What's new in...

Ciliopathies

Albert CM Ong
Marie-Claire Gubler

Primary cilia are hair-like organelles expressed by almost every cell in the body. Although they were first recognized at the end of the 19th century, their functions remained obscure until the last decade. It is increasingly recognized that disorders of cilia structure or function underlie a number of rare human genetic diseases that affect the kidney and other organs, the so-called 'ciliopathies'. Study of these cilia proteins is giving us new insights into how primary cilia function in normal human physiology and development and is shedding light on the pathogenesis of more common diseases, such as obesity, hypertension and organ fibrosis.

Keywords ADPKD; ARPKD; Bardet-Biedl syndrome; centrosomes; cilia; ciliopathies; cysts; Meckel-Gruber syndrome; nephronopthisis; polycystic kidney disease

Primary cilia – basic functions and link to human disease

What are primary cilia?

Unlike motile cilia, primary cilia are non-motile, though they are sensitive to bending or flow. Evolutionarily, they are structurally related to (motile) flagella expressed by single-cell organisms such as the green algae, *Chlamydomonas*. Remarkably, proteins involved in the formation and maintenance of flagella structure, a microtubule-dependent process called intraflagellar transport (IFT) are also expressed in mammalian cilia.

Cilia and human disease

The link between cilia and human disease came from several coincidental findings.[1] A group studying a *Chlamydomonas* IFT gene, IFT88, associated with flagella defects discovered that it was homologous to Tg737, the gene mutated in *orpk*, a mouse mutant with polycystic kidney disease (PKD). Tg737 mice were found to express shortened cilia associated with other extrarenal features, such as *situs inversus* and retinal degeneration. Secondly, researchers studying male mating behaviour in nematodes (*C. elegans*) discovered that mutants of the worm pkd1 homologue (Lov-1) had functional defects in the mechano-sensory neuronal response required for male mating. Cilia expressed by the subset of neurons responsible for this behaviour were structurally normal but functionally defective. Thirdly, mammalian kidney cells defective for the ADPKD proteins, polycystin-1 or polycystin-2, had defects in flow-activated calcium currents. These findings clearly linked ciliary structure and function to kidney morphogenesis and to polycystin function.

Cilia, centrosomes, cysts and the cell cycle

Since these seminal discoveries, many more proteins associated with a cystic kidney phenotype have been localized to primary cilia and their associated structures, the centrosomes (Figure 1). This has led to the formulation of the 'ciliary hypothesis' as a unifying pathogenic mechanism for cyst formation.[2,3] A link to cell cycle control is also implied by the fact that the mother centriole enucleates the primary cilium (present in non-dividing cells) and that both centrioles also form the mitotic spindle poles (in dividing cells). These cilia-associated diseases have been termed 'ciliopathies'.

Nodal cilia and asymmetry generation

The embryonic node is a transient structure found during gastrulation that is critical for the determination of body-axis asymmetry through the action of nodal cilia (primary cilia that are motile). A further link between cilia function and disease came from the finding that Pkd2 null mice developed defects in left–right body axis leading to *situs inversus*.[4] Polycystin-2, the Pkd2 protein, was clearly localized to a subset of nodal cilia, and associated with the flow-activated symmetry-breaking Ca^{2+} signal. *Situs inversus* has been described in some though not all ciliopathies (Table 1).

Kidney cilia and planar cell polarity

Orientated cell division (OCD) and planar cell polarity (PCP) are two processes that are important in maintaining tubular

Albert CM Ong *DM MA FRCP is Professor of Renal Medicine and Head of the Academic Nephrology Unit at the University of Sheffield Medical School, Sheffield, UK. Competing interests: none declared.*

Marie Claire Gubler *MD is Director of Research Emeritus, Hôpital Necker Enfants Malades, Université Paris Descartes, Paris, France. Competing interests: none declared.*

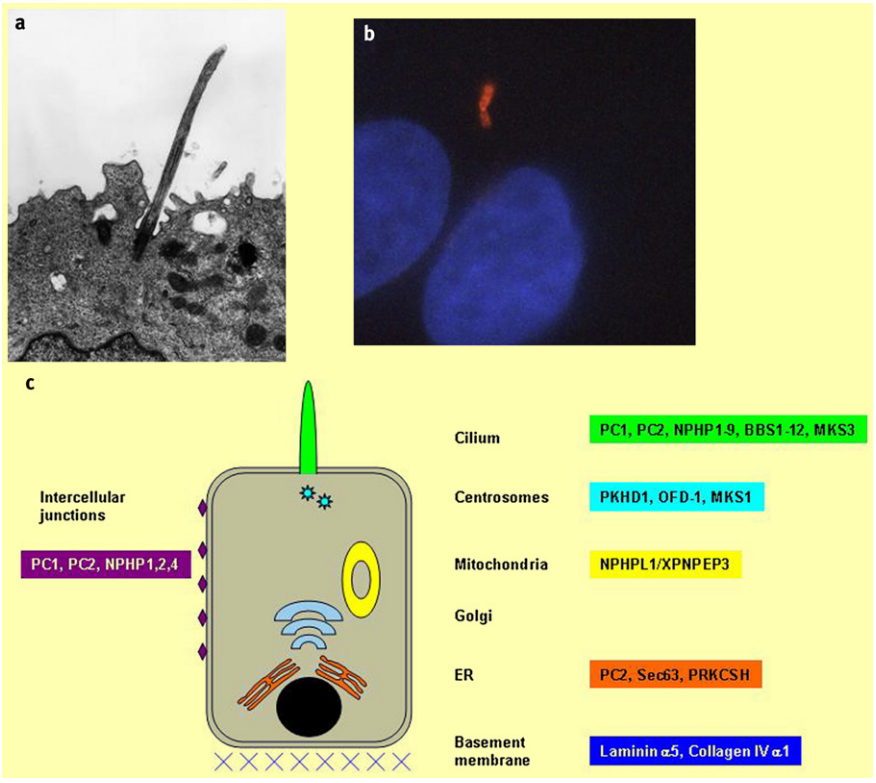

Figure 1 Primary cilia and the localization of major cystic proteins in epithelial cells. **a** Electron micrograph of an apical cilium in a normal human kidney tubule. **b** Cilia expression (red) detected by Immunofluorescence staining in cultured tubular cells; nuclear (blue) counter stain. **c** Cystic proteins and their subcellular localization in epithelial cells.

diameter during tubular elongation.[5] Defects in both have been reported in some murine PKD models though the primary role of OCD in cyst initiation has been challenged.[6] The fidelity of OCD is clearly dependent on accurate positioning of the mitotic spindle in relation to the longitudinal axis of the tubule but how this is regulated by cilia signalling is unknown. Experimental evidence linking cilia (flow)-regulated Wnt signalling to PCP has been shown.[7]

Extrarenal cilia and other phenotypes

The pathogenesis of liver and pancreatic cysts is thought to be similar to that of kidney cysts. Endothelial cells express primary cilia in some vascular beds and these have been shown to act as flow sensors (via the polycystin proteins) for nitric oxide synthesis.[8] This may be the cellular basis for the prevalence of hypertension in ADPKD and other ciliopathies. The connecting cilium is a modified primary cilium that transports rhodopsin from the inner to the outer retinal segment. A striking feature of some ciliopathies is the presence of retinitis pigmentosa. Digital abnormalities such as polydactyly (related to Hedgehog signalling) are present in some of the ciliopathies.[3] Other rare phenotypes include CNS malformations, anosmia, obesity and hypogonadism (Table 1).

Ciliary signalling and function

Although primary cilia are non-motile, bending or flow can activate Ca^{2+} transients *in vitro*.[9] The initial extracellular Ca^{2+} spike leads to Ca^{2+} release from internal stores, resulting in signal amplification and activation of Ca^{2+}-sensitive signalling cascades. Other potential pathways currently implicated in cilia signalling are Wnt, Stat6/AP6, Akt and Hedgehog, though these are likely to be tissue- and function-specific. Linking different signalling pathways to specific functions is still work in progress.

Cystic genes and ciliary proteins – is it all in the cilia?

The strongest evidence linking cilia and centrosome function to human disease is a cystic kidney or PKD phenotype. Nonetheless, much of the evidence is based on the immuno-localization of these proteins to primary cilia rather than function. Two observations should be borne in mind. First, the subcellular localization of some cystic proteins may not be exclusive to primary cilia (Figure 1c). For instance, polycystin-1 clearly localizes to the basolateral membrane in kidney epithelial cells and mediates cell–cell adhesion.[10] On the other hand, polycystin-2 is predominantly localized to the ER.[11] Similarly, the nephronophthisis proteins (e.g. NPHP1, 2 and 4) show localization to cell–cell junctions and may mediate junction integrity.[12] Secondly, mutations in genes that clearly do not encode ciliary proteins can also lead to cystic disease. Examples of these include the ER-located proteins (Sec63 and PRKCSH) that are implicated in autosomal dominant polycystic liver disease,[13,14] the basement membrane-located proteins (Laminin α5 and collagen IV α1)[15,16] and the mitochondrial protein (XPNPEP3) that causes nephronophthisis.[17]

Phenotypic variability in the ciliopathies

Genotype–phenotype correlations and additional mutations in other ciliary genes could modify the renal and extrarenal disease phenotype (see later). In addition, certain ciliary genes may have other ciliary-unrelated functions, modify specific subsets of cilia or only alter cilia substructure.[18] All these features may contribute to the diversity of ciliopathy phenotypes.

Clinical features and pathological features

Ciliopathies encompass a large group of diseases heterogeneous at the clinical, pathological, and genetic levels. Renal involvement, present in most of them, may be the prominent feature, as in polycystic kidney diseases, or may be part of a complex syndromic disease (Table 1).

Polycystic kidney diseases

Clinical features

Polycystic kidney diseases (PKD) are characterized by the large size of the kidneys owing to the presence of renal cysts.

In the frequent **autosomal dominant polycystic kidney disease (ADPKD)** (prevalence 1/400–1/1000) accounting for 8–10% of the cases of end-stage renal disease (ESRD) worldwide, two genes,