2X and P2Y purinoceptors (P2) are present in kidney cells and are located on, or near the primary cilium to transduce this autocrine ATP signal.[51–54]

Loss of a cilium-dependent autocrine purinergic signaling system has been proposed to be a critical event that underlies the etiology of PKD.[49] In support of this hypothesis, the flow-induced calcium response of isolated mouse aortas restrained in a capillary to avoid stretching is strongly reduced by the hydrolysis of external ATP by apyrase or by knockdown of *Pkd2*.[11] In addition, the calcium response and nitric oxide release by endothelial cells in response to fluid flow is impaired in *P2x4*-knockout mice, but is rescued by overexpression of *P2x4*.[55] Furthermore, vessel dilation (as a consequence of nitric oxide release) induced by acute increases in blood flow is markedly suppressed in *P2x4*-knockout mice. Consequently, *P2x4*-knockout mice have higher blood pressure and excrete smaller amounts of nitric-oxide-derived products in their urine than do wildtype

mice.[55] Finally, no adaptive vascular remodeling (that is, a decrease in vessel size in response to a chronic decrease in blood flow) is observed in *P2x4*-knockout mice.[55] These findings indicate that both renal epithelial cells and endothelial cells may respond to shear stress through primary cilium-dependent and polycystin-dependent calcium influx, which leads to an increase in intracellular calcium level, to release of ATP (presumably by vesicular mechanisms), and to autocrine and/or paracrine stimulation of purinergic receptors (Figure 5).

Cilium-independent pressure activation

In addition to experiencing subtle changes in fluid flow rates, renal epithelial cells are also subjected to stretch. For instance, epithelial cells in the distal part of the collecting duct are exposed to distension during papillary contractions.[56] Moreover, abnormal fluid accumulation in renal cysts also causes the cyst wall to stretch. Indeed, in patients with autosomal dominant PKD, renal cysts may be several centimeters in diameter and cyst pressures may range from 6 mmHg to 70 mmHg.[57] Cyst walls are in particular under great tension because tension correlates with cyst radius according to the law of Laplace: tension = (pressure × radius)/2 × wall thickness.

Epithelial stretch, together with other predisposing factors, are stimuli that may contribute to cell proliferation and cyst enlargement.[58] Both stretch and *Pkd1* inactivation are in fact particularly effective stimuli for proliferation in poorly differentiated cells or in cells that are inclined to enter the cell cycle in the developing kidney.[34,59,60] Indeed, ligation of the ureter in rabbit fetuses (unlike in the adult) produces cystic changes throughout the nephron[60] and stretching MDCK monolayers by 25% approximately doubles the percentage of cells that synthesize DNA.[58] Mechanical stretch of tubular cells is also a key primary insult in obstructive nephropathy.[61] Therefore, pressure-induced membrane stretch is relevant to both physiological and pathophysiological kidney conditions.

In perfused kidney tubules, increasing the inflow perfusion pressure provokes sudden distension of the tubule (that is, stretch of tubular cells), nucleotide release, and calcium signaling via P2Y2 receptors.[62] Accordingly, in MDCK cells, an increase in trans-epithelial pressure triggers a rise in intracellular calcium level.[56] The amplitude of the calcium response correlates with the level of the applied pressure. The pressure response can be produced in immature non-confluent MDCK cells or in cells treated by chloral hydrate that do not possess primary cilia.[56] Moreover, the application of pressure either to the apical side of MDCK cells or to the basolateral side, which does not contain a primary cilium, results in a similar calcium response, demonstrating this response to be cilium independent. The cilium-independent response to such pressure is rapid, does not critically require extracellular calcium, and can be repeated with no desensitization, unlike the cilium-dependent response to fluid flow.[56,62] Importantly apyrase and suramin also inhibit the pressure response, indicating the involvement of purinergic receptors.[56] Therefore, the pressure response of renal epithelial cells does not require the primary cilium, but similar to the response to fluid flow, it involves the release of nucleotides and the autocrine and/or paracrine stimulation of purinergic receptors.

A subset of fluids from the cysts of patients with autosomal dominant PKD contain very high concentrations of ATP in the range of 0.5–10 μM.[63] In contrast to flow-induced ATP release, the release of ATP that is induced by either constitutive (that is, basal) or hypotonic (that is, swelling) conditions is greater in renal epithelial cells from patients with autosomal dominant or autosomal recessive PKD than in renal epithelial cells from healthy individuals.[64] Nucleotide release and nucleotide signaling have been proposed to contribute detrimentally to the gradual expansion of cyst fluid volume.[63,65,66] Together these findings indicate that pressure-induced ATP or nucleotide release, purinergic stimulation, and calcium signaling may have important functional roles in kidney pathophysiology.

Pressure-induced ATP release has also been described in vascular cells. For instance, in freely moving mouse aortas, a stepwise increase in flow rate (resulting in both shear stress and an increase in lumen diameter caused by stretch of the arterial wall) induces a sustained cytosolic calcium response that is greatly decreased by apyrase or by knockdown of *Pkd2*.[11]

Stretch-activated ion channels

Polycystins are abundantly expressed in arterial myocytes that respond to pressure rather than flow.[38,39] An increase in intraluminal pressure causes the gradual depolarization of vascular smooth muscle cells (VSMCs) that is linked to the opening of non-selective SACs. This process is followed by the opening of voltage-gated calcium channels, which results in an increase in intracellular calcium concentration and myocyte constriction, also known as myogenic tone or the Bayliss response.[67,68]

We recently demonstrated that polycystin-2 inhibits SACs in a variety of cells, including vascular myocytes and renal epithelial cells.[14] This specific effect does not occur with other TRP channels such as TRPC6 or TRPV4, and is in fact reversed by co-expression of polycystin-1, indicating that it is the ratio of polycystin-1 to polycystin-2 that regulates SAC opening. Moreover, deletion of *Pkd1* in smooth muscle cells reduces SAC activity and arterial myogenic tone.[14] Conversely, knocking down *Pkd2* in *Pkd1*-deficient arteries rescues both SAC opening and the myogenic response.[14] Finally, polycystin-2 interacts with filamin A—an actin cross-linking protein that is critical for SAC regulation by polycystins (Figure 6).[14] Filamin A stiffens the cell cortex by cross-linking adjacent actin filaments (reviewed in detail elsewhere[69]). Consequently, localized increases in filamin A may cause an accumulation of cross-linked F-actin in the cell cortex and reduce SAC activity.[14] The subcellular location of polycystin-2 does not seem to affect its ability to inhibit SAC, as both the wildtype form of the protein that is predominantly found in the endoplasmic reticulum and the mutant Δ742 form (Figure 1), which lacks an

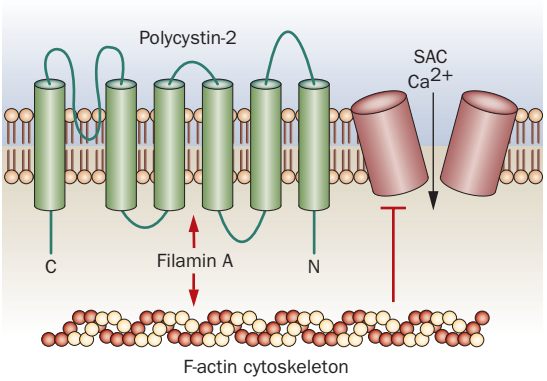

Figure 6 | Role of polycystins in the regulation of stretch-activated ion channels. Expression of polycystin-2 inhibits calcium-permeable stretch-activated ion channels opening via the filamin A–F actin pathway; by contrast, polycystin-1 reverses this effect (not shown). Abbreviation: SAC, stretch-activated ion channel.

endoplasmic reticulum retention signal and is therefore more efficiently targeted to the plasma membrane, have inhibitory function.[14,70,71] Whether alterations in SAC activity are involved in the arterial defects associated with autosomal dominant PKD, including brain aneurysms and aortic dissections, remains to be experimentally determined. A loss of myogenic tone is anticipated to result in an increase in wall tension (owing to a larger arterial radius for a given pressure according to the law of Laplace), which would possibly contribute to aneurysm formation.

These findings are in contrast to the proposed role of the polycystin-1/polycystin-2 complex as a flow sensor in the primary cilium of renal epithelial and endothelial cells, where inactivation of either polycystin-1 or polycystin-2 impairs flow sensing.[9,10] In VSMCs, it is the ratio of polycystin-1 to polycystin-2 that regulates the activity of SACs.[14] Myocytes in the arterial wall are not subjected to blood flow, although they possess a primary cilium. Thus, this organelle may have another role in arterial myocytes independent of flow sensing. Indeed, it should be remembered that the primary cilium is also centrally involved in various signaling pathways, including the Wnt and hedgehog pathways and thus may also fulfill an important chemosensory function.[15,17]

In the endothelium, polycystins are thought to be responsible for flow sensing, whereas in arterial myocytes they regulate pressure sensing. Arterial autoregulation induced by flow-mediated dilation and myogenic responses are anticipated to profoundly affect renal vasoreactivity and may therefore also influence renal disease progression.

Conclusions

The findings discussed in this Review indicate a key role for polycystins in cellular mechanosensory transduction. Polycystin-1 and polycystin-2 are proposed to be involved in flow sensing by the primary cilium.[3] The resulting calcium signal is amplified by nucleotide release and by the autocrine and/or paracrine stimulation of purinergic receptors (Figure 5).[72] Loss of flow sensing may be involved in the pathogenesis of cystogenesis and may also contribute to hypertension associated with autosomal dominant PKD.[9–11] Although the elegant *in vitro* findings obtained by studies in various cell lines are strongly supportive of a role for polycystins as flow sensors in the primary cilium, no *in vivo* demonstration of this role has yet been provided. Moreover, several observations from the past three years may seriously challenge this view. For instance, the delayed, mild presence or absence of cyst formation associated with inactivation of *Pkd1*, *Ift88* or *Kif3a* at later stages of development or in the adult indicates that cystogenesis in autosomal dominant PKD is not simply caused by a loss of flow sensing.[34,35]

Pressure responses are independent of the primary cilium, but similar to flow responses involve ATP-dependent calcium signaling and may also contribute detrimentally to the expansion of cysts.[63–65] Finally, the ratio of polycystin-1 to polycystin-2 regulates SAC opening through the actin cytoskeleton and tunes arterial pressure sensing (Figure 6).[14] A loss of myogenic tone may contribute to aneurysm formation owing to an increase in arterial wall stress.

Although the role of polycystins in mechanotransduction is established at least *in vitro*, several important mechanistic questions remain to be answered: Is polycystin-1 the main flow sensor in kidney cells? What conformational changes are induced by fluid flow? How does the flow-activated polycystin-1 gate polycystin-2? What is the mechanism of flow-induced polycystin-1 cleavage? How does polycystin-2 sense nodal flow in the absence of polycystin-1? What is the mechanism of ATP release? Is flow-induced ATP release involved in left–right asymmetry? What is the molecular identity of SACs? At the clinical level, the key question that remains is whether defects in mechanosensory transduction are indeed involved in the pathogenesis of PKD. The answers to these questions will enhance our understanding of the molecular physiology of mechanosensory transduction and most importantly will better define the etiology of PKD.

Review criteria

The papers cited in this manuscript were selected because they were considered to be either recent, important reviews in the field of kidney disease, primary cilium or ion channels, or original papers that demonstrate key roles for polycystins and/or nucleotide release in mechanosensory transduction (that is, the flow and pressure sensing) of renal epithelial cells, nodal cells or vascular cells. The mechanisms of ATP release and the different P2 purinoceptors expressed in the kidney and the vasculature were not reviewed due to space limitations, and instead relevant reviews are cited. No specific databases were searched. Rather, the material was identified based on the authors' knowledge of the field. We apologize if there are any important papers that we have missed.

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Author contributions
A. Patel and E. Honoré contributed equally to researching data for the article, discussing content, writing, and reviewing/editing the manuscript before submission.