Primary cilia and renal cysts: does length matter?

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It is now nearly 400 years since Antonie van Leeuwenhoek noticed the first motile organisms moving by flagella using the earliest microscopes. He would have been surprised to note the fascination that nephrologists have developed with this tiny but versatile organelle over the past 15 years. Flagella appear to be the evolutionarily conserved precursors of both motile and non-motile (or primary) cilia in higher organisms including mammals [1, 2]. One was the recognition that a swimming defective mutant of the biflagellate algae *Chlamydomonas reinhardtii* was orthologous to an insertional mouse mutant, Tg737 or Oak Ridge Polycystic Kidney (orpk), which provided the critical clue as to the biological importance of cilia [3]. *Orpk/Tg737* mice developed polycystic kidneys but also other systemic features now known to be associated with cilia defects such as heterotaxia and retinal and neural tube defects [4]. The *orpk* mutation was mapped to a gene (*Tg737*) which encodes for the protein polaris, which in turn is orthologous to the *Chlamydomonas* agellate transport protein 88 (ift88), identified as essential for cilia assembly (as reviewed in [5]). Intraflagellar transport (IFT) was first observed in *Chlamydomonas* and shown to be essential for flagellar assembly and maintenance [6]. It is comprised of two multimeric protein complexes, IFT protein complex (‘B’) involved in anterograde cilia transport and a second IFT complex (‘A’) responsible for retrograde transport: IFT88 is a member of Complex B. Mutations in mammalian IFT proteins are now known to be associated with structural defects in cilia and human disease. The phenotype of these ciliary diseases or ‘ciliopathies’ is pleiotropic reflecting the ubiquitous expression of primary cilia by different cell types throughout the body [7].

The localization of the ADPKD proteins, polycystin-1 (PC1) and polycystin-2 (PC2), to primary cilia was the next important clue in directly linking cilia to renal cysts [8]. Unlike IFT mutants, cilia length is normal in *PKD1* and *PKD2* mutant cells and organisms implying a defect in function rather than structure. Credence was given to this hypothesis by the observation that *Pkd1* and *Pkd2* mutants in the nematode *Caenorhabditis elegans* have defects in male mating behaviour, a process known to be dependent on mechanosensation or chemosensation mediated by a small number of ciliated neuronal cells; these mutants expressed structurally normal but functionally defective cilia [9]. The demonstration that the mammalian PC1 and PC2 proteins in kidney epithelium are essential for transducing a cilia-based mechanosensory Ca2+ signal confirmed that polycystin-mediated cilia function was also likely to apply to epithelial cell behaviour [10]. An important difference between PC1 and PC2, however, is the essential requirement for PC2 in embryonic nodal cilia signalling, a function not required for PC1 [11].

We now know that the vast majority of mutated proteins linked to renal cystic diseases can be localized to the primary cilium or its associated structures such as the basal body, centrosomes or ciliary transition zone [12]. There is no doubt that cilia structure, function and stability are all essential for normal kidney development and maintenance. However, it is uncertain that an exclusive ‘cilia hypothesis’ can explain the highly variable renal phenotypic spectrum seen in different diseases, which range from dysplasia to degeneration or fibrosis to cysts [13]. The relative importance of cilia function during organ development as opposed to tissue maintenance in the mature organ has also been debated [14, 15].

In this issue, Mergen et al. report a new mechanism by which cilia disassembly could be regulated by the NPHP2/Inv protein, inversin. Previous work had shown that inversin can act as a flow-activated molecular switch between the canonical and non-canonical Wnt signalling pathways by binding to the common effector protein, Dishevelled (Dvl1), targeting it to the proteasome for degradation [16]. This process can be antagonized by the NPHP8 protein, RPGRIP1L, which forms a complex with both inversin and the NPHP4 protein [17]. In the absence of inversin, canonical Wnt signalling is activated with a concomitant failure to activate non-canonical Wnt activity. The consequences of this would be an increase in cell proliferation with an associated loss of planar cell polarity, likely mechanisms leading to cyst formation. In this latest study, they show that inversin has another function, i.e. it can bind and inhibit the kinase, Aurora A (AurA), which is known to play a pivotal role in regulating cilia stability [18]. AurA
phosphorylates and activates the histone deacetylase HDAC6, which in turn deacetylates α-tubulin, resulting in cilia disassembly [18]. The presence of an intact cilium is characteristic of non-dividing cells, and cilia disassembly is a key event in triggering cell cycle re-entry (as reviewed in [19]). NPHP2 mutants were predicted to have an increased rate of cilia disassembly due to loss of AurA inhibition, possibly leading to shorter cilia. Indeed, using an in vitro lentiviral-mediated inducible shRNA knockdown system, the authors demonstrate that knockdown of NPHP2 can result in shorter cilia in MDCK cells, and that this phenotype could be partially rescued by chemical inhibitors of AurA or HDAC6. In support of their observation, they also show that the NPHP1 protein, nephrocystin-1, has a similar effect, suggesting that this may be a more common mechanism operative in other forms of nephronophthisis.

This study reports a previously unrecognized role for inversin in regulating the cilia length. However, certain caveats should be borne in mind. The authors present no in vivo evidence to support their findings. Previous studies of cilia length in NPHP2/Inv mice have reported a normal cilia length although these studies were limited to post-natal tissues [20]; it is possible that shorter cilia might be detected during embryonic development. Second, inversin has been localized to the proximal ciliary shaft in quiescent cells, in association with the NPHP3 and NPHP9 proteins, in the so-called ‘inv’ compartment, whereas AurA localizes prominently to the basal bodies during the interphase [21]. It is possible that there is a small subpopulation of inversin molecules at the basal body where it could exert its inhibitory effect on AurA although the authors were unable to demonstrate this by using available antibodies. Other groups have shown that inversin can be localized to mitotic spindles and lateral cell–cell junctions in subconfluent cells, supporting the possibility that it could shuttle between subcellular compartments during different phases of the cell cycle [22, 23]. Similarly, AurA relocates to the spindle poles during mitosis and could play other roles during interphase such as regulating PC2-dependent Ca2+ ER release and PC2-regulated G1/S transition [24, 25]. Live cell imaging of these two molecules could shed further light into their dynamic interactions during both ciliary assembly and disassembly.

Finally, it is apparent that rather than shorten, renal tubular cilia can paradoxically lengthen in several hypomorphic mouse mutants (Nphp3<sup>bry</sup>/<sup>bry</sup>, Nphp9<sup>jk1/jk1, Pkd1<sup>R3277C</sup></sup>) [26–28]. Intriguingly, this has also been observed following acute tubular necrosis in the human kidney [29]. The functional consequences of cilia elongation as opposed to cilia shortening are less clear. Following injury, it is likely that this represents a compensatory organ repair mechanism (to permit cellular proliferation and repair) or alternatively, a transient loss of fidelity of cilia length control (e.g. due to inflammation). In this regard, it is worth noting that Pkd1 and Pkd2 heterozygous mice are more sensitive to acute ischaemia-reperfusion renal injury than their wild-type littermates [30, 31]. If better understood, this could be a regulatory system that could be exploited for restoring the cilia length in disease. Clearly, we still have much to learn about this tiny but fascinating organelle.

### CONFLICT OF INTEREST STATEMENT

None declared


### REFERENCES

The patient perspective and physician’s role in making decisions on instituting dialysis

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Shared decision making describes an approach to medical decision making that lies between paternalism and autonomy [1]. This model of decision making, described recently as the ‘pinnacle of patient-centered care’ [2] is quickly becoming the preferred approach to medical decisions, where there is no one ‘best’ treatment option. In these circumstances, involvement of patients in decision making helps us to ensure that treatment decisions reflect the patient’s preferences and values.