Recent advances in the molecular pathology, cell biology and genetics of ciliopathies

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ABSTRACT

Primary cilia have a broad tissue distribution and are present on most cell types in the human body. Until recently, they were considered to be redundant organelles, but progress over the past 5 years has led to an understanding of their role in normal mammalian development. The class of inherited disorders that involve aberrant ciliary function are known as ciliopathies, and although their range of severity can vary, they share some common and unexpected clinical phenotypes. The aim of this review is to assess recent insights into the structure, function and formation of primary cilia, and relate this to the pathology, molecular genetics and cell biology of the ciliopathies.

The name "cilia" derives from the Latin for "eyelash". Zimmermann is reported as the first person to observe cilia and, in the 19th century, was the first to describe the primary cilium.[1] Cilia can be subdivided based on both their function and structure as either motile cilia/flagella or primary cilia. Both subtypes consist of a basal body located below the cell surface, and an axoneme that extends away from the cell. The basal body is analogous to the centrioles (a radial array of nine triplet microtubules) of the centrosome, and forms when the centrosome docks at the apical surface of the cell. The structure of the axoneme determines the subtype of cilium: in motile cilia and flagella the axoneme has a 9+2 arrangement of microtubules, whereas primary cilia have a 9+0 arrangement and are immotile (reviewed in Dawe *et al*). Primary cilia have a broad tissue distribution and, until recently, many scientists considered them to be redundant organelles. However, over the past 5 years, progress has been made in understanding the role of these organelles in normal mammalian development. Key to this has been the positional cloning of genes that encode structural or functional components of primary cilia, in some instances from rat or mouse models of polycystic kidney disease (PKD), bioinformatic and phylogenetic analysis of known or inferred ciliary genes, and the characterisation of ciliary proteomes.[3–6]

Mutations in ciliary genes give rise to a multitude of human monogenic disorders that are now collectively known as "ciliopathies" (reviewed in Badano *et al*[7]), but that often share common phenotypic features. Primary cilia are now acknowledged as vital cellular components, with probable functions as chemosensors or mechanosensors of the extracellular environment. The focus of this review will therefore be a concise overview of recent progress in the molecular genetics of ciliopathies, and a discussion of the possible functions of ciliary proteins in the context of molecular pathology and cell biology. As many of the recent advances have concerned pleiotropic conditions with an underlying primary cilium/basal body dysfunction, we will not deal with disorders of motile cilia, such as primary ciliary dyskinesis, or ciliary disorders associated with a situs defect. The pre-eminent example of the latter is Kartagener syndrome, which arises from dysfunctional nodal cilia (a specialised form of motile primary cilia that localise at the embryonic node to establish asymmetries along the left–right axis). Further details of the molecular genetics and aetiology of these disorders are described in a number of excellent recent reviews[8 9] and the references cited within them.

CILIOPATHY PHENOTYPES

Ciliopathies have a broad range of phenotypes encompassing a number of different autosomal recessive and dominant syndromes of previously unknown aetiology. In a previous review for the *Journal of Medical Genetics*, we described the common clinical features in the so-called "congenital hepatorenal fibrocystic" spectrum of phenotypes, with cystic dysplasia of the kidneys as being common feature for most conditions.[10] At the time, we suggested that these phenotypic similarities arose from a common molecular pathogenesis, and 5 years on, this prediction has been proven to be largely correct. Many of the recently identified ciliary genes (table 1) encode either structural or functional components of primary cilia or basal bodies. Although the functional role of many of the encoded proteins still remains an active area of research, it is clear that the clinical aspects of many of the ciliopathy phenotypes can be explained by a ciliary defect.

Ciliated cells are usually a highly specialised, post-mitotic cell type, and are therefore non-proliferating, differentiated cells. However, cilia can also form on quiescent cells, which may undergo later rounds of cell division, and on proliferating cells. Almost all epithelial cells are ciliated, and they commonly exist as a sheet of polarised cells forming a tube or tubule with the cilia projecting into the lumen. The cilia are then exposed to the contents of the lumen where they can provide a sensory role mediating specific signalling cues, including soluble factors in the external cell environment, a secretory role in which a soluble protein is released to have an effect downstream of the fluid flow, and mediation of fluid flow if the cilia are motile.[11 12] The cell biology of the ciliopathies can be considered in terms of functional loss or complete absence of a particular

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Table 1 A summary of the ciliopathies for each inherited disorder

Inherited disorder	Loci	Gene(s)	Location	Protein product	Function and other comments	Interactors	References
PKD (autosomal dominant)							
Adult type 1	PKD1	PKD1	16p13.3	PC1	Cell–cell or cell–matrix interactions; interacts with PC-2 to produce calcium-permeable nonselective cation currents	PC-2	
Adult type 2	PKD2 PKD3?	PKD2	4q22.1	PC2	Probable channel protein; interacts with PC-1 Further genetic heterogeneity suggested but not proved	PC-1	
PKHD (autosomal recessive)							
Infantile type	PKHD1	PKHD1	6p12.2	Fibrocystin, polyductin	Probable receptor protein that acts in collecting duct and biliary differentiation; colocalises with PC-2 at the basal bodies of primary cilia		
NPHP, SLS							
Juvenile, type 1	NPHP1, SLSN1, JBTS4	NPHP1	2q13	Nephrocystin	Adaptor protein; associates with signalling molecules involved in cell adhesion and actin cytoskeleton organisation, and with β-tubulin, a major component of primary cilia	Nephrocystin-4, p130Cas/BCAR1, signal-rich PTK2B, TNS, β-tubulin	47, 95
Infantile, type 2	NPHP2	INVS	9q31.1	Inversin	Primary cilia function and involvement in the cell cycle; possible molecular switch between different Wnt signalling cascades; left–right axis determination	Nephrocystin, APC2	95–97
Adolescent, type 3	NPHP3, SLSN3	NPHP3	3q22.1	Nephrocystin-3	With nephrocystin and nephrocytin-4, may mediate a common developmental pathway in the primary cilia of renal epithelial cells	Nephrocystin	98
Juvenile, type 4	NPHP4, SLSN4, JBTS4	NPHP4	1p36	Nephrocystin-4, nephroretinin	Possible roles in signal transduction, cell–cell adhesion and actin cytoskeleton organisation and biogenesis; mutations in RPGRIP1 (associated with Leber congenital amaurosis type 6) disrupt the interaction with nephrocystin-4	Nephrocystin, RPGRIP1	47, 64, 95
	SLSN5	IQCB1	3q21.1	IQCB1, nephrocystin-5	IQCB1 and RPGR may participate in a common pathway in connecting cilia of photoreceptors and to primary cilia of renal epithelial cells	RPGR, calmodulin	95
JBTS and related disorders/SLS phenotypes	NPHP6, SLSN6, JBTS5, LCA10, MKS4	CEP290, NPHP6	12q21.3	CEP290	Localised at the nuclei of renal epithelial cells in a cell cycle-dependent manner, in connecting cilia of photoreceptors, centrosomes and primary cilia; modulates the activity of ATF4, a transcription factor implicated in cAMP-dependent renal cyst formation	ATF4	34–38
MKS							
	MKS1	MKS1, FLJ20345	17q22	MKS1	MKS1 is a member of the flagellar apparatus basal body proteome; contains a B9 domain of unknown function; localises to basal bodies and centrosomes, but interacts with meckelin	Meckelin	14, 15
	MKS2		11q13		Causative gene has not been identified		99
	MKS3, JBTS6	MKS3, TMEM67	8q22.1	Meckelin	Meckelin localises to the cell surface and primary cilia; putative transmembrane receptor with an extracellular cysteine-rich domain that may have similarities to those in Frizzled-type receptors	MKS1	14, 36, 40
Meckel-like cerebro-reno-digital syndrome	MKS4, JBTS5	CEP290, NPHP6	12q21.3	CEP290	See entry above for CEP290; mutations also cause cerebro-reno-digital syndrome, with a phenotype between that of MKS and JBTS		36
CORS, JPTS type B	MKS5, JBTS7, CORS3	RPGRIP1L, KIAA1005	16q12.2	RPGRIP1L	Colocalises at the basal body and centrosomes with CEP290, and interacts with nephrocystin-4; in mice, Ftm/Rpgrip1l is necessary for the establishment of left–right asymmetry and patterning of the neural tube and the limbs; may mediate cilium-related Shh signalling	Nephrocystin-4	39, 63, 100
JPTS, CORS							
CORS type 1	JBTS1, CORS1		9q34.3		Causative gene has not been identified		28, 29
CORS type 2	JBTS2, CORS2		11p12–q13.3		Causative gene has not been identified		29–31
	JBTS3	AHI1	6q23.3	AAHI1 protein homologue, jouberin	Unknown function; AHI1 expression may contribute to the development of specific types of human leukaemia; may be associated with susceptibility to schizophrenia		32, 101
	JBTS4	NPHP1	2q13	Nephrocystin	See entry above for NPHP1; deletions of the *NPHP1* gene are a rare cause of JBTS		33

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Table 1 Continued

Inherited disorder	Loci	Gene(s)	Location	Protein product	Function and other comments	Interactors	References
	JBTS5, MKS4	CEP290, NPHP6	12q21.3	CEP290	See entry above for CEP290		34, 35
	JBTS6, MKS3	MKS3, TMEM67	8q22.1	Meckelin	See entry above for MKS3		36
CORS type 3	JBTS7, CORS3, MKS5	RPGRIP1L, KIAA1005	16q12.2	RPGRIP1L	See entry above for RPGRIP1L		39, 63
LCA							
	LCA10	CEP290, NPHP6	12q21.3	CEP290	See entry above for CEP290		37
	LCA5	LCA5, C6orf152	6q14.1	Lebercilin	Localises to the microtubules, centrioles and primary cilia, and to the connecting cilia of photoreceptors	Dynein light chains 1 and 2, $p150^{glued}$ and p50-dynamitin subunits of dynactin, nucleophosmin, nucleolin, 14-3-3eta, HSP70	13
BBS							
Major form (type 1)	BBS1	BBS1, BBS2L2	11q13.1	BBS1	Unknown function; probable role in eye, limb, cardiac and reproductive system development		102
	BBS2	BBS2	16q13	BBS2	Unknown function		103
	BBS3	ARL6	3q11.2	ARL	Member of the ARL subgroup of the Ras superfamily; may regulate diverse cellular functions, including regulation of intracellular traffic; in *C. elegans* ARL6 undergoes intraflagellar transport in the ciliary axoneme, implicating it in ciliary transport		104, 105
	BBS4	BBS4	15q24.1	BBS4	Contains tetratricopeptide repeats; localises to the centriolar satellites of centrosomes and basal bodies of primary cilia; adaptor of the p150(glued) subunit of the dynein transport machinery; may be required for microtubule anchoring and cell cycle progression	PCM1, $p150^{glued}$ subunit of dynactin	61, 102
	BBS5	BBS5	2q31.1	BBS5	Localises to basal bodies; in *C. elegans* it is necessary for the formation of both cilia and flagella		5
MKKS	MKKS, BBS6	MKKS	20p12.2	MKKS/BBS6 putative chaperonin	Group II chaperonin-like protein that localises to pericentriolar material; centrosomal component required for cytokinesis; possible role in protein processing in limb, cardiac and reproductive system development		62, 106–108
	BBS7	BBS7, BBS2L1	4q27	BBS7	Unknown function; protein contains a six-bladed β-propeller motif, which shares homology to regions in the BBS1 and BBS2 proteins		79
	BBS8	TTC8	14q31.3	Tetratricopeptide repeat domain 8	Localises to centrosomes and basal bodies; colocalises with γ-tubulin and BBS4 in centrosomes, and interacts with PCM1, a protein probably involved in ciliogenesis; in *C. elegans*, bbs-8 is required for the stability of intraflagellar transport complexes	PCM1	50, 109
	BBS9	PTHB1	7p14.3	PTHB1	Unknown function; may be involved in parathyroid hormone action in bones; expressed in ciliated cells in *C. elegans*		110
	BBS10	BBS10, C12orf58	12q21.2	BBS10	Unknown function; group II chaperonin-like protein		80
	BBS11	TRIM32	9q33.1	tripartite motif-containing 32	Contains a RING finger domain and may be a E3 ubiquitin-protein ligase; possible role in proteasome degradation pathway	HIV-1 Tat protein, TRIM 23 and 27, PDE9A, UBQLN1	111
	BBS12	BBS12, C4orf24	4q27	BBS12	Unknown function; group II chaperonin-like protein; shares distant homology to the BBS6 and BBS10 proteins		112
ALMS							
	ALMS1	ALMS1	2p13	ALMS1	Localises to centrosome and basal body; in vivo phenotypes of *Alms1* gene-trap mice include a lack of sperm flagella and defective rhodopsin transport through the connecting cilia of photoreceptor cells; possible role in intracellular trafficking	MEGF10	19–22

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Table 1 Continued

Inherited disorder	Loci	Gene(s)	Location	Protein product	Function and other comments	Interactors	References
OFD syndrome							
OFD type 1	OFD1	OFD1, CXorf5	Xp22.2	OFD1	Localises to centrosomes and basal bodies; defective primary ciliary and left-right asymmetry in *Ofd1* knock-out mice, with impaired patterning of the neural tube and altered expression		24, 25, 113
ATD, Jeune syndrome							
	ATD1	IFT80, WDR56	3q26.1	IFT80	Localises to the basal body and the ciliary axoneme; possible role in Shh signalling	In *Chlamydomonas reinhardtii*, components of IFT complex B	74, 114

AHI1, Abelson helper integration site 1; ALMS, Alström syndrome; APC2, anaphase-promoting complex subunit-2; ARL-6, ADP-ribosylation factor-like 6; ATD, asphyxiating thoracic dystrophy; ATF4, activating transcription factor 4; BBS, Bardet–Biedl syndrome; *C. elegans, Caenorhabdits elegans*; CEP290, Centrosomal protein 290 kDa; CORS, cerebello-oculo-renal syndrome; GTP, guanidine triphosphate; HIV, human immunodeficiency virus; HSP, heat shock protein; IFT80, Intraflagellar transport 80 kDa protein homologue; IQCB1, IQ motif-containing protein B1; JBTS, Joubert syndrome; LCA, Leber congenital amaurosis; MEGF-10 multiple epidermal growth factor-like-domains 10; MKKS, McKusick–Kaufman syndrome; MKS, Meckel–Gruber syndrome; NPHP, nephronophthisis; OFD, orofaciodigital; PC, polycystin; PCM1, Pericentriolar material 1; PDE9A, phosphodiesterase 9A; PKD, polycystic kidney disease; PKHD, polycystic kidney and hepatic disease; PTHB1, Parathyroid hormone-responsive B1; PTK2B, protein tyrosine kinase 2B; Ref., reference; RPGR, retinitis pigmentosa GTPase regulator; RPGRIP1, retinitis pigmentosa GTPase regulator-interacting protein; SLS, Senior–Løken syndrome; TNS, tensin; TRIM, tripartite motif-containing; UBQLN1, ubiquilin 1.

protein. For example, the outer segments of retinal rod cells are modified primary cilia, and compromised protein transport across the photoreceptor-connecting cilium causes either retinitis pigmentosa or blindness due to retinal degeneration (retinal dystrophy). Alternatively, a failure of mechanosensation in the primary cilia of renal tubular cells causes PKD.[11 13]

Many ciliary proteins are components of large, multi-subunit complexes, and individual proteins interact directly or indirectly within the complex. The absence or dysfunction of one component affects the function of the whole, to a greater or lesser extent, resulting in a range of phenotypic severity. Furthermore, many ciliary proteins also exist at sites other than the cilium or basal body, which implies that they may have other roles elsewhere in the cell. A second explanation could be that these proteins are produced as inactive precursors before either the formation of cilia or transport to the mature cilium.

Ciliopathies can be classified according to whether there is aberrant function in an intact cilium or complete absence/partial loss of the mature cilium. The latter is the case with the more severe multiorgan phenotypes such as Meckel–Gruber syndrome (MKS), but a more specific defect occurring in intact cilia is seen in the late-onset cystic kidney diseases.[14–16] Alternatively, more specific defects may be due to loss of a protein or protein isoform with a highly specialised role exclusive to the eye or kidney, and both retinal degeneration and PKD are common clinical features associated with ciliopathies. Other less common features include anosmia, ataxia, liver fibrosis, cardiac defects, infertility, obesity, central nervous system (CNS) abnormalities and skeletal dysplasia. These additional features are indicative of the diverse functional roles of primary cilia during the development of the many tissues affected by the phenotypes.[17] The aetiology of anosmia, for example, can be explained as an occasional manifestation of ciliopathies, because the odour receptors of the olfactory epithelium are positioned on the cilia of olfactory neurons.[18]

Two diseases that are known to be caused by proteins associated with primary cilia are Alström syndrome (ALMS) and orofaciodigital syndrome type 1, and we discuss these briefly as representative examples of the ciliopathy phenotype. ALMS is an autosomal recessive disorder characterised by childhood obesity associated with type 2 diabetes mellitus, chronic hyperglycaemia, cone–rod retinal dystrophy and neurosensory deficits. The single causative gene on chromosome 2p13, *ALMS1*, encodes a novel protein that contains coiled-coil domains and a putative nuclear localisation signal.[19 20] *ALMS1* is widely expressed, and localises to centrosomes and basal bodies of primary cilia.[21] However, the disruption of the gene in either a patient with ALMS or a gene-trap *Alms1* mutant mouse strain results in normal primary cilia and microtubule cytoskeleton formation, suggesting that the ALMS phenotype results from impaired function rather than abnormal development or absence of these organelles.[21 22] These data support the findings for Bardet–Biedl syndrome (BBS; see below), indicating a unifying pathway involving primary cilia that underlies these overlapping disorders. Recent work, using small interfering RNA-mediated loss of *Alms1* expression has shown that this gene is required for ciliogenesis in a ciliated cell line (mouse IMCD3 cells). Furthermore, in support of the putative functional roles of the polycystins (see below), loss of *Alms1* expression prevented flow-induced Ca^{2+} influx into the cytosol of individual cells, thus implicating the ALMS1 protein in mechanosensation.[23]

Oro-facial-digital type 1 (OFD1) syndrome is an X-linked dominant condition with lethality in males. It is characterised by malformations of the face, oral cavity and digits, but is also associated with malformation of the brain and polycystic kidneys. The underlying gene, *OFD1*, on chromosome Xp22, encodes a protein containing coiled-coil α-helical domains and an N-terminal Lis homology (LisH) motif.[24] OFD1 is a core component of the human centrosome, and the coiled-coil domains are crucial for localising the protein to the centrosome. Importantly, most reported *OFD1* mutations are predicted to cause protein truncation with loss of coiled-coil domains, presumably leading to loss of centrosomal localisation. OFD1 also localises to the basal bodies of renal epithelial cells.[25]

A PHENOTYPIC SPECTRUM OF DISEASE SEVERITY: JOUBERT SYNDROME AND MECKEL–GRUBER SYNDROME

Joubert syndrome (JBTS) is a ciliopathy with extensive genetic heterogeneity and variability in phenotypic severity. It was first described in 1969 in a family containing four siblings with ataxia, mental retardation, oculomotor apraxia, hypotonia and neonatal breathing dysfunction.[26] The "molar tooth sign" (MTS) seen on MRI scans is characteristic and diagnostic of JBTS, and is caused by a complex midbrain–hindbrain abnormality.[27] Five loci have been identified to date *(JBTS1-5)*. *JBTS1* has been mapped to chromosome 9q34.3 but no causative gene

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has yet been identified.[28] Patients with *JBTS1* linkage tend to have features restricted to the CNS, particularly the cerebellar and the midbrain–hindbrain junction.[29] A novel locus for JBTS, *JBST2*, has been mapped to chromosome 11p11.2–q12.3.[30 31] Valente *et al* showed that, unlike patients with *JBTS1* linkage, patients with *JBST2* linkage present with multi-organ involvement including the kidney, brain and retina, in addition to the typical CNS abnormalities, and the phenotype has also been described as "cerebellar-ocular-renal syndrome" (CORS).[29] No causative gene at this locus has yet been identified. *JBTS3* is found at chromosome 6q23.3, with pathogenic mutations identified in the gene *AHI1*, which encodes a novel protein, jouberin, that contains a N-terminal coiled-coil domain.[32] Patients with mutations in *AHI1* typically have features that are restricted to the CNS. The fourth JBTS locus was identified in a subset of patients presenting with juvenile nephronophthisis (NPHP) in combination with typical CNS abnormalities. The gene *NPHP1*, on chromosome 2q13, was identified as causing this distinctive phenotype.[33] A fifth JBTS locus, *JBTS5*, has been mapped to chromosome 12q21.3, because of the recent identification of mutations in the *NPHP6/CEP290* gene, and encodes the 290 kDa centrosomal protein CEP290.[33 34] Patients with *NPHP6/CEP290* mutations have a variable phenotype with neurological and neuroradiological features of JBTS, but also severe retinal and renal involvement.[34] Remarkably, mutations in *NPHP6/CEP290* have also been associated with a range of phenotypes that include MKS and Leber congenital amaurosis (LCA).[36 37]

A variety of other abnormalities has been described in children with JBTS, with different levels of severity, including a characteristic facial appearance, delayed language, hypersensitivity to noise, autism, ocular and oculomotor abnormalities, meningoencephaloceles, microcephaly, low-set ears, polydactyly, retinal dysplasia, kidney abnormalities (renal cysts), soft-tissue tumours of the tongue, liver disease (including fibrocystic changes) and duodenal atresia.[38] Several of these features overlap with those described for MKS including, in particular, the classic MKS features of polydactyly, fibrocystic changes to the liver and encephalocele. Owing to the phenotypic overlap between MKS and JBTS, it was hypothesised that the two separate diseases might in fact be allelic, with pathogenic mutations in the same gene. This has subsequently been shown for the *NPHP6/CEP290* gene, with mutations causing a Meckel-like cerebrorenodigital syndrome. A novel form of CORS, with additional craniofacial abnormalities such as cleft palate and small jaw, is caused by mutations in the *RPGRIP1L/KIAA1005* gene.[36 39] Furthermore, mutations in the *MKS3/TMEM67* gene can cause both an MKS phenotype and JBTS.[40 41] In addition, there is a strong genotype–phenotype correlation for mutations at the *MKS3* and *MKS1*, with marked differences in the frequency of polydactyly and the severity of CNS malformations.[42 43] The severity of the MKS and JBTS spectrum of phenotypes has been suggested to be due to a complete absence of cilia in affected tissues.[44] A very recent and unexpected finding has come from two genome-wide association studies for both adult and childhood obesity.[45 46] Frayling *et al* studied type 2 diabetes and found that a common variant in the *FTO* (fat mass and obesity associated) gene contributed to obesity.[45] They pointed out that the first intron of *FTO* is also part of the control region for the *RPGRIP1L/KIAA1005* gene. *RPGRIP1L/KIAA1005* may therefore be implicated in not only the rare, mendelian disorders of MKS and JBTS, but also a common, multifactorial disease such as obesity.

THE ROLE OF CILIARY PROTEINS IN CILIOGENESIS

For a cilium to form in the right locale and to function correctly, several criteria have to be met. In the first instance, the cell has to exhibit a polar orientation with respect to the external cell environment (fig 1). This is established by the formation of intercellular junctions with adjacent cells along the basolateral surface, creating a sheet of epithelial cells. These junctions, known as tight junctions in vertebrates, serve to separate membrane compartments and thus create a diffusion barrier between those compartments. In the kidney, these sheets form tubules through which urine flow is monitored by primary cilia. Proteins implicated in ciliopathies have been shown to exist in complexes with tight junction proteins or other junctional complexes (such as adherens junctions), or to have an association with the basolateral surface before cilia formation. The protein nephrocystin, encoded by the *NPHP1* gene (table 1), has a partial localisation with β-catenin, and isoforms of inversin (encoded by the *INVS/NPHP2* gene) also associate with N-cadherin and catenins.[47 48] This overlap in subcellular location implies that these proteins exist as part of conserved multifunctional complexes, a common theme in many ciliopathies.[49 50] Evidence for a "nephrocystin complex" is provided by in vitro biochemical experiments that show specific interactions of nephrocystin with nephrocystin-4.[47] Proteins lost or defective in PKD may also be involved in cell–cell contacts, as both fibrocystin/polyductin and polycystin-2 localise with E-cadherin at adherens junctions and may be involved in stabilisation of the actin cytoskeleton across the epithelial cell layer.[16]

Tight junction complexes are crucial components for maintenance of cell polarity, and serve as anchoring points for the cell's cytoskeletal architecture. The cytoskeleton consists of

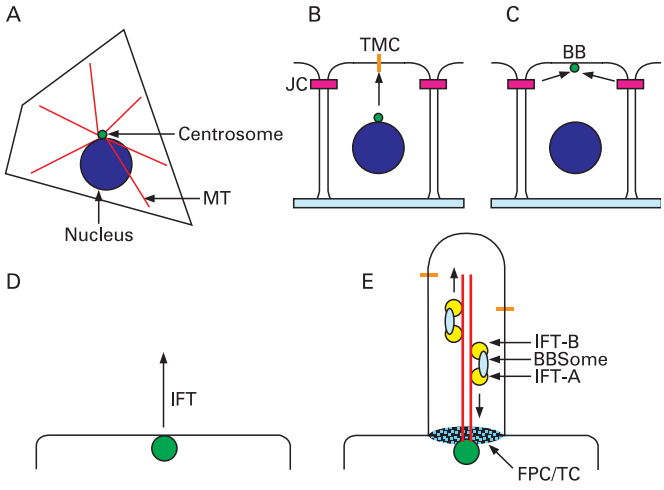


Figure 1 Ciliogenesis and structure of the mature cilium (A) A non-polarised epithelial cell showing positions of the nucleus (blue), the centrosome (green) and a simplified microtubule array focused on the centrosome or microtubule organising centre (MTOC; red). (B). A polarised post-mitotic epithelial cell forming part of a cell layer anchored into place by junctional complexes (JC; pink), such as tight junctions. The centrosome migrates towards the apical cell surface (arrow) where it anchors with a transmembrane protein or complex (TMC; orange) to form the basal body (BB), seen in position in (C). Cilia precursor proteins then migrate from the junctional complexes to the basal body (arrows) where they initiate cilia growth via intraflagellar transport (IFT) (D, arrow). An intact mature cilium is seen in (F) showing the location of the flagellar pore complex/transition zone (FPC/TZ) and retrograde/anti-retrograde movement (arrows) of IFT complexes A and B, linked via the BBSome.

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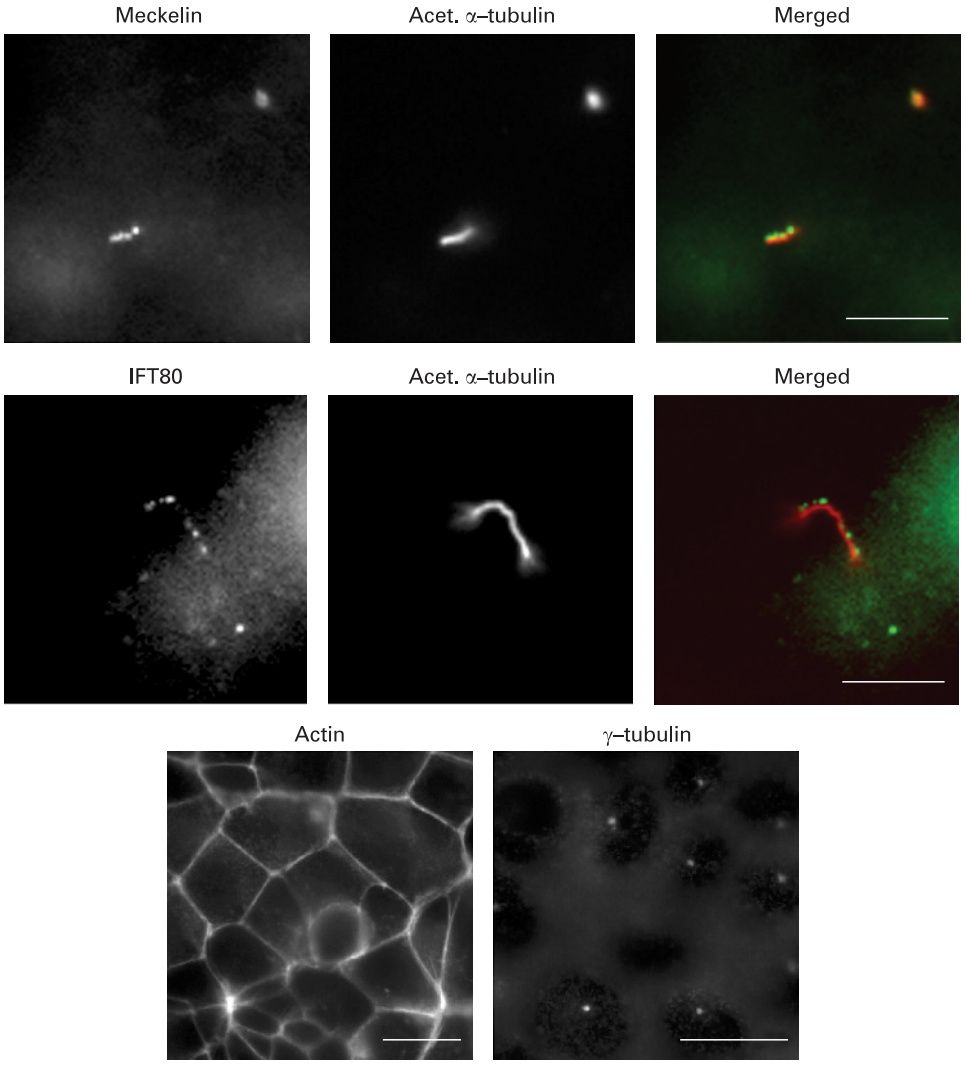


Figure 2 Immunofluoresence microscopy of ciliated IMCD3 cells. Top, location of (left) meckelin; (middle) acetylated-α-tubulin (the axoneme) and (right) a merged image confirming presence of meckelin (green) along the axoneme (red) (bar: 5 μm). Middle, the location of (left) IFT80, (middle) acetylated-α-tubulin (the axoneme) and (right) a merged image confirming punctate distribution of IFT particles (green) along the axoneme (red) (bar: 5 μm). Bottom, distribution of (left) basolateral actin (bar: 10 μm) and (right) γ-tubulin, indicating position of the basal body (bar: 10 μm).

actin filaments, intermediate filaments and microtubules, and both the actin and microtubule networks have specific roles in cilia or ciliogenesis. Of particular importance is the microtubule network, which forms an array centred around the centrosome; this array is considered the microtubule organising centre (MTOC; fig 1). In proliferating cells, the centrosome is crucial in mediating mitosis and forming the poles of the mitotic spindle. Although aberrant proliferation occurs in the development of cysts within the kidney, and inversin and polycystin localise to the mitotic spindle, it is unclear whether there is a direct role for a nephrocystin complex in proliferation. More convincing is a trafficking role towards the centrosome/basal body, mediated by the microtubule motor protein kinesin 2.[51 52] KIF3A is a subunit of kinesin 2, which, when inactivated in mice, results in PKD, whereas a second subunit, KIF3B, exists as a complex with polycystin 2 and fibrocystin/polyductin. Kinesins also have a role in intact cilia, and mediate transport along the axoneme.[53–55]

After the establishment of polarity, the centrosome is required to migrate to the apical cell surface where it docks before basal body formation and cilia growth (fig 1). This occurs by an unknown process, but in multiciliated airway epithelia, the actin cytoskeleton is implicated in basal body migration.[56] In addition, as the interphase centrosome is positioned by microtubules, a role for the microtubule cytoskeleton cannot be discounted. Centrosomal migration may also be mediated by proteins involved in cell polarity.[57] Loss of MKS1 or meckelin negates centrosome movement and docking at the apical surface. MKS1 has been shown to localise to the centrosome/basal body although meckelin, at the early stages of ciliogenesis, localises to the apical cell surface. Meckelin is a transmembrane receptor, and co-immunoprecipitation experiments have shown that it can exist in the same complex as MKS1. This interaction implies that meckelin may be responsible for docking of the centrosome at the apical surface.[14] Loss of this interaction results in complete absence of the cilia and may therefore account for the relative severity of the MKS phenotype.[44] The CEP290 protein (table 1) has also been shown to localise to the pericentriolar matrix (PCM) in preciliated cells and to the basal body in ciliated cells.[35] This is in common with other PCM proteins, which translocate to a more focussed point after basal body formation.[58] Polycystin 2 has been shown to associate with pericentrin at the basal body, which may direct the nephrocystin/polycystin complex to the cilia after it is transported from the basolateral surface.[59 60] Centriolar/basal body components may also serve to direct BBS4 and BBS6 to the cilium.[61 62] Nephrocytin-4 also interacts with the protein produced by the *RPGRIP1L* gene (table 1) at the basal body.[63 64]

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INTRAFLAGELLAR TRANSPORT AND ASPHYXIATING THORACIC DYSTROPHY

The apical localisation of cilia-related proteins and precursors at the basal body may be necessary for cilia initiation, and is a process that relies upon intraflagellar transport (IFT) by kinesins.[16 65] IFT is the process by which protein complexes, called IFT particles, are transported bidirectionally along the axoneme of cilia and flagella by the coordinated action of IFT motors (figs 1, 2).[53–55 65 66] A probable initial stage in ciliogenesis is that the basal body forms the template for the ciliary axoneme, and the structure of the basal body complex accounts for the radial array of microtubules within the cilium. The axoneme is essentially the cilia "backbone", which remains attached in intact cilia and consists of a polarised array of microtubules extending from the basal body to the ciliary tip. The tubulin within the axoneme is a long-lived stable subtype, which is post-translationally modified by acetylation (fig 2). Cilia growth and maintenance is achieved by the movement of proteins along this acetylated tubulin track as part of large vesicular complexes or particles, which comprise the IFT complexes.[50 53–55 65 66] Ciliary protein precursors enter the cilia through a flagellar pore complex or transition zone at the base of the cilia (fig 1). It has been suggested that nephrocystin has a role at this location because of the overlapping distribution of nephrocystins at the basal body/transition zone, which may be a loading point before transport through the flagellar pores.[47 67 68] Proteins are then associated with IFT particles and are transported along the axoneme via microtubule motor complexes; kinesins towards the tip (anterograde transport) and dyneins to the base for recycling (retrograde transport). Certain cell types, such as photoreceptor cells, rely on IFT for transport of proteins and lipids between the inner and outer segments along the connecting cilium.[69] It is therefore not surprising that defects in intersegmental transport in photoreceptors, due to dysfunction or absence of a ciliary or basal body protein, can give rise to a loss-of-vision phenotype in many ciliopathies. Retinitis pigmentosa or blindness due to retinal degeneration is a feature of BBS, the CORS form of JBTS and the Senior–Løken syndrome (SLS) form of nephronophthisis (table 1).

IFT proteins were first identified as essential factors for the growth and maintenance of flagella in the single-celled algae *Chlamydomonas reinhardtii*. The flagellum of this eukaryote is composed of >360 proteins that must be transported and assembled into various structures precisely along the microtubules.[69 70] IFT particles can be isolated by subcellular fractionation and separated into two classes by sucrose gradient centrifugation. These size classes are called complexes A and B.[66] Complex A is composed of at least five polypeptides (IFT144, IFT140, IFT139, IFT122A, IFT122B and possibly IFT43), and complex B is composed of at least 12 different polypeptides (IFT172, IFT88, IFT81, IFT80, IFT74, IFT75, IFT55, IFT57, IFT52, IFT46, IFT27 and IFT20), of relative mass ranging from 20 to 172 kDa (reviewed by Cole[71]). Mutations in either complex A or complex B genes cause short, absent or stumpy flagella. For example, mutant *C. reinhardtii* lacking IFT88 have a normal cell phenotype but their flagella do not assemble and they are described as "bald".[69] An insertion mutation in the mouse homologue, *Tg737*, encoding the protein polaris, gave rise to a phenotype resembling ARPKD, first described in the *orpk* (Oak Ridge polycystic kidney disease) mouse.[72] The phenotype of the homozygous mutated mice included bilateral polycystic kidneys, abnormal outer segment development and retinal degeneration, which resulted in premature death within a few weeks of birth.[69] Subsequently, the human homologue, *TG737/TTC10*, at chromosome 13q12.1, was characterised and suggested as a candidate gene for ARPKD, but subsequent screening of patients with ARPKD has found no mutations in this gene.[73]

To date, asphyxiating thoracic dystrophy (ATD; also known as Jeune syndrome) is the only human ciliopathy that is associated with mutations in an IFT protein homologue. ATD is an autosomal recessive chondrodysplasia, which often leads to death in infancy because of a severely constricted thoracic cage and respiratory insufficiency. Retinal degeneration, cystic renal disease and polydactyly are other common features. Mutations in *IFT80/WDR56* were identified in a small subset (10%) of ATD families without extraskeletal manifestations, which suggests that further genetic heterogeneity in ATD is likely to be caused by mutations in other IFT-associated genes.[74] A possible candidate is *IFT57*, which is reported to localise with polycystin-2.[59]

GENETIC HETEROGENEITY: NEPHRONOPHTHISIS AND BARDET-BIEDL SYNDROME

One obvious characteristic of the ciliopathies is the extensive genetic heterogeneity for conditions such as nephronophthisis (NPHP) and BBS (BBS; table 1). NPHP is an autosomal recessive cystic kidney disease and is the most common genetic cause of end-stage renal failure in first three decades of life. Five causative genes have been identified to date (table 1; reviewed by Hildebrandt *et al*[75]). BBS is an autosomal recessive pleiotropic condition with multiorgan involvement and variable severity. It is characterised by obesity, retinal degeneration, polydactyly, renal and gonadal malformation and behavioural and developmental problems.[76] Twelve BBS-associated genes have been identified to date (table 1). At least seven of the encoded proteins (BBS1, 3, 4, 5, 6, 7 and 8) are known to associate with either the centriole/basal body or the axoneme of primary cilia. In both of these examples, the genetic heterogeneity implies that the encoded NPHP or BBS proteins could be components of either a common pathway or a multisubunit complex.[77] The BBS proteins have now been shown to interact in a multisubunit complex known as the "BBSome"; BBS4 forms a complex with BBS 1, 2, 5, 7, 8, and 9, with BBS9 acting as a possible organising subunit.[44] This observation provides a mechanistic explanation for both the potential epistatic interactions of BBS genes, and the wide variability in the severity of the phenotype. Katsanis *et al.* identified three mutations in both the *BBS2* and *BBS6* genes in affected members of three pedigrees, with an inheritance pattern that they suggested was triallelic.[78] BBS can also be considered a monogenic recessive disease, with heterozygous mutations at a second BBS gene modifying severity of the phenotype. For example, three families, with two mutations in either *BBS1* or *BBS2*, had some but not all patients carrying a third heterozygous mutation in *BBS1*, *BBS2* or *BBS6*.[79] Stoetzel *et al.* found that a minority (18%) of families with *BBS10* mutations also had mutations or recognised variants (predominantly missense changes) at another BBS locus.[80] The most compelling examples from this report were two families that each had affected patients with two *BBS10* mutations and one additional pathogenic *BBS1* mutation, and, a third family that, conversely, had a single *BBS10* frameshift mutation carrying two pathogenic *BBS1* mutations. Further evidence of the oligogenic inheritance pattern of BBS has come from the identification of a novel locus, *MGC1203/CCDC28B*, which contributes epistatic alleles to BBS.[81] The encoded protein, coiled-coil domain-containing protein 28B (CCDC28B), is a pericentriolar protein, and interacts with the BBS1, 2, 4, 5, 6, 7 and 8. It is possible that

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the penetrance of other ciliopathies may be modified in a similar fashion.

The BBS7 and BBS8 proteins have been shown to be crucial for normal IFT of components along the axoneme of primary cilia, and the distribution of BBS4 shows motility along the axoneme similar to IFT (fig 1).[82] A possible role for BBS proteins in ciliary assembly and disassembly is suggested from observations of BBS proteins at centriolar satellites in non-ciliated cells, followed by translocation during cilia formation to primary cilia. BBS4 localises with PCM1, which is normally absent from cilia, and the loss of PCM1 leads to the disappearance of centriolar satellites, disruption of centrosomes and diminished cilia formation.[44 58] BBS1 associates with the vesicular trafficking protein Rabin-8, the loss of which leads to depleted ciliation and disruption of BBS4 localisation in pre-ciliated cells. The normal function of Rabin-8 at the basal body appears to be the activation of proteins that migrate to the cilia and are involved in cilia elongation. Depletion of BBS1 and BBS5 proteins result in the most severe phenotype, with PCM1 and BBS4 still localised to centriolar satellites, and centrin and pericentrin are still targeted to the centrosome, but ciliation being dramatically reduced. Once inside the cilium, it is plausible that the BBSsome may act as an IFT cohesion factor responsible for linking IFT-A with IFT-B (fig 1).[44]

SIGNALLING PATHWAYS MEDIATED BY PRIMARY CILIA

It is now understood that IFT not only serves to assemble and maintain the structure of the cilia and flagella, but is also involved in signalling. Cole *et al* showed that mutations affecting IFT particle polypeptides in *C. elegans* result in defects in the sensory cilia located on sensory neurons.[66] Recent studies have suggested that IFT has an important role in vertebrate Sonic Hedgehog (Shh) signalling. Two mouse mutants, wimple (wim) and flexo (fxo), lack ventral neural cell types and show other phenotypes characteristic of defects in Shh signalling. Both mutations disrupt IFT proteins: the wim mutation is an allele of the previously uncharacterised mouse homologue of IFT172, and fxo is a hypomorphic allele of polaris, the mouse homologue of IFT88. Genetic analysis showed that Wim, Polaris and the IFT motor protein Kif3a are required for Hedgehog signalling at a step downstream of Patched1 (the Hedgehog receptor) and upstream of direct targets of Hedgehog signalling.[83] Beales *et al* also investigated the role of ift80 during zebrafish embryonic development, and suggested that it acts downstream of ptc1 (the zebrafish Hedgehog receptor) in the Shh pathway.[74] Cilia are therefore clearly used as sensory organelles in lower eukaryotes, and are likely to have the same role in mammals. Aberrant signalling is also hypothesised as a pathogenesis for MKS. Meckelin is a ciliary surface transmembrane protein (fig 2) with predicted topological similarity to the frizzled family of receptor proteins, which have diverse roles in signal transduction and tissue morphogenesis.

Polycystin-1 and fibrocystin/polyductin are both implicated in signal transduction pathways, albeit with different effects. Loss of polycystin-1 activates a signalling pathway, whereas loss of fibrocystin inactivates a pathway and results in loss of ligand secretion. Polycystin-1 undergoes proteolytic cleavage, resulting in nuclear translocation of cytoplasmic tail. This domain interacts with transcription factor STAT6 and the coactivator P100 to stimulate STAT6-dependent gene expression and epithelial cell proliferation. In intact cilia with normally functioning polycystins, STAT6 is localised along the axoneme. During cyst formation, renal epithelial cells proliferate and eventually replace most of the normal renal tissue, resulting in loss of apical fluid flow and in aberrant mechanosenation. In cyst-lining cells, STAT6 predominantly has a nuclear localisation.[12 84] Fibrocystin is a membrane-spanning protein that also undergoes post-translational proteolytic cleavage. Through the activity of ADAM sheddases, the external domain is released into the extracellular space, where it may act as a ligand for other receptors. In addition, the fibrocystin C-terminus is also cleaved but translocates to the nucleus. Importantly, the proteolysis of fibrocystin is dependent upon the influx of intracellular calcium, a process requiring intact functioning and localisation of the polycystins. Polycystin-2 is an ion channel, the activity of which is necessary for calcium influx during sensation of fluid flow.[11 85 86] This activity may be regulated by its binding partners, polycystin-1 and fibrocystin.[16 65] The function of this complex at the plasma membrane and the cilium may be highly tissue-specific and depend upon the level of expression. High expression is seen in the kidney and may account for the presentation of kidney-specific defects when these proteins are mutated.[87 88] The function of this complex underlies a key mechanosensory role for cilia, which ultimately regulate tissue morphogenesis. Shear stress caused by fluid flow in the kidney tubule causes bending of the cilia at a point where this complex is localised. A conformational change within the complex may activate polycystin-2, resulting in the influx of calcium, which mediates signalling to adjacent cells via intercellular junctions. Alterations in the subtle relationships between polycystins and fibrocystin account for the similar phenotypes when these proteins are mutated in PKD. Dysfunction results in the formation of renal cysts as the cells cannot sense normal mechanical cues. An intriguing observation is that this loss of mechanosensation does not affect ciliogenesis or cilia length, which is a situation also seen with the nephrocystins.[16 67 89 90] The implication of this, alongside the kidney-specific pathologies, is that mutations in these complexes may not affect cilia function in most other organs. However, there is evidence that the function of this complex may be aberrant within other ciliopathies. An association of polycystin-2 with IFT57, which would indirectly associate this complex with the BBS phenotype, has been reported, and localisation with pericentrin at the centrosome/basal body has implications for many other ciliopathies.[59]

CONCLUSIONS AND PERSPECTIVES

Primary cilia are vital cellular components for chemosensation and mechanosensation. Ciliopathies, such as MKS, JBTS and ALMS, are characterised by the complete or partial loss of these cilia, and their expressed phenotypes vary accordingly. The broad spectrum of phenotypes observed in the ciliopathies is indicative of the extensive roles cilia play in the development of many tissue types, including kidney, brain, liver, eyes and bone. Genetic heterogeneity is a common feature in ciliopathies, with mutant genes giving rise to differing disorders, thus making their identification and characterisation all the more elusive. At least 12 genes are associated with BBS, leading to speculation that their products are components of a common pathway or complex. Moreover, it is likely that all of the proteins involved in the ciliopathies are linked through their interactions in the same pathways and complexes. Ciliary proteins tend to exist in complexes at various cellular locations, mediating processes such as the formation of intercellular junctions, cell–cell contacts, and centrosome movement. IFT motors coordinate the transportation of ciliary protein complexes, thereby facilitating cilia growth and maintenance. Mutations in IFT genes result in absent or shortened cilia, but ATD (Jeune syndrome) is

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the only characterised example of this in humans. IFT also appears to be involved in Sonic Hedgehog signalling, any disruption of which can have potentially severe developmental consequences.

A number of well-characterised flagellar proteomes have been described recently (reviewed in Gherman *et al*[6]; also refer to www.ciliaproteome.org). These include the flagellar proteome of the flagellated protozoan parasite *Trypanosoma brucei* (the trypanosome flagellar proteome), a human airway proteome, and the *Tetrahymena* proteome.[91–93] Although primary cilia have not yet been analysed at the proteomics level, a recent study has identified 1968 proteins in the specialised mouse photoreceptor sensory cilium or outer segment.[94] In addition, Pazour *et al*[5] used a comparative genomics strategy to identify 688 genes present exclusively in organisms with flagella and basal bodies, which led to the identification of the *BBS5* gene. These proteomes and datasets will be invaluable for future studies of how cilia are built and maintained, and how these processes are disrupted in human disease. In addition, they will direct future gene identification studies for several developmental disorders that are presumed to be ciliopathies, but for which causative genes have not yet been identified. Probable such ciliopathies include Ivemark syndrome (renal–hepatic pancreatic dysplasia) and, by analogy with ATD, the short rib polydactyly syndromes (SRPSs). Even for the known ciliopathies, the extensive genetic heterogeneity of most of the conditions means that there are still new loci to be mapped and new genes waiting to be identified. The remarkable progress made in the past 5 years guarantees that we can look forward to some exciting insights in the future.

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