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Vasopressin and interactive calcium, cyclic AMP and purinergic signaling in Polycystic Kidney Disease

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common monogenic kidney disease and the fourth leading cause of end-stage renal disease, responsible for 5–10% of cases. The disease is characterized by relentless development and growth of cysts causing progressive kidney enlargement associated with hypertension, pain, reduced quality of life, and eventually kidney failure. It is caused by mutations to *PKD1* or *PKD2*, encoding polycystin-1 and polycystin-2, respectively. Their function and the molecular mechanisms responsible for the development of polycystic kidney disease are not well understood. The objective of this review is to synthesize a large body of literature that examines how reduction of functional PC1 or PC2 at the primary cilia and/or the endoplasmic reticulum directly disrupts intracellular calcium signaling and indirectly disrupts calcium regulated cAMP and purinergic signaling. We propose a hypothetical model where dysregulated metabolism of cAMP and purinergic signaling increase the sensitivity of principal cells in collecting ducts and of tubular epithelial cells in the distal nephron to the constant tonic action of vasopressin. The resulting magnified response to vasopressin further enhances the disruption of calcium signaling initiated by mutations to PC1 or PC2 and activates downstream signaling pathways responsible for impaired tubulogenesis, cell proliferation, increased fluid secretion and interstitial inflammation.

Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common monogenic kidney disease and the fourth leading cause of end-stage renal disease, responsible for 5–10% of cases.^{1,2} It affects all ethnic groups and is a major burden for public health. It is genetically heterogeneous with two loci identified, *PKD1* (16p13.3), encoding polycystin-1 (PC1) and *PKD2* (4q22), encoding PC2. The disease is characterized by relentless development and growth of cysts causing progressive kidney enlargement associated with hypertension, abdominal fullness and pain, episodes of cyst hemorrhage, gross hematuria, nephrolithiasis, cyst infections, and reduced quality of life. Despite continuous destruction of the parenchyma, compensatory hyperfiltration of the surviving glomeruli maintains renal function within the normal range for decades. Only when the majority of nephrons have been destroyed, renal function declines, typically after the fourth decade of life, and end-

stage renal disease eventually ensues in the majority of patients. Medical advances have improved the treatment of patients with ADPKD, but today this remains restricted to the detection and treatment of complications and renal replacement therapy. Research on Polycystic Kidney Disease (PKD) increased exponentially after the discovery of the *PKD1* and *PKD2* genes in 1994 and 1996. Targets for intervention have been identified and tested in animal models and some clinical trials have provided modest but encouraging results.³⁻⁵ Nevertheless, the function of the polycystins and the molecular mechanisms responsible for the development of PKD remain poorly understood and better therapies are needed.

The objective of this review is to synthesize a large body of literature that examines how reduction of functional PC1 or PC2 (1) directly disrupts calcium signaling at specific cellular compartments, i.e primary cilia (2) and/or endoplasmic reticulum (3), and indirectly disrupts calcium regulated cyclic adenosine monophosphate (cAMP) (4) and purinergic signaling (5). We propose a hypothetical model where dysregulated cAMP metabolism and purinergic signaling increase the sensitivity of principal cells in collecting ducts and of tubular epithelial cells in the distal nephron to the continuous tonic action of vasopressin (6), further enhancing the disruption of calcium signaling initiated by mutations to PC1 or PC2 (7) and activating downstream signaling pathways responsible for impaired tubulogenesis, cell proliferation, increased fluid secretion and interstitial inflammation (8). *In vivo* studies addressing the role of calcium and cAMP in PKD are consistent with this hypothetical model (9). The following nine sections examine the different components of this hypothesis in detail. A cartoon illustrating this hypothesis is presented in Figure 1.

1. Polycystins and calcium signaling

PC1 (4303aa; ~600kDa, uncleaved and glycosylated) is a receptor-like protein with a large extracellular region (3074aa) that comprises a number of domains involved in protein-protein and protein-carbohydrate interactions (Figure 2). PC1 also has 11 transmembrane domains and a cytoplasmic tail. The last six transmembrane spans of PC1 share sequence homology with PC2 although PC1 has not been shown to function directly as a channel protein. Auto-proteolytic cleavage of PC1 at the G protein coupled receptor proteolytic site (GPS) domain is an important step to form a functional protein.⁶⁻⁸ After embryonic development, the full-length protein is rarely seen, with a ~130kDa C-terminal (CT) and two large N-terminal (NT) products; NT and CT are thought to stay associated after cleavage. The two NT products are glycoforms, one mature form that traffics through the Golgi and is thought to be localized at the plasma membrane/cilia or secreted and one that may be retained in the endoplasmic reticulum.⁹⁻¹¹ PC1 has been proposed to undergo further proteolytic cleavages, one that generates a P100 intramembranous fragment with six membrane spanning domains that interacts with Stromal Interaction Molecule 1 (STIM1) and inhibits store operated calcium entry. The other two generate COOH-terminal tail (CTT) fragments capable of entering the nucleus and either binding to transcription factors T-cell factor or TCF and CCAAT-enhancer-binding protein homologous protein or CHOP (35 kDa CTT fragment) preventing coactivation by P300 or binding to coactivator P100 (17 kDa fragment) and activating the transcription factor signal transducer and activator of transcription 6 (STAT6).¹²⁻¹⁶ The importance of these latter cleaved products to the pathogenesis of ADPKD has not been elucidated. Several studies have used exogenous

expression of chimeric proteins containing the CTT of PC1 fused to a membrane-targeted protein. The results of these studies are difficult to interpret and in some cases may recapitulate the function of the native protein while in other cases have dominant negative effects (Table 1).^{17–24}

PC2 (968aa; ~110kDa) is a six transmembrane, large conductance, calcium permeable, non-selective cation channel of the transient receptor potential (TRP) family.^{25,26} PC1 and PC2 interact via their C-terminal tails. The resulting PC complex is thought to localize to primary cilia and play a role in intracellular calcium regulation.^{6,27–29} PC2 is mainly localized in the endoplasmic reticulum with its interaction with PC1 important for its translocation to the plasma membrane and primary cilia. Recent evidence indicates that this interaction is essential for PC1 maturation.^{30,31} The interaction of PC1 and PC2 with other calcium channel and sensor proteins provides support to the hypothesis that the polycystins function in the regulation of intracellular calcium homeostasis. For example, PC1 interacts with the inositol 1,4,5-trisphosphate receptor (IP3R) and the stromal interaction molecule 1 (STIM1), while PC2 interacts with IP3R, the ryanodine receptor (RyR), TRPV4 and TRPC1, 3, 4 and 7.^{32–39}

2. Role of the polycystins in primary cilia

The function of the polycystins in primary cilia has been proposed to be critical in the pathogenesis of PKD. Data supporting this view are that LOV1 or location of vulva 1 (homolog of PC1) and PC2 in *C. elegans* are found in primary cilia of male-specific sensory neurons and mediate male sensory behaviors, that mice homozygous for a hypomorphic *Ift88^{Tg737Rpw}* mutation resulting in absent or stunted primary cilia have polycystic kidneys, and that bending of the primary cilium by fluid flow or directly with a micropipette elicits an intracellular calcium signaling response.^{40–42} Additional experiments showed that PC1 and PC2, influx of extracellular calcium, and RyR-mediated release of intracellular calcium mediate the transduction of fluid flow shear stress into calcium transients in kidney epithelial cells.⁴³ These observations led to the ciliocentric hypothesis of PKD, according to which PC1 senses the bending of the primary cilium and activates tightly associated PC2 calcium channels. The local calcium influx in the cilium subsequently triggers calcium induced calcium release from intracellular stores and store operated calcium entry. The absence of flow sensing in renal epithelia causes PKD.

Recent observations have challenged the validity of this hypothesis. Knockdown of TRPV4, which forms mechanosensitive heteromeric complexes with PC2, abolishes the intracellular calcium response to fluid flow shear stress but does not result in a cystic phenotype in mice or zebrafish.³⁸ Studies using genetically engineered calcium indicators have shown that primary cilia are specialized calcium signaling organelles with calcium concentrations much higher than those in the cytoplasm and that the amount of calcium that can diffuse from the cilia to the cytoplasm in response to bending of the cilia is too miniscule to trigger calcium induced calcium release from intracellular stores.⁴⁴ Changes of calcium concentration in the cilia can be dissociated from those in the cytoplasm and a lag time between the calcium increase in the cilia and in the cytoplasm raises the possibility of an intermediate step.⁴⁵ The shear stress of flow on epithelial cell monolayers differs from the mechanical effect of flow

in isolated perfused tubules which includes stretch in addition to shear stress.⁴⁶ Extracellular adenosine triphosphate (ATP) acting on purinergic (P2) receptors has recently emerged as a potential candidate to mediate both shear- and stretch-induced calcium responses.^{46–48} A recent study using Madin-Darby canine kidney (MDCK) cells showed that the intracellular calcium response to shear stress was already present in unciliated cells on culture day 1 and was magnified in ciliated cells on day 7, but that this enhanced response was dependent on higher expression of purinergic P2Y2 and P2×7 receptors rather than the presence of cilia per se.⁴⁹

3. Role of the polycystins in the endoplasmic/sarcoplasmic reticulum

PC1 and particularly PC2 are found in the endoplasmic/sarcoplasmic reticulum membrane and contribute to the regulation of calcium release from intracellular stores. Studies aimed at elucidating the role of the polycystins in this process and how it is altered in ADPKD are summarized in Table 2.^{12,32–34,39,50–65} The results of these studies are not always consistent, likely due to different experimental conditions. For example, reduced IP3-induced calcium release has been observed in experiments where PC1 was either knocked down or overexpressed.^{32,33,60} While some studies have shown that PC1 or PC2 haploinsufficiency disrupts intracellular calcium homeostasis,^{66–68} particularly in myocytes,^{51,54,65} others have found no evidence for this.⁶⁹

a) Polycystin-2—PC2 functions as an endoplasmic reticulum calcium release channel activated by increases in calcium concentration.⁵⁰ Constitutive phosphorylation by casein kinase II at Ser812 increases the sensitivity of the channel to calcium activation on the cytosolic side.⁵² Aurora A kinase and protein kinase A (PKA) phosphorylate PC2 at Ser829, enhancing the amplitude of ATP-induced calcium release.^{62,63} In contrast, co-transfection of Aurora kinase A in PC2 transfected human embryonic kidney (HEK) cells reduced the amplitude of vasopressin induced calcium transients, but the effect of Ser829 phosphorylation was not directly tested.⁶³ Syntaxin-5 binds to and prevents leaking of calcium from endoplasmic reticulum stores.⁵⁷ PC2 interacts physically and functionally with IP3R and RyR.^{34,39} In most studies PC2 has been found to enhance the amplitude of IP3R and RyR mediated calcium transients and to inhibit the leakage of calcium from endoplasmic reticulum stores,³⁴ although in one study exogenous expression of PC2 was associated with increased leakage of calcium from the endoplasmic reticulum.⁵⁹ Another study suggests that the simultaneous presence of PC1 and PC2 is required to amplify IP3-induced calcium release.⁶⁰ Interestingly, knockdown of IP3R1 or IP3R3, like the knockdown of PC2, induces cell proliferation, apoptosis, and cyst formation in a collagen-matrigel three-dimensional culture system using pig kidney epithelial (LLC-PK) cells.⁷⁰ Several studies have shown that store operated calcium entry is reduced in PC2 defective cells^{34,51,55,57,71} and it has been suggested that the interaction between STIM1 (a transmembrane sensor for calcium concentration in the endoplasmic reticulum lumen) and ORAi (the pore-forming unit of a plasma membrane channel that permits calcium entry in response to a reduction in the endoplasmic reticulum calcium concentration) is uncoupled in these cells.⁷¹ Although PC2 is mostly located in the endoplasmic/sarcoplasmic reticulum membrane, truncated forms lacking the endoplasmic/sarcoplasmic reticulum retention domain translocate to the plasma membrane.⁵⁰

b) Polycystin-1—The function of PC1 in the endoplasmic/sarcoplasmic reticulum is complex and not well understood. PC1 has been shown to interact with the IP3R and STIM1 in the endoplasmic/sarcoplasmic reticulum.^{12,32,33} Exogenous expression of full-length PC1 decreased the ATP-induced, IP3R-mediated increase in cytosolic calcium in MDCK cells.³² In contrast, the simultaneous presence of PC1 and PC2 was required to elicit an IP3-induced calcium release in conditionally immortalized, plasma membrane-permeabilized human proximal-tubule epithelial cells.⁶⁰ In another study PC1 was shown to inhibit calcium leak across the endoplasmic reticulum membrane.⁵⁸ More recently PC1 has been proposed to play a more complex role in the regulation of calcium release from the endoplasmic reticulum through its interactions with PC2 and STIM1.³³ Exogenous expression of PC1 reduces the interaction of IP3R with PC2 and enhances the interaction of STIM1 with IP3R leading to inhibition of endoplasmic reticulum calcium release, enhancement of PC2 traffic to the plasma membrane and entry of extracellular calcium, and inhibition of store operated calcium entry. According to this model, mutations in PC1 and PC2 lead to depletion of endoplasmic reticulum calcium by different mechanisms. PC1 mutations increase the interaction between PC2 and IP3R, increase calcium release from the endoplasmic reticulum, and reduce calcium influx through the plasma membrane, while PC2 mutations reduce calcium influx across the plasma membrane and enhance the interaction of PC1 and STIM1, thus inhibiting store operated calcium entry.

Despite the diversity of conclusions reached in these studies, most studies are consistent with the hypothesis that the polycystins by themselves and through their interaction with other calcium channels in the endoplasmic/sarcoplasmic reticulum function to prevent the leaking of calcium across the endoplasmic/sarcoplasmic reticulum membrane and depletion of intracellular stores, thus maintaining the amplitude of physiological calcium oscillations.^{34,58} Either loss or gain of function may disrupt the physiological role of the polycystins and lead to a cystic phenotype.^{11,72–75}

4. Calcium and dysregulation of cAMP signaling in PKD

Numerous animal models of PKD show elevated levels of cAMP in the kidney^{11,76–79} as well as in cholangiocytes,⁸⁰ vascular smooth muscle cells,⁸¹ and choroid plexus.⁸² Tissue levels of cAMP are determined by the activities of membrane-bound and soluble adenylyl cyclases (ACs) and phosphodiesterases (PDEs). Both are subject to complex regulatory mechanisms, e.g, membrane bound ACs are under the positive or negative control of G protein-coupled receptors [GPCRs] and extracellular ligands.

Several hypotheses have been advanced to account for the increased levels of cAMP in PKD tissues, most directly linked to the dysregulation of intracellular calcium signaling. A prevailing hypothesis contends that reduced calcium in PKD affects both cAMP synthesis and hydrolysis. cAMP synthesis is increased in PKD due to activation of calcium-inhibitable AC6. cAMP hydrolysis is decreased in PKD due to inhibition of calcium/calmodulin-dependent PDE1.^{77,81,83} Levels of cyclic guanosine monophosphate also increase with limited PDE1 activity, and contribute to elevated cAMP levels by inhibiting cyclic guanosine monophosphate-inhibitable PDE3.

The importance of AC6 to PKD is demonstrated by the occurrence of a milder cystic disease in mice with a collecting duct-specific knockout of both *AC6* and *Pkd1* compared to knockout of *Pkd1* alone.⁸⁴ The induction of pronephric cysts, hydrocephalus, and body curvature in *pde1a* zebrafish morphants, its rescue by human PDE1A RNA and PKA inhibitors, the aggravation of the cystic phenotype in *pkd2* morphants by morpholino induced depletion of PDE1, and the partial rescue of *pkd2* morphants by *PDE1A* RNA support the role of PDE1 in PKD.⁸⁵ Notably, the pool of cAMP generated in response to vasopressin is mainly catabolized by PDE1 and the accumulation of cAMP in response to vasopressin is markedly increased when intracellular calcium is reduced, mainly due to lower PDE1 activity.^{86,87} PDE3, which localizes to ER membranes,^{88,89} and catabolizes cAMP pools that control cell proliferation^{90–92} and cystic fibrosis transmembrane conductance regulator (CFTR) driven chloride secretion,^{93,94} may also be involved. Other hypothetical mechanisms linked to disrupted calcium signaling include: 1) Dysfunction of a ciliary protein complex comprising A-kinase anchoring protein 150, AC5/6, PC2, PDE4C, and PKA, such that decreased PC-2 mediated calcium entry activates AC5/6 and inhibits PDE4C.⁹⁵ 2) Depletion of the ER calcium stores, such that STIM1 oligomerizes, translocates to the plasma membrane, and recruits and activates AC6.⁷¹

5. Calcium and dysregulation of purinergic signaling in PKD

Renal tubular epithelial cells, like neuronal and neuroendocrine cells, exhibit constitutive ATP release and spontaneous calcium oscillations.^{46–48} These two processes are reciprocally regulated since ATP release occurs by calcium dependent vesicular exocytosis while the calcium oscillations are abolished by ATP scavengers or P2 receptor antagonists. ATP acts on ligand-gated P2X receptors and on G protein-coupled P2Y receptors. P2Y2 and P2×7 are expressed in collecting ducts and in MDCK cells. P2X receptors are calcium-permeable, nonselective cation channels that, when activated, increase intracellular calcium. P2Y receptors are Gq/phospholipase C (PLC)-coupled receptors; PLC cleaves phosphatidylinositol (4,5)-biphosphate (PIP2) into IP3 and diacylglycerol. IP3 acts on IP3R to release calcium and diacylglycerol activates protein kinase C (PKC). P2 receptors require approximately 1 to 10 μM of extracellular nucleotides for full activation. High concentrations of released ATP sufficient to activate purinergic receptors likely exist close to the cell membrane. Primary and immortalized ADPKD cyst cells exhibit markedly reduced flow-sensitive ATP release.⁹⁶ Furthermore, the intracellular calcium response to exogenously added ATP is moderately reduced in immortalized ADPKD cyst cells, possibly due to reduced mRNA and protein levels of P2×7 as also observed in immature, unciliated MDCK cells. ATP release in response to shear stress has also been shown to be reduced in collecting duct principal cells derived from an Oak Ridge polycystic kidney (*orpkTg737*) mouse model that lack normal apical cilia as compared to rescued cells exhibiting intact primary cilia.⁹⁷ Knockdown of PC2 markedly blunts the ATP-dependent increase in intracellular calcium elicited by shear stress or by mechanical stretch in endothelial cells.⁹⁸ It is plausible but not proven that the defective release of ATP in ADPKD derived cyst cells is a consequence of the altered intracellular calcium homeostasis in PKD.

6. Continuous, tonic effect of vasopressin in polycystic kidney disease

As a result of terrestrial adaptation, tetrapods are constantly subjected to the tonic action of vasopressin on V2 receptors. This effect is enhanced in patients with ADPKD because the circulating levels of vasopressin are often increased in patients with this disease.^{99–101} Plasma levels of copeptin, a surrogate for vasopressin, have also been found to be elevated in ADPKD patients compared to healthy individuals after donation of a kidney for transplantation despite higher glomerular filtration rate (GFR) in the ADPKD patients.¹⁰² Furthermore, plasma copeptin levels have been shown to correlate with the severity of PKD in cross-sectional studies and with the rate of disease progression in prospective studies.^{103–105} The cause of the elevated levels of vasopressin and copeptin is thought to be impaired urinary-concentrating capacity, which is one of the earliest if not the earliest manifestation of ADPKD and can be detected in patients with normal glomerular filtration rate and even in children with ADPKD.^{99,106} The cause of this concentration defect has not been fully elucidated. It cannot be explained by reduced generation of cAMP or expression of aquaporin-2, as both cAMP level and aquaporin-2 expression are increased in animal models of PKD.^{77,107,108} It is interesting to note that urine-concentrating capacity is not reduced, or is even slightly increased, in *Pkd1*^{+/-} mice which have very few renal cysts.⁶⁸ In contrast, in a rapidly progressive PKD model, the cpk mouse, the development of polyuria occurs alongside overexpression of genes involved in urinary concentration in collecting ducts, and this overexpression precedes the development of cysts.¹⁰⁷ Similarly, in diphenylamine- or diphenylthiazole-induced PKD models, polyuria occurs before collecting duct dilation.^{109,110} Additionally, renal ciliopathies in which macroscopic cysts feature less prominently, such as autosomal recessive PKD, nephronophthisis, and Bardet-Biedl syndrome, are also characterized by the early development of impaired renal concentrating capacity. Some studies have shown an abnormal intracellular distribution of aquaporin-2, suggesting that its trafficking or recycling may be altered in PKD.^{107,111} In the final analysis, alteration of molecular pathways within collecting duct cells paired with cystic distortion of the countercurrent mechanism, probably underlie the urine-concentrating defect.

a) Vasopressin and cAMP signaling—Studies in the last decade have shown that circulating vasopressin acting on vasopressin V2 receptors in the basolateral cell membranes and/or, less likely, urine vasopressin acting on V2 receptors in the primary cilia exert a strong modulatory effect on the development of PKD. Vasopressin is the main agonist of AC in freshly dissociated collecting ducts.¹¹² Administration of the V2 receptor agonist DDAVP increases the renal levels of cAMP and aggravates the development of PKD rodent models orthologous to autosomal recessive PKD (ARPKD, PCK rat) and ADPKD caused by *PKD1* (*Pkd1*^{RC/RC} mouse) or *PKD2* (*Pkd2*^{WS25/-} mouse) mutations.¹¹³ Genetic elimination of circulating vasopressin markedly inhibits the development of the renal cystic disease in a rat model (PCK rat) while exogenous administration of DDAVP fully recovers the cystic phenotype.¹¹⁴ Vasopressin V2 receptor antagonists have been shown to inhibit the development of the disease in five rodent models orthologous to human ARPKD, ADPKD and nephronophthisis.^{77,108,115–117} Finally, a randomized double blind clinical trial showed that the V2 receptor antagonist tolvaptan, given over 3 years, slowed the increase in kidney volume and the decline in kidney function and lowered the frequency of disease related

events in 18–50 year-old patients with ADPKD, baseline estimated creatinine clearance 60 ml/min, and total kidney volume 750 ml.³

b) Vasopressin and purinergic signaling—Physiological concentrations of vasopressin induce RyR-dependent calcium oscillations in inner medullary collecting duct (IMCD) cells, trafficking and exocytotic insertion of aquaporin-2 into the apical membrane, and release of ATP in sufficient concentrations to stimulate the luminal P2 receptors.^{118,119} Vasopressin V2 receptor antagonists reduce the urinary ATP concentration in mice.¹²⁰ Release of ATP may initiate a negative feedback loop that limits the effect of vasopressin since ATP inhibits vasopressin induced water permeability in the collecting duct. This is due to activation of the phosphoinositide signaling pathway and a PKC and Gi-dependent mechanism.^{121,122} Possibly calcium-dependent activation of phospholipase A2 resulting in increased generation of arachidonic acid and prostaglandin E2 (PGE2) acting on Prostaglandin E Receptor 3 (EP3) may also play a role.¹²³ Genetic deletion of the P2Y2 receptor results in increased expression of aquaporin-2 and urea transporters (UT)-A1 and UT-A2, and in increased urine concentration under basal conditions and following the administration of vasopressin.¹²⁴ This feedback mechanism may be defective in the cystic epithelium, further up-regulating cAMP and PKA signaling which in the setting of reduced intracellular calcium leads to the proliferative and secretory phenotype of the cystic epithelium.

7. Self-perpetuating disruption of calcium signaling in ADPKD

While dysregulation of intracellular calcium homeostasis directly linked to mutated PC1 or PC2 may be responsible for the upregulation of cAMP and PKA signaling, sustained activation of PKA may result in a maladaptive response that further disrupts calcium signaling. PKA is a component of the IP3R macromolecular signaling complex, and PKA phosphorylation of IP3R increases its channel activity in planar lipid bilayers.^{125,126} PKA is also a component of RyR macromolecular complexes, and PKA phosphorylation of RyR promotes the dissociation of the inhibitory protein calstabin enhancing the channel activity.^{127,128} The physical interaction of PC2 with the RyR2 receptor has been shown to stabilize the RyR2 receptor in the closed position, and the knockdown of PC2 enhances the leak of calcium from the sarcoplasmic reticulum leading to cardiomyopathy.⁶¹ PKA also phosphorylates and enhances the channel activity of PC2.⁶² Dephosphorylation of PC2 is mediated by PC1 binding through the recruitment of protein phosphatase-1 alpha and unopposed cAMP stimulated hyperphosphorylation of PC2 in the absence of functional PC1 may contribute to cyst initiation in PKD1 patients⁶². Sustained maladaptive upregulation of cAMP and PKA signaling may lead to hyperphosphorylation of IP3R, RyR and PC2, depletion of endoplasmic reticulum calcium stores, reduced calcium transients, and development of PKD. Thus, PKD may bear some similarity to heart failure and muscle fatigue¹²⁹ where persistent catecholaminergic stimulation and PKA induced hyperphosphorylation of RyR2 or RyR1 makes these channels leaky and depletes the SR calcium stores,^{130–132} causing heart failure or muscle fatigue, respectively.

8. Downstream effects from the dysregulation of intracellular calcium and cAMP signaling

The dysregulation of intracellular calcium and cAMP signaling may be responsible for many of the features characteristic of the cystic phenotype, reviewed in detail elsewhere.¹³³ The cross-talk of calcium and cAMP signaling seems to be particularly important for the control of cell proliferation. cAMP exerts opposite effects on cell proliferation in different cell types. Whereas cAMP and PKA signaling inhibit cellular proliferation in normal human kidney cortical cells, they activate pro-proliferative pathways (extracellular signal-regulated kinase [ERK]) in cells derived from polycystic kidneys.^{134,135} Treatment of normal human kidney or murine collecting duct cells with calcium channel blockers replicates the proliferative response of the ADPKD cells to cAMP.¹³⁶ Conversely, calcium-elevating treatments (calcium channel activators or ionophores) restore the antimitogenic response to cAMP of ADPKD or ARPKD cyst-derived cells. Thus, there is overwhelming evidence that intracellular calcium controls the cellular proliferative response to cAMP. Understanding the mechanisms involved is critical to understanding the pathogenesis of PKD. Yamaguchi et al showed that inhibition of calcium-dependent phosphatidylinositol 3-kinase activity and downstream inhibition of Akt in mouse collecting duct epithelial cells allows B rapidly accelerated fibrosarcoma (B-Raf) and ERK to be activated in a PKA-, Src-, and Ras-dependent manner and underlie the proliferative response to cAMP in the setting of reduced calcium.¹¹⁹ Consistent with these observations other studies have shown that knockdown of PC1 or PC2, reduction in intracellular calcium and downregulation of calcium calmodulin kinase II (CaMKII) inhibit Akt and activate ERK,^{137,138} and that knockdown of CaMKII induces pronephric cysts in zebrafish.¹³⁹ Similarly, inhibition of PKA-associated proliferation by elevation of intracellular calcium in cholangiocytes derived from PCK rats has been shown to be dependent on the activation of PI3K and Akt.¹⁴⁰ On the other hand, enhanced phosphorylation of PI3K and Akt have been found in cystic tissues from patients and rodent models of PKD.^{141–145} Activation of mammalian target of rapamycin (mTOR) signaling downstream of PKA through ERK-mediated phosphorylation of tuberin^{146,147} has been linked to transcriptional activation of aerobic glycolysis, increased levels of ATP, and together with ERK-dependent inhibition of liver kinase B1, inhibition of AMP kinase, which may further enhance mTOR signaling.^{148–151} Phosphorylation and inhibition of glycogen synthase kinase 3 β and direct phosphorylation and stabilization of β -catenin by PKA enhance Wnt/ β -catenin signaling may contribute to the development of PKD.^{152–155} PKA-dependent upregulation of cAMP response element binding transcription factor, paired box gene 2, and signal transducer and activator of transcription 3 also contribute to the proliferative phenotype of the cystic epithelium.^{156–161}

Upregulation of PKA-stimulated, CFTR-mediated, chloride-driven fluid secretion plays an important role in the progression of the cystic disease.^{162,163} Recent studies suggest that the calcium-dependent chloride channel anoctamin-1 (ANO1) activated through stimulation of Gq-coupled purinergic receptors (P2YR) may also play an important role.^{164,165} ANO1 is strongly expressed in human ADPKD kidneys. Inhibitors of anoctamin ion channels and knockdown of ANO1 inhibit forskolin-driven MDCK or metanephric organ culture cystogenesis. Possibly, upregulation of CFTR- and ANO1-driven chloride secretion act synergistically downstream from PKA activation since forskolin stimulates the translocation of ANO1 to the apical membrane and PKA phosphorylation enhances the sensitivity of the

IP3R to IP3. It is also possible that the relative importance of CFTR- and ANO1-driven chloride secretion in PKD depends on the animal species as it does in cystic fibrosis.¹⁶⁶

9. *In vivo* studies addressing the role of calcium in Polycystic Kidney Disease

The literature reviewed here provides strong support to the prevailing belief that disruption of intracellular calcium signaling plays a central role in the pathogenesis of ADPKD. This view is supported by an increasing number of *in vivo* studies that have focused on the ability of calcium aimed interventions to modify the course of PKD as summarized in Table 2.^{38,167–171,138,172–176}

Treatments that increase intracellular calcium have in general shown a protective effect. Cyclic AMP-induced cystogenesis in metanephric organ culture is inhibited by a calcium ionophore.¹⁷⁵ Although the knockout of TRPV4 does not induce a cystic phenotype, TRPV4 activators significantly alleviate cystic disease development in PCK rats.^{173,176} The administration of triptolide, a drug that activates the PC2 channel by a poorly understood mechanism, has an inhibitory effect on the development of PKD in *Pkd1*^{-/-} embryos and in conditional and kidney specific *Pkd1* knockout mice.^{167,168,172} Calcimimetic agents have a beneficial effect in Cy/+ Han:SPRD rats and pcy mice,^{170,174} but no significant effect (except for reduced fibrosis) in PCK rats and *Pkd2*^{WS25/-} mice, possibly because the potential beneficial effect of the drug was offset by marked hypocalcemia.^{171,177} On the other hand, interventions that lower intracellular calcium aggravate the development of PKD. The administration of verapamil aggravated cyst development in Cy/+ Han:SPRD rats.^{ENREF_134}¹⁶⁹ CaV1.2 zebrafish morphants have dilated pronephric ducts.¹³⁸ Lentiviral transfection of CaV1.2 shRNA aggravates the cystic phenotype in *Pkd1*^{+/-} mice.¹³⁸

Conclusions

ADPKD, a leading cause of end-stage kidney disease, is caused by mutations to *PKD1* or *PKD2* encoding PC1 and PC2, respectively. The function of the polycystins and the molecular mechanisms responsible for the development of the disease are not well understood. Substantial evidence indicates that a reduction of functional PC1 or PC2 at the primary cilia and/or the endoplasmic reticulum directly disrupts intracellular calcium signaling and indirectly disrupts calcium regulated cAMP and purinergic signaling. This review presents a hypothetical model where dysregulated metabolism of cAMP and purinergic signaling increase the sensitivity of principal cells in collecting ducts and of tubular epithelial cells in the distal nephron to the constant tonic action of vasopressin, further enhancing the disruption of calcium signaling initiated by mutations to PC1 or PC2 and activating downstream signaling pathways responsible for impaired tubulogenesis, cell proliferation, increased fluid secretion and interstitial inflammation.

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Biographies

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Key points

- Polycystin 1 and polycystin 2 in the primary cilia and endoplasmic reticulum regulate intracellular calcium signaling.
- In Polycystic Kidney Disease, reduced intracellular calcium enhances the generation and inhibits the destruction of cAMP, and blunts the release of ATP and purinergic signaling.
- Altered cAMP metabolism and purinergic signaling in collecting duct principal cells and distal nephron epithelial cells markedly increases the sensitivity of these cells to the constant tonic effects of vasopressin.
- Undefined cellular mechanisms and/or disruption of medullary architecture by cysts interfere with urine concentration and increase circulating levels of vasopressin.
- Vasopressin and cAMP driven activation of protein kinase A enhances the phosphorylation of polycystin-2, ryanodine receptors and inositol 1,4,5-trisphosphate receptors, increases the leakage of calcium across the endoplasmic reticulum membrane, and further disrupts intracellular calcium signaling.
- The reduction in intracellular calcium that results from *PKD1* or *PKD2* mutations causes a striking switch in the cellular response to cAMP from suppression to marked stimulation of proliferation.
- A better appreciation of the mechanisms responsible for this phenotypic switch is critical for the understanding of Polycystic Kidney Disease.
- Preclinical trials targeting calcium or cAMP signaling and clinical trials targeting cAMP signaling have provided encouraging results, providing support for their pathophysiological relevance in Polycystic Kidney Disease.

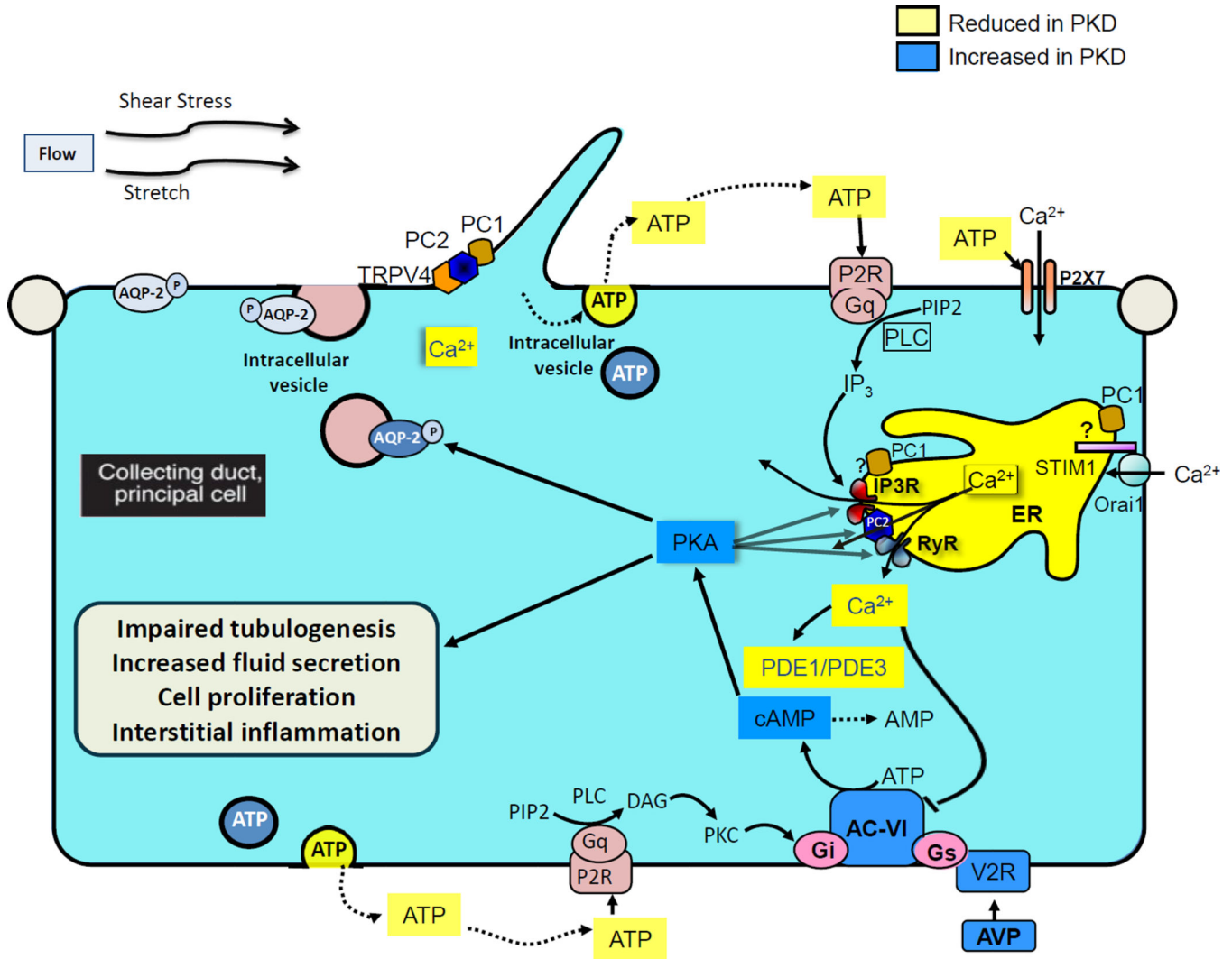


Figure 1. Hypothetical roles of calcium, vasopressin and purinergic signaling in ADPKD
 Polycystin-1 and polycystin-2 the primary cilia and endoplasmic reticulum regulate intracellular calcium signaling directly and through their interaction with other calcium channel proteins. Reduced intracellular calcium caused by mutations to polycystin-1 or polycystin-2 enhances the generation of cAMP by calcium inhibitable adenylyl cyclase 6 and inhibits the destruction of cAMP by calcium dependent phosphodiesterase 1 and cyclic guanosine monophosphate (cGMP) inhibited phosphodiesterase 3 which control the cAMP pool responsive to vasopressin stimulation. The reduction of physiological calcium oscillations also blunts the release of ATP and its action on P2Y2, thus abolishing a negative feedback loop that normally limits the vasopressin V2 receptor-dependent activation of adenylyl cyclase 6. Undefined cellular mechanisms (possibly disruption of the calcium dependent insertion of aquaporin-2 into the apical membrane) and/or disruption of medullary architecture by cysts interfere with urine concentration and increase circulating levels of vasopressin. Thus, altered cAMP metabolism and purinergic signaling, along with increased levels of circulating vasopressin, markedly enhanced the constant tonic effect of vasopressin on the V2 receptors in collecting duct principal cells and distal nephron

epithelial cells. Vasopressin-driven activation of protein kinase A enhances the phosphorylation of polycystin-2, ryanodine receptors and inositol 1,4,5-trisphosphate receptors, increases the leakage of calcium across the endoplasmic reticulum membrane, and further disrupts intracellular calcium signaling. The reduction in intracellular calcium determines a striking switch in the cellular response to cAMP from suppression to marked stimulation of proliferation. Thus, in the setting of reduced intracellular calcium sustained activation of cAMP and protein kinase A activate downstream signaling pathways responsible for impaired tubulogenesis, cell proliferation, increased fluid secretion and interstitial inflammation, characteristic of the cystic phenotype.

AC6: adenylyl cyclase 6 ; PDE: Phosphodiesterase; PKA: Protein kinase A; RYR: Ryanodine receptor; AQP2: Aquaporin 2; PLC: Phospholipase C; PIP2: phosphatidylinositol (4,5)-bisphosphate ; IP3: Inositol 1,4,5-trisphosphate; DAG: Diacylglycerol; PKC: Protein Kinase C; Gs and Gi refer to guanosine nucleotide-binding proteins s and i, respectively. Yellow indicates molecules that are reduced in PKD; blue indicates molecules that are increased in PKD.

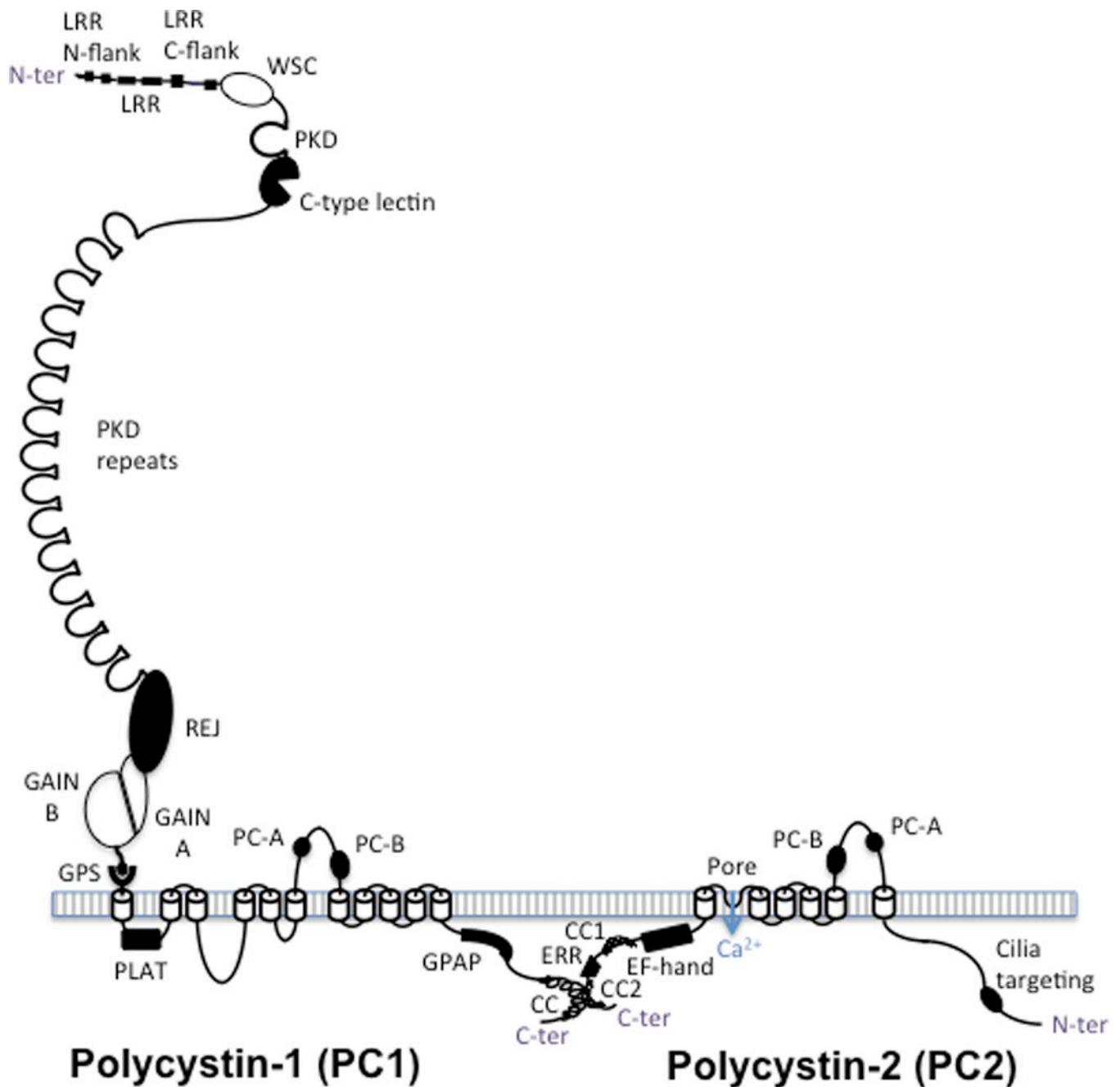


Figure 2. Predicted structures of polycystin-1 (PC1), left, and polycystin-2 (PC2), right
 PC1 has a large ectodomain with a number of recognized domains sequentially located from the N terminus of the protein (after the signal peptide is cleaved): leucine rich repeats (LRR), flanked by N and C terminal domains, the cell wall integrity and stress-response component (WSC) domain, polycystic kidney disease (PKD) protein repeats, a C-type lectin, the receptor for egg jelly (REJ) domain, the G-protein coupled receptor (GPCR) proteolysis site (GPS), where PC1 is cleaved, and the associated GPCR autoproteolysis inducing (GAIN) domain, with A and B subdomains. PC1 is associated with the membrane with 11 transmembrane domains with the PC1, lipoygenase, alpha-toxin (PLAT) domain

found in the first cytoplasmic loop. The last 6 transmembrane region of PC1 is homologous to the transmembrane region of PC2, with the polycystin homologous motifs A and B found in the large extracellular loops of both proteins. The ~200aa cytoplasmic tail contains a G-protein activating peptide (GPAP) and a coiled coil (CC) domain that interacts with the second coiled coil domain (CC2) found in the C-ter tail of PC2. PC2 is a TRP-like calcium channel that has an EF-hand and an endoplasmic reticulum retention (ERR) signal also in the C-tail and a proposed cilia targeting sequence in the N-terminal tail.

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Table 1

Articles using plasma membrane anchored chimeric proteins to study the function of the COOH-terminal tail of Polycystin 1

Reference	Model system	Findings
Sutters 2001 ¹⁷	M1 cells; dexamethasone inducible sIg7-PC1-193	sIg7-PC1-193 transforms the response to cAMP from growth inhibition to growth stimulation.
Vandorpe 2002 ¹⁸	Xenopus oocytes and HEK cells; CD16.7-PC1(CTT constructs)	CD16.7- <i>PKDI</i> (1–226), (115–226), (115–203) or (115–189), but not CD16.7- <i>PKDI</i> (1–92), (1–115), (1–155) or CD16.7 (control) up-regulate a nonselective Ca ²⁺ -permeable cation conductance.
Aguiari 2003 ¹⁹	HEK-293 and HeLa cells; Trk-PC1(CTT constructs)	Trk-PC1-1-226 or 26-226 enhance ATP-evoked (HEK) and histamine-evoked (HeLa) cytosolic Ca ²⁺ transients (upregulation of PC2 or other intracellular cation channel activity?).
Hooper 2003 ²¹	M1 cells; dexamethasone inducible sIg7-PC1-193	sIg7-PC1-193 enhances ATP induced, but not forskolin induced, chloride secretory currents.
Wildman 2003 ²⁰	M1 cells; dexamethasone inducible sIg7-PC1-193	sIg7-PC1-193 prolongs the decay phase of ATP induced Ca ²⁺ transients and the duration of ATP stimulated Cl ⁻ conductance.
Puri 2004 ²²	HEK cells; sIg7-PC1(CTT constructs)	PC1 constructs containing the heterotrimeric G protein binding and activation domain activate Gαq and PLC, releasing of Ca ²⁺ from intracellular stores, activating store-operated Ca ²⁺ entry, and activating calcineurin and NFAT.
Manzati 2005 ²³	HEK-293 cells; Trk-PC1(26–226)	Trk-PC1(26–226) expressing cells have higher basal Ca ²⁺ concentration and 1% FBS or ATP induced Ca ²⁺ transients and proliferative responses.
Xu 2007 ²⁴	NK cells; CD16.7-PC1(115–226).	CD16.7- <i>PKDI</i> (115–226) accelerates the decay of flow-induced Ca ²⁺ transients.

Table 2

Articles using endogenous, exogenously expressed or knockdown of polycystins to study intracellular calcium stores and release.

Reference	Model system	Findings
Koulen 2002 ⁵⁰	LLC-PK1 cells. Exogenous expression of full-length PC2, C-terminal truncated PC2 or missense PC2 mutant. Microsomes reconstituted onto lipid bilayers.	WT PC2 functions as calcium-activated high conductance, divalent cation permeable channel. Truncated forms of PC2 traffic to the plasma membrane and retain channel activity. Missense PC2 mutant retains the subcellular localization in the ER but has no channel activity. Vasopressin (70 nM) induces IP3R-mediated [Ca ²⁺] _i transients in LLC-PK1 cells overexpressing PC2 of greater amplitude and duration compared to cells transfected with empty vector.
Qian 2003 ⁵¹	Endogenous PC2. Mouse <i>Pkd2</i> ^{+/-} and <i>+/+</i> VSMCs.	Mouse <i>Pkd2</i> ^{+/-} VSMCs have lower basal [Ca ²⁺] _i , sarcoplasmic reticulum Ca ²⁺ store, and store operated Ca ²⁺ channel activity compared with wild-type VSMCs.
Cai 2004 ⁵²	LLC-PK1 cells. Exogenous expression of PC2 or PC-2 S812A. Microsomes reconstituted onto lipid bilayers.	Constitutive phosphorylation at Ser812 makes the PC2 channel more sensitive to activation by calcium and the duration and amplitude of vasopressin (100 nM) induced intracellular Ca ²⁺ transients.
Aguiari 2004 ⁵³	Endogenous PC1 and PC2. B-lymphoblastoid cells.	Lower platelet-activating factor induced Ca ²⁺ transients and cell proliferation in PC1 or PC2 deficient cells.
Gao 2004 ⁵⁴	WT, <i>Pkd2</i> null and RyR mutant <i>Drosophila</i> larvae.	PC2 cooperates with the RyR to mediate optimal rate of larval body wall contraction. <i>Pkd2</i> haploinsufficiency lowers smooth muscle contractility by 53%.
Koulen 2005 ⁵⁵	WT and <i>pkd2</i> null <i>C. elegans</i> ER vesicles incorporated onto lipid bilayers.	<i>C. elegans</i> pc2 is activated by 100 nM-1 mM cytosolic Ca ²⁺ and inactivated by higher levels. Lower IP3R- or RyR-mediated Ca ²⁺ release from pc2 deficient compared to WT microsomes. Longer half-rise and half-decay of Ca ²⁺ transients in pc2 null cells.
Li 2005 ⁵⁶	Xenopus oocytes. Exogenous expression of PC2 (WT, mutant, C-terminus) and IP3R. UV photolysis of caged IP3 in the absence of extracellular Ca ²⁺ .	PC2 interacts with IP3R through its C terminus. Exogenous PC2 (but not PC2 mutants) prolongs IP3-dependent Ca ²⁺ release from intracellular stores. A channel dead mutant lowers the amplitude of IP3-induced Ca ²⁺ transients. Expression of PC2 C-terminus prolongs the half-decay time (due to dysfunctional closure of IP3R?) but reduces transient amplitude (due to ER Ca ²⁺ depletion?).
Hooper 2005 ⁶⁴	MDCK cells. Exogenous expression of full length PC1	PC1 overexpression accelerates the decay of Ca ²⁺ transients in response to ATP.
Anyatonwu 2007 ³⁴	Embryonic mouse <i>Pkd2</i> ^{-/-} and <i>+/+</i> cardiomyocytes	PC2 interacts with RyR2, stabilizes closed state and inhibits Ca ²⁺ release. Loss of inhibition of RyR2 by PC2 in PC2-deficient cardiac myocytes reduces amplitude and increases frequency of Ca ²⁺ oscillations and reduces SR calcium stores.
Geng 2008 ⁵⁷	LLC-PK1 cells. Exogenous expression of WT PC2, (5-72)PC2 or empty vector. ER vesicles incorporated onto lipid bilayers.	Syntaxin-5 interacts with PC2 and blocks its channel activity. Expression of PC2 increases resting Ca ²⁺ above control. Expression of a PC2 mutant that does not bind Stx5 further increases resting cytosolic Ca ²⁺ levels, decreases ER Ca ²⁺ stores, and reduces vasopressin (200 nM)-induced Ca ²⁺ release from ER stores.
Weber 2008 ⁵⁸	MDCK cells. Exogenous expression of PC1. Fura and fura 2-AM microfluorimetry.	PC1 attenuates the Ca ²⁺ peak and rate of decay of luminal ER calcium following inhibition of SERCA (with no effect on mitochondrial Ca ²⁺ uptake or extrusion of Ca ²⁺ across the plasma membrane). PC1 inhibits Ca ²⁺ leak across the ER membrane.
Wegierski 2009 ⁵⁹	Xenopus oocytes; HEK, HeLA cells (exogenous PC2 expression). MDCK cells (shRNA <i>Pkd2</i> knockdown).	PC2 transfection or depletion of PC2 has opposite effects (decrease or increase) ER Ca ²⁺ stores. PC2 regulates the ER Ca ²⁺ gateway to apoptosis by decreasing the Ca ²⁺ concentration in the ER.
Morel 2009 ⁶⁵	Mouse <i>Pkd1</i> ^{+/+} and <i>Pkd1</i> ^{-/-} VSMCs	Denuded aortic muscle from <i>Pkd1</i> ^{+/-} mice has lower basal intracellular Ca ²⁺ compared to that of <i>Pkd1</i> ^{+/+} mice. Ca ²⁺ entry after caffeine induced SR Ca ²⁺ depletion was reduced in <i>Pkd1</i> ^{+/-} aortic muscle, but basal SR Ca ²⁺ store was not reduced.

Reference	Model system	Findings
Li 2009 ³²	Xenopus oocytes, MDCK cells. Exogenous PC1 expression (fragments or full-length).	A PC1 fragment consisting (last six TM domains and C-terminal tail) interacts with the IP3-binding domain of IP3R and reduces the amplitude of IP3-induced Ca ²⁺ transients. WT PC1 decreases ATP-induced Ca ²⁺ transients.
Miyagi 2009 ³⁹	LLC-PK1 cells. Exogenous expression of WT PC2 and PC2 with a C-terminus truncating mutation.	WT PC2 localizes to ER; PC2 with truncated C-terminus localizes to plasma membrane. PC2 (but not truncated PC2) enhances acetylcholine-induced, ER Ca ²⁺ release in the absence of external Ca ²⁺ . Enhanced Ca ²⁺ entry after reintroduction of external Ca ²⁺ in cells expressing truncated PC2 and co-expressing TRPC3 or TRPC7.
Plotnikova 2010 ⁶³	HEK cells. Exogenous expression of PC2 and Aurora A kinase.	Cotransfection of Aurora kinase A in PC2 transfected cells reduces the amplitude of vasopressin induced calcium transients
Woodward 2010 ¹²	Xenopus oocytes, MDCK and CHO cells. Expression of WT PC1, P100 (fragment generated by cleavage within the third intracellular loop).	PC1 and P100 (but not a mutant P100 lacking the C-terminal 76 amino acids) interfere with STIM1 puncta formation after ER Ca ²⁺ depletion and inhibit SOC.
Santoso 2011 ³³	MCDK, M1 cells. Exogenous expression of WT PC1 or PC1R4227X.	PC2 and STIM1 interact with IP3R, enhancing inhibiting ER Ca ²⁺ release. PC1 reduces the interaction of IP3R with PC2 and enhances the interaction of STIM1 with IP3R thus 1) inhibiting ER Ca ²⁺ release, 2) enhancing traffic of PC2 to the plasma membrane and entry of extracellular Ca ²⁺ , and 3) inhibiting store operated calcium entry. Mutations in PC1 may increase the interaction between PC2 and IP3R, increase ER release of Ca ²⁺ , reduce Ca ²⁺ influx through the plasma membrane and lead to depletion of ER Ca ²⁺ . Mutations in PC2 may reduce Ca ²⁺ influx across the plasma membrane and store operated calcium entry (increased interaction of PC1 and STIM1) leading to low intracellular Ca ²⁺ .
Mekahli 2012 ⁶⁰	Permeabilized, non-mitochondrial calcium store loaded, conditionally immortalized human PTE cells. <i>PKD1</i> and/or <i>PKD2</i> knockdown.	Simultaneous presence of PC1 and PC2 to amplify IP3-induced ER Ca ²⁺ release. PC1 and possibly PC2 inhibit the passive Ca ²⁺ leak from the ER.
Paavola 2013 ⁶¹	<i>Pkd2</i> ^{-/-} and <i>pkd2</i> ^{+/+} zebrafish. Microdissected hearts.	<i>Pkd2</i> null cardiac myocytes display prolonged Ca ²⁺ transient rise and decay times, slow Ca ²⁺ cycling, and reduced SR Ca ²⁺ stores, leading to reduced heart rate and stroke volume, pericardial and abdominal edema, pulse alternans, and AV block.
Streets 2013 ⁶²	HEK and MDCK cells; Xenopus. Exogenous WT PC2, PC2(S829A) or PC2(S829D).	PKA phosphorylates PC2 at Ser829. PC1 through its interaction with PC2 and PP1a facilitates the dephosphorylation of pSer829. PC2 Ser829 phosphorylation is increased in cystic human and mouse tissue. Phosphorylation of PC2 at Ser829 is necessary to rescue a <i>pkd2</i> knockdown phenotype in Xenopus. The phosphomimic S829D mutant shows increased ATP-stimulated Ca ²⁺ transients and loss of growth suppression. PC2 while hyperphosphorylation may contribute to cystogenesis.

Table 3Articles examining the role of calcium in Polycystic Kidney Disease *in vivo*.

Reference	Model system	Findings
Leuenroth 2007 ¹⁶⁷	<i>Pkd1</i> KO mice. Triptolide.	<i>Pkd2</i> ^{-/-} epithelial cells are less susceptible to triptolide induced cell death. Triptolide binds to PC2, induces IP3R dependent Ca ²⁺ release in <i>Pkd1</i> ^{+/-} and <i>Pkd1</i> ^{-/-} but not in <i>Pkd2</i> ^{-/-} cells, increases p21 expression and arrests growth of <i>Pkd1</i> ^{-/-} kidney cells. Treatment of pregnant mice from E10.5 lowers the cyst burden of <i>Pkd1</i> ^{-/-} embryonic kidneys.
Kottgen 2008 ³⁸	Xenopus oocytes, MDCK and HEK cells. Trpv4 and <i>Pkd2</i> mutant mice.	Knockdown of Trpv4 (lentiviral siRNA) abolishes flow induced Ca ²⁺ transients. Trpv4 morpholinos by themselves or in combination with low concentrations of <i>pkd2</i> morpholinos do not induce cyst formation in zebrafish pronephros. Trpv4-deficient mice do not develop cysts.
Leuenroth 2008 ¹⁶⁸	<i>Pkd1</i> ^{flox/-} ;Ksp-Cre mice. Triptolide.	Treatment with triptolide from P1 through P8 lowers kidney weight, cyst burden, epithelial cell proliferation, and BUN levels at postnatal day 8. Treatment from P8 through P12 achieves a slight but significant reduction in the number of cysts.
Nagao 2008 ¹⁶⁹	Cy/+ Han:SPRD rats. Verapamil.	Verapamil increases B-Raf and ERK phosphorylation, tubular epithelial cell proliferation and apoptosis, kidney weight and cyst burden.
Gattone 2009 ¹⁷⁰	Cy/+ Han:SPRD rats. Calcimimetic R-568, high calcium or both.	R-568 and to a lesser extent high calcium lower kidney volume, cyst burden, fibrosis and serum BUN at 38, but not at 34 weeks of age.
Wang 2009 ¹⁷¹	PCK rats and <i>Pkd2</i> ^{WS25/-} mice. Calcimimetic R -568.	No effect on kidney weight, cyst volume or plasma BUN. Significant reduction in renal interstitial fibrosis in PCK rats. Potential benefit from R-568 might have been offset by marked hypocalcemia.
Leuenroth 2010 ¹⁷²	<i>Pkd1</i> ^{flox/flox} ;Mx1Cre mice. Triptolide.	Treatment with triptolide from P16 to P35 lowers tubular epithelial cell proliferation, cyst burden and BUN levels at P35.
Gradilone 2010 ¹⁷³	WT and PCK cholangiocyte cell lines and primary cultures; bile ducts; PCK rats. TRPV4 activators.	Trpv4 stimulation increases cytosolic Ca ²⁺ , activates PI3K and Akt, inhibits ERK, and decreases cell proliferation and cyst growth <i>in vitro</i> . Trpv4 activation decreases cyst growth and fibrosis <i>in vivo</i> .
Chen 2011 ¹⁷⁴	Immortalized human ARPKD renal epithelial cells; pcy mice. Calcimimetic R-568.	R-568 increased intracellular Ca ²⁺ and decreases cAMP accumulation and cell proliferation. R-568 treatment from 4 to 15 weeks decreases kidney cAMP, weight, cyst volume, and fibrosis when given. Treatment at later stages is less effective.
Mahajan 2012 ¹⁷⁵	Metanephric organ culture. Ca ²⁺ ionophores and channel agonists.	A23187 and phorbol-12-myristate-13-acetate inhibit 8-Br-cAMP induced cystogenesis in metanephric organ culture.
Zaica 2013 ¹⁷⁶	PCK and WT SD rats. Freshly microdissected cyst walls and split-open collecting ducts. TRPV4 activator.	Lower resting cytosolic Ca ²⁺ in cyst cells. TRPV4 activator diminishes cyst development and growth in PCK rats.
Jin 2014 ¹³⁸	Zebrafish; WT and <i>Pkd1</i> ^{+/-} mice. Transfection with CaV1.2 shRNA-lentivirus.	Cav1.2 zebrafish morphants have dilated pronephric ducts. Lentiviral CaV1.2 shRNA transfection aggravates the cystic phenotype in <i>Pkd1</i> ^{+/-} mice.