

## The Function of Polycystin-1 and Polycystin-2 in Cardiovascular System

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### Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) caused by mutations in polycystic kidney disease 1 or 2 (Pkd1 or Pkd2) gene is the most common inherited renal disease and is characterized by cardiovascular abnormalities like hypertension and aneurysms. Polycystin-1 (PC1) and polycystin-2 (PC2), encoded by Pkd1 and Pkd2 genes, respectively, are detected in the cardiovascular system, indicating that PC1 or PC2 may play an important role in cardiovascular function. In the present review, we summarize current findings of PC1 and PC2 in cardiovascular regulation, such as hypertension and aneurysms associated with ADPKD and embryonic development.

**Keywords:** Polycystin; Hypertension; Aneurysm; Cardiovascular

### Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disease caused by mutations in Pkd1 or Pkd2 [1]. This disease is characterized by the formation of fluid-filled cysts in the kidneys as well as cardiovascular abnormalities such as hypertension and aneurysms, which have emerged as a major cause of death in patients with ADPKD [2-4]. Polycystin-1 (PC1) and polycystin-2 (PC2), encoded by the Pkd1 and Pkd2 gene, respectively, are found in various vascular tissues including aorta, intracranial arteries, afferent arterioles, mesenteric arteries, portal vein, hepatic arteries and placental, where they may play an important role in the pathogenesis of cardiovascular abnormalities [5-10].

This review describes the basic structure and function, as well as the expression of polycystins (PCs) in the vascular system, and summarizes evidence demonstrating that PCs are important for hypertension and aneurysms associated with ADPKD. The role of PCs in embryo development will also be addressed.

### Structure and Function of PC1 and PC2

PC1 is a large integral membrane protein (~460 kDa) that includes 11 transmembrane segments with extracellular N- and intracellular C-terminal domains. PC1 contains 4,302 amino acids and possesses a large extracellular domain of approximately 3,000 amino acids (Figure 1). A number of characteristic protein motifs are contained in the extracellular domain including leucine-rich repeats flanked by cysteine-rich domains, a C-type lectin and WSC (cell-wall and stress-response component) domains, low-density lipoprotein-like domain (LDL), and PKD repeats with homology to immunoglobulins. The interaction between these specific domains and extracellular matrix proteins such as collagen type I, fibronectin, and laminin, indicates the potential role of PC1 in cell-matrix and cell-cell interactions [11]. A receptor for the egg jelly (REJ) domain, implicated as a novel effector site for normal PC1 function, follows downstream of the PKD repeats. A GPS domain, the potential proteolytic cleavage site, is situated between the REJ and the first transmembrane segment of PC1 [12,13]. The 11 transmembrane segments are suggested to interact and convey specific protein conformations to PC2 [14]. In addition, the first and second transmembrane domains resemble the PLAT domain of the lipoxigenases [13]. The cytosolic C-terminal domain includes a coiled-coil domain that has been implicated in interactions with the C-terminus of PC2 as well as a variety of other proteins involved in cellular signaling [14-16]. Most PC1 proteins also contain a G protein

activation site, suggesting a potential role in the regulation of G protein intracellular signaling [17].

PC2, encoded by Pkd2, contains 968 amino acids (~110 kDa) and includes six transmembrane segments with intracellular N- and C-terminal domains (Figure 1). The PC2 proteins form a tetramer which serves as a Ca<sup>2+</sup>-permeable cationic channel that conducts both monovalent (Na<sup>+</sup>, K<sup>+</sup>) and divalent (Ca<sup>2+</sup>) ions, with a slightly increased selectivity for Ca<sup>2+</sup> over monovalent cations [16,18]. A "sensor" region is expected to be present between transmembrane segment 1 and transmembrane segment 4 as it is present in other six transmembrane channels [19]. However, this remains to be proven. Transmembrane segments 5 and 6 are expected to form the channel pore by homo- or hetero-tetramer with the P-loop in the center [20,21]. PC2 contains two cytosolic extremities with two EF-hands (helix-loop-helix domains that bind calcium), two coiled-coil domains, and an Endoplasmic Reticulum (ER) retention signal in the C-terminus [22-25]. The EF-hands provide a regulatory region for voltage-gated channel inactivation while the ER retention motif contains two putative phosphorylation sites with consensus motifs for PKA and PKC [14]. Beside these, cytoskeletal proteins such as troponin I and tropomyosin-1 can interact directly with the C-terminal tail of PC2 [26,27].

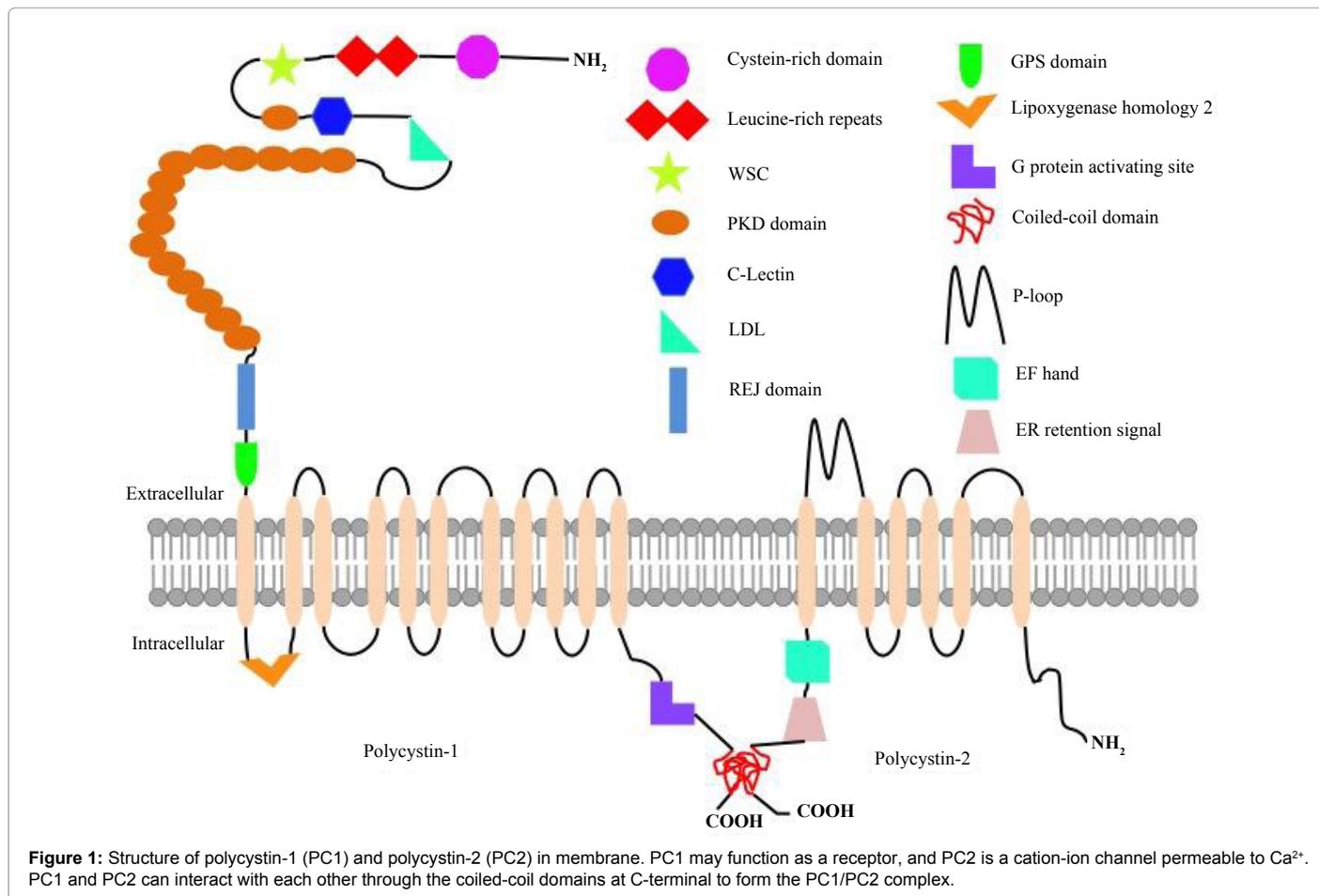
The interaction between PC1 and PC2 is important for the Ca<sup>2+</sup> channel function. PC1 is localized at the plasma membrane, whereas PC2 is localized both at the plasma membrane and the endoplasmic reticulum. PC1 and PC2 can interact with each other via their coiled-coil domains to form the PC1/PC2 complex [22-25]. Co-expression of PC1 and PC2 is necessary to form the functional channel complex while elimination of the cytoplasmic domain of either PC1 or PC2 obliterates the Ca<sup>2+</sup>-permeable cation conductance in Chinese hamster ovary (CHO) cells [16]. Xu et al. observed that isolated PC2 channel was inactivated either spontaneously or by switching the holding potentials to negative values [28]. This inhibition could be reversed

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by adding the coiled-coil domain of PC1. Thus, the C-terminal of PC1 may be an agonist for helping stabilize PC2 channel function [28].

The PC1/PC2 complex functions as a flow sensor in mouse embryonic renal epithelial cells [29]. Flow-induced PC2-mediated Ca<sup>2+</sup> influx was observed in mouse embryonic renal epithelial cells with a well-developed primary cilia where wild type PC1 and PC2 were co-localized [29]. Neither PC1 deficient cells nor wild type cells with antibodies against either PC1 or PC2 responded to shear stress [29,30]. The PC1/PC2 complex may function as a “receptor-ion channel” with PC1 transducing environmental signals to PC2 while PC2 mediates Ca<sup>2+</sup> influx [15,31]. PCs may also have important functions in cardiovascular biology. PC1 and PC2 were found in the primary cilia of vascular endothelial cells and in vascular smooth muscle cells (VSMCs). Similar to renal epithelial cells, endothelial cells lacking PC1 or PC2 did not respond to shear stress (see section entitled “Polycystins and Hypertension” for detail) [32,33]. Although interaction between PC1 and PC2 was also found in VSMCs, the expression levels of PC1 and PC2 were dynamic during development [34]. PC1 is expressed at significantly higher levels in VSMCs at E17 and newborn compared with the adult stage, while PC2 expression is relatively unchanged throughout development [34]. This suggests that besides functioning as the PC1/PC2 complex, PC1 and PC2 may have additional independent roles in the cardiovascular system [35].

### Polycystins and Hypertension

Hypertension is common in ADPKD and occurs before the loss

of kidney function in more than 60% of affected individuals. Pkd1 patients typically have an early onset of hypertension and occur with more frequency than in patients with Pkd2 [31,36]. The average age of onset of hypertension is between 30 and 34 years, with men more commonly affected than women [2,37,38]. Genetic factors may influence hypertension in ADPKD since an increased frequency and earlier age of onset of hypertension in ADPKD offspring is associated with having an affected parent. Blood pressures are 4 to 6 mmHg higher on average in ADPKD children as compared to unaffected age- and sex-matched controls [39,40].

The pathogenesis of hypertension in ADPKD is complex and depends on many interconnected factors. Two possible theories have been proposed to describe the pathogenesis of hypertension in ADPKD. In one theory, cilia dysfunction leads to decreased Nitric Oxide (NO) production, which causes abnormal thickening of blood vessels [41]. In support of this theory, lower plasma concentration of NO was detected in PKD patients [42,43]. Dopamine has been suggested as potential therapeutic target for PKD patients with hypertension [44]. In a dopamine receptor study, the dopamine receptor type 5 (DR5) has been shown to play an important role in managing cilia function [45]. DR5 activation has been shown to restore cilia function in cells isolated from the Tg737<sup>orp<sup>k</sup>/orp<sup>k</sup></sup> (Oak ridge polycystic kidney disease mice with mutations in Tg737, an orthologous gene of Chlamydomonas flagella IFT88 which results in cells with no or shortened cilia) and Pkd1<sup>-/-</sup> mice. In contrast, silencing of DR5 abolishes mechano-ciliary function in wild type cells. The other theory favors a role of cysts formation, where

enlargement of the renal cysts cause distortion of renal architecture, resulting in intra-renal ischemia and activation of the Renin-Angiotensin-Aldosterone System (RAAS) [42,46]. Activated RAAS has been proposed to cause cardiovascular remodeling and contribute to hypertension in PKD patients and PKD mouse models [47-49]. The angiotensin-converting enzyme inhibitor and/or the angiotensin receptor blocker are still being used for treating PKD patients [50]. Endothelin is implicated in cyst formation and hypertension progress and plasma concentration of endothelin in ADPKD patients has been reported to be higher compared with healthy volunteers, while no differences were found between normotensive and hypertensive patients in ADPKD group [51,52]. This observation suggests that the pathogenesis of hypertension in ADPKD is complex and depends on many interconnected factors.

Similar to its function in kidney epithelial cells, the PC1/PC2 complex has been implicated in sensing blood flow in vascular endothelial cells [32,33]. Interaction between PC1 and PC2 has been confirmed by immunoprecipitation study in vascular endothelial cells [33]. Embryonic vascular endothelial cells with Pkd1<sup>-/-</sup> mutation failed to transmit extracellular shear stress sensing into intracellular Ca<sup>2+</sup> signaling and NO synthesis while increased cytosolic Ca<sup>2+</sup> and NO production were seen in wild type cells [32]. In a study on PC2, mouse endothelial cells with knockdown or knockout of Pkd2 were incapable of generating NO under fluid shear stress [33]. Abnormal localization of either PC1 or PC2 to cilia also impairs flow sensing [32,33]. PC1 localized in the basal body of Tg737<sup>orp/ork</sup> endothelial cells is insufficient to respond to shear stress [32]. Loss of ciliary PC2 in isolated endothelial cells from ADPKD patient kidneys also showed minimal Ca<sup>2+</sup> and NO response to shear stress [33].

Although Pkd1<sup>-/-</sup> and Pkd2<sup>-/-</sup> endothelial cells failed to respond to shear stress, they still showed response to other pharmacological stimulation [32,33]. No significant differences in increased cytosolic Ca<sup>2+</sup> and NO were observed between wild type and Pkd1<sup>-/-</sup> endothelial cells when treated with Acetylcholine (ACh) [32]. Similarly, Pkd2<sup>-/-</sup> endothelial cells did not lose their responsiveness to ATP [33]. These studies suggest that ciliary PC1 and PC2 are shear stress-specific molecules.

In contrast with Pkd2<sup>-/-</sup> endothelial cells, Pkd2<sup>+/-</sup> endothelial cells are responsive to shear stress. Increased cytosolic Ca<sup>2+</sup> was followed by NO production in Pkd2<sup>+/-</sup> endothelial cells [33]. Similar to the “second-hit” mechanism has been suggested in cyst formation in ADPKD, Pkd2 heterozygous vascular endothelia may also require a “second hit” to lose their shear response [33,53,54].

Interestingly, a marked defect in ACh stimulated endothelial dependent vasodilatation was still found in Pkd2<sup>+/-</sup> mesenteric arterioles while these arterioles still exhibited normal responses to Sodium Nitroprusside (SNP), Phenylephrine (PE), Potassium Chloride (KCl) and pressure, indicating this response is vascular smooth muscle independent [55]. However, isolated Pkd2<sup>+/-</sup> endothelial cells showed normal Ca<sup>2+</sup> and NO production stimulated by ACh while isolated Pkd2<sup>+/-</sup> VSMCs displayed basal increases in superoxide and SNP-stimulated peroxynitrite formation [55]. Moreover, serum nitrite levels were similar between wild type and Pkd2<sup>+/-</sup> mice [55]. A further study showed that the NO bioavailability was reduced in Pkd2<sup>+/-</sup> vessels with defects in vasodilatation [55].

The de-endothelial arterial vasculatures including aorta and resistance (fourth order mesenteric) arteries in Pkd2<sup>+/-</sup> mice exhibit an exaggerated contractile response and increased sensitivity to PE

as compared to wild type vessels [56]. Further studies showed that elevated Smooth Muscle A-Actin (SMA) expression was observed in Pkd2<sup>+/-</sup> arterial smooth muscle cells [56]. Hui et al. reported that these abnormal vascular contractions and SMA expression are influenced by RhoA hyperactivation and defects in cellular filamentous-to-globular (F-to-G)-actin dynamics in Pkd2<sup>+/-</sup> arteries [57].

Taken together, Pkd<sup>-/-</sup> endothelial cells lose their ability to generate NO in response to fluid shear stress, which may promote high blood pressure [32,33]. Nevertheless, these still need to be confirmed in Pkd endothelial cell conditional knockout mice. On the other hand, haploinsufficiency of Pkd2 is sufficient to elevate SMA expression and increase superoxide and SNP-stimulated peroxynitrite formation in VSMCs, inducing increased vascular contraction and reduced vasodilatation [55-57]. However, no significant differences in blood pressure was found between wild type mice and Pkd2<sup>+/-</sup> mice [55]. Again, these questions may be better addressed in the Pkd2 VSMC specific knockout mice to gain a better understanding of Pkd function in VSMCs in hypertension.

## Polycystins and Aneurysms

ADPKD may also cause arterial aneurysms, such as intracranial berry aneurysms, which are the leading cause of death in these patients [58]. Aneurysms associated with the aorta, coronary arteries, and splenic arteries have also been reported in ADPKD patients [59-62].

Genetic factors likely influence the occurrence of aneurysms in ADPKD. Familial clustering of intracranial aneurysms has been observed where incidences of aneurysms has been reported to be almost as five times higher in patients with a family history of ruptured intracranial aneurysms than in those without a family history [63,64]. Furthermore, ADPKD accounts for 9% of familial intracranial aneurysms [65].

Aneurysms can occur in patients with mutations in either the Pkd1 and Pkd2 genes. These mutations can be missense, splice site changes, small deletions, or small insertions [66]. The position of the germline mutation in Pkd1 is an important factor in aneurysm development. In the subsets of the ADPKD patients with aneurysmal rupture, the median mutation goes further 5' compared to those without vascular phenotype [67]. Abou Alaiwi et al. found that loss of Pkd1 or Pkd2 in endothelial cells results in the dysfunction of primary cilia, which leads to the abnormal cell differentiation and cellular division. The abnormality in cell division is associated with polyploidy formation in vascular endothelia, which might be the pathophysiological mark for aneurysms [68].

Pkd1 and Pkd2 are also important for maintaining vessel wall structural integrity, and mutations in these genes results in abnormal vascular wall integrity which can also lead to aneurysms. Reductions in Pkd1 transcription levels induce degenerative alterations in both the intima and media, resulting in a dissecting aneurysm [69]. Furthermore, gross edema and hemorrhage, indicative of severely compromised vessel wall integrity, were reported in the Pkd1<sup>-/-</sup> and Pkd2<sup>-/-</sup> knockout mouse embryos [8,30,70-72].

It has been proposed that loss of Pkd1 or Pkd2 can result in overproduction of matrix proteins, which is the common cause for the weakening of blood vessel walls. Increased deposition of proteoglycans and fibronectin, leading to the thickening of vessel wall media, was observed in Pkd1 hypomorphs [69]. The N-terminal extracellular domain has been proposed to participate in focal adhesion and cell adhesion. Direct binding between isolated PC1 leucine-rich repeats

fusion protein and matrix proteins collagen I, fibronectin, and laminin has been observed, indicating that PC1 can function as an elastic linkage between cells or between cells and extracellular matrix [11,73,74]. In addition, overproduction of matrix collagen was also found in Pkd1 and Pkd2 deficiency zebrafish [75].

Vascular leakage and aneurysms can also be caused by weakened VSMCs as a result of apoptosis under fluid mechanical stress. Haploinsufficiency of human Pkd2 function leads to decreased VSMCs contractility and function [76]. Pkd2<sup>-/-</sup> VSMCs have defective intracellular Ca<sup>2+</sup> regulation followed by higher cyclic 3': 5'-adenosine monophosphate (cAMP) concentration compared with wild type vessels. The intracellular Ca<sup>2+</sup> reduction and cAMP accumulation may cause an increase in both cellular proliferation and apoptosis, resulting in aneurysms [77]. Interactions between PC1 and PC2 have been shown in VSMCs in the sarcoplasmic membrane, suggesting that PC1 may also be involved in regulating intracellular Ca<sup>2+</sup> levels [34].

PC1 and PC2 can influence Stretch-Activated Channel (SAC) activity to help regulate pressure sensing in arterial myocytes [5]. Abnormal SAC activity may contribute to aneurysm formation because of increased wall tension. Interestingly, it has been reported that the ratio of PC1 to PC2 is important in the regulation of SAC activity [5]. SAC activity can be inhibited by PC2 and this was reversed by co-expression with PC1 [5]. This study further showed that the actin crosslinking protein FLNa is necessary for the SAC inhibition by PC2 [5]. Thus, PC1 and PC2 may influence aneurysm formation by regulating intracellular Ca<sup>2+</sup> concentration induced by pressure via SAC in arterial myocytes. However, this hypothesis is remains to be experimentally determined.

## Polycystins and Embryo Development

PC1 and PC2 are associated with embryo development. Developmental retardation of the labyrinth layer of Pkd1<sup>-/-</sup> placentas is apparent as early as E11.5 with increased severity at later time points [10]. In other studies, mice with targeted Pkd1 null mutations (at exons 17-21 or exons 43-46) or Pkd2 null mutations (exon1) die at mid-embryonic stages with massive hemorrhage or cardiac defects [8,30,70-72]. The placenta is one of three major organs responsible for Ca<sup>2+</sup> homeostasis, and a loss of Pkd1 or Pkd2 may disrupt Ca<sup>2+</sup> homeostasis leading to induce abnormal placenta development [78].

It has been reported that selective inactivation of Pkd1 and Pkd2 in endothelial cells resulted in polyhydramnios and abnormalities similar to those observed in Pkd1<sup>-/-</sup> placentas [10]. Increased death in utero was observed in the Pkd1 and Pkd2 endothelial specific knockout, however, in our hands, the Pkd1 or Pkd2 endothelial knockout mice, generated by crossing with the Tie2-Cre (B6.Cg-Tg(Tek-Cre)12Flv) transgenic line, were born in Mendelian ratio (unpublished data) [10]. This discrepancy could partially due to different Cre mouse lines used to induce deletion of loxP flanked PKD1/PKD2 and/or the timing of the targeted gene deletion. More detailed future studies are needed to resolve the discrepancy.

It seems that PCs are essential for the development of both trophoblast and fetal vascular compartment of placenta. Moreover, PCs are important in the development and maintenance of the myoelastic structural organization of the vasculature.

## Conclusions

The findings discussed in this review indicate important functions of PC1 and PC2 in the development and homeostasis of cardiovascular

system. PCs have pivotal roles in hypertension and aneurysms associated with ADPKD as well as during placentation development. PCs can function as a complex or independently. Abnormal expression or localization of PC1 or PC2 is associated with cardiovascular defects. However, several important questions remain to be answered: What is the direct role of PC1 and PC2 in the ADPKD associated cardiovascular complications? What are the roles of PC1 and/or PC2 in vascular wall cells (endothelial cells, vascular smooth muscle cells and pericytes) *in vivo*. Do the cardiovascular defects observed in ADPKD patients occur independent of kidney abnormalities? The answers of these questions will enhance our understanding of PCs in cardiovascular system and may initiate the discovery of new pharmacological targets that will benefit ADPKD patients.

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## Review Criteria

The papers cited in this manuscript were selected because they were considered to be either recent, important reviews in the field of autosomal dominant polycystic kidney disease, primary cilium or ion channels, or original papers that demonstrate key functions for polycystins in cardiovascular system or mechanosensory transduction of endothelial cells and vascular smooth muscle cells. No specific databases were searched. Rather, the material was identified based on the authors' knowledge of the field.

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