HHS Public Access

Author manuscript

Cell Signal. Author manuscript; available in PMC 2020 December 23.

Published in final edited form as:

Cell Signal. ; : 109519. doi:10.1016/j.cellsig.2019.109519.

Cilia in Cystic Kidney and Other Diseases

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Abstract

Epithelial cells lining the ducts and tubules of the kidney nephron and collecting duct have a single non-motile cilium projecting from their surface into the lumen of the tubule. These organelles were long considered vestigial remnants left as a result of evolution from a ciliated ancestor, but we now recognize them as critical sensory antennae. In the kidney, the polycystins and fibrocystin, products of the major human polycystic kidney disease genes, localize to this organelle. The polycystins and fibrocystin, through an unknown mechanism, monitor the diameter of the kidney tubules and regulate the proliferation and differentiation of the cells lining the tubule. When the polycystins, fibrocystin or cilia themselves are defective, the cell perceives this as a pro-proliferative signal, which leads to tubule dilation and cystic disease. In addition to critical roles in preventing cyst formation in the kidney, cilia are also important in cystic and fibrotic diseases of the liver and pancreas, and ciliary defects lead to a variety of developmental abnormalities that cause structural birth defects in most organs.

Keywords

intraflagellar transport; polycystic kidney disease; kidney; cilia

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CRediT statements

Gregory Pazour: Writing - Original Draft, Writing - Review & Editing. **Lynne Quarmby:** Writing - Original Draft, Writing - Review & Editing. **Paurav Desai:** Visualization, Writing - Review & Editing. **Abigail Smith:** Visualization, Writing - Review & Editing. **Miriam Schmidts:** Writing - Original Draft, Writing - Review & Editing.

Conflict of interest

The authors have no conflicts of interest.

Introduction to Cilia

The cilium is central to cystic kidney disease. Products of many of the human disease genes localize to cilia and defects in a large number of ciliary proteins cause the disease. Historically, the recognized functions of cilia in human biology were limited to lung health, male fertility and preventing *situs inversus*. A convergence of studies in model organisms with human genetics has produced a new view of the cilium as a key player in vertebrate development and disease. It is now recognized that ciliary dysfunction underlies a wide range of human disease. These “ciliopathies” encompass a diversity of symptoms that reflect the many roles of cilia in motility and sensory perception [1]. Proteomic analysis suggests that cilia consist of 500 to 1000 or more distinct proteins [2]. From model organism research and human genetics, we know that mutations in many of these result in ciliary dysfunction that can cause death or disease. Ciliary dysfunction underlies cystic diseases of kidney, liver and pancreas, retinal degeneration or blindness, olfaction defects, skeletal abnormalities including cranial facial defects, polydactyly, and rib cage abnormalities, along with brain malformations and hydrocephaly. In addition, disturbances of the left-right axis have been expanded beyond the relatively benign *situs inversus*, to include structural birth defects in the heart, lungs and abdominal organs. While this review will focus on basic biology of cilia and their involvement in cystic disease of the kidney (Figure 1), extensive reviews on the role of cilia in many organ systems and development are available including skeleton [3], brain and neural tube [4], vision [5] and heart [6].

With only a few exceptions, cells in vertebrate organisms assemble cilia at some point in development. In some cell types, the cilia are only present during development and regress once development is complete. In other cell types, including the tubular epithelium of the kidney, cilia remain through the life of the individual. Cilia can be either motile or non-motile. Motile cilia have both motility and sensory functions while non-motile cilia have only sensory functions. The types of sensory inputs that can be detected by cilia include light detection by the rod and cone outer segments of the retina, odorants by the olfactory cilia, and fluid flow by kidney cilia along with diverse hormonal and chemical signals by cilia on neuronal and other cells throughout the body. Typically, motile cilia use the energy of ATP to move cells through the environment or to move fluid over the surface of cells. In mammals, defects in the sperm flagellum (cilium) can impair sperm motility and cause male infertility. In tissues such as lung, brain, and the oviduct, epithelial cells have multiple cilia that use a coordinated beat to move mucus with trapped debris, cerebral spinal fluid or oocytes. Ciliary motility defects in the respiratory epithelium typically cause chronic lung disease due to insufficient mucociliary clearance. In mice, defects in the cilia on the ependymal cells of the brain cause a high incidence of hydrocephaly, and motility defects appear to be a risk factor for hydrocephaly in humans [7]. Both the motility and sensory functions of the mono-cilia (or primary cilia) on the node of mammalian embryos are critical for establishment of the left-right axis during development. These cilia are unusual in that most primary cilia are non-motile and serve only as sensory organelles.

The sensory functions of cilia rely on the localization of specific receptors in the ciliary membrane and the organization of signal transduction cascades around the cilium and the centrosome. The number of receptors known to localize to the ciliary membrane continues to

grow, revealing a variety of signal transduction pathways modulated by the cilium. In the kidney, the polycystins and fibrocystin localize to cilia but the ligands and pathways in which they participate in are not fully understood. During development, cilia have been proposed to participate in PDGF, Wnt, planar cell polarity, G-protein coupled receptor (GPCR), hippo and hedgehog signaling. In PDGF signaling, the PDGFαα receptor localizes to cilia and regulates the Mek1/2-Erk1/2 pathway in growth arrested fibroblasts [8]. Cilia are implicated in Wnt signaling because ciliary dysfunction causes upregulation of canonical Wnt signaling [9–13]. However, this is controversial as Ocbina *et al.* did not find evidence for altered Wnt signaling in a cilium mutant [14]. In addition, polycystin-1 can bind Wnt ligands and has been proposed to be a Wnt receptor [15] but the mechanism of ciliary control of the Wnt pathway has not been established. Cilia also play a role in planar cell polarity. Polycystins are required for oriented cell division in the kidney and the homologues of numerous *Drosophila* planar cell polarity proteins are required for ciliogenesis in vertebrates [16, 17]. The participation of cilia in GPCR signaling is evidenced by the large number of seven-transmembrane receptors found in cilia. These include the somatostatin receptor 3, the 5HT6 serotonin receptor, and three isoforms of the dopamine receptor along with a variety of other receptors [18]. The hippo pathway is regulated by the nephronophthisis cystic kidney disease gene products NPHP4 [19] and NEK8/NPHP9 [20, 21]. Additionally, knockout of the hippo pathway component TAZ results in multicystic kidneys with embryonic onset [22]. Hedgehog signaling is the best understood of the pathways that are organized around the cilium.

Hedgehog signaling involves the dynamic movements of receptors, cofactors and transcription factors into and out of cilia. In the basal, non-stimulated state, patched1, a twelve transmembrane protein resides in the ciliary membrane and represses the pathway. Upon activation, patched-1 exits the cilium allowing the seven-transmembrane receptor smoothened to enter the cilium [23, 24] along with the Gli transcription factors. These transcription factors are thought to be processed in the ciliary compartment and then directed to the nucleus to control gene expression [25]. The hedgehog pathway is critical for mammalian development and many of the structural birth defects that are caused by ciliary dysfunction can be traced to defective hedgehog signaling. Hedgehog signaling plays critical roles in the development of the kidney and urinary tract with mutations in hedgehog genes leading to renal aplasia, hydronephrosis, hydroureter, and duplex kidneys among other phenotypes [26, 27]. The loss of hedgehog components does not lead to cystic disease [26–28] however, inhibiting the hedgehog pathway reduced cyst growth driven by the loss of an IFT-A gene [29]. Mutations in Glis2, which is structurally related to the Gli transcription factors of the hedgehog pathway cause nephronophthisis. Curiously, the Glis2 transcription factor localizes to cilia [30] but whether it participates in a hedgehog-like pathway remains to be determined.

Left-right patterning is another critical developmental pathway that is controlled by cilia and cystic kidney disease genes. The early vertebrate embryo is left right symmetrical and this symmetry is broken by action at the node. The node contains two populations of ciliated cells. The cilia on the cells in the center of the node have a rotary-like motility. The direction of rotation is controlled by the chirality of the basal body and the cilia are tipped towards the posterior end of the embryo. This results in net left ward flow of fluid across the node. This

flow is detected by non-motile cilia on the edge of the node. Polycystin-2 and polycystin-1L1 defects lead to disturbances of left-right patterning indicating that they are involved in detecting or transmitting the signal generated by nodal flow [31, 32]. The signal transmitted by nodal flow is controversial but it activates the nodal signal transduction cascade to cause differential expression of genes on the left and right sides of the embryos [33]. These gene expression cascades influence cell type determination, proliferation, migration and apoptosis to generate the proper placement and development of the internal organs. *Situs inversus* is a complete reversal of the left-right axis and causes little detriment to the individual. In contrast, the heterotaxies, where incomplete axis reversal occurs, can cause severe developmental defects and death. The heterotaxies include right and left isomerisms, midline placement of organs and more subtle alterations in left-right patterning. This can lead to blood vessel and organ anomalies including congenital heart malformations. Mouse embryos lacking cilia have severe structural heart defects and die at mid-gestation (embryonic day 10.5), shortly after the initiation of heart beat [34]. It is likely that heart defects are major contributors to death, but placental or other defects have not been ruled out. In human development, heartbeat is initiated at approximately embryonic day 22 suggesting that lack of cilia is likely to cause first trimester miscarriages. This is supported by the finding that to date, no human individuals completely lacking cilia have been identified. Mutations that affect ciliary motility, but do not prevent ciliary assembly, such as those found in Primary Ciliary Dyskinesia (PCD) also can cause heterotaxy. However, when only motility is affected, the heterotaxy is less severe and prenatal development is not greatly impaired. Less severe ciliary defects are likely contributors to the structural heart defects seen in the clinic. Cardiac malformations are found in as many as 1% of newborns [35] and evidence for ciliary dysfunction is prevalent in this population [36, 37]. Interestingly, several studies have found that patients with cardiac malformations often have kidney and urinary tract abnormalities [38]. In addition to heart defects, left right patterning defects can lead to structural lung malformations, asplenia or polysplenia along with liver lobation defects. In the kidney, left-right patterning does not play a role in organ development but places the kidneys along the cranialcaudal axis. In mice, the right kidney is located more cranially while the left is positioned more craniallyin humans and mutations that affect left-right patterning alter kidney placement.

Cilia Structure

Cilia consist of a microtubule-based cytoskeleton called the axoneme that is surrounded by an extension of the plasma membrane and associated cytoplasm or ciliary matrix. The axoneme serves as a scaffold to anchor and organize the proteins that carry out the sensory and motile functions of cilia. The typical axoneme consists of nine doublet microtubules arranged around the circumference of a cylinder (Figure 2). A pair of singlet microtubules is usually present in the center of the axoneme of motile cilia but absent from non-motile cilia. The doublet microtubules are templated from the distal end of the basal body or mother centriole, which is embedded within the centrosome. In addition to templating the microtubules of the axoneme, the centriole organizes the interphase microtubule network and the mitotic spindle. The centriole is composed of nine triplet microtubules, two of which are continuous with the doublets of the axoneme. In addition to multiple tubulin isoforms

that make up the triplets, more than thirty different proteins contribute to the internal structure of the centriole and form distal and subdistal appendages [39–41]. The distal appendages link the centriole to the membrane through structures known as the transition fibers. Defects in centriolar proteins can cause ciliopathy-like diseases (oral facial digital syndrome, hydrolethalus syndrome) and the phenotypically distinct disease, microcephaly. The ciliopathy-like syndromes are probably caused by an inability of the affected centriole to assemble cilia. *OFD1*, which is defective in oral facial digital syndrome patients, is required for the formation of the distal appendages [42], which are needed to anchor the cilium to the membrane and may provide docking sites for intraflagellar transport proteins [43]. Similarly, the hydrolethalus syndrome gene product *HYLS1* is a component of the centriole wall that may be specifically involved in anchoring the centriole to the membrane for ciliogenesis [44]. The centriolar proteins (STIL, CPAP, CEP152, CEP135 and CEP63) mutated in primary microcephaly are likely to affect non-ciliary functions of the centriole such as orientation of the mitotic spindle, which is critical for neurogenesis [4, 41]. Interestingly other microcephaly gene products associate with the centrosome that surrounds the centriole and are also likely to be involved in mitotic spindle orientation [45]. Like the centriole, the centrosome is important for organizing the interphase microtubule network and the mitotic spindle and it is rich in microtubule nucleation and anchoring proteins. The centrosome also is enriched for a large number of signaling components suggesting that it influences a variety of signaling pathways [46]. Centrosomal defects also contribute to ciliary dysfunction as RNAi knockdown of a number of centrosomal proteins reduced assembly of primary cilia [47].

The ciliary axoneme is surrounded by an extension of the plasma membrane of the cell. While the ciliary membrane is continuous with the cell membrane, it is a unique compartment and home to specific receptors. The base of the cilium contains a barrier that isolates the ciliary membrane from the rest of the plasma membrane. It appears that this barrier is largely achieved by the transition zone and an associated septin ring [48], but lipid composition may also contribute [49, 50]. The transition zone also serves to separate the ciliary cytoplasm or matrix from the cytoplasm in the cell body. The protein composition of the ciliary matrix is distinct from the cytoplasm in the cell body [51] but the function of matrix proteins has not been extensively studied. The matrix is important in signal transduction as the concentrations of second messengers like Ca^{2+} and cAMP can be controlled differentially in the cilium compared to the cell body and the small volume of the ciliary matrix means that small changes in messenger number can represent large changes in concentration [52, 53].

The ciliary transition zone is a domain at the base of the cilium between the basal body and the cilium proper. A distinct set of proteins are associated with the axoneme in this region including an extensive network that connects the axoneme to the ciliary membrane. The structure of the transition zone varies from species to species but typically contains a “Y” or champagne glass-shaped structure that connects the outer double microtubules to the membrane. At the membrane, the ends of the Y-shaped connectors are embedded in the bilayer and can be observed in freeze fracture electron microscopy as rows of transmembrane proteins known as the ciliary necklace [54]. Recently, the transition zone has become the focus of intense study as a large number of human ciliopathy gene products have

been found to localize to this structure. In particular, it is the gene products associated with nephronophthisis, retinal degeneration, Joubert and Meckel-Gruber syndromes that are enriched in the transition zone. Genetic, proteomic and high-resolution microscopy studies indicate that the transition zone is organized in interconnecting modules. One large module consists of Mks1, Tctn1, Tctn2, Tmem216, Tmem67, Cep290, B9d1, and Cc2d2 [55]. The proteins in this module may make up the Y-shaped structure as this linker is missing from *Chlamydomonas* Cep290 mutants [56]. Tctn2, Tmem216 and Tmem67 are transmembrane proteins and so could provide the connection to the membrane. Another module containing Nphp1/Nphp4/Nphp8(RpgriplL) was found at both the transition zone and at cell-cell contacts [57]. In *C. elegans*, the two modules may function redundantly because single mutations in components of either module do not block ciliogenesis, but when genes from both modules carry mutations, cilia are not built [58]. Mouse mutations in components of the transition zone have tissue-specific effects on ciliogenesis whereby cilia are missing from some cells but remain on others. The cilia that remain have reduced levels of ciliary membrane proteins suggesting that the barrier is less effective [55]. The renal phenotype observed in humans carrying biallelic mutations in MKS, BBS, Joubert or NPHP genes as well as syndromal IFT-gene mutations typically present with no or only small cysts and early progressive fibrosis rather than classical enlarged polycystic kidneys observed in IFT mouse models and humans with *PKD1*, *PKD2* or *PKHD1* mutations (Figure 3 A,B,C,K). End stage renal disease usually occurs in childhood or adolescence. Due to ethical concerns, renal biopsies are not usually performed on affected children making renal tissue for functional analyses difficult to obtain. The underlying and likely shared molecular mechanisms of pathology remain largely unknown.

Ciliary Assembly

Mammalian cilia, like most eukaryotic cilia, are assembled inside membrane projections from the surface of the cell. Because cilia have no ribosomes, all proteins needed to build the structure must be synthesized in the cell body and then carried into the cilium. Intraflagellar transport (IFT) is the driving force for this process. IFT involves the movement of large protein complexes termed IFT particles along the cilium [59]. The particles are composed of IFT-A, IFT-B and BBSome subcomplexes, which are organized from about 30 unique proteins [60, 61]. The IFT particles are not structural components of cilia but are cargo adaptors that allow the molecular motors to carry proteins into the cilium for assembly and maintenance of the organelle. The particles are transported from the cell body to the tip of the cilium by kinesin-2 motors [60] and returned back to the cell body by cytoplasmic dynein 2 [62].

Null alleles of IFT-B genes typically cause a complete loss of cilia, reflecting the fundamental and critical role of most IFT-B proteins in ciliary assembly. For example, null alleles of *Ift88* in *Chlamydomonas*, *C. elegans* and mouse completely fail to assemble cilia [63–65]. The mice carrying this null allele die at embryonic day 11.5 while hypomorphic animals survive to adulthood [64]. Similarly, mice carrying biallelic loss of function alleles for *Ift172* [66], *Ift80* [67], *Ift57* [68], *Ift54* [69], *Ift52* [70], *Ift46* [71], *Ift38* [72] and *Ift20* [12] die *in utero*. However, not all IFT-B genes are required for ciliary assembly. In *C. elegans*, Ift22 appears dispensable for ciliary assembly [73] and in mouse, Ift25 and Ift27 are

not needed for assembly of primary cilia [74, 75]. Mice carrying putative null mutations in *Ift25* or *Ift27* survive gestation but die soon after birth with lung, heart and brain defects. Interestingly, the loss of Ift25 or Ift27 cause hedgehog signaling defects much like other IFT-B mutations even though the cilia are structurally normal [74, 75]. Another IFT-B component Ift56 shows similar properties. The *Ttc26*^{hop} allele truncates 125 amino acids from the C-terminal end of Ift56. Mice carrying this allele mostly survive gestation but have polydactyly, hydrocephaly and male sterility. Similar to *Ift25* and *Ift27* mutants, ciliary assembly in the *Ift56*^{hop} mutants is not greatly affected, but hedgehog signaling is disrupted [76, 77].

Biallelic human mutations in genes encoding the IFT-B components IFT80, IFT172 and IFT27 have been identified in individuals diagnosed with Jeune asphyxiating thoracic dystrophy (JATD) / Mainzer-Saldino Syndrome (MZSDS) (OMIM #611263, #615630) and Bardet-Biedl Syndrome (BBS, OMIM #615996). *IFT172* mutations can cause JATD/MZSDS with mild thoracic dysplasia but with renal and retinal disease [78], isolated retinal degeneration [79] as well as BBS with renal and retinal involvement [80]. *IFT80* mutations have been identified in JATD cases with mild thoracic dysplasia without extraskeletal findings [67] as well as short rib polydactyly syndrome (SRPS) [81]. *IFT27* dysfunction causes BBS [82] as well as a SRPS-like ciliopathy form with renal agenesis and imperforate anus [83]. No human cases (postnatal or fetal) carrying two null alleles for IFT-B components have been identified to date, indicating this would be incompatible with development. Renal phenotypes described for IFT-B defects in human include nephronophthisis-like presentations with small or no cysts, increased renal fibrosis as well as renal agenesis in the one *IFT27* fetal case [83].

In contrast to IFT-B genes, mutations in IFT-A genes typically do not completely block ciliary assembly but instead produce bulbous cilia enriched in IFT proteins. This was originally observed in *C. elegans* [84] and is found in *Chlamydomonas* [85] and mice. In mice, null alleles of IFT-A genes including *Ift144* [86], *Ift140* [13], *Ift139* [87], *Ift122* [88, 89] and *Ift121* [90] affected ciliary structure and caused mid gestational or neonatal death.

The human phenotypes observed for hypomorphic IFT-A loss of function mutations resemble hypomorphic IFT-B alleles with mild thoracic dysplasia that is not usually lethal, along with childhood or adolescence-onset renal and retinal degeneration. Cranioectodermal Dysplasia (CED, OMIM #218330), JATD and SRPS share the phenotypes of short long bones and ribs, polydactyly in some cases (Figure 3D–G), as well as extraskeletal symptoms such as retinal involvement (Figure 3J), nephronophthisis (Figure 3C,K) and renal, liver and pancreatic cysts. JATD/MZSDS is considered the mild end of the SRPS spectrum and prenatal clinical differentiation of fetal phenotypes can be difficult. *IFT121*/*WDR35*, *IFT122*, *IFT144*/*WDR19* and *IFT43* mutations cause CED. CED patients share common features like mild thoracic dysplasia, nephronophthisis-like renal disease and retinal degeneration with JATD/MZSD, however patients have additional ectodermal features including coarse and slow growing hair, dysplastic finger and toe nails, sagittal craniosynostosis and a characteristic facial *gestalt* [91] (Figure 3G,H). While *IFT122*, *IFT43* and *IFT121/WDR35* most often cause CED, individuals with fetal SRPS have been reported [92–96]. Mutations in *IFT144* cause both CED and JATD/MZSD [97]. *IFT139B/TTC21B*

dysfunction can cause JATD as well as non-syndromic nephronophthisis [98] and a single fetal case of SRPS has been reported [96]. All known cases, including fetal ones, presenting with CED, JATD/MZSDS or SRPS always harbor at least one partial loss of function allele such as a missense mutation or a non-consensus splice variation. Where examined, fibroblasts derived from affected patients do not show significant changes in cilia length or frequency but do show accumulations of IFT proteins in cilia [94, 99, 100].

The IFT particles are transported from the cell body to the ciliary tip by kinesin-2. The *C elegans* and mammalian genomes encode heterotrimeric and homodimeric forms of this motor. The heterotrimeric form is composed of two motor subunits (Kif3a and Kif3b) and an accessory subunit (KAP or KIFAP3). In mouse, mutations in genes encoding the heterotrimeric motor subunits completely block ciliary assembly and cause mid-gestational lethality [101, 102] much like mutations in most IFT-B genes. The homodimeric kinesin is important to ciliary assembly in *C. elegans* [103], but the *Chlamydomonas* genome does not encode a homodimeric motor and mice with mutations in the homodimeric motor do not have ciliopathy phenotypes [104]. In humans, no kinesin-2 mutations have been identified. This likely reflects the critical role that kinesin-2 plays in ciliary assembly such that severe defects would cause first trimester lethality. In addition, kinesin-2 plays non-ciliary roles in neuronal transport and cell body vesicular transport, activities that may complicate phenotypes [105].

Cytoplasmic dynein 2 carries IFT particles from the ciliary tip to the cell body. Currently the motor is thought to be composed of heavy, intermediate, light-intermediate and light chains. In *Chlamydomonas,* mutations in the various subunits cause the cilia to be very short and packed with IFT particle proteins [62, 106–108]. In mouse, mutations in the heavy [109] and light intermediate chains [110] block ciliogenesis and cause mid gestational lethality.

Mutations in the heavy chain *DYNC2H1* [111, 112], intermediate chains *WDR34* [113, 114] and *WDR60* [115], the light intermediate chain *DYNC2LI1* [116] and the light chain *TCTEX1D2* [100] have been identified in patients with JATD and SRPS. *TCTEX1D2* seems to have a partially redundant function in human embryonic development as biallelic null alleles have been identified in humans born alive while such alleles in other dynein genes are usually lethal around mid-gestation [100]. Immunofluorescence analyses on patient fibroblasts indicate that loss of function of dynein-2 results in shortened bulbous cilia containing accumulated IFT proteins, compatible with a retrograde IFT defect as observed in model organisms [99, 100]. The accumulation of IFT particle proteins in cilia is similar to what is observed in IFT-A patient fibroblast cilia [94, 97]. However clinically, dynein-2 patients (except for *TCTEX1D2*) present with a more severe thoracic phenotype than IFT-A patients. Dynein-2 mutations typically cause 20–30% lethality in the first two years of life whereas IFT-A patients develop retinal degeneration and a nephronophthisis-like renal phenotype not observed with hypomorphic dynein-2 variants [117].

IFT-subunits are rich in WD40, TPR and coiled-coil domains. Human IFT gene mutations often, but not exclusively, affect WD40 domains. These domains fold into circular beta-propeller structures that facilitate protein-protein interactions. Potentially, the human mutations could disrupt specific interactions with ciliary cargos and thus account for the

allele-specific phenotypes that are observed with particular IFT-A mutations. For example, mutations in *IFT122*, *IFT43, IFT144* and *IFT121/WDR35* cause ectodermal defects but these are not seen in *IFT140*, dynein-2 or IFT-B components. In addition, nephronophthisis-like renal disease and retinal degeneration are observed in CED and JATD patients with IFT-A, IFT-B but not dynein-2 mutations. Work to establish ciliary cargos for specific IFT proteins is in its infancy but IFT-A is important for the delivery of GPCRs and other membrane proteins to cilia. This appears to work through TULP3, which binds IFT-A and ciliary targeting sequences in membrane proteins [118, 119]. In *Chlamydomonas*, IFT140 is required for ciliary assembly, but the ciliary assembly of a null allele can be partially rescued with a truncated version lacking the N-terminal WD40 repeats. These cilia are about half-length and have a largely normal protein composition except for a notable reduction in proteins predicted to be myristoylated or prenylated [120]. Ciliary trafficking of lipid modified proteins typically involves carrier proteins in the RhoGDI family such as UNC119 and Pde6delta [121, 122] suggesting that the IFT140 N-terminal WD repeats may interact with RhoGDI-like chaperones.

In addition to IFT-A and IFT-B, a third complex called the BBSome is carried along the cilium by IFT. Unlike the IFT proteins, which were first identified by cell biological approaches in green algae, the BBSome components were first identified as disease genes in human patients with Bardet-Biedl syndrome (BBS). Later it was shown that these proteins traffic along cilia with IFT particles [123] and assemble into a large complex [61]. In *Chlamydomonas*, null mutations in the BBSome components BBS1, BBS4 and BBS7 do not affect ciliary assembly but alter cell behavior and cause a time-dependent accumulation of proteins in the cilium [124]. In *C. elegans*, the BBSome appears to hold IFT-A and IFT-B together. Defects in BBS7 and BBS8 affect ciliary structure and alter behavior [123, 125]. In the mouse, mutations in BBSome components *Bbs1* [126], *Bbs2* [127], *Bbs4* [126, 128], *Bbs7* [129, 130], *Ttc8/Bbs8* [131] and *Bbip1* [132] cause phenotypes that are milder than what is observed with IFT mutants and at least some homozygous animals survived to adulthood in each of the lines. In general, the mouse BBS mutations modeled the obesity, olfactory and retinal degeneration phenotypes seen in the human BBS patients. Cilia formation has not been fully documented in most of the mouse lines, but in *Bbs4* animals, sperm did not form flagella, but other cilia appeared normal [128]. Interestingly, *Bbs2* and *Bbs4* mutant animals failed to localize the seven transmembrane receptors Sstr3 and Mchr1 to neuronal cilia [133]. This would suggest that the BBSome is not required for ciliary assembly but likely plays critical roles in the trafficking of specific cargos into cilia. Careful analysis of respiratory cilia in *Bbs2* and *Bbs4* lines indicated that these proteins are not required for ciliary assembly but a fraction of the mutant cilia had bulges and other structural abnormalities. These abnormalities may increase with time suggesting that the BBSome is needed for maintenance rather than initial assembly [134]. This ideas is consistent with the observation that rod and cone outer segments appeared to form normally in *Bbs4* mice but then degenerate with time [128] and the finding in *Chlamydomonas* that BBS-mutant cilia accumulated abnormal proteins with time suggesting that the BBSome is primarily used for removal of proteins from cilia [124].

Similar to what is observed regarding phenotype severity in mouse BBS mutants, human individuals with biallelic BBS gene-loss-of-function variants typically survive to birth and

usually survive into mid or late adulthood. The human phenotype strongly resembles what is observed in mice. After a period of muscle hypotonia, often associated with feeding problems during the first year of life, affected individuals develop severe obesity (Figure 3I) with metabolic derangement including hypercholesterinemia and diabetes mellitus. Postaxial polydactyly is a common feature at birth and most affected individuals develop progressive retinal degeneration starting from childhood or adolescence. Many become legally blind in adulthood. Developmental delay of varying degree is another phenotypic feature. Male individuals often suffer from hypogenitalism. A subset of patients develops a renal cystic or non-cystic nephronophthisis-like phenotype with marked tubulointerstitial fibrosis with childhood or adolescent onset. Rarely polycystic kidneys are observed during prenatal ultrasound with normalization after birth [135, 136]. To date, mutations in 22 genes have been identified to cause BBS, namely *BBS1*, *BBS2*, *ARL6* (BBS3), *BBS4*, *BBS5*, *MKKS* (BBS6), *BBS7*, *TTC8* (BBS8), *BBS9*, *BBS10*, *TRIM32* (*BBS11*), *BBS12* MKS1 (*BBS13*), *CEP290* (BBS14), *WDPCP* (BBS15), *SDCCAG8* (BBS16), *LZTFL1* (BBS17), *BBIP1* (BBS18), *IFT27* (BBS19), *IFT172* (BBS20), *C3orf37* (BBS21) and *IFT74* [136]. Eight of these genes (*BBS1*, *BBS2*, *BBS4*, *BBS5*, *BBS7*, *TTC8/BBS8*, *BBS9* and *BBIP1*) encode subunits of the BBSome and two encode BBSome binding proteins (*ARL6/BBS3* and *LZTFL1/BBS17*) while the remaining encode IFT and other ciliary proteins. *BBS1* p.M390R, a widespread founder allele in the Caucasian population seems to result in a mild phenotype with slower progression of retinal disease, less pronounced metabolic disturbances and infrequent end stage renal disease [137, 138]. Interestingly, apparently unaffected individuals homozygous for the M390R variant have been described and it has been suggested that in some families carrying M390R, a variant in a second BBS gene is required to produce the BBS phenotype (so-called triallelic inheritance) [139, 140]. However, the majority of human patient alleles identified to date are null alleles and tri-allelic inheritance is rare [141, 142]. For example, mutations in *BBS10* and *BBS12* seems to cause a more pronounced renal phenotype than mutations in BBS1 [137, 143]. IFT-A gene mutations do not seem to cause BBS but individuals with CED and JATD/MZSDS carrying biallelic IFT-A and IFT-B mutations share phenotypic features of renal and retinal disease with BBS patients while JATD patients with dynein mutations present with a more severe skeletal phenotype but usually do not develop extraskeletal findings (at least not until mid-adulthood).

Alstrom Syndrome is an autosomal recessively inherited ciliopathy, phenotypically very similarto BBS. Biallelic null alleles in *ALMS1* have been identified as the sole cause to date [144]. In addition to the polydactyly, obesity, developmental delay, renal and retinal disease that are observed in BBS patients, Alstrom patients also develop pronounced liver disease, hearing loss and cardiomyopathy. Cardiac complications limit life expectancy [145] and may result from impaired cardiomyocyte terminal differentiation with increased postnatal proliferation rates. Normally cardiomyocyte expansion is high during the embryonic period but declines postnatally and cells terminally differentiate into cardiac muscle [146]. However, in siRNA mediated *ALMS1* knockdown cardiomyocytes, in *Alms1*$^{-/-}$ mice as well as in *ALMS1* patients undergoing heart transplantation, increased cardiomyocyte proliferation rates were noted compared to controls. This suggests that in Alstrom syndrome, cardiomyocytes remain proliferative beyond the normal window of postnatal development

[147]. As in BBS, renal fibrosis is frequently observed rather than a true polycystic renal phenotype [148]. ALMS1 localizes to centrioles [149] and ALMS1 loss of function results in truncated cilia [150]. *Alms1*$^{-/-}$ mice recapitulate the human phenotype. In these animals, shortened cilia were noted in the kidney epithelia cells with increased apoptosis in proximal tubules [151] but the mechanism behind the renal phenotype remains unclear.

Cilia in Non-Syndromic Cystic Kidney Disease

As described in other chapters in this volume, there are two major types of non-syndromic cystic kidney disease. The most common being adult onset or autosomal dominant PKD (ADPKD), which affects 1 in 1000 or more adults worldwide and causes end stage renal disease in about half of affected individuals. ADPKD kidneys can grow to an enormous size due to the developing cysts (Figure 3A). The other being infantile or autosomal recessive PKD (ARPKD), which affects about 1 in 20,000 individuals. The recessive disease causes neonatal death in about one third of affected individuals due to lung insufficiency and leads to kidney, liver and pancreas cysts along with organ fibrosis in the survivors. Whereas it is clear that cilia are key to the syndromic forms of cystic disease, the role of cilia in autosomal dominant and autosomal recessive PKD continues to be debated.

Autosomal dominant polycystic kidney disease is caused by mutations in *Pkd1* and *Pkd2*, which encode polycystin-1 and polycystin-2. Polycystin-2 is a clear member of the Trp family of cation channels. The transmembrane region of polycystin-1 shares sequence homology with polycystin-2 and other Trp family channels but has a large N-terminal extension that extends into the extracellular space. The polycystins have been localized to various sites in the cell but are prominently localized to cilia [152–155]. The function of the ciliary polycystins is not clear with the main model suggesting that they detect flow as a measure of tubule diameter [156]. Other work has not been able to detect flow induced calcium currents from the polycystins or cilia [157] raising questions that will require more study [158]. Furthermore intravital imaging shows that in mice, the kidney cilia are bent along the wall of the tubule and do not oscillate under normal conditions [159]. Other models for the function of polycystins include polycystin-1 being a receptor for Wnt ligands [15]. This is an attractive model as Wnt signaling is critical to kidney development and is likely to be important in the etiology of cystic disease [160].

Mutations in the *Pkhd1* gene lead to autosomal recessive PKD. The encoded protein, fibrocystin or polyductin, is a single pass transmembrane protein with a large extracellular N-terminal domain and a short C-terminal tail residing in the cytoplasm [161]. Based on the presence of the large extracellular domain, it is expected that fibrocystin is a receptor but the ligand is unknown. Whether this protein localizes to cilia has not been completely resolved. The C-terminal end adjacent to the transmembrane domain encodes a strong ciliary targeting sequence suggesting that it is a ciliary protein [162]. In addition, a *Chlamydomonas* paralog of fibrocystin was found in the ciliary proteome [2]. In mammalian cells, endogenous fibrocystin has been localized to cilia [163, 164] and the centrosome at the base of the cilium [165, 166]. Localization studies with new antibodies always suffer from concerns about whether the staining represents the true localization of the protein. To overcome this problem, epitope tagged fibrocystin constructs have been made. In one study, the C-terminal

end was tagged with HA. This construct localized to vesicle-like structures at the apical ends of the cell. It is not clear if these structures represent the centrosome, but no ciliary localization was seen [167]. In another study, the N-terminus, just downstream of the signal sequence, was tagged with SV5-PK tags. This form of fibrocystin localized to cilia, although it was proposed that the fibrocystin was secreted in exosome-like vesicles and then delivered to the ciliary membrane from the extracellular space [168]. Understanding the true fibrocystin localization will require more extensive studies with well-characterized antibodies along with *Pkhd1*-null cells as negative controls.

In humans, autosomal-recessive polycystic kidney disease is caused by *PKHD1* loss of function mutations. *PKHD1* is expressed in a number of epithelia including renal, neural, hepatic and intestinal tubules during embryonic development. Mutations have been described along the full length of the gene without any evident clustering pattern. However, phenotype severity correlates with mutation type as individuals carrying two null alleles often present with earlier onset and faster disease progression than individuals with missense mutations [169]. In severe cases, renal function is impaired prenatally resulting in insufficient urine production by the fetus. This results in the Potter sequence where the lack of urine production causes oligo- or anhydramnios, which in turn causes severe pulmonary hypoplasia as the lungs are no longer fluid filled during development. Affected children often do not survive due to respiratory insufficiency. Early loss of renal function is commonly observed in ARPKD with many individuals requiring dialysis and/or renal transplantation in childhood. In addition, development of renal cysts can be accompanied by hepatic cysts and fibrosis, damaging the liver and likewise rendering liver transplantation necessary. Compared to cysts in ADPKD, ARPKD cysts are smaller, and in ultrasound imaging the kidneys present with a typical “salt and pepper” pattern (Figure 3B).

The fact that ciliary dysfunction or loss of the polycystins cause cystic disease suggests a simple model where ciliary localization of the polycystins is required for their activity. Thus, losing either the polycystins or the site of polycystin activity would cause cystic disease. However, studies removing both cilia and the polycystins suggest a much more complex relationship. Ma and colleagues combined floxed alleles of the polycystin genes with floxed alleles of IFT components such that they could produce animals lacking only the polycystins, both the polycystins and cilia, or cilia alone. Kidneys lacking the polycystins became massively cystic. Kidneys lacking cilia also became cystic but the severity was much reduced compared to those lacking the polycystins. Unexpectedly, the loss of both the polycystins and cilia produced a mild form of the disease suggesting that the loss of cilia compensated for the loss of the polycystins. This suggests that the cilium produces a procystic signal that is normally inhibited by the polycystins [170]. The identity of this procystic signal is unknown but its identification has great potential for elucidating the pathway downstream of the polycystins.

Summary

In the two decades since the discovery of a connection between cilia and polycystic kidney disease, we have learned much about the structure and assembly mechanisms of cilia and identified a plethora of human diseases caused by ciliary dysfunction. Major questions that

remain in the field are what signals are detected by cilia to control tubule diameter and prevent cyst formation and what signal transduction pathways carry this information from the ciliary receptors to the nucleus to control proliferation. Answers to these questions will advance the field towards a cure for this important cause of end stage renal disease.

Acknowledgments

This work was supported by National Institute of Health grants GM060992 and DK103632 to G.J.P., the European Research Council (ERC, StG TREATCilia, grant agreement number 716344) and the German Research Foundation (DFG, CRC1140, KIDGEM) to M.S.

Abbreviations:

ADPKD	autosomal dominant polycystic kidney disease
ARPKD	autosomal recessive polycystic kidney disease
BBS	Bardet Biedl Syndrome
CED	Cranioectodermal Dysplasia
GPCR	G-protein coupled receptor
IFT	intraflagellar transport
JATD	Jeune asphyxiating thoracic dystrophy
MZSDS	Mainzer-Saldino Syndrome
PCD	Primary Ciliary Dyskinesia
PKD	polycystic kidney disease
SRPS	short-rib polydactyly syndrome

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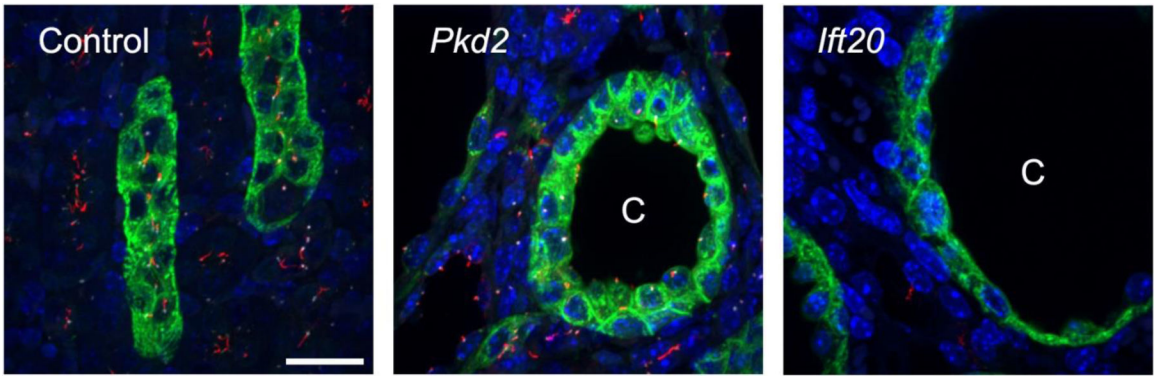

Figure 1. Cystic Disease Caused by Loss of Polycystin-2 or Ift20.

During the development of cystic kidney disease, the normal tubular architecture seen in control animals is lost and large fluid filled cysts (C) form. The loss of polycystin-2 does not affect cilia formation (acetylated alpha tubulin, red; gamma tubulin, white) and the cilia remain in the cysts whereas the loss of Ift20 prevents cilia formation. Green (Dolichos Biflorus Agglutinin) marks collecting ducts. Scale bar is 20 microns.

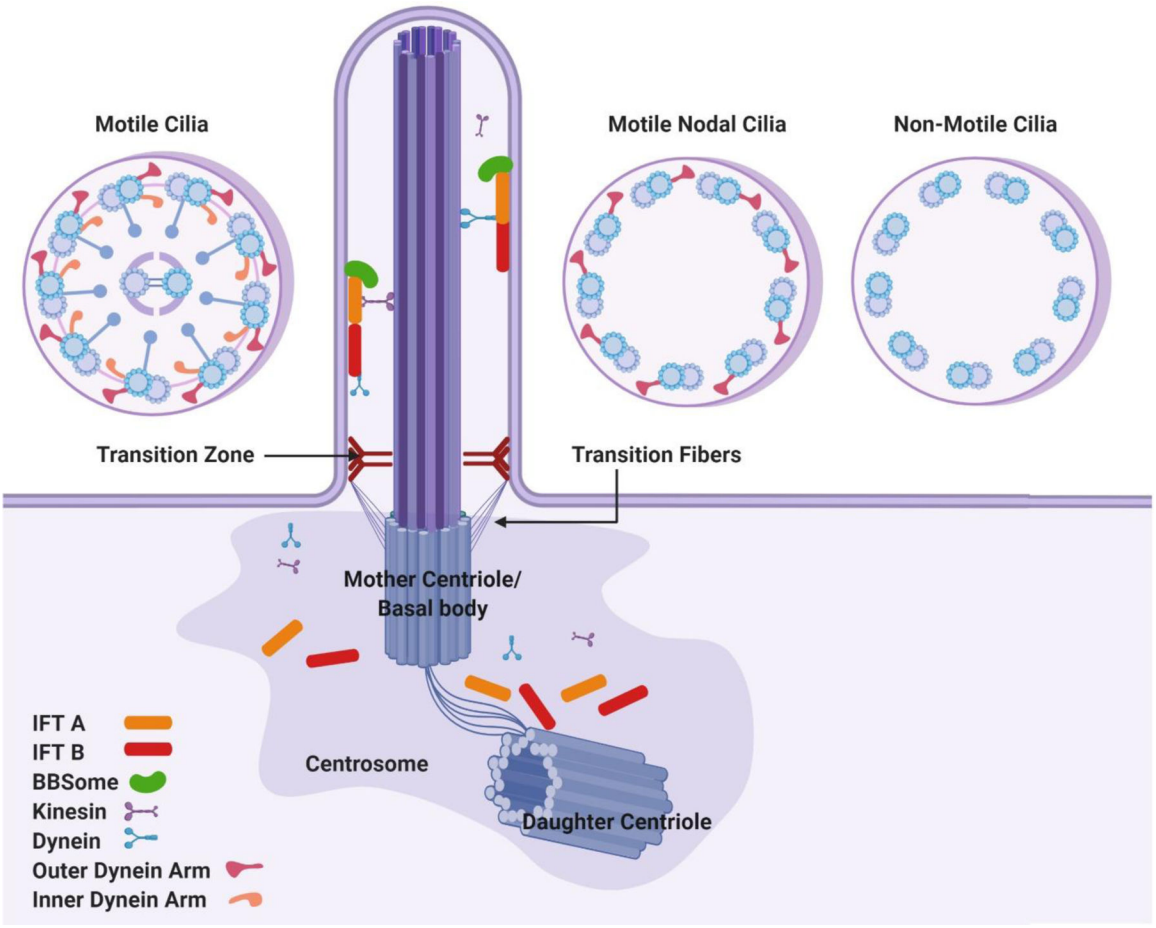

Figure 2. Structure of the cilium, centriole and centrosome.

The cilium is composed of 1000 or more proteins organized around a microtubule-based cytoskeleton called the axoneme and in the ciliary membrane, an extension of the plasma membrane that surrounds the axoneme. The microtubules are templated from the mother centriole or basal body, which is embedded within the centrosome. In addition to the centrioles, the centrosome also contains microtubule binding and nucleating proteins and a variety of signal transduction components. The human axoneme has a stereotypical arrangement of microtubules. These are referred to as 9+2 or 9+0 depending on whether the central pair is present or not. The central pair is critical for generating complex waveforms and is present in most motile cilia but is absent in non-motile cilia. This structure is either missing or significantly underdeveloped in the motile cilia of the embryonic node, which have a relatively simple waveform. Force generation is provided by the inner and outer dynein arms in response to signals generated by the central pair and transmitted through the radial spokes. These structures are missing from non-motile cilia but outer dynein arms are found in nodal cilia. The cilium is anchored to the plasma membrane through appendages from the centriole called the transition fibers and through an extensive network of cytoskeletal to membrane linking proteins at the base of the cilium. The latter structure is called the transition zone and appears to be an important part of the diffusional barrier that separates the ciliary membrane compartment from the bulk plasma membrane. Ciliary

assembly is driven by the intraflagellar transport (IFT) system that involves the movement of large protein complexes along the ciliary axoneme. The large protein complexes or IFT particles, are built from the IFA-A. IFT-B, and BBSome subcomplexes. The outward movement (anterograde direction) is powered by kinesin-2 while the inward movement (retrograde direction) is powered by cytoplasmic dynein 2. The IFT particles are motor adaptors that are needed to couple the molecular motors to cargos made in the cell body and transported into the cilium. Figure prepared with Biorender.

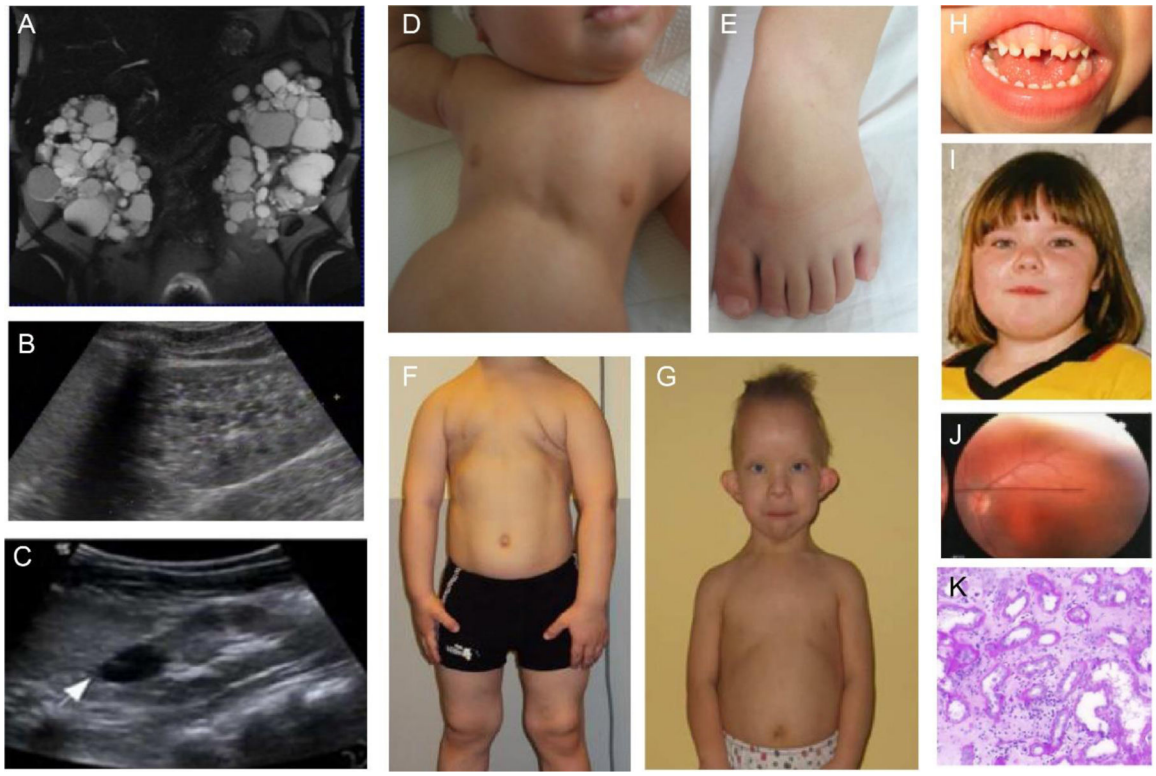

Figure 3. Clinical features of human ciliopathy syndromes with renal involvement.

(**A**) MRI image of enlarged polycystic kidneys in autosomal dominant polycystic kidney disease. (**B**) Ultrasound image showing a “salt and pepper” pattern and cysts in an enlarged autosomal recessive polycystic kidney disease kidney. (**C**) Small hyperechogenic kidney in nephronophthisis with a single cysts (arrow) visualized by ultrasound. (**D**) Substantially narrowed thorax in an infant with Jeune asphyxiating thoracic dystrophy. (**E**) Polydactyly of the feet observed in Jeune asphyxiating thoracic dystrophy. (**F**) Mildly narrowed thorax in a boy with Jeune asphyxiating thoracic dystrophy / Mainzer-Saldino Syndrome resulting from biallelic IFT140 dysfunction. (**G**) Mildly narrowed thorax, facial dysmorphism with low set ears, small mouth, downwards slanting palpebral fissures, sparse hair, dolichocephalous due to craniosynostosis and (**H**) teeth abnormalities in Sensenbrenner syndrome or Cranioectodermal dysplasia. (**I**) Facial features and obesity in Bardet-Biedl-Syndrome. (**J**) Retinal degeneration occurs in Jeune asphyxiating thoracic dystrophy / Mainzer-Saldino Syndrome, Cranioectodermal dysplasia and Bardet-Biedl-Syndrome. (**K**) Histological pattern of a renal biopsy in Jeune asphyxiating thoracic dystrophy with renal disease / Mainzer-Saldino Syndrome due to biallelic IFT172 dysfunction (hematoxylin and eosin staining) showing ectatic tubules but no larger cysts. Image A was a courtesy of Dr. Gerd Walz, University Hospital Freiburg. Image B was a courtesy of Dr. Martin Pohl, University Hospital Freiburg Images C-K have been reprinted with permission [171], [78], [100], [171], [95], [172], [173], [78] respectively.