NEPHROLOGY

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ISSN 2380-0445 Open Journal http://dx.doi.org/10.17140/NPOJ-SE-1-102

Special Edition
"*Global Health Policy and Kidney Disease*"

*Mini Review*

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***Special Edition 1***
***Article Ref. #: 1000NPOJSE1102***

***Article History***
**Received:** April 3rd, 2017
**Accepted:** May 1st, 2017
**Published:** May 1st, 2017

***Citation***
Guseynova A, Vijayakumar P, Vijayakumar S. The role of primary cilia in autosomal dominant polycystic disease revisited. *Nephrol Open J*. 2017; SE(1): S4-S7. doi: 10.17140/NPOJ-SE-1-102



# The Role of Primary Cilia in Autosomal Dominant Polycystic Disease Revisited

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## ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease that causes significant morbidity and mortality. The main manifestation of the disease is cyst formation in the nephron and liver, the pathogenesis of which involves the primary cilia. Recently, the mechanosensory ability of primary cilia which leads to calcium signaling has come into question. It is hypothesized that failed control of a physiologic process called cyst-dependent cyst activation (CDCA) contributes to cyst formation in ADPKD. Studies into the dysregulation of CDCA may reveal the exact mechanism for cystogenesis in ADPKD.

**KEY WORDS:** Polycystic Kidney Disease; ADPKD; Primarly cilia; Mechanosensor hypothesis; Calcium influx; Hedgehog signaling.

**ABBREVIATIONS:** ADPKD: Autosomal Dominant Polycystic Kidney Disease; CDCA: Cyst-Dependent Cyst Activation; ESRD: End-Stage Renal Disease; PC: Polycystin; mTOR: mammalian Target of Rapamycin; cAMP: cyclic Adenosine Monophosphate.

## INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) caused by mutations in either of two genes, *PKD1* or *PKD2*, is the 4th leading cause of end-stage renal disease (ESRD) in adults, affecting approximately 12.5 million people worldwide.[1] ADPKD is characterized by the development of epithelial-lined cysts in the kidney, liver, and pancreas and the presence of connective tissue abnormalities. At the cellular level, changes in cell polarity and structural variation to the extracellular matrix are also seen.[2] The protein products of *PKD1* and *PKD2* are transmembrane proteins called polycystin 1 (PC1) and polycystin 2 (PC2), respectively.[3] PC1 is a glycoprotein and is found in the luminal membrane of renal tubular cells, at apical junctions and in the primary cilium. PC1 has a large extracytoplasmic domain and a short cytoplasmic tail. The N-terminal region is located at the extracytoplasmic domain and the C-terminal region is at the cytoplasmic tail. When proteins bind to PC1, it is usually at the cytoplasmic tail. PC1 interacts with PC2 through the C-terminal region. This interaction forms a $Ca^{2+}$-permeable mechanosensitive ion channel. PC1 has been hypothesized to have receptor type function on primary cilia. For a long time, PC1 has also been thought to have a sensory function; however, recent studies contradict that notion.[4] PC1 was found to play a role in $Ca^{2+}$ signaling when it was shown that cells lacking PC1 had little or no $Ca^{2+}$ influx to their cytoplasm after the application of shear stress to the cells' cilia. However, the mechanism of $Ca^{2+}$ influx and the function of PC1 has come into question in recent studies.[4,5] A full understanding of the function of polycystins, how they are controlled, and the roles of PC1 and PC2 has yet to be reached.[6] The exact cause of cyst formation in ADPKD is also not yet known. Primary cilia play an important role in the pathogenesis of ADPKD; however, not in the way originally thought.[4] Further, investigations of the various signaling pathways associated with primary cilia will most likely uncover the

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ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-SE-1-102

specific mechanism for cystogenesis.

### Role of Primary Cilia in Cystogenesis

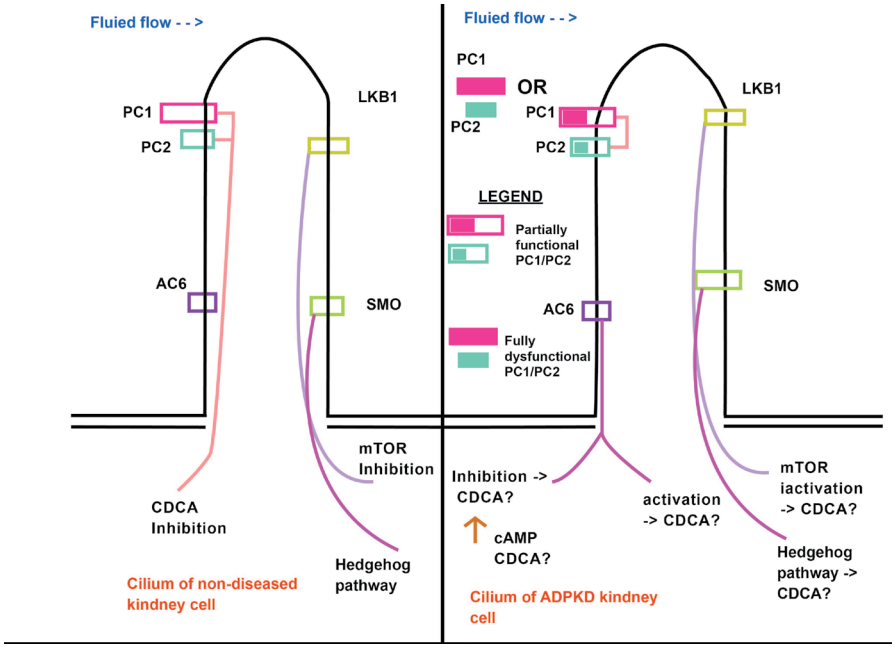
The cilium is connected to the apical membrane of an epithelial cell *via* the basal body and the transition zone which functionally separate the cilia compartment and its overlying membrane from the rest of the cell body. Structural and functional abnormalities in the primary cilia of kidney epithelial cells have been shown to regulate cystogenesis in several studies.[3] Genetic studies of various animal disease models resulted in cyst formation and fibrosis in different organs under three separate conditions: when there was a complete loss of cilia, when the cilia structure was altered, or when the protein composition of a cilia's membranes was changed.[3] It should be noted that in *PKD1* and *PKD2* mutations in kidney and liver cells, the removal of cilia in mouse models slowed down the growth of cysts in both organs in both the early and adult-inactivation models.[3] Polycystin mutants and cilia-polystin double mutants all showed an increased proliferation of cysts in ADPKD models.[3]

### Mechanosensor Hypothesis for Polycystins in Cilia

The mechanosensor hypothesis introduced over a decade ago suggested that the fluid flow outside the cell is recognized by primary cilia and causes a chemical signal.[1,5] These studies described that PC1 would turn the mechanical sensory ciliary signal into a chemical one by binding to PC2 and causing a release of calcium into the cilia *via* the opening of PC2's calcium channels.[5] The bending of primary cilia in studies measuring calcium infux was usually achieved by using a perfusion chamber and increasing the rate of the perfusate, or by suction using a micropipette.[1]

The mechanosensory properties of primary cilia in kidney cells have recently come into question along with PC1's function as a flow sensor.[5-7] In a recent study, measurements of calcium influx in cilia of cultured kidney epithelial cells and thick ascending tubules were taken after application of mechanical pressure to primary cilia.[3] Primary cilia extracted from cells of kidney tubules and embryonic node were bent using a custom-made jet flow system.[5] *Via* a micropipette, flow of liquid was generated at velocities that imitated both physiological and supraphysiological ones.[5] There was no calcium influx in cilia observed in the allowed time frame of 10 μs to 100 ms, when the mechanosensitive channels open.[4] Calcium increase in the cilia was seen approximately 10-20 seconds after stimulation.[4] It was concluded that the cell body was responsible for the calcium rise initially.[4] The calcium would then end up in the cilium after diffusing from the cytoplasm.[4] Past studies could have wrongly accounted the time for calcium influx into the cilia since calcium could diffuse in less than 200 ms from the cytoplasm to the cilia.[4] Also, motion and light-path dependent artifacts could have accounted for the error in previous studies.[4] Also, mutations in PC1 and the use of antibodies against PC2 did not result in flow-induced calcium signaling and influx of calcium, respectively.[5] It is suggested that if mechanosensory abilities for cilia exist, they could induce mediators and not initiate cell calcium signaling.[3] The importance of intact cilia along with the presence of fully functional polycystins to stop initiation of cysts in ADPKD points to a probable unknown signal occurring within cilia that is termed cilia-dependent cyst activation (CDCA) (Figure 1).[3]

**Figure 1**: Possible Signaling Mechanisms Involved in Cilia-Dependent Cyst Activation.



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ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-SE-1-102

### Cilia-Dependent Cyst Activation

CDCA is hypothesized to control kidney tubule lumen diameter and the shape of the lining of the tubule epithelial cells.[3] The complexity of the CDCA may be compared to the signal cascade of the mammalian Hedgehog signaling pathway.[3] Of note, cilia play a role in the Hedgehog signaling pathway.

When polycystins fail to bind to cilia, ADPKD can result from the poor control of CDCA.[3] However, it is important to note that the disturbance of cilia in diseased patients can stop progression even with the lack of polycystins.[3] CDCA is inhibited by the function of the PC1-PC2 complex, therefore, mutation of either or both results in the dysregulation of CDCA.[3,7,8] Previous studies have proposed that PC1 is the rate limiting constituent of CDCA and the hypothesized activation of PC2 by PC1 can make mutants responsible for sending a poor signal to inhibit CDCA.[3,5,7,11]

### Signaling Pathways in Cilia that could Result in CDCA

Cilia participate in a number of signaling pathways and one or more of them could potentially be responsible for CDCA.[2] One example of a signaling pathway is the Hedgehog pathway. The transcriptional target genes of the Hedgehog pathway were elevated in one study in kidneys that are cystic along with in kidneys of the *PKD1* early inactivation model.[3,12] In another study, *PKD1* mutant mice had a low penetrance of a gut looping chirality defect, a process that results from the Hedgehog pathway.[3,13,14] The mechanism in how the Hedgehog pathway causes cyst formation is unknown.[3]

There has been an association observed between elevated cyclic adenosine monophosphate (cAMP) levels and ADPKD.[3,9,10] Vasopressin receptor 2 antagonist administration has been shown to slow down cyst growth in mouse models.[3,9,10] The ciliary A-kinase anchoring protein complex has been found to be abnormal in a study with *PKD2* mutants and the protein's components were enzymes of the cAMP family.[3] This complex may be involved in CDCA activation.[3] When adenylyl cyclase, a cAMP protein expressed by cilia, was naturally accompanied in the collecting duct by an inactivated *PKD1*, a reduction in the number of cysts occurred in a model of early-onset ADPKD.[3]

Liver kinase B1 (LKB1) is found within cilia and regulates cell size in reaction to flow while at the same time controlling mammalian target activity (mTOR).[3] Cell growth and proliferation are regulated by mTOR.[3] In mouse models, mTOR inhibition decreased the number of cysts; however, it is unlikely that mTOR signaling is involved with CDCA.[3]

The MAPK/EPK signaling pathway was limited to the distal nephron and to later stages of cyst formation in previous ADPKD models, and therefore is probably not a cause of CDCA.[3] The inactivation of β-1 integrin in the extracellular matrix slowed down cyst formation in a developmental model of ADPKD and in a matrix-gel model that had *PKD1* knockdown cells, and therefore could play a role in CDCA activation.[3]

## CONCLUSION

Primary cilia have an important function in the pathogenesis of ADPKD as seen in animal models from previous experiments.[3] Cilia-dependent cyst activation results in ADPKD, however, the specific signaling pathway that initiates the dysregulation of this process is unknown and needs to be further explored. Future studies should concentrate on the mechanism of how primary cilia specifically contribute to cystogenesis and perhaps whether the diffusion of calcium from the cell body to the cilia is related.[4]

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Praetorius HA. Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membrane Biol*. 2001: 184: 71-79. doi: 10.1007/s00232-001-0075-4

2. Yoder BK, Hou X, Guay-Woodford LM. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *J Am Soc Nephrol*. 2002; 13: 2508-2516. doi: 10.1097/01.ASN.0000029587.47950.25

3. Ma M, Gallagher A-R, Somlo S. Ciliary mechanisms of cyst formation in polycystic kidney disease. *Cold Spring Harb Perspect Biol*. 2017. doi: 10.1101/cshperspect.a028209

4. Delling M, Indzhykulian AA, Liu X, et al. Primary cilia are not calcium-responsive mechanosensors. *Nature*. 2016; 531: 656-660. doi: 10.1038/nature17426

5. Nauli SM, Alenghat FJ, Luo Y. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet*. 2003; 33: 129-137. doi: 10.1038/ng1076

6. Doerr N, Wang Y, Kipp KR, et al. Regulation of Polycystin-1 Function by Calmodulin Binding. *PLoS One*. 2016; 11(8): e0161525. doi: 10.1371/journal.pone.0161525

7. Fedeles SV, Tian X, Gallagher AR, et al. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. *Nat Genet*. 2011; 43: 639-647. doi: 10.1038/ng.860

8. Hopp K, Ward CJ, Hommerding CJ, et al. Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity. *J Clin Invest*. 2012; 122(11): 4257-4273. doi: 10.1172/JCI64313

9. Gattone VH 2nd, Wang X, Harris PC, Torres VE. Inhibition of

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ISSN 2380-0445 http://dx.doi.org/10.17140/NPOJ-SE-1-102

renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat Med*. 2003; 9: 1323-1326. doi: 10.1038/nm935

10. Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH 2nd. Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat Med*. 2004; 10: 363-364. doi: 10.1038/nm1004

11. Fedeles SV, Gallagher AR, Somlo S. Polycystin-1: A master regulator of intersecting cystic pathways. *Trends Mol Med*. 2014; 20: 251-260. doi: 10.1016/j.molmed.2014.01.004

12. Tran PV, Talbott GC, Turbe-Doan A, et al. Downregulating hedgehog signaling reduces renal cystogenic potential of mouse models. *J Am Soc Nephrol*. 2014; 25: 2201-2212. doi: 10.1681/ASN.2013070735

13. Zhang XM, Ramalho-Santos M, McMahon AP. Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R asymmetry by the mouse node. *Cell*. 2001; 105: 781-792. doi: 10.1016/S0092-8674(01)00385-3

14. Delling M, DeCaen PG, Doerner JF, Febvay S, Clapham DE. Primary cilia are specialized calcium signalling or- ganelles. *Nature*. 2013; 504: 311-314. doi: 10.1038/nature12833