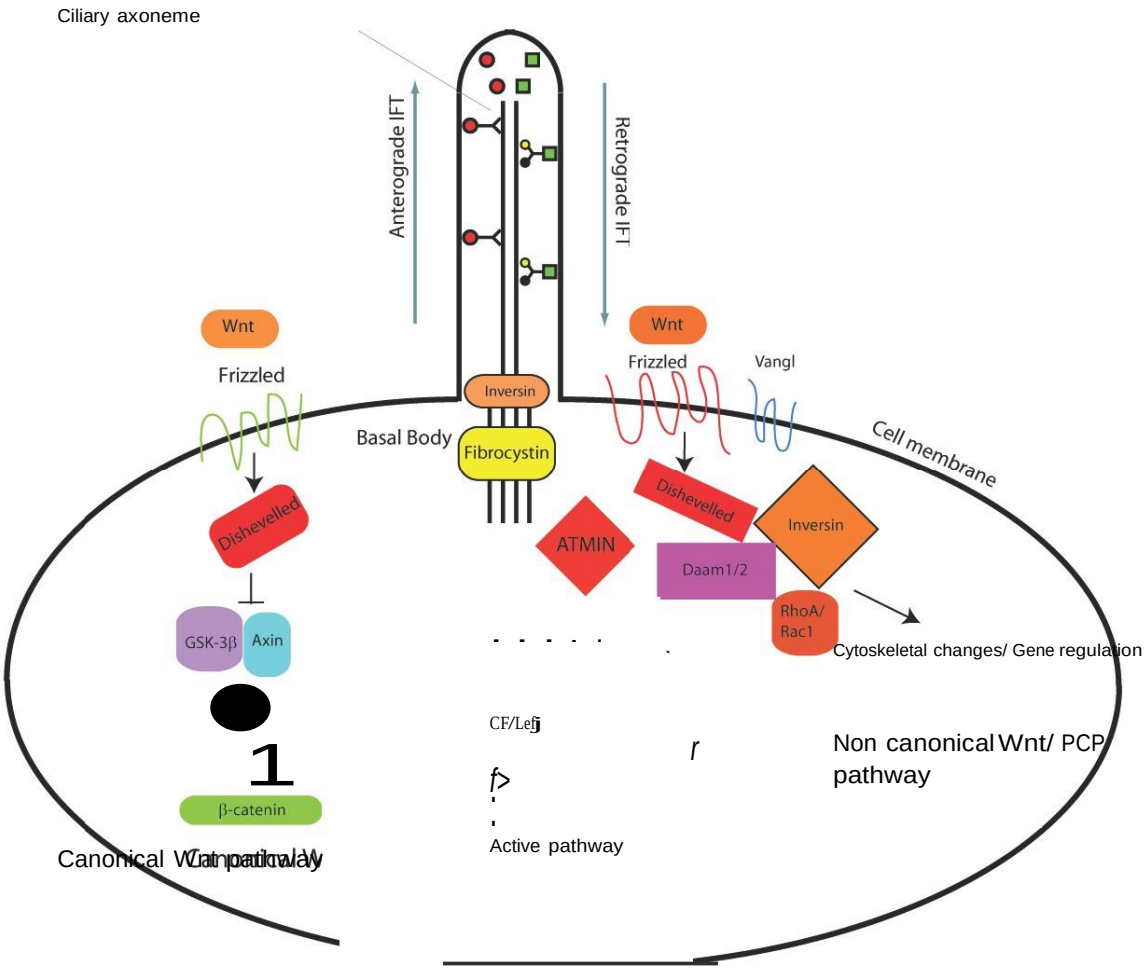
Ciliary axoneme
Anterograde IFT
Retrograde IFT
Wnt
Frizzled
Inversin
Basal Body
Fibrocystin
Wnt
Frizzled
Vangl
Cell membrane
Dishevelled
ATMIN
Dishevelled
Inversin
Daam1/2
RhoA/
Rac1
Cytoskeletal changes/ Gene regulation
GSK-3β
Axin
β-catenin
CF/Lef
Non canonical Wnt/ PCP
pathway
Active pathway

Novel biomarkers in kidney disease: ~~the~~ role~~s~~ ~~o~~for cilia~~, and~~ Wnt signalling and ATMIN in polycystic kidney disease

Paraskevi Goggolidou[1,2*], Patricia D. Wilson[2]

1. Faculty of Science, Engineering and Computing, Kingston University, Kingston upon Thames, KT1 2EE, UK.
2. Centre for Nephrology, University College London~~Medical School~~, Royal Free Campus, ~~London,~~ NW3 2PF, UK.

*Corresponding author

Running title: Cilia, Wnt signalling and ATMIN in the kidney

Key words: Wnt Signalling, Polycystic Kidney Disease, Cilia, ATMIN

**Abstract**

Biomarkers, the measurable indicators of biological conditions, are fast becoming a popular approach in providing information to track disease processes that could lead to novel therapeutic interventions for chronic conditions. Inherited, chronic kidney disease affects millions of people worldwide and although pharmacological treatments exist for some conditions, there are still patients whose only option is kidney dialysis and kidney transplantation. In the past ten years, certain chronic kidney diseases have been re- classified as ciliopathies. Cilia in the kidney are antenna-like, sensory organelles that are required for signal transduction. One of the signalling pathways that requires the primary cilium in the kidney is Wnt signalling and it has three components: canonical Wnt, non- canonical Wnt/Planar Cell Polarity (PCP) and non-canonical Wnt/$Ca^{+2}$ signalling. Identification of the novel role of ATMIN as an effector molecule in the non-canonical Wnt/PCP pathway has intrigued us to investigate its potential role in chronic kidney disease. ATMIN could thus be an important biomarker in disease prognosis and treatment that might lighten the burden of chronic kidney disease and also impact on its progression.

The cilium is an evolutionarily-conserved, microtubule-based organelle, the existence of which was discovered more than 200 years ago. Although it contains similarities with the prokaryotic flagellum, its role and functional importance was unclear until recently. It is now known that the eukaryotic cilium is an apical, cellular protrusion that forms in almost all cell types and can be motile or immotile [1]. Although not in the scope of this mini-review, it should be noted that cilia form in the G0 or G1 phases of the cell cycle and that since protein synthesis does not take place in the cilium, its formation and resorption depends on anterograde and retrograde motor proteins and intraflagellar transport (IFT) proteins. When IFT trafficking is disrupted, defective ciliogenesis or deficient cilia function ensues [2].

In the kidney in particular, only immotile, primary cilia have been discovered. They are found on the surfaces of non-proliferating cells and their length varies according to the stage of kidney development. In the renal vesicles, primary cilia are 0.59 μm long and further lengthen to 0.81 μm in S-shaped nephrons, finally extending to over 3 μm in human fetal and post-natal~~mature~~ nephrons [3]. Many proteins important for correct kidney development and function have been shown to localise to the primary cilium or to require the primary cilium in order to be able to exert their role. Further, loss of cilia, malformed cilia or mutations in IFT genes have been demonstrated to cause proliferative defects, affect fluid secretion and result in cystic renal disease [4,5]. Signalling pathways, such as the Sonic Hedgehog (Shh) and Platelet Derived Growth Factor (PDGF) have been shown to require the cilium for timely regulation and efficient activation [6] and in the kidney, the primary cilium plays an important role in Wnt signalling responses.

**The Wnt signalling pathway**

Wnts are glycoproteins and more than 19 of them exist in mammals [7]. Wnt signalling has three constituents: canonical Wnt, non-canonical Wnt/Planar Cell polarity (PCP) and non- canonical Wnt/$Ca^{+2}$ signalling. The canonical Wnt signalling pathway initiates when a canonical Wnt ligand binds to a Frizzled (Fz) receptor in the presence of lipoprotein receptor-

related protein 5 or 6 (LRP5 or 6). The outcome of the binding is Dishevelled (Dvl) activation and inhibition of the β-catenin destruction complex (Glycogen synthase kinase 3β (GSK3β), adenomatous polyposis coli (APC), Axin and casein kinase 1 (CK1)). Activated canonical Wnt signalling results in β-catenin accumulation in the cytosol and the transcriptional activation of *Wnt* target genes. When the pathway is inactive, β catenin is targeted through phosphorylation for proteosomal degradation (Figure 1).

In the past ten years, detailed studies have been conducted on the characterisation of proteins involved in the non-canonical Wnt/ PCP pathway. This pathway is also activated when a Wnt ligand behinds to a Fz receptor, but in this case, activation of Dvl leads to downstream cytoskeletal rearrangements or transcriptional activation through the stimulation of *RhoA* or *Rac1.* Core PCP proteins such as Van Gogh-like (Vangl), Prickle and Celserus (Celsr) are important for non-canonical Wnt/PCP pathway signal transduction [8]. It should also be noted that there is a third component to Wnt signalling, the non-canonical Wnt/$Ca^{+2}$ signalling that upon Dvl stimulation results in increased intracellular calcium concentration and activated calcineurin, protein kinase C and $Ca^{+2}$/calmodulin-dependent protein kinase II
**(**CAMKII) and thisat has formed the basis for other reviews [9].

Although few Wnt ligands have been shown to exclusively activate the canonical or the non- canonical/PCP pathway, most Wnt ligands have been shown to be able to trigger both pathways and it is still unclear how timely regulation of the two pathways is achieved. Significantly, the interaction of inversin with Dvl is key for the activation of the non-canonical Wnt/PCP pathway in the kidney [10] and the primary cilium has also been demonstrated to be required for the timely regulation of Wnt signalling [11]. It is proposed that an active cilium acts as a repressor of the canonical Wnt pathway, permitting signalling from the non- canonical Wnt pathway and thus acting as a switch between the two [12], although the exact mechanism of this switch has not been determined.

**Canonical and non-canonical Wnt signalling: its role in kidney disease**

A number of PCP proteins have been shown to play a role in kidney development and function. Vangl2 is important for normal morphogenesis of the ureteric bud and metanephric mesenchyme-derived structures [13]; it localizes to the base of the cilium in kidney cells [14]. Loss of Fat4 leads to cystic kidney disease by disrupting oriented cell division and tubule elongation in kidney development [15]. Further, targeted mutations in Fz4 and Fz8 cause maller kidneys and reduced ureteric bud growth [16], while increased Fz3 expression was observed in post-natal polycystic kidney disease (Pkd)1 inactivated cystic kidneys [17]. Wnt ligands such as Wnt9b and Wnt11 also have important, recognised roles in kidney development and function [3]. Furthermore, frizzled-related-protein (FRP) 4 is upregulated in human Autosomal Dominant Polycystic Kidney Disease (ADPKD) and in ADPKD mouse models [18]. Increased serum FRP4 levels were recently detected in ADPKD patients [19], while DKK3, a β-catenin antagonist, is a potential modifier of ADPKD [20]. Further evidence linking cilia to Wnt signalling in the kidney comes from inversin, loss of function of which causes nephronophthisis. Inversin targets Dvl1 for degradation, inhibiting canonical Wnt signalling and regulates convergent extension in *Xenopus* [12]. It acts downstream of Fz8 in pronephros morphogenesis [10], localises to the primary cilium and is required for Dvl recruitment to the plasma membrane after Fz activation, instigating PCP signalling [21].

Although defective Wnt signalling has been implicated in a number of diseases, including cancer, osteoporosis and Alzheimer's disease [7] as well as ADPKD, researchers have mostly concentrated on the canonical Wnt signalling pathway. Recently, decreased β- catenin phosphorylation and increased β-catenin expression was demonstrated in aquaporin-1-null PKD mice, implicating aquaporin-1 in renal cyst development and ADPKD by modulation of canonical Wnt signalling [22]. A very interesting study has just been published that demonstrates that Wnts bind to the extracellular domain of PKD1, resulting in the induction of $Ca^{+2}$ influx dependent on PKD2 and exhibiting the involvement of non-canonical $Wnt/Ca^{+2}$ signalling in ADPKD [23].

**ATMIN, a protein with diverse functions**

We have recently identified the novel role of a PCP effector protein, ATM INteractor (ATMIN) in kidney development and we are intrigued by its potential involvement in chronic kidney disease. ATMIN was initially discovered as a DNA damage response protein, interacting with the ATM protein upon replicative and hypotonic stress [24, 25] and is also essential for RAD51 localisation after DNA methylation damage [26]. Oxidative damage accumulated in the ageing brain of ATMIN-null mice [27] and developing B-cell survival was regulated by ATMIN through Dynein light chain LC8-type 1 (DYNLL1) expression activation and Bim- dependent apoptosis [28]. After the discovery that ATMIN carried an ATM-dependent function, work from our group and others showed that ATMIN was also a transcription factor with ATM-independent roles. In particular, our own published data has identified the novel role of Atmin in ciliogenesis and lung morphogenesis. Generation of the *Atmin*$^{gpg6/gpg6}$ mutant mouse resulted in embryonic lethality at mid-gestation, accompanied by gross oedema, exencephaly, micrognathia and severe pulmonary hypoplasia [29]. The *Atmin*$^{gpg6/gpg6}$ mouse mutant has got elements of a ciliopathy and close examination of the embryonic node revealed defects in cilia number and length that also extended to the neural tube and limb bud. These defects were caused by the transcriptional regulation of Atmin on a retrograde IFT protein, Dynll1 and identified a novel series of interactions between Dynll1, Wdr34, a mammalian homologue of the Chlamydomonas cytoplasmic dynein 2 intermediate chain and Atmin. Further work on the *Atmin*$^{gpg6/gpg6}$ mouse revealed abnormal embryonic kidney morphogenesis, associated with defective Wnt signalling [30]. *Atmin*$^{gpg6/gpg6}$ kidneys manifested reduced numbers of renal vesicles and ureteric bud tips, abnormal cytoskeleton and mis-oriented cell division, although cilia length, numbers and Hh signalling were unaffected in the mouse embryonic kidney. Additional analysis showed reduced *Wnt9b*, *Wnt11* and *Dvl1* mRNA expression and increased *Daam2* expression. Intercrosses between the *Atmin*$^{gpg6/+}$ and *Vangl2*$^{lp/+}$ mice revealed a genetic interaction between Atmin and Vangl2, identifying Atmin as a novel effector molecule in the non-canonical Wnt/PCP pathway. It has

been challenging to determine whether ATMIN directly interacts with Wnt signalling or if its effects are mediated through the primary cilium, but future investigations should be able to shed further light on this dilemma.

Subsequently, ATMIN was shown to also play a role in B-cell lymphoma, T-cell activation and neuroblastoma. In the context of lymphomagenesis, ATMIN was shown to be critical for MYC-driven lymphoma development that originated from bone marrow B-cell precursors [31]. The role of ATMIN in MYC-driven lymphomagenesis was demonstrated to be driven through its downstream transcriptional target, DYNLL1 and both genes were shown to be critical for pre-leukemic immature B-cell survival in *E*μ-*Myc* mice before progression to malignant lymphoma. Intriguingly, loss of ATMIN, together with another ATM co-factor, NBS1 caused increased DNA damage and peripheral T-cell hyperactivation that led to severe intestinal inflammation, colitis and premature death in the ATMIN-deficient, NBS1- deficient mice [32]. Significantly, it was recently shown that the increased Pdgfra expression observed in Trp53-null mice was normalised when the mice were crossed with Atmin deficient mice, suggesting that ATMIN inhibitors might have therapeutic roles in Glioblastoma multiforme [33]. Although the authors suggested that this was due to ATMIN- dependent ATM signalling, loss of ATM did not restore Pdgfra expression as significantly, hinting at the ATM-independent functions of ATMIN. Pdgfra has been linked to many human tumours, such as gliomas and gastrointestinal stromal tumours and it is important to note that Pdgfra localises to and signals through the primary cilium [34]. As we have shown that loss of Atmin disrupts ciliogenesis [29], it is possible that this defect is also relevant to abnormalities in neural cilia formation that could lead to reduced Pdgfra signalling.

**Understanding Autosomal Recessive Polycystic Kidney Disease**

The ATM-dependent and ATM-independent roles of ATMIN create very exciting opportunities in the context of modifying ATMIN levels as a therapeutic approach in human

disease. We focus our work on understanding the molecular mechanisms of Autosomal Recessive Polycystic Kidney Disease (ARPKD), a rare paediatric disease for which no pharmacological treatment currently exists. ARPKD is a genetic disorder affecting ~1:20,000 and is a common cause of perinatal death [4]. It manifests as extreme bilateral enlargement of cystic kidneys *in utero*, associated with hepatic ductal plate abnormalities and pulmonary hypoplasia [35,36]. In those patients who survive the perinatal period, the majority will require renal replacement therapy (kidney dialysis or transplantation) within the first decade of life [37]. Recently, however, ARPKD patients have been diagnosed in their 30s with relatively mild renal insufficiency, suggesting a previously unrecognised, wide spectrum of disease severity [38].

ARPKD is caused by mutations in polycystic kidney and hepatic disease 1 (*PKHD1),* which encodes the large membrane protein, fibrocystin that is required for normal branching morphogenesis of the ureteric bud during embryonic renal development [39-41]. In ARPKD kidneys, cystic dilation is restricted to the ureteric bud-derived collecting tubules (CTs) associated with increased epithelial cell proliferation and luminal fluid secretion [42], together with abnormalities in apoptosis [43,44], epithelial cell polarity [45] and matrix interactions [46]. Fibrocystin is localised in primary cilia protruding from the apical surface of CT lumens [47,48], together with Polycystins (PC-)1 and -2 [49], the protein products of the ADPKD- associated genes *PKD1* and *PKD-2* [35]. PC-1 is thought to act as a mechanosensory membrane receptor forming multi-protein complexes with PC-2 [50], a calcium-permeable channel protein [51], β-catenin [52] and α2β1-integrin [53]. Since fibrocystin can interact directly with PC-2/1 at cell and ciliary membranes [49], it might act as part of this important mechanosensory pathway to control normal renal development. It should be noted, however, that although the majority of evidence suggest that cilium bending is an important mechanism for sensing mechanical force, recent studies have questioned this as an absolute [54].

Loss of fibrocystin is also associated with focal adhesion abnormalities, such that loss of focal adhesion kinase (FAK) activation as well as increased FAK inhibition and c-Src activation are observed in fibrocystin deficient cells [46]. Loss of a component of the canonical Wnt signalling pathway, GSK3β inhibited cAMP generation and cellular proliferation of collecting duct cells in an ARPKD mouse model, delaying disease progression [55]. A binding site for the hepatocyte nuclear factor (HNF)-1β is found on the *Pkhd1* promoter and it drives *Pkhd1* expression in the kidney, intrahepatic bile ducts and the male reproductive tract [56]. In addition, Arf4, a small G protein of a subfamily of Ras-related small G proteins, binds to the ciliary targeting sequence of fibrocystin and is required for its effective trafficking to the cilia [57].

It thus becomes apparent that evidence of a *PKHD1* transcriptional network and of fibrocystin protein interactions is fast accumulating, although the direct interacting partners remain largely unknown. Our published work links ATMIN to kidney development and places it as a novel effector molecule in non-canonical Wnt/PCP signalling [30]. Understanding kidney development will help us address kidney disease and PKD remains one of the chronic, heritable kidney conditions that greatly impacts on the population and the healthcare systems worldwide. Although Wnt signalling has been investigated in ADPKD, no work has yet been published on the impact of Wnt signalling in ARPKD. We are thus interested in investigating the role of PCP proteins in ARPKD and finding out whether they can provide insights into ARPKD prognosis and treatment. As no pharmacological treatment currently exists for ARPKD, such discoveries will greatly impact on the young patients and their families and will provide alternatives to kidney dialysis and transplantation.

Figure 1. Schematic representation of the canonical and non-canonical Wnt/Planar Cell Polarity (PCP) signalling pathways. Activated canonical Wnt signalling in the cell nucleus (dashed lines) results in transcriptional activation of downstream target genes. Key canonical and non-canonical Wnt proteins are represented and the localisation of fibrocystin and

inversin in the cilium is highlighted. ~~Dashed lines represent suggested interactions between~~ ATMIN is shown as a red diamond and its suggested interaction with ~~and~~ Fibrocystin~~, which~~ could involve either the primary cilium or non-canonical Wnt/PCP. Anterograde (red) and retrograde (green) intraflagellar transport along the cilium is facilitated by the kinesin and dynein motor proteins respectively.

**Funding**

PG's work was funded by the PKD charity UK.

**References**

1. Hildebrandt, F., Benzing, T. and Katsanis, N. (2011) Ciliopathies. *N. Engl. J. Med.* 364(16), 1533-1543
2. Rosenbaum, J.L. and Witman, G.B. (2002) Intraflagellar transport. *Nat. Rev. Mol. Cell. Biol.* 3(11), 813-825
3. Goggolidou, P. (2014) Wnt and planar cell polarity signaling in cystic renal disease. *Organogenesis* 10(1), 86-95
4. Harris, P.C. and Torres, V.E. (2009) Polycystic kidney disease. *Annu. Rev. Med.* 60, 321-337
5. Jonassen, J.A., SanAgustin, J., Baker, S.P. and Pazour, G.J. (2012) Disruption of IFT complex A causes cystic kidneys without mitotic spindle misorientation. *J. Am. Soc. Nephrol.* 23(4), 641-651
6. Eggenschwiler, J.T. and Anderson, K.V. (2007) Cilia and developmental signalling. *Annu. Rev. Cell. Dev. Biol.* 23, 345-373
7. Kahn, M. (2014) Can we safely target the WNT pathway? *Nat. Rev. Drug. Discov.* 13(7), 513-532
8. Gao, B. (2012) Wnt regulation of planar cell polarity (PCP). *Curr. Top. Dev. Biol.* 101, 263-295
9. Li, C., Bellusci, S., Borok, Z. and Minoo, P. (2015) Non-canonical WNT signalling in the lung. *J. Biochem.* 158(5), 355-365
10. Lienkamp, S., Ganner, A., Boehlke, C., Schmidt, T., Arnold, S.J., Schafer, T., Romaker, D., Schuler, J., Hoff, S., Powelske, C., Eifler, A., Kronig, C., Bullerkotte, A., Nitschke, R., Kuehn, E.W., Kim, E., Burkhardt, H., Brox, T., Ronneberger, O., Gloy,
J. and Walz, G. (2010) Inversin relays Frizzled-8 signals to promote proximal pronephros development. *Proc. Natl. Acad. Sci. USA.* 107(47), 20388-20393
11. Wallingford, J.B. and Mitchell, B. (2011) Strange as it may seem: the many links between Wnt signalling, planar cell polarity, and cilia. *Genes Dev.* 25(3), 201-213
12. Simons, M., Gloy, J., Ganner, A., Bullerkotte, A., Bashkurov, M., Kronig, C., Schermer, B., Benzing, T., Cabello, O.A., Jenny, A., Mlodzik, M., Polok, B., Driever, W., Obara, T. and Walz, G. (2005) Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signalling pathways. *Nat. Genet.* 37(5), 537-543
13. Yates, L.L., Papakrivopoulou, J., Long, D.A., Goggolidou, P., Connolly, J.O., Woolf, A.S. and Dean, C.H. (2010) The planar cell polarity gene Vangl2 is required for

mammalian kidney-branching morphogenesis and glomerular maturation. *Hum. Mol. Genet*. 19(23), 4663-4676
14. Ross, A.J., May-Simera, H., Eichers, E.R., Kai, M., Hill, J., Jagger, D.J., Leitch, C.C., Chapple, J.P., Munro, P.M., Fisher,S., Tan, P.L., Phillips, H.M., Leroux, M.R., Henderson, D.J., Murdoch, J.N., Copp, A.J., Eliot, M.M., Lupski, J.R., Kemp, D.T., Dollfus, H., Tada, M., Katsanis, N., Forge, A. and Beales, P.L. (2005) Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. *Nat. Genet*. 37, 1135–1140
15. Saburi, S., Hester, I., Fischer, E., Pontoglio, M., Eremina, V., Gessler, M., Quaggin, S.E., Harrison, R., Mount, R. and McNeill, H. (2008) Loss of Fat4 disrupts PCP signalling and oriented cell division and leads to cystic kidney disease. *Nat. Genet.* 40(8), 1010-1015
16. Ye, X., Wang, Y., Rattner, A. and Nathans, J. (2011) Genetic mosaic analysis reveals a major role for frizzled 4 and frizzled 8 in controlling ureteric bud growth in the developing kidney. *Development.* 138(6), 1161-1172
17. Luyten, A., Su, X., Gondela, S., Chen, Y., Rompani, S., Takakura, A. and Zhou, J. (2010) Aberrant regulation of planar cell polarity in polycystic kidney disease. *J. Am. Soc. Nephrol.* 21(9), 1521-1532
18. Romaker, D., Puetz, M., Teschner, S., Donauer, J., Geyer, M., Gerke, P., Rumberger, B., Dworniczak, B., Pennekamp, P., Buchholz, B., Neumann, H.P., Kumar, R., Gloy, J., Eckardt, K.U. and Walz, G. *et al.* (2009) Increased expression of secreted Frizzled-related protein 4 in polycystic kidneys. *J. Am. Soc. Nephrol.* 20, 48-56
19. Zschiedrich, S., Budde, K., Nurnberger, J., Wanner, C., Sommerer, C., Kunzendorf, U., Banas, B., Hoerl, W.H., Obermuller, N., Arns, W., Pavenstadt, H., Gaedeke, J., Lindner, T.H., Faerber, L., Wimmer, P., Stork, R., Eckardt, K.U. and Walz, G. (2016) Secreted frizzled-related protein 4 predicts progression of autosomal dominant polycystic kidney disease. *Nephrol. Dial. Transplant.* 31(2), 284-289
20. Liu, M., Shi, S., Senthilnathan, S., Yu, J., Wu, E., Bergmann, C., Zerres, K., Bogdanova, N., Coto, E., Deltas, C., Pierides, A., Demetriou, K., Devuyst, O., Gitomer, B., Laakso, M., Lamnissou, K., Magistroni, R., Parfrey, P., Breuning, M., Peters, D.J., Torra,R., Winearls, C.G., Torres, V.E., Harris, P.C., Paterson, A.D. and Pei, Y. (2010) Genetic variation of DKK3 may modify renal disease severity in ADPKD. *J. Am. Soc. Nephrol.* 21, 1510-1520
21. Lienkamp, S., Ganner, A. and Walz, G. (2012) Inversin, Wnt signalling and primary cilia. *Differentiation.* 83(2), S49-S55
22. Wang, W., Li, F., Sun, Y., Lei, L., Zhou, H., Lei, T., Xia, Y., Verkman, A.S. and Yang, B. (2015) Aquaporin-1 retards renal cyst development in polycystic kidney disease by inhibition of Wnt signalling. *FASEB J.* 29(4), 1551-1563
23. Kim, S., Nie, H., Nesin, V., Tran, U., Outeda, P., Bai, C.X., Keeling, J., Maskey, D., Watnick, T., Wessely, O. and Tsiokas, L. (2016) The polycystin complex mediates Wnt/Ca2+ signalling. *Nat. Cell. Biol.* doi:10.1038/ncb3363
24. Kanu, N. and Behrens, A. (2007) ATMIN defines an NBS1-independent pathway of ATM signalling. EMBO J. 26(12), 2933-2941
25. Schmidt, L., Wiedner, M., Velimezi, G., Prochazkova, J., Owusu, M., Bauer, S. and Loizou, J.I. (2014) ATMIN is required for the ATM-mediated signalling and recruitment of 53BP1 to DNA damage sites upon replication stress. DNA Repair (Amst). 24, 122-130
26. McNees, C.J., Conlan, L.A., Tenis, N. and Heierhorst, J. (2005) ASCIZ regulates lesion-specific Rad51 locus formation and apoptosis after methylating DNA damage. EMBO J. 24(13), 2447-2457.

27. Kanu, N., Penicud, K., Hristova, M., Wong, B., Irvine, E., Plattner, F., Raivich, G. and Behrens, A. (2010) The ATM cofactor ATMIN protects against oxidative stress and accumulation of DNA damage in the ageing brain. J Biol Chem. 285(49), 38534-
38542.
28. Jurado, S., Conlan, L.A., Baker, E.K., Ng, J.L., Tenis, N., Hoch, N.C., Gleeson, K., Smeets, M., Izon, D. and Heirhorst, J. (2012) ATM substrate Chk2-ineracting Zn2+ finger (ASCIZ) is a bi-functional transcriptional activator and feedback sensor in the regulation of the dynein light chain (DYNLL1) expression. J Biol Chem. 287(5), 3156-
3164.
29. Goggolidou, P., Stevens, J.L., Agueci, F., Keynton, J., Wheway, G., Grimes, D.T., Patel, S.H., Hilton, H., Morthorst, S.K., DiPaolo, A., Williams, D.J., Sanderson, J., Khoronenkova, S.V., Powles-Glover, N., Ermakov, A., Esapa, C.T., Romero, R., Dianov, G.L., Briscoe, J., Johnson, C.A., Pedersen, L.B. and Norris, D.P. (2014) ATMIN is a transcriptional regulator of both lung morphogenesis and ciliogenesis. *Development.* 141(20), 3966-3977
30. Goggolidou, P., Hadjirin, N.F., Bak, A., Papakrivipoulou, E., Hilton, H., Norris, D.P. and Dean, C.H. (2014) Atmin mediates kidney morphogenesis by modulating Wnt signalling. *Hum. Mol. Genet.* 23(20), 5303-5316.
31. Wong, D.M., Li, L., Jurado, S., King, A., Bamford, R., Wall, M., Walia, M.K., Kelly, G.L., Walkley, C.R., Tarlinton, D.M., Strasser, A. and Heierhorst, J. (2016) The transcription factor ASCIZ and its target DYNLL1 are essential for the development and expansion of MYC-driven B cell lymphoma. Cell Rep. 14(6), 1488-1499
32. Prochazkova, J., Sakaguchi, S., Owusu, M., Mazouzi, A., Wiedner, M., Velimezi, G., Moder, M., Turchinovich, G., Hladik, A., Gurnhofer, E., Hayday, A., Behrens, A., Knapp, S., Kenner, L., Ellmeier, W. and Loizou, J.I. (2015) DNA repair cofactors ATMIN and NBS1 are required to suppress T cell activation. *PLoS Genet.*11(11), e1005645
33. Blake, S.M., Stricker, S.H., Halavach, H., Poetsch, A.R, Cresswell, G., Kelly, G., Kanu, N., Marino, S., Lunscombe, N.M., Pollard, S.M. and Behrens, A. (2016) Inactivation of the ATMIN/ATM pathway protects against glioblastoma formation. Elife. 5, e08711
34. Michaud, E.J. and Yoder, B.K. (2006) The primary cilium in cell signalling and cancer. Cancer Res. 66(13), 6463-6467
35. Wilson, P.D and Goilav, B. (2007) Cystic disease of the kidney. *Annu. Rev. Pathol*. 2, 341-368
36. Wilson, P.D. (2008) Mouse models of polycystic kidney disease. *Curr. Top. Dev. Biol*. 84, 311-350
37. Bacallao, R.L. and McNeill, H. (2009) Cystic kidney diseases and planar cell polarity signaling. *Clin. Genet.* 75, 107-117
38. Rossetti, S. and Harris, P.C. (2007) Genotype-phenotype correlations in autosomal dominant and autosomal recessive polycystic kidney disease. *J. Am. Soc. Nephrol*. 18(5), 1374-1380
39. Onuchic, L.F., Furu, L., Nagasawa, Y., Hou, X., Eggermann, T., Ren, Z., Bergmann, C., Senderek, J., Equivel, E., Zeltner, R., Rudnik-Schoneborn, S., Mrug, M., Sweeney, W., Avner, E.D., Zerres, K., Guay-Woodford, L.M., Somlo, S. and Germino, G.G. (2002) PKHD1, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin- transcription factor domains and parallel beta-helix 1 repeats. *Am. J. Hum. Genet.* 70, 1305-1317
40. Ward, C.J., Hogan, M.C., Rossetti, S., Walker, D., Sneddon, T., Wang, X., Kubly, V., Cunningham, J.M., Bacallao, R., Ishibashi, M., Milliner, D.S., Torres, V.E. and Harris,

P.C. (2002) The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat. Genet*. 30, 259-269
41. Mai, W., Chen, D., Ding, T., Kim, I., Park, S., Cho, S.Y., Chu, J.S., Liang, D., Wang, N., Wu, D., Li, S., Zhao, P., Zent, R. and Wu, G. (2005) Inhibition of Pkhd1 impairs tubulomorphogenesis of cultured IMCD3 cells. *Mol. Biol. Cell*. 16, 4398-4409
42. Wilson, P.D. and Falkenstein, D. (1995) The pathology of human renal cystic disease. *Curr. Top. Pathol*. 88, 1-50
43. Hu, B., He, X., Li, A., Qiu, Q., Li, C., Liang, D., Zhao, P., Ma, J., Coffey, R.J., Zhan, Q. and Wu, G. (2011) Cystogenesis in ARPKD results from increased apoptosis in collecting duct epithelial cells of Pkhd1 mutant kidneys. *Exp. Cell. Res*. 317(2), 173-187
44. Goilav, B. (2011) Apoptosis in polycystic kidney disease. *Biochim. Biophys. Acta*. 1812(10), 1272-1280
45. Ryan, S., Verghese, S., Cianciola, N.L., Cotton, C.U. and Carlin, C.R. (2010) Autosomal recessive polycystic kidney disease epithelial cell model reveals multiple basolateral epidermal growth factor receptor sorting pathways. *Mol. Cell. Biol*. 21(15), 2732-2745
46. Israeli, S., Amsler, K., Zheleznova, N. and Wilson, P.D. (2010) Abnormalities in focal adhesion complex formation, regulation, and function in human autosomal recessive polycystic kidney disease epithelial cells. *Am. J. Physiol. Cell. Physiol.* 298, C831- C846
47. Wang, S., Luo, Y., Wilson, P.D., Witman, G.B. and Zhou, J. (2004) The autosomal recessive polycystic kidney disease protein is localised to primary cilia, with concentration in the basal body area. *J. Am. Soc. Nephrol*. 15, 592-602
48. Zhang, M.Z., Mai, W., Li, C., Cho, S.Y., Hao, C., Moeckel, G., Zhao, R., Kim, I., Wang, J., Xiong, H., Wang, H., Sato, Y., Wu, Y., Nakanuma, Y., Lilova, M., Pei, Y., Harris, R.C., Li, S., Coffey, R.J., Sun, L., Wu, D., Chen, X.Z. and Breyer, M.D. (2004) PKHD1 protein encoded by the gene for autosomal recessive polycystic kidney disease associates with basal bodies and primary cilia in renal epithelial cells. *Proc. Natl. Acad. Sci. USA*. 101 (8), 2311-2316
49. Wang, S., Zhang, J., Nauli, S.M., Li, X., Starremans, P.G., Luo, Y., Roberts, K.A. and Zhou, J. (2007) Fibrocystin/polyductin, found in the same protein complex with polycystin-2, regulates calcium responses in kidney epithelia. *Mol. Cell. Biol*. 27(8), 3241-3252
50. Nauli, S.M., Alenghat, F.J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A.E., Lu, W., Brown, E.M., Quinn, S.J., Ingber, D.E. and Zhou, J. (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet*. 33(2), 129-137
51. Vassilev, P.M., Guo, L., Chen, X.Z., Segal, Y., Peng, J.B., Basora, N., Babakhanlou, H., Cruger, G., Kanazirska, M., Ye, Cp., Brown, E.M., Hediger, M.A. and Zhou, J. (2001) Polycystin-2 is a novel cation channel implicated in defective intracellular Ca(2+) homeostasis in polycystic kidney disease. *Biochem. Biophys. Res. Commun*. 282(1), 341-50
52. Kim, E., Arnould, T., Sellin, L.K., Benzing, T., Fan, M.J., Gruning, W., Sokol, S.Y., Drummond, I. and Walz, G. (1999) The polycystic kidney disease 1 gene product modulates Wnt signalling. *J. Biol. Chem*. 274(8), 4947-4953
53. Wilson, P.D., Geng, L. and Burrow, C.R. (1999) The PKD1 gene product, "polycystin-1," is a tyrosine phosphorylated protein that colocalizes with alpha2/beta1-integrin in focal clusters in adherent renal epithelia. *Lab Invest.* 79(10), 1311–1323

~~53.5~~4. Delling, M., Indzhykulian, A.A., Liu, X., Li, Y., Xie, T., Corey, D.P. and Clapham, D.E. (2016) Primary cilia are not calcium-responsive mechanosensors. *Nature* 531(7596), 656-660

~~54.5~~5. Tao, S., Kakade, V.R., Woodgett, J.R., Pandey, P., Suderman, E.D., Rajagopal, M. and Rao, R. (2015) Glycogen synthase kinase-3b promotes cyst expansion in polycystic kidney disease. *Kidney Int.* 87(6), 1164-1175

~~55.5~~6. Williams, S.S., Cobo-Stark, P., Hajarnis, S., Aboudehen, K., Shao, X., Richardson, J.A., Patel, V. and Igarashi, P. (2014) Tissue-specific regulation of the mouse Pkhd1 (ARPKD) gene promoter. *Am. J. Physiol. Renal Physiol.* 307(3), F356-368

~~56.5~~7. Follit, J.A., San Agustin, J.T., Jonassen, J.A., Huang, T., Rivera-Perez, J.A., Tremblay, K.D. and Pazour, G.J. (2014) Arf4 is required for mammalian development but dispensable for ciliary assembly. *PLoS Genet.* 10(2), e1004170