Chapter 16

Implications of Dysfunction of Mechanosensory Cilia in Polycystic Kidney Disease

Hannah C. Saternos, Wissam A. AbouAlaiwi

Department of Pharmacology and Experimental Therapeutics, The University of Toledo, USA

Author for correspondence: Wissam A. AbouAlaiwi PhD, Assistant Professor, Department of Pharmacology and Experimental Therapeutics; MS 1015, College of Pharmacy and Pharmaceutical Sciences, The University of Toledo, Health Science Campus, Frederic and Mary Wolfe Center, Room 282E, 3000 Arlington Avenue, Toledo, Ohio 43614, USA. Email: Wissam.Abou-Alaiwi@UToledo.Edu

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a multisystemic disorder characterized by numerous fluid-filled renal cysts that eventually destroy the kidney architecture and lead to end-stage kidney disease (ESKD). Although the formation of bilateral cystic kidneys is the hallmark of the disease, patients with ADPKD also suffer from extra-renal manifestations and cardiovascular complications. ADPKD is considered
a ciliopathy disease due to defects in mechanosensory polycystins, localized to primary cilia, which have been recognized as mechanosensory organelles due to their involvement in ADPKD only within the past decade. Our recent studies focus on the fluid mechanosensory functions of primary cilia using cultured cells, animal models, and tissue from ADPKD patients. Growing evidence from these studies suggests that aberrant expression or localization of polycystins to cilia could promote high blood pressure due to the inability to synthesize nitric oxide in response to an increase in shear stress, and alteration of function of cilia could contribute to vascular and renal abnormalities in ADPKD. Our results have led us to propose that drugs targeting primary ciliary function could be a novel therapeutic approach to slow the progression of pathogenesis in ADPKD. In this chapter, in order to explain the involvement of primary cilia in ADPKD, the structure of primary cilia and their mechanosensory function will be described and their contribution to diseases of the kidney and cardiovascular system will be discussed in regards to ADPKD.

**Key words:** Fluid flow; Mechanosensing; Polycystic kidney; Primary cilium; Shear stress

**Introduction**

Polycystic kidney disease (PKD) is the most widely inherited kidney disease and a leading cause of end stage kidney disease (ESKD) in both adults and children (1, 2). Affecting 1:400 to 1:1000 people, it is primarily characterized by renal cystogenesis; however, multisystemic complications are seen in both cystic and non-cystic phenotypes (3). PKD can be categorized into two types, autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). ADPKD has the highest occurrence rate, but clinical manifestations do not develop until 30 or 40 years of age. It is caused by a mutation in either **PKD1** or **PKD2** genes encoding for polycystin-1 (PC-1) or polycystin-2 (PC-2), respectively (4, 5). ARPKD is significantly less common (1 in 20,000 live births), predominantly affecting children (6). ARPKD is caused by a mutation in **PKHD1**, the gene encoding for fibrocystin/polyductin (FPC). Though the chief clinical manifestation is still bilateral cyst development, this mutation, in combination with others, may be the cause of the increased severity of the disease and the broad phenotypic variance (7). One of the key facets of PKD is ciliary dysfunction, which contributes to different cellular pathologies related to planar cell polarity, cellular differentiation, cell signaling mechanisms, and fluid sensing and transport (8). Ciliary alterations not only cause renal manifestations but also lead to extra-renal complications in the liver and cardiovascular system (9, 10).
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Etiology

ADPKD is a completely penetrative heterogeneous disease associated with mutations at two loci, \textit{PKD1} on chromosome 16 or \textit{PKD2} on chromosome 4 \((2, 11)\). Mutations in \textit{PKD1} are responsible for about 85% of all PKD cases while mutations in \textit{PKD2} only cause about 15% of the cases. The PC-1 and PC-2 proteins form a mechanosensory complex in the ciliary membrane that requires both proteins in order to function properly. Henceforth, either mutation will display identical phenotypes \((12)\). PC-1 is a large transmembrane protein that functions as a mechanosensor and/or a chemosensor. PC-2, on the other hand, is a calcium channel that requires PC-1 to function properly \((13)\).

A mutation in the \textit{PKHD1} gene on chromosome 6 leads to ARPKD, a rare pediatric form of polycystic kidney disease. The \textit{PKHD1} gene product, FPC, has also been found to localize to the cilia. It remains uncertain what the exact function of fibrocystin is; however, several studies show that FPC interacts with PC-2 but some suggest there may also be an interaction with PC-1 \((6, 14, 15)\). Due to the wide genotypic variance in \textit{PKHD1}, the clinical phenotypes also vary, even amongst family members \((16)\). Renal cysts also characterize the clinical manifestations, and most patients will progress to ESKD. Unlike ADPKD, in which patients may or may not develop hepatic cysts, all patients with ARPKD will exhibit hepatic phenotypes, specifically a ductal plate malformation. This malformation in turn causes the biliary duct to dilate and macroscopic cysts to form \((6)\).

Clinical manifestations

The formation of bilateral cystic kidneys is the hallmark of the disease. However, patients with PKD also suffer from extra-renal manifestations and cardiovascular complications.

Kidney

Due to cyst growth, the renal parenchyma is disturbed, altering normal kidney architecture and leading to variations in size \((150 \text{ cm}^3 \text{ to } >1500 \text{ cm}^3)\) as well as marked asymmetry between the kidney pairs \((17, 18)\) (Figure 1). An increased expression of proto-oncogenes in the kidney leads to uncontrolled cellular proliferation of the cells lining the lumen, initiating cystogenesis. Glomerular filtrate accumulates within the cysts while still connected to the originating tubule. Once the cyst separates from the lumen, fluid accumulates through the secretion of transepithelial fluid, leading to continued cyst enlargement. In ADPKD patients, the cysts are located through the cortical and medullar regions of the kidney and are only produced by a small fraction
of nephrons. Generally, the fluid within the developed cysts is similar to urine in appearance, but there are cases in which the fluid is very dark with a paste-like consistency (17).

ARPKD typically presents prenatally and the kidney phenotype is characterized by nonobstructive cystogenesis occurring through symmetric dilatation as well as an elongation of the collecting duct by 10-90%. In stark contrast to ADPKD, the kidneys in ARPKD maintain their integrity mainly due to the fact that the cysts are typically smaller and the malformation is primarily caused by the increase in the collecting duct size (19). Though parts of the cellular proliferation pathways are thought to be similar in both ADPKD and ARPKD, the distinct anatomical difference in cysts suggests a different secretion pathway. Currently, it is thought that cystogenesis in ARPKD patients is caused by a decrease in sodium absorption leading to altered fluid secretion (20, 21).

Hematuria is seen in more than 40% of ADPKD patients and usually resolves itself within a week. This diagnosis can be based on a variety of presentations such as hemorrhagic cysts, renal stones, urinary tract infections, or abdominal trauma. Hypertensive ADPKD patients are significantly more likely to experience hematuria than are normotensive ADPKD patients (10).

Figure 1. Cystic kidney. Comparison between a normal human kidney (left) and a cystic kidney (right). This image was adopted from (77) with permission.
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Nephrolithiasis occurs more frequently in ADPKD patients due to the presence of cysts that hinder the urinary collection process. However, metabolic alterations also play a role in stone development. ADPKD patients are more likely to have metabolic problems such as hypocitraturia or hyperuricemia, leading to renal stones with specific compositions (22).

Poor fluid management, such as nocturia, is also commonly seen in ADPKD patients. In the normal population, a loss of ability to concentrate urine is typically associated with old age; however, in the ADPKD population, it displays as an early symptom of the disease (17).

Liver

One of the more common extra-renal manifestations in ADPKD is the formation of hepatic cysts, which develop later than the renal cysts and for which incidence rates increase with age (5). The cysts develop due to a combination of a malformation in the ductal plate and dysfunctional primary cilia. The ductal plate is a single layer of hepatoblasts that surround the portal vein; through a sequence of growth and apoptosis, the hepatoblasts form a double layer that eventually becomes the bile duct (23). The cystic abnormality occurs when intralobular bile ductules remain disconnected (von Meyenburg complexes) and begin to dilate, due to the inability to undergo apoptosis (24, 25). Cilia in the liver stem from specific cells called cholangiocytes. The cilia have a mechanosensory function. Cilia bending in response to fluid flow help regulate intracellular levels of cAMP and calcium. Thus, deficient cilia result in altered levels of calcium and cAMP, causing increased proliferation of cholangiocytes. They also function to detect the osmolality and composition of bile. As such, the defective cilia are also unable to regulate the fluid flow, absorption, and secretion within the lumen (26).

Cardiovascular

Cardiovascular complications such as hypertension, aneurysm, left ventricular hypertrophy, and mitral valve prolapse are the leading cause of death for ADPKD patients (27). The overall cardiovascular abnormalities contribute significantly to morbidity and mortality in ADPKD patients (27, 28). Due to the focus on the extreme pathology of the renal system in ADPKD patients, the cardiovascular prevalence in ADPKD has unfortunately not been well studied.

Hypertension is the most common risk factor in ADPKD. It can be used as a diagnostic marker for ESKD simply due to its occurrence before the onset of renal dysfunction (29). The incidence of hypertension in ADPKD patients is usually one to two decades earlier than in the general population and differs between males and females. Increased blood pressure has been reported among children with ADPKD with an association with target organ damage, specifically if at least one of the parents is hypertensive (27, 30). Hypertensive ADPKD
patients often exhibit left ventricular hypertrophy (LVH), biventricular diastolic dysfunction and impaired coronary blood flow (31). However, the incidence of hypertension alone is not sufficient to explain these cardiovascular phenotypes, as a significant fraction of normotensive ADPKD patients exhibit some cardiovascular abnormalities. Thus, it is safe to assume that additional factors contribute to the pathogenesis of cardiovascular abnormalities. In addition, hypertension is associated with increased kidney volume and decreased kidney function in ADPKD. Evidence suggests the possible involvement of the renin angiotensin aldosterone system (RAAS) in this association, though the exact mechanism is unknown. Taken together, early diagnosis and treatment of hypertension will definitely lead to a decrease in the prevalence of target organ damage in ADPKD patients.

Intracranial aneurysms are significantly more common in ADPKD patients than in the general population and are responsible for 4-7% of the deaths in ADPKD patients. Furthermore, in ADPKD patients, intracranial aneurysmal development and rupture both occur at a younger age than in the general population. ADPKD-associated aneurysms are not limited to the cranial arteries, but have also been reported in the coronary arteries, abdominal aorta, renal artery, and splenic artery. With the expression of PC-1 and PC-2 in vascular smooth muscle cells, it stands to reason that these proteins have a potential role in the pathogenesis of aneurysms. Currently, clinical testing and diagnosis is routinely performed in ADPKD patients with a family history of aneurysms, since the incidence rate of aneurysm formation is double that of the ADPKD population with no known history. Nevertheless, patients who have no prior history of aneurysms, but have experienced a subarachnoid hemorrhage, are also screened (5).

LVH has been reported in roughly half of hypertensive ADPKD patients. In ADPKD patients, an increase in left ventricular mass index (LVMI) has been associated with poor renal prognosis as well as negative overall outcomes. Increased LVMI has also been reported in normotensive ADPKD patients with preserved renal function, which is suggestive of diastolic dysfunction, an abnormality seen in both normotensive and hypertensive ADPKD patients (31).

Cardiac valvular abnormalities have also been reported in ADPKD patients, with mitral valve prolapse being one of the more common malformations. Several studies have reported an approximate 25% incidence rate of mitral valve prolapse and a 30% incidence rate of mitral incompetence (27).

**Screening, diagnosis and therapies**

ADPKD is relatively easy to diagnose, given the characteristic renal cyst development. Familial history also aids in the diagnostic process and can be useful for a presymptomatic
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diagnosis. An enlarged kidney or liver and cardiovascular issues such as hypertension or mitral valve prolapse in patients with a known family history of ADPKD is highly suggestive of a positive diagnosis. For those whose family history is unknown, bilateral renal enlargement and cysts, along with an absence of phenotypes suggesting other cystic diseases, leads to a more ostensive diagnosis requiring further tests. A diagnosis is confirmed through either imaging or genetic testing, regardless of familial history (32). Most commonly, an ultrasound is used to detect the classic symptoms such as bilateral cysts; however, imaging becomes unreliable under certain circumstances. Children suffering from early stages of ADPKD have smaller cysts that may escape sonographic detection; this is also frequently the case for patients suffering from the milder ADPKD type 2 (33). An age-dependent algorithm for ADPKD type 1 was developed for at-risk patients to assess a potential diagnosis based on the number of cysts an individual has at a certain age. This algorithm was later modified for more stringent parameters to aid in the diagnosis of ADPKD type 2 patients. For ADPKD type 1 patients, two unilateral or bilateral cysts between the ages of 15-29 years is a valid indication of a potential diagnosis, while for ADPKD type 2 patients aged 15-39 years, at least three unilateral or bilateral cysts is the parameter (33, 34). Computerized tomography (CT) scans and magnetic resonance imaging (MRI) are occasionally used in diagnosis, since their detection sensitivity is much greater than that of ultrasound; however, they are more expensive (35, 36). In addition to imaging techniques, genetic tests such as sequence analysis and duplication/deletions analysis are used to confirm PKD diagnosis. Sequence analysis detects small insertions/deletions, missense, and splice-site mutations that account for genetic variances from benign to pathogenic. Larger mutations such as duplications or deletions of a complete gene are not detected in this method but can be detected through duplication/deletion analysis (32).

Though there are no targeted ADPKD therapies that are clinically approved, the current treatment strategies focus on slowing cyst formation and treating the associated complications. Mammalian target of rapamycin (mTOR) inhibitors have been considered as potential therapeutic agents for ADPKD due to their ability to inhibit cellular proliferation and cyst growth. In a retrospective study, rapamycin proved to be more effective than cyclosporine in preventing kidney enlargement after renal transplants in ADPKD patients, which supported mTOR inhibitors as potential therapies. Unfortunately, upon clinical trials in ADPKD patients, mTOR inhibitors have shown disappointing results. An 18-month study of rapamycin therapy in ADPKD patients had no effect on the total kidney volume (TKV), but seemed to slow the decline of renal function (37). Worse yet, a two-year study with everolimus (a rapamycin analog) showed an increase in TKV and worsened renal function.

Somatostatin has also been studied as a therapeutic target for ADPKD and polycystic liver disease (PLD) due to its ability to decrease cAMP levels in tubular epithelial cells and
cholangiocytes. One study provided octreotide, a somatostatin analogue, to ADPKD and PLD patients over the course of one year. Compared to the control group, there was a significant decrease in changes in liver volume, but TKV remained stable. The patients in the octreotide group reported less pain and were able to increase their physical activity (1).

Currently, Tolvaptan therapy has the most promising results. Arginine vasopressin stimulates cAMP production in the distal nephron and collecting duct; Tolvaptan antagonizes vasopressin V2 receptor, thereby inhibiting cAMP production. Tolvaptan is well into the clinical trial process, with phase-2 results reporting that it is well tolerated in ADPKD patients. After a three-year study with the therapy, the rate of increasing TKV and renal function decline was significantly slowed; however, adverse effects caused 25% of the patients to discontinue the drug (38).

Unfortunately, the treatment options for those who progress to ESKD are limited to dialysis and renal transplantation (39). Patients with ESKD generally respond well to peritoneal dialysis, assuming there is enough intraperitoneal space within the kidneys to support the increased fluid volume. The lack of space due to kidney enlargement increases the risk of hernia development; therefore, it is often not the primary choice for treatment of ADPKD-induced ESKD (40). The primary course of treatment is renal transplant surgery, considering that the surgery risks for ADPKD patients are no greater than those for patients with ESKD from other diseases (41).

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ADPKD is a pathology associated with ciliary dysfunction, also known as ciliopathy. Ciliopathy is a general term used to collectively describe genetic disorders caused by mutations that affect the structure and function of the cilia or the basal bodies.

Ciliary structure

Non-motile primary cilia, as both chemosensory and mechanosensory organelles, act as an antenna on the apical surface of most cells to sense and transmit information from the extracellular matrix (ECM) to the cell interior. The primary cilium is a microtubule-based organelle that originates from the basal body of the mature centriole in quiescent cells (42). During cell division, the cilium is reabsorbed into the cell, allowing the centrioles to reform into the centrosome. As the cell re-enters the G₀ phase of cell division, the mature centriole migrates towards the cell surface to anchor just below the cell membrane, where it becomes the basal body (43). The basal body is a cylindrically-arranged group of nine triplet
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Microtubules (A, B, and C) responsible for both producing the ciliary skeleton and anchoring the organelle to the cell (44). The termination of the basal body is marked by the transition of the C microtubule into transition fibers that attach the basal body to the cell membrane. Microtubules A and B remain and continue to elongate through the process of intraflagellar transport (45), creating the concentric nine doublet microtubules of the cilium structure, known as the axoneme (46, 47). IFT is a process that uses the bidirectional movement of cargo proteins located between the axoneme and the ciliary membrane. Kinesin-2 motors are responsible for anterograde movement associated with cilia assembly, while dynein-2 motor proteins transport particles from the tip to the base of the cilium for recycling. The IFT system is essential for ciliogenesis as well as for the signaling cascades elicited through normal function of cilia (48). The ciliary membrane, distinct from the cell membrane, encases the microtubules of the axoneme. The ciliary necklace constitutes a series of proteins at the transition zone that separate the ciliary and cell membranes (Figure 2) (49, 50).

Ciliary function

Although primary cilia have been reported in the literature since 1968, it was only recently that cilia were considered to be more than vestigial organelles (51, 52). The normal length of primary cilia is between 5 to 10 μm, and cilia extend into the extracellular environment, making them ideal chemosensory and mechanosensory organelles (43). Studies have demonstrated the roles of primary cilia in various organs and structures, including but not limited to the kidneys (53), blood vessels (8), heart (54), liver (55), bone (56), retina (57), nose (58), and inner ear (59).

The mechanosensory function of primary cilia in the kidney is one of their most widely studied functions. Many receptors have been localized to renal primary cilia, thus implying a chemosensory function also involved in the complex signaling cascades for cell and tissue homeostasis (53). Cilia sense fluid flow, a function that is essential for proper kidney function (60); however, detection of fluid flow requires the functioning mechanosensory complex that forms between PC-1 and PC-2. Primary cilia are activated in response to sheer stress. This in turn leads to the primary cilia bending and the opening of the PC-2 calcium channel, resulting in the influx of calcium ions and the increase in intracellular calcium concentration. Intracellular calcium acts as a second messenger for multiple signaling pathways in the cell (Figure 3). Following the cessation of the stimulus, PC-1 is cleaved and acts as a transcription activator in conjunction with several other pathways (61). It is therefore not surprising that a mutation in genes responsible for PC-1, PC-2 or fibrocystin would result in polycystic kidney disease. This further suggests that dysfunction of renal cilia in response to urine flow would result in polycystic kidney disease.
Dysfunctional primary cilia can contribute to the pathogenesis of polycystic kidney disease through planar cell polarity (PCP), an organized arrangement of cells in a plane of tissue perpendicular to the apical-basal axis as a direction for the orientation of cell division (62-64). One of the signaling pathways regulated by cilia activation is the Wnt (Wingless type mouse mammary tumor virus) signaling pathway, which can be subdivided into two types, canonical and non-canonical pathways. The canonical pathway stabilizes β-catenin, a transcription factor that helps regulate gene expression. The canonical pathway also helps regulate cellular proliferation, differentiation, and fate. The non-canonical pathway (PCP), in contrast, degrades β-catenin but also influences cytoskeletal organization and morphogenesis. The receptors responsible for determining which pathway is activated have been localized to primary cilia (43, 65). Using different mouse models of cystic kidney disease, defects in any ciliary protein lead to abnormal orientation during cell division. It is therefore thought that inactivation of ciliary proteins would result in abnormal PCP, which in turn triggers an increase in tubular diameter in the kidney (Figure 4). The net result is cystic formation in the kidney.
Cilia are mechanosensory organelles. Cilia are sensory organelles that sense fluid-shear stress on the apical membrane of the cells. Fluid flow that produces enough drag-force on the cells will bend sensory cilia. The diagram illustrates the mechanism that polycystin-1 (PC1), polycystin-2 (PC2), signaling proteins, molecules and other receptors exert on signaling pathways, leading to cyst formation. The blue box indicates the reduced molecules and signaling proteins in ADPKD. The orange box indicates the increased signaling proteins in ADPKD, which are thought to be responsible for an increase in cell proliferation, including cAMP, Ras/Raf/ERK, adenylate cyclase (AC), and mTOR activity. In addition, EGFR activation is also enhanced by amphiregulin (AR), which is abnormally expressed in cystic cells through cAMP, CREB and API signaling (not shown). The sphingolipid, Na+/K+ ATPase, Wnt and P2x7 purinergic receptors are also involved in the regulation of mTOR and TSC1/TSC2 complex activity. Other receptors that are involved in ADPKD include adenosine receptor-3A (A3AR) and somatostatin receptor (10), which regulate the activity of AC. This illustration was adopted from (53).

Another pathway that is linked to primary cilia function is Hedgehog (Hh) signaling which is essential for proper cell proliferation, differentiation, and general tissue homeostasis. The Patched 1 receptor (PTCH1), responsible for binding sonic hedgehog ligand, has been localized to primary cilia. Once the receptor is activated, it diminishes from the cilia and initiates a sequence of complex processes enabling normal renal development and homeostasis. Hh also regulates gene expression through suppression and activation of several transcription factors that have been localized to primary cilia. Several studies...
provided evidence for the implication of Hh in renal cyst formation. Evidence suggests that renal cystogenesis is associated with enhanced Hh activity and that Sonic Hh mutant mice are characterized by abnormal kidney development (66, 67).

**Figure 4.** Cyst formation. a. Normal kidney tubule structure; urine can easily pass. The cilia are able to sense the fluid flow and respond accordingly. b. Mutations in the cilia due to genetic disorders such as ADPKD prevent the cilia from functioning properly and thereby hinder response pathways. c. Due to the mutation, planar cell polarity is lost, inhibiting the cells from dividing in the proper orientation. d. The tubular diameter increases and begins to fill with fluid. e. The cyst begins to bud and separate from the nephron. f. The cyst continues to fill with fluid through absorption and secretion processes, causing continued enlargement and reducing the diameter of the nephron. Figure is reproduced under license from the original publisher (78).
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Although the molecular mechanisms by which Hh signaling might contribute to kidney cyst formation are still under investigation, numerous reports suggest that decreased Hh activity assists renal epithelial cells in sustaining differentiation (68). Other studies suggest a link between calcium levels and renal cystogenesis through Hh signaling (67, 69). It has been demonstrated that Hh activity can modulate calcium signaling at both the intracellular and the primary ciliary levels.

Notch signaling uses surface receptors to link the fate of one cell to its neighbor, thereby influencing differentiation, proliferation, and apoptosis. Though notch is known to be essential for proper kidney development, it has not been widely studied in PKD patients. Ligand binding leads to the activation of notch receptors and results in the cleavage of their intracellular domains. These domains translocate to the nucleus and activate target gene expression through a series of complex protein binding interactions. Only recently, notch signaling has been shown to be regulated by primary cilia. Notch3 receptor was localized to the primary cilia of skin cells and kif3a knockout, a mutation that prevents cilia formation, caused the ablation of the nuclear localization of notch intracellular domains. In addition, ift88 knockdown led to the differentiation failure in mouse keratinocytes and decreased notch activity.

The presence and the function of primary cilia in vascular endothelial cells lining the inner wall of blood vessels have been previously demonstrated (8). Primary endothelial cilia function as fluid shear stress mechanosensors to sense changes in blood flow and pressure and convert these mechanical changes into biochemical signals that regulate smooth muscle tone and blood pressure. The presence of endothelial cell primary cilia has been demonstrated in mouse aortic endothelial cells in vitro, in isolated mouse arteries ex vivo, and in mouse models as well as in blood vessels from human patients in vivo (5, 7). Primary endothelial cilia can detect changes in blood pressure or shear stress through the mechanosensory complex PC-1/PC-2. This in turn leads to the influx of calcium ions through the calcium channel, PC-2. As a result, an increase in intracellular calcium concentration triggers a series of signaling cascades that result in the activation of endothelial nitric oxide synthase (eNOS) and the release of NO gas, which diffuses to the surrounding vascular smooth muscle cells, causing vasodilation. Therefore, the inability of ciliary polycystins to sense fluid shear stress due to mutations might contribute in part to hypertension in ADPKD patients. In addition, our recent studies have provided evidence for the involvement of primary endothelial cilia in signaling pathways that regulate vascular architecture and have shown that cilia dysfunction can contribute to aneurysm formation through downregulation of survivin (70). All in all, this led us to propose primary endothelial cilia as a potential therapeutic target for vascular disease (Figure 5).
Figure 5. The role of mechanosensory cilia and nitric oxide production in ADPKD. The illustration depicts the activation of nitric oxide (NO) during fluid shear stress. The biochemical production and release of NO is dependent on the function of endothelial cilia in the vasculature. In ADPKD, dysfunctional cilia are not able to sense blood flow mechanically, and NO is not produced, resulting in increased blood pressure. The bending of cilia by fluid-shear stress activates the mechanosensory polycystins complex and initiates biochemical synthesis and the release of NO. This biochemical cascade involves extracellular calcium influx, followed by the activation of various calcium-dependent proteins, including calmodulin (79), protein kinase C (PKC) and Akt/PKB. Figure is modified from (80).

Vascular smooth muscle cells (VSMC) are constantly under stress due to blood flow, arterial wall stretching, and constriction. This dynamic environment requires transmission of mechanical and chemical perturbations to the VSMC through the ECM (71). VSMCs are ciliated, though they do not come into direct contact with fluid flow unless there is an injury to the arterial cell wall. The VSMC cilia have a mechanosensory function and are specifically oriented along the arterial axis at a 60° angle (72, 73). It is thought that the angle assists in enhancing the signal transduction by increasing the contact area with the ECM. These cilia are implicated in cellular migration and respond to fluid flow, suggesting a role in intracellular calcium levels (72). Upon injury to the arterial wall, a study found that deciliated VSMC showed a reduced cell migration and thus a reduction in healing efficacy (71).
mechanical force placed on cilia cause them to bend, initiating a calcium influx through the PC-2 channel that is proportional to the degree of cilia bending.

Cellular signaling pathways in the heart are regulated by cardiac primary cilia that localize in cardiomyocytes. In the developing heart, cardiac primary cilia have been implicated in signaling cascades associated with morphogenesis, differentiation, and maturation (54). For example, primary cilia may be involved in the highly conserved left-right (LR) asymmetry that manifests in the heart, as well as throughout the body. A type of motile cilia called nodal cilia primarily dictates LR asymmetry by moving in a rotatory motion, pushing embryonic fluid (nodal flow) toward the left. It is suggested that the nodal flow activates the PC-2 calcium channel of non-motile primary cilia (74). As the heart continues to develop, it is also suggested that the primary cilia begin to reabsorb from the endothelium of the endocardial cushion in response to shear stress. Shear stress is essential for proper cardiomyocyte proliferation and development of the conduction system. Mutations in cilia structure and/or polycystins have led to impaired fluid flow sensing, causing aberrations in cardiac maturation (54).

As mentioned earlier, cholangiocyte cilia are preferentially oriented towards the bile duct lumen to enable proper monitoring of bile flow and changes in bile composition. These changes are in turn associated with signaling cascades responsible for cholangiocyte proliferation and secretion (55). The multisensory functions and implications of cholangiocyte cilia are being investigated fervently. The polycystins are mechanosensory molecules crucial for calcium signaling associated with primary cilia; however, cholangiocyte cilia also host calcium-inhibitable adenylyl cyclase 6 (AC6), an axoneme-localized protein involved in cAMP signaling (75).

In summary, it has been well documented that cilia bending in response to fluid shear stress, trigger intracellular calcium signaling. However, recent studies from Nauli’s group have shown that calcium signaling triggered by fluid shear stress is initiated rather in the cilioplasm and can be spatially and temporally differentiated from the subsequent intracellular cytosolic calcium. The authors suggest that the flow-induced calcium signaling is mainly dependent on the ciliary mechanosensor, PC-2. This is mainly supported by data demonstrating that ciliary activation by the dopamine receptor-5 (DR-5)-specific agonist induces calcium signaling only in the cilioplasm while thrombin treatment induced cytosolic signaling through the IP_3 receptors. This led the authors to conclude that cilium-dependent signaling can either be contained within the cilioplasm or spread to the cytoplasm. Henceforth, proposing cilia as a calcium signaling compartment in addition to its function as a sensory organelle (76).

In contrast to the above hypothesis, the Clapham group has recently proposed cilia as unique calcium compartments regulated by a heteromeric TRP channel, PKD1-L1/PKD2-L1.
According to their studies, ciliary calcium concentrations fluctuates in response to external stimuli, such as rupturing the ciliary membrane at the tip of the cilia with a laser pulse, without substantial effect on cytosolic calcium levels (69). While both studies concluded that cilia can function as a calcium compartment, it remains difficult to compare the difference in the overarching conclusions regarding changes in ciliary and subsequent cytosolic calcium levels simply due to the difference in the approaches employed to study ciliary calcium signaling.

**Conclusion**

ADPKD is a ciliopathy disease characterized by the formation of kidney cysts, vascular aneurysms and hypertension. Surprisingly, these complications in the kidney tubules and blood vessels are associated with one another. Abnormal renal epithelial ciliary function in sensing urine flow can lead to kidney cyst formation. The inability of vascular endothelial cilia to sense blood flow can induce hypertension and vascular aneurysms. However, the formation of renal cysts or vascular aneurysms may or may not be dependent on the incidence of hypertension. Primary cilia, as newly-discovered mechanosensory and chemosensory organelles, have important roles in disease and development. Defects in primary cilia can trigger a wide range of pathologies in the kidney, heart, vasculatures and many other organs. The importance of sensory cilia in other organ systems is yet to be discovered, and many more cilia-related diseases are still to be identified. There is no doubt that the physiological roles of primary cilia will continue to be debated in years to come.

**Conflict of interest**

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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