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Genes and molecular pathways underpinning ciliopathies

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Abstract

Motile and non-motile (primary) cilia are nearly ubiquitous cellular organelles. The dysfunction of cilia causes diseases known as ciliopathies. The number of reported ciliopathies (currently 35) is increasing, as is the number of established (187) and candidate (241) ciliopathy-associated genes. The characterization of ciliopathy-associated proteins and phenotypes has improved our knowledge of ciliary functions. In particular, investigating ciliopathies has helped us to understand the molecular mechanisms by which the cilium-associated basal body functions in early ciliogenesis, as well as how the transition zone functions in ciliary gating, and how intraflagellar transport enables cargo trafficking and signalling. Both basic biological and clinical studies are uncovering novel ciliopathies and the ciliary proteins involved. The assignment of these proteins to different ciliary structures, processes and ciliopathy subclasses (first order and second order) provides insights into how this versatile organelle is built, compartmentalized and functions in diverse ways that are essential for human health.

Many people are introduced to cilia together with microscopy. A compound microscope — or, increasingly, a smartphone fitted with a ball lens — allows young schoolchildren to see protists such as *Paramecium* using cilia (also known as flagella) to swim. Antonie van Leeuwenhoek first observed these thin ‘nimbly moving feet’, and even early microscopists appreciated that cilia can exist as either solitary or multiple structures on a single eukaryotic cell, and that they can be either motile or immotile (FIG. 1); these criteria are still used to discriminate between different types of cilia.

Like protists, most vertebrate cells have either a single non-motile (‘primary’) cilium or multiple cilia, as found in kidney and olfactory epithelial cells, respectively. Cilia can be either actively motile, as observed at the embryonic node or in sperm, or immotile, as in photoreceptor cells or olfactory neurons. Immotile cilia function in transducing signals from the environment or from other cells, whereas motile cilia propel cells (such as sperm cells) or move extracellular fluids (for example, to clear mucus and debris from the lung) (FIG. 1).

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

The dysfunction of motile or immotile cilia is associated with a wide range of human diseases that are known as ciliopathies (FIG. 2). As the physiological consequences of defects in motile and immotile cilia are different, we discuss these two classes of ciliopathies separately (BOX 1). In addition, we distinguish between first-order ciliopathies, which are caused by the disruption of ciliary proteins, and second-order ciliopathies which result from the disruption of non-ciliary proteins that are required for ciliary function (BOX 1).

Many ciliary proteins are known to have essential roles in human physiology, signalling and development, and their importance is striking when we consider their collective contribution to the ciliopathy disease spectrum. Supplementary information S1 (table) lists 187 genes that have been implicated in 35 established ciliopathies, and at least another 241 genes that have been associated with ciliary structures and/or functions that could potentially result in known or novel ciliopathies if disrupted in humans.

In this Review, we briefly describe the structural features of motile and non-motile cilia, and how they are assembled to form discrete, compartmentalized organelles. We then summarize the functions of these two classes of cilia, and elaborate on how the impairment of different cilium-associated processes and structures — namely, signalling, ciliogenesis, compartmentalization (or gating) and dynamic trafficking — results in ciliopathies with distinct phenotypes. We also discuss evidence that nonciliary proteins influence ciliary functions, and that some ciliary proteins can have non-ciliary roles. Finally, we conclude by describing approaches for identifying the full complement of ciliary and ciliopathy-associated proteins.

Conserved ciliary structures

The first details of ciliary structure were described in the mid-20th century, using transmission electron microscopy (TEM). The ability to resolve subcellular structures led to the realization that cilia from diverse organisms and tissues can look different and can have different accessory substructures, but can also have many structural commonalities.

Basal body and axoneme

The most stereotypical features of cilia are the basal body and the axoneme[1,2] (FIG. 1). The basal body describes the mother centriole when it is associated with a cilium. Most basal bodies comprise a barrel of nine triplet microtubules, subdistal appendages and nine strut-like structures, known as distal appendages or transition fibres, which connect to the membrane at the base of the cilium. The skeleton of the ciliary shaft, or axoneme, consists of doublet microtubules that originate from the basal body. Additional structures that are often found in motile cilia include a central pair of microtubules and axonemal inner and outer dynein arms that power ciliary movement (FIG. 1). Given their intimate relationship[1,2], the basal body and cilium are considered in this Review as a functional unit.

Transition zone: compartmentalisation of signalling and motility functions

To move and/or to function as a signalling device, cilia must contain the motility and/or signal transduction machineries, and must have a composition that is distinct from the rest of the cell[3–5] (FIGS 1,3). Unlike most other organelles, the ciliary membrane is contiguous

with the plasma membrane. To achieve such compartmentalization and to thus maintain the distinct composition of the cilium, the proximal-most region of the axoneme consists of a transition zone (FIG. 1) that controls which proteins can enter and leave the cilium[4,5]. The transition zone features prominent Y-shaped structures that connect the ciliary membrane to the underlying axoneme and that are thought to form or to organize a diffusion barrier for membrane-associated soluble proteins[6–10].

Control of selective entry into cilia probably involves other structural and functional features. For example, septins may form part of the membrane diffusion barrier[11], and the basal body distal appendages may prevent the inappropriate entry of vesicles into the cilia. Furthermore, a sieve-like functionality at the base of the cilium — potentially within the transition zone — controls the access of soluble proteins, restricting the entry of large (~70 kDa) proteins into the ciliary compartment[6,12–14]. It is unclear whether the different gate components have partially overlapping functions or how they help to regulate the entry of distinct ciliary components.

Key stages of ciliogenesis

As early as 1959, a study of vertebrate ciliary photoreceptor biogenesis described the key steps in ciliogenesis[15]. In brief, the mother centriole is modified to become a basal body and attaches to the plasma membrane through its distal appendages; the transition zone (known as the connecting cilium in photoreceptor cells (FIG. 4)) then forms, and finally, the rest of the axoneme extends out from the cell body (FIGS 4,5).

Therefore, the separation of the ciliary compartment from the cell body by the transition zone occurs early during ciliogenesis[4,10] (FIG. 4a). The axoneme is then constructed and maintained by the intraflagellar transport (IFT) machinery[16] (FIG. 5a). The IFT machinery comprises several subcomplexes, including heterotrimeric kinesin-2, which moves anterogradely from the transition fibres to the ciliary tip, and dynein-2, which moves retrogradely to return the IFT complex to the base. The IFT complex that binds to the motors consists of two core subcomplexes, IFT-A and IFT-B, and an accessory module that contains Bardet–Biedl syndrome (BBS) proteins (known as the BBSome) (FIG. 5a); together, these IFT components traffic various ciliary proteins, including α-/β-tubulin ‘building blocks’ of the axoneme and signalling proteins[17,18]. There is some evidence that the transition zone proteins work together with the IFT trafficking machinery to dynamically deliver or to remove ciliary components[19], which warrants further investigation.

Motile ciliopathies

Motile cilia on different cell types have different waveforms and functions[20]. Similar to choanoflagellates, sperm use a specialized cilium (flagellum) to locomote. Other motile cilia do not propel cells but instead move the overlying fluid. Such cilia are found on airway epithelial cells, oviduct cells, ependymal cells that line the brain ventricles, and on the node, which is a transient developmental structure that is crucial for left–right axis determination[21].

Primary Ciliary Dyskinesia (PCD)

Impaired ciliary motility almost exclusively results in a motile ciliopathy (BOX 1) that is known as primary ciliary dyskinesia (PCD)[20] (FIG. 3). PCD results in situs inversus (a left–right patterning anomaly), chronic bronchitis, sinusitis and atelectasis (attributable to defective clearance of lung mucus) and male infertility (owing to defective sperm locomotion)[20]. Less common manifestations of PCD include decreased female fertility (resulting from improper oocyte transport through the oviducts) and a disposition to headaches and hydrocephalus (presumably resulting from impaired cerebrospinal fluid movement by ependymal cilia).

PCD is an inherited autosomal recessive disease. It is usually caused by the impaired formation or function of the inner or outer dynein arms, dynein regulatory complex or central pair, all of which are required for ciliary motility[20], and does not normally affect ciliary signalling (FIG. 1). As in many ciliopathies, PCD displays considerable genetic heterogeneity, and mutations in at least 37 separate loci have been linked to the syndrome (FIG. 3; see Supplementary information S1 (table)). Such heterogeneity probably reflects the complexity of the structures required to generate ciliary motility.

First-order and second-order PCD ciliopathies

Most forms of PCD are linked to genes that encode ciliary components that are directly required for motility, and thus can be considered first-order ciliopathies (BOX 1). However, not all PCD-associated genes encode ciliary proteins. For example, mutations in *DNAAF2, DNAAF3* and *DYX1C1* cause PCD but encode cytoplasmic proteins that are involved in the pre-assembly of axonemal dynein complexes before their import into cilia[22–24]. PCDs that are a result of the loss of these non-ciliary components are examples of second-order ciliopathies (BOX 1).

Sensory ciliopathies

Sensory ciliopathies result specifically from defects in the sensory and/or signalling functions of cilia, and are primarily caused by defects in non-motile cilia (although either motile or non-motile cilia can be involved) (FIG. 3).

Defects in primary cilium structure or signalling cause sensory ciliopathies

Although motile cilia also have sensory capabilities, such as sensing noxious chemicals in the respiratory airway[25], the phenotypic presentations of PCD are distinct from those exhibited when nonmotile primary cilia functions are disrupted. Metazoan non-motile primary cilia have evolved different sensory modalities for environmental cues and intercellular cues. Therefore, defects in primary cilia function lead to more varied sensory, physiological and developmental anomalies than do defects in motile cilia (FIGS 1,3). Sensory ciliopathies have several possible molecular aetiologies, including impaired cilium formation or maintenance; abrogation of ciliary signal transduction pathway components; or trafficking defects that prevent the signalling machinery from being localized to, or removed from, cilia.

Sensory ciliopathies can impair the perception of environmental cues

Different cell types make use of different forms of ciliary signalling. The determinants of this ciliary signalling specificity are only beginning to be understood, but are probably determined by the nature of the signal transduction machinery that is expressed by different cell types.

For example, in the human retina, photoreceptor cells that are responsible for vision express proteins that are specialized for phototransduction. In the photoreceptor outer segment, which is part of a modified cilium[26], light interacts with opsin to activate cGMP-specific phosphodiesterase via the G protein transducin; this reduces intracellular levels of cGMP and leads to the closing of ion channels on the cell membrane. Consequent decreases in the intracellular calcium concentration outside the outer segment then promote neurotransmitter release. Therefore, for vision, the cilium is the site of both signal reception and initial transduction, with the subsequent transmission of the information to the cell body being required for interpretation and communication with other cells.

Similarly, in olfaction[27], an odorant is detected by its G protein-coupled receptor (GPCR) on the ciliary membrane. However, instead of being transmitted via cGMP, the activation of the olfactory receptor stimulates an adenylate cyclase (ADCY3) to generate a different second messenger, cAMP. In further contrast to vision, olfaction uses the G protein Golf and opens ion channels to trigger an action potential. Thus, although the machinery is different, sight and smell rely on conceptually similar mechanisms for signal reception and transduction.

Defects in ciliary signalling through opsins and olfactory receptors are linked to sensory ciliopathies such as retinal degeneration and anosmia (olfaction impairment), respectively[26–28]. Three different molecular aetiologies for a sensory ciliopathy can be observed in retinal degeneration (retinitis pigmentosa (RP)): cilium formation or length control may be impaired[29]; an enzyme that lowers ciliary cGMP concentration may be disrupted[30]; or mislocalization of opsin or other phototransduction components may occur[31,32]. In these cases, the ciliary defects promote apoptotic cell death through a mechanism that is unknown but may involve the accumulation of opsin in the endoplasmic reticulum and the subsequent activation of the unfolded protein response[33].

Sensory defects can also result from anomalies in the structure or signalling functions of olfactory epithelial cell cilia[34]. In olfactory cells, mistrafficking of ciliary proteins does not cause apoptosis but impairs their function, resulting in anosmia. Consistently, anosmia is one hallmark of ciliopathies, such as BBS[35] or those caused by transition zone dysfunction[36].

Impaired ciliary signalling impacts development

Sensory ciliopathies extend beyond defects in interpreting environmental cues. Primary cilia also regulate intercellular signalling pathways (FIG. 1) that, when impaired, result in defects that affect physiology (for example, weight control) or the function of various organs, including the heart, kidney, skeleton and brain (FIG. 2).

Although cilia participate in multiple intercellular signalling pathways (FIG. 1), Hedgehog signalling is one of the pathways that has been most strongly linked to ciliary function.

Hedgehog is a lipoprotein morphogen that participates in the developmental patterning of many vertebrate tissues, including the neural tube and limb buds[37]. We focus below on the Hedgehog signal transduction pathway, as its relationship to cilia function may elucidate general principles by which other signalling systems use cilia. Many components of the Hedgehog signal transduction pathway, including the Hedgehog receptor PTCH1, localize to cilia[38]. Binding of Hedgehog to PTCH1 allows the downstream seven-pass transmembrane protein SMO to accumulate inside cilia, where it converts its transcriptional effectors, the GLI proteins, from repressors to activators[39]. GLI proteins regulate transcription in the nucleus, but also localize to cilia to function in signalling[40,41]. As discussed below, both IFT (trafficking) and the transition zone (gating) have important roles in dynamically modulating the localization of Hedgehog (and other) signalling components to the cilia. Therefore, many developmental abnormalities that are associated with syndromic ciliopathies, such as polydactyly in BBS and the neural tube defects of Meckel syndrome (MKS), may result from compromised ciliary Hedgehog signalling[42].

Other ciliary proteins help to modulate the output of Hedgehog signalling. These include the GPCR GPR161, disruption of which can cause pituitary stalk interruption syndrome, and the EVC–EVC2 complex, dysfunction of which can cause two ciliopathies that are associated with skeletal malformations: Ellis–van Creveld syndrome (EVC) and Weyers acrofacial dysostosis[43–45] (FIG. 3).

Hedgehog signalling in *Drosophila melanogaster* imaginal discs and cuticle does not require cilia, indicating that at least some organisms have evolved ciliumindependent mechanisms for Hedgehog signalling[37]. Cilia form on some sensory neurons in *D. melanogaster*, including those involved in olfaction, and these neurons use cilium-dependent Hedgehog signalling[46]. Thus, within a single organism, a signal transduction pathway can be deployed in both ciliary-dependent and ciliary-independent manners. *In vitro* evidence suggests that mammals may also be able to interpret Hedgehog signals through a cilium-independent mechanism, with different outputs from those of cilium-dependent signalling[47].

Early ciliogenis is linked to ciliopathies

Defects in specific ciliary signal transduction components, such as those required for olfaction, phototransduction and Hedgehog signalling, can result in ciliopathies without impairing cilium structure. However, many ciliopathies are caused by the disruption of a specific aspect of the ciliogenic programme, such as the transcriptional regulation of ciliogenesis, basal body formation and the early ciliogenesis pathway, the formation of the transition zone (ciliary gate), and the trafficking machinery responsible for building the ciliary axoneme.

Initiation of the ciliogenic programme

In metazoans, ciliogenesis is initiated by a transcriptional cascade that involves one or more RFX transcription factors, namely, RFX2, RFX3, RFX4 and RFX7 in vertebrates, DAF-19 in *Caenorhabditis elegans* and Rfx in *D. melanogaster*[48–55]. These transcription factors bind to X box regulatory motifs to activate the transcription of many genes that are required to build cilia[52,55,56]. RFX targets include genes that encode components of the transition zone

and the IFT–BBSome system. The formation of specialized forms of cilia also requires other transcriptional regulators that may cooperate with RFX transcription factors[48]. Examples include forkhead box protein J1 (FOXJ1) for generating motile cilia, GEMC1 (also known as GMNC) and MCIDAS for producing multiciliated cells, the homeobox transcription factor NOTO for generating nodal cilia, and CRX for producing photoreceptors[57–60]. CRX is associated with two retinal ciliopathies: Leber congenital amaurosis (LCA) and cone–rod dystrophy (CRD)[61]. Recently, the TAp73 isoform of TP73 was found to function upstream of RFX and FOXJ1 to contribute to motile multiciliogenesis[62]. At least two proteins that are needed for multiciliated cell differentiation, MCIDAS and the regulator of centriole duplication cyclin O (CCNO), underlie motile ciliopathies[63,64] (FIG. 3; see Supplementary information S1 (table)). Additional regulators of ciliogenesis probably await discovery, and it is possible that mutations in gene-regulatory elements or in non-coding genes (BOX 1) may also cause ciliopathies.

Basal body and initiation of ciliogenesis

Ciliogenesis has long been known to involve basal body docking to the incipient ciliary membrane, after which the transition zone forms and IFT extends the axoneme (FIGS 4,5). Further mechanistic details have described, for example, how binding of a ciliary vesicle to distal appendages may be a prerequisite for basal body migration to the cell surface[65]. More recent analyses of ciliogenesis have uncovered proteins that functionally connect basal bodies via their distal appendages to membranes, including small GTPases that regulate vesicular trafficking (such as RAB8 and RAB11)[66–68], proteins that shape membranes (such as EHD1 and EHD3)[69] and proteins that promote membrane fusion (for example, the exocyst complex)[70,71]. Consistent with their essential roles in an early step in ciliogenesis, five distal appendage components (CEP164, CEP89, CEP83 (also known as CCDC41), FBF1 and SCLT1) are required for ciliogenesis[72–74].

Mutations in genes encoding distal appendage components can cause a variety of ciliopathies. For example, the disruption of *CEP164* or *CEP83* causes nephronophthisis (NPHP)[75,76], a cystic kidney disease, and mutations in *SCLT1* may result in orofaciodigital syndrome (OFD)[77], which is characterized by polydactyly and craniofacial abnormalities (FIG. 4).

Other ciliopathy-associated proteins localize to the distal basal body region and are essential for distal appendage formation or function (FIG. 4). One such protein, HYLS1, is associated with hydrolethalus syndrome, which is a perinatal lethal syndrome that is characterized by hydrocephalus and brain malformation, or the milder ciliopathy Joubert syndrome (JBTS)[78–80]. Other proteins include OFD1 (associated with JBTS, RP, OFD and Simpson–Golabi–Behmel syndrome[81–83]) and C2CD3 (which is linked to OFD[84]). Yet another distal basal body component, TALPID3 (also known as KIAA0586), supports ciliogenesis and underlies some cases of JBTS, hydrolethalus syndrome and short-rib polydactyly syndrome[85].

How mutations in genes encoding either distal appendage components or distal basal body components engender such a pleiotropic range of human syndromes is unclear. Hydrolethalus syndrome may result from strong loss-of-function alleles, as the absence of

distal appendages in mice severely impairs ciliogenesis and is incompatible with life[85]. Other non-lethal ciliopathies are likely to be caused by hypomorphic alleles or mosaicism (BOX 2). For example, null alleles of mouse *Ofd1*, an X-linked gene, are lethal in males but recapitulate many human OFD phenotypes in heterozygous females, which are epigenetic mosaics owing to X inactivation[81].

Centriolar satellites

In addition to the distal centriole, OFD1, C2CD3 and TALPID3 also localize to centriolar satellites[86–88] (FIG. 4). Various centrosomal and ciliary proteins partially localize to these regions, which are found near centrosomes or basal bodies[89]. Like OFD1 and C2CD3, many centriolar satellite proteins are essential for cilium formation[89–91]. However, it remains unclear whether such proteins simply localize within the centriolar satellites before their transit to other locations that are directly relevant to ciliogenesis. Some proteins that are involved in centriole duplication and microcephaly, a disorder that is mainly associated with centrosomal dysfunction and with possible ramifications for ciliary signalling[92,93], also require centriolar satellites[94]. Thus, centriolar satellites have roles that are both relevant to, and potentially independent of, ciliary function.

In summary, basal body-associated defects can compromise cilium formation or function, resulting in diverse ciliopathies that are often characterized by developmental abnormalities (FIG. 4). Numerous basal body proteins that are relevant to cilia function have been identified[95–98], and we anticipate that some of these — as well as centriolar satellite proteins — will be implicated in other ciliopathies (Supplementary information S1 (table)).

Transition zone is a hotspot for ciliopathies

Once the basal body has docked to a membrane, the nascent cilium becomes a separate compartment that is separated from the cytosol by the transition zone[4,5]. Like the distal basal body, components of the transition zone have been extensively implicated in ciliopathies (FIG. 4; see Supplementary information S1 (table)). Transition zone-associated ciliopathies have effects that are generally restricted to single organs, such as effects in NPHP, but can also have pleiotropic effects, as in MKS[4,5].

The transition zone influences ciliary composition

A large network of proteins that are present at the transition zone modulates the composition of the cilium in diverse organisms, including vertebrates, *C. elegans, D. melanogaster* and *Chlamydomonas reinhardtii*[6–10,99]. The MKS complex and the NPHP complex are the two main functional modules of the transition zone, which are associated with, respectively, MKS and NPHP ciliopathies[10,100]. In mammals, the disruption of the MKS complex reduces the ciliary abundance of membraneassociated ciliary proteins, including ARL13B, INPP5E, ADCY3 and the central Hedgehog signal transduction component SMO[8,9,101,102]. As Hedgehog signalling is crucial for specifying digit number and central nervous system development[37], compromised SMO localization to cilia may well be sufficient to account for several of the ciliopathy-associated developmental defects.

Transition zone proteins are also crucial for the ciliary localization of polycystin 2 (PKD2), which is a transmembrane protein that interacts with PKD1 (REF. 9). As mutations in either *PKD1* or *PKD2* cause autosomal dominant polycystic kidney disease (ADPKD) in humans (FIG. 3; see Supplementary information S1 (table)), decreased ciliary localization of PKD2 may account for the kidney cysts in MKS[103]. Therefore, although it is possible to build cilia without a transition zone[9,99], transition zone-associated ciliopathies are probably the result of the altered distribution of one or more ciliary signalling proteins.

Transition zone proteins are also critical for the ciliary localisation of PKD2, a transmembrane protein that interacts with PKD1[9]. As mutations in either *PKD1* or *PKD2* cause autosomal dominant polycystic kidney disease (ADPKD) in humans (Fig. 3; Table S1), decreased ciliary localisation of PKD2 may account for the kidney cysts in MKS[103]. Hence, although it is possible to build cilia without a transition zone[9,99], transition zone-associated ciliopathies are probably due to the altered distribution of one or more ciliary signalling proteins.

Transition zone involvement in ciliopathies

In addition to MKS, mutations in genes that encode components of the MKS complex are associated with JBTS and COACH (cerebellar vermis hypo/aplasia, oligophrenia (mental retardation), ataxia, ocular coloboma, and hepatic fibrosis) syndromes, whereas mutations that affect the NPHP complex also cause Senior–Løken syndrome (SLSN), which is characterized by NPHP with RP (FIG. 4; see Supplementary information S1 (table)). Some mutations may bridge these complexes, as exemplified by the minority of JBTS-affected individuals who have NPHP and RP in addition to the pathognomonic cerebellar defects[104]. Similar to the basal body-associated ciliopathies discussed above, the phenotypic diversity that is caused by transition zone dysfunction may result from alleles of different strengths (BOX 2). Perhaps MKSassociated alleles compromise MKS complex function to such an extent that ciliogenesis is compromised, whereas JBTS-associated alleles spare ciliogenesis but alter ciliary membrane composition (and thus ciliary signalling).

Another non-exclusive possibility to explain the pleiotropy of ciliopathies is the alteration of phenotypic outcomes of Mendelian-inherited ciliopathies by modifiers (BOX 2). For example, the BBSome, NPHP and MKS complexes may have partially overlapping roles in promoting the ciliary localization of membrane proteins, as exemplified by the finding that BBS can be caused by mutations in core transition zone proteins such as MKS1 (BBS13, a subtype of BBS) and CEP290 (BBS14)[105] (FIGS 4,5; see Supplementary information S1 (table)). Thus, modest effects on one complex may phenocopy the consequences caused by the disruption of another complex. In *C. elegans* and mice, disruption of both the MKS complex and the NPHP complex, or both the MKS complex and the BBS complex, has a synthetic (synergistic) effect on the phenotypes[10,102,106]. Whether similar genetic interactions affect the manifestations of human ciliopathies will require careful phenotyping of large pedigrees.

With their moderate level of allelism and easily quantifiable discrete phenotypic outcomes, ciliopathies may represent a particularly tractable ‘sweet spot’ between strictly Mendelian and complex polygenic disorders. For example, genome-wide association studies (GWAS)

cannot account for more than a small proportion of the estimated heritability of polygenic traits, leading to searches for the ‘missing heritability’ (REF. 107). At least in ciliopathies, specific genetic interactions between distinct functional complexes, such as the MKS and NPHP complexes, which GWAS fail to detect, could help to account for this missing heritability (BOX 2). In support of this possibility, NPHPor BBS-affected individuals can have lesions in multiple genes[108,109].

Is the transition zone a lipid gate for ciliary trafficking?

How the transition zone is organized and functions to control ciliary composition is mostly unknown, but will be key to understanding various ciliopathies. A plausible hypothesis is that structural proteins that form the Y-links organize protein complexes at the transition zone membrane (the so-called ciliary necklace) establish a lipid microdomain that is involved in partitioning the ciliary domains from the extraciliary domains[4,99].

The presence of a barrier at the base of cilia implies that there are trafficking systems involved in transiting this partition. One such trafficking system, which we refer to here as lipidated protein intraflagellar targeting (LIFT), is specific for proteins that are modified with lipids (for example, farnesylation and myristoylation). LIFT involves several components, including UNC119, PDE6D, RP2, ARL3 and ARL13B[30,110], and is disrupted in ciliopathies such as RP, rod–cone dystrophy and JBTS (FIG. 5; see Supplementary information S1 (table)). Interestingly, JBTS is associated with multiple transition zone proteins[100,102,111–113], suggesting a functional association between the transition zone and this lipidated protein trafficking system. Another key trafficking system that transits the transition zone is IFT.

IFT–BBSome trafficking defects in ciliopathies—In 1993, the Rosenbaum laboratory observed that in *C. reinhardtii* flagella, particles moved bidirectionally between the basal body and the tip of the axoneme[114]. The machinery involved in this process, IFT, was subsequently found to be powered by kinesin and dynein molecular motors, and to comprise two ‘core’ multiprotein subcomplexes (IFT-A and IFT-B), as well as an associated BBSome complex that mediates ciliary cargo transport[16,18,115] (FIG. 5).

Most IFT subcomplex A/B subunits are linked to ciliopathies

In vertebrates, IFT is essential for cilium biogenesis and, consequently, embryonic development[16,18,116]. Many mouse mutations that affect IFT components cause embryonic midor late-gestation arrest with mispatterning of Hedgehog-dependent tissues, such as the neural tube and limb buds[116]. In humans, mutations in IFT genes that cause ciliopathies often affect the skeletal system (FIG. 5; see mation S1 (table)). Mutations in genes encoding several components of the IFT retrograde motor dynein-2 (DYNC2H1, DYNC2LI1, TCTEX1D2, WDR34 and WDR60) and the IFT-A subcomplex (IFT43, IFT121 (also known as WDR35), IFT122, IFT139 (also known as TTC21B), IFT140 and IFT144) are associated with several skeletal ciliopathies, including Jeune asphyxiating thoracic dystrophy (JATD), cranioectodermal dysplasia (CED; also known as Sensenbrenner syndrome) and short-rib polydactyly syndrome[117–125]. Mutations in IFT subunits are also linked to other diseases, including RP, NPHP and SLSN[126,127] (Supplementary information S1 (table)).

The disruption of several IFT-B subunits is similarly associated with an overlapping subset of ciliopathies (FIG. 5). For example, hypomorphic mutations in *IFT172* result in the skeletal ciliopathies JATD and Mainzer–Saldino syndrome[128], or VACTERL (vertebral anomalies, anal atresia, cardiac defects, tracheoesophageal fistula and/or esophageal atresia, renal and radial anomalies and limb defects) associated with hydrocephalus[129]. Mutations in *IFT52* and *IFT80* also cause skeletal ciliopathies[130,131]. The disruption of *IFT57* is associated with OFD, as well as short stature and brachymesophalangia[132].

To understand the molecular basis of different IFTassociated ciliopathies, researchers are studying how mutations in different IFT subunits cause defects in the transport of specific cargo[16,115]. For example, the IFT-Aassociated protein TULP3 facilitates the transport of specific GPCRs to cilia[133]. LZTFL1 and IFT27, which are both associated with the IFT-B subcomplex, are implicated in the transport of Hedgehog signalling proteins[134,135]. Of note, both LZTFL1 and IFT27 are linked to BBS in humans[136,137].

Possible additional links between IFT proteins and ciliopathies

Mouse models suggest that genes encoding other IFT components are good candidates for orphan ciliopathies (Supplementary information S1 (table)). For example, mouse IFT46 is essential for brain, neural tube and heart development[138]. A hypomorphic mutation in mouse *Ift88* results in kidney cyst formation, suggesting that cilia modulate kidney epithelial growth and organization[139]. IFT components (DYNC2H1, IFT74 and IFT140) were also uncovered in a mouse mutagenesis screen for congenital heart defects[140]. Of note, these were among a high proportion of ciliary genes to be identified, which confirms the importance of motile and non-motile cilia, and the specification of left–right asymmetry, in the origin of congenital heart defects[141,142].

BBS proteins: connecting signalling defects to ciliopathies

BBS arises from the disruption of BBSome components (BBS1, BBS2, BBS4, BBS5, BBS7 and BBS8), or disruption of BBSome trafficking (ARL6; also known as BBS3)[143] or assembly (BBS6, BBS10 and BBS12)[42] (FIG. 5; see Supplementary information S1 (table)). The broad phenotypic range of BBS — which includes retinal degeneration, cystic kidneys, obesity, polydactyly and cognitive impairment — may be explained by its crucial role in the transport of diverse ciliary cargoes.

Three BBS-dependent cargoes are dopamine receptor 1 (DR1)[144], somatostatin receptor 3 (SSTR3)[145] and melanin-concentrating hormone receptor 1 (MCHR1)[145]. Aberrant ciliary protein localization is the probable aetiology of BBS-associated phenotypes; for example, polydactyly may arise from impaired Hedgehog signalling[146]. However, many clinical presentations still have unclear molecular aetiologies and could be multifactorial. For example, obesity in BBS may result from hypothalamic dysfunction and satiety defects owing to the mislocalization of the NPY receptor MCHR1 and, potentially, the mislocalization of the leptin receptor[145,147,148]. Similarly, retinal degeneration may be caused by inefficient opsin trafficking[31].

Regulation of IFT-BBSome trafficking and links to ciliopathies

Understanding the molecular basis of ciliopathies will require a deeper understanding of how IFT particles and the BBSome assemble and function to regulate the trafficking of ciliary cargoes, including GPCRs. Evidence that several IFT and BBSome proteins are evolutionarily related to vesicle coat proteins may be instructive, as protein functions may have parallels to vesicle trafficking[18,149,150]. The study of ciliopathies is likely to identify new core or regulatory players in these processes and to lead to important insights. For example, kinases that influence cilium length by regulating IFT include ICK, MAK and MOK, with ICK associated with lethal endocrine-cerebro-osteodysplasia and shortrib polydactyly syndrome, and MAK associated with RP[151,152] (FIGS 3,5). Mutations in *NEK1* cause short-rib polydactyly syndrome with brain malformations and kidney cysts[153], pointing to an additional possible association between this NIMA-related kinase and IFT. The ciliopathy-associated ciliogenesis and planar polarity effector (CPLANE) complex, which participates in basal body recruitment of the IFT machinery, was recently associated with JBTS, OFD and SRPS[154]; this respresents another example of how the genetics of ciliopathies and cell biological insights into ciliogenesis inform each other.

Second-order ciliopathies

Most ciliogenic and ciliopathy proteins are components of the cilium, basal body or centriolar satellites. We use the term first-order ciliopathies for those associated with these proteins to reflect their local requirement at the basal body or cilium (BOX 1). However, non-ciliary proteins can also participate in ciliary functions and can be associated with ciliopathies. For example, transcription factors (such as RFX2, RFX3 and RFX4) that regulate the expression of ciliary genes are not cilium-localized but are crucial for cilium formation and function[48]. As another example, some ciliary complexes must be pre-assembled in the cytosol before being incorporated into the cilium. The PCD-associated proteins DNAAF2, DNAAF3 and DYX1C1 mediate the cytosolic assembly of axonemal dynein complexes that are crucial for ciliary motility[22–24]. These ciliopathies can be regarded as being secondary (second-order) to ciliary processes (BOX 1).

Second-order ciliopathies will continue to be uncovered. For example, mutations in the gene encoding the Golgi-localized glycosyltransferase GALNT11 perturb Notch signalling and alter motile and non-motile cilia ratios in *Xenopus laevis*, leading to laterality and heart defects[155].

Ciliary proteins with extra-ciliary functions

Given the wide distribution of cilia in extant phyla, the last eukaryotic common ancestor (LECA) probably had cilia with essentially complete IFT–BBSome and transition zone systems[1,156]. Interestingly, there is some evidence that, as metazoans evolved specialized cell types, ancient ciliary proteins acquired novel functions.

For example, EFHC1 is widely conserved in ciliated eukaryotes that have motile cilia and is required for ciliary motility in mammalian cells[157]. However, *C. elegans*, which lacks motile cilia, has an orthologue of EFHC1 (REF. 158), and the *D. melanogaster* EFHC1 orthologue

regulates the morphogenesis of neurons that lack cilia[159]. As in *C. elegans*, mammalian EFHC1 is expressed by cells with non-motile cilia, including those in the brain[160]. The function of EFHC1 in neurons is unclear but important, as mutations in this protein predispose humans to juvenile epilepsy[157]. Thus, the ancient roles of EFHC1 within motile cilia may have been more recently adapted in several non-ciliary functions that are relevant to neuronal or brain function.

Similarly, other ciliary proteins may have acquired extraciliary functions; for example, IFT20 may transport PKD2 from the Golgi to the cilium, and may enable trafficking to the immunological synapse[161,162]. When a cytotoxic T cell, which is unciliated, engages a target cell, its centrosome docks at the cell periphery using distal appendages, similar to those of the basal body. At this subcellular position, the centrosome directs polarized vesicle trafficking to create a functional immunological synapse[163]. Therefore, structures and proteins that have been implicated in cilium function — distal appendages, IFT20, and the small GTPase RAB29 that colocalizes with RAB8, RAB11 and IFT20 — are also associated with immunological synapse assembly and function[164]. At least in the mouse, the disruption of an established ciliary protein — surprisingly, one associated with cilium motility (SPAG6) — impairs immune synapse function[165].

However, the cytotoxic T cell centrosome does not build a transition zone or extend an axoneme, so although there are similarities in the organization of the cilium and the immunological synapse and they use some of the same machinery, there are also pronounced structural and functional differences. It will be interesting to determine whether other cell type-specific centrosome-associated functions represent divergent functions for the ciliary machinery. IFT20 may be particularly versatile in its functions as, in addition to its roles in trafficking cargo within the cilium and to the immunological synapse, it contributes to intracellular transport of collagen[166].

Thus, the analysis of proteins with established roles in cilia may need to take into consideration the possibility that such proteins participate in other cellular processes in both ciliated and non-ciliated cells. Shedding light on the combination of ciliary and cilium-independent functions of proteins may be helpful in explaining the complete molecular aetiology of the associated diseases.

Discovery of ciliopathy-associated proteins

Using the list of manually curated cilium-associated components (the current gold standard) published by the SysCilia consortium as a starting point, we compiled a list of 428 human proteins that are associated with cilia (by localization and/or function), and found that 187 of them are linked to ciliopathies (Supplementary information S1 (table)). Of note, since the publication of this gold standard list in 2013, at least 50 additional cilium-associated proteins have been identified; approximately 50% are linked to ciliopathies, highlighting the crucial importance of cilia in human disease.

Identification of ciliary proteins and ciliopathy candidates

A wide range of complementary studies aimed at uncovering the ‘ciliome’ suggest that additional basal body and ciliary proteins will be discovered, and some of these proteins might be associated with one or more ciliopathies. Such studies, compiled in the CilDB database[167], include proteomics studies of isolated motile and non-motile cilia[97,168,169], comparative genomics studies of ciliated versus non-ciliated organisms[96,150], gene expression studies showing the upregulation of genes during cilium formation or changes in expression in mutants[170,171], and the identification of RFX transcription factor target genes (through bioinformatic searches for X-box regulatory motifs and uncovering genes regulated by the nematode RFX transcription factor orthologue DAF-19)[56,170]. The CilDB database can be searched using Boolean logic for the presence or absence of a given protein in different studies and organisms, and can help to identify candidate ciliary proteins and ciliopathy proteins.

The refinement of the ciliome, which now comprises more than 420 proteins (mostly, but not exclusively human) (Supplementary information S1 (table)), is on-going. Furthermore, studies in model organisms continue to identify ciliopathy candidates and to provide insights into ciliary function. For example, the discoveries that *C. elegans* TMEM-218 functions at the transition zone[172], and that the mouse *Tmem218* mutant exhibits kidney cysts and retinal degeneration[173], suggest that this gene is an excellent candidate gene to underlie SLSN or a related ciliopathy. Interactomes of established ciliary proteins can also identify new ciliary proteins and ciliopathy candidates[100,174].

The various approaches for identifying ciliary proteins all have limitations. TMEM80, for example, was not implicated as a ciliary protein in any study included in the CilDB database before being revealed as a transition zone component, based on its homology to known ciliary proteins (TMEM17 and TMEM216)[172]. Thus, complementary and novel approaches are useful for identifying new ciliary proteins. A promising technique is proximity-dependent protein identification, in which a given protein is fused to an enzyme that can tag (for example, biotinylate) nearby interaction partners for subsequent identification by mass spectrometry[98]. Similarly, model organism genetic or genome-wide RNA interference (RNAi) screens can uncover, in an unbiased manner, new genes that are required for cilia function[140,175,176].

Confirmation of novel ciliopathy genes and ciliopathies

As whole-genome sequencing continues to become more tractable, we expect that novel mutations that are associated with ciliopathies will be readily identified. Baker and Beales[177] predicted in 2009 that more than 72 syndromes were possible ciliopathies. Some of their candidates have since been confirmed to be linked to ciliary dysfunction, including hydrolethalus syndrome, which is caused by mutations in *TALPID3, KIF7* and *HYLS1* (REFS 78,80,85,178,179). The endocrine-cerebroosteodysplasia syndrome was shown to result from mutations in *ICK*[178], which encodes a kinase that is involved in the control of IFT[180]. Walker–Warburg (WWS) syndrome was a suspected but unproven ciliopathy; the B3GNT1 (also known as B4GNT1) glycosyltransferase implicated in this disorder is now known to influence ciliated cell function in *C. elegans*[175]. The 241 candidates listed in

Supplementary information S1 (table) may reveal additional connections to known or novel ciliary disorders. Functional analysis of novel ciliopathy proteins will further increase our knowledge of the signalling, physiological and developmental functions of cilia.

Conclusions and perspectives

The known connections between cilia and human disease will continue to increase, and are likely to include additional diseases that are not specifically — or traditionally — thought of as ciliopathies, such as cancer and congenital heart defects. Ciliopathy research will provide new, valuable insights into the fundamental biology of cilia. Furthermore, the discovery of rare disease variants of essential genes may help to unveil unanticipated roles in ciliogenesis. For example, mutations in the BUBR1 mitotic spindle checkpoint regulator are associated with aneuploidy, cancer predisposition and impaired ciliogenesis[181]. New tools (beyond loss-of-function approaches) may be required to understand whether non-ciliary proteins underlying common diseases, such as cancer, have ciliary functions.

Notwithstanding such important advances, understanding the molecular functions of human ciliopathyassociated proteins, and deciphering their mechanistic roles within a complex, network and pathway, remains challenging, and the use of model organisms to dissect the roles of ciliary proteins and to model the effects of mutations remains essential. Studies in mammalian (mouse) and vertebrate (zebrafish) model systems must continue to be complemented by research in *C. elegans* and *D. melanogaster*, and in ciliated protists such as *C. reinhardtii, Trypanosoma brucei* and *Tetrahymena thermophila*. Connections between clinician scientists and model organism researchers through organizations such as the Rare Diseases: Models & Mechanisms (RDMM) network[182] will help to use human clinical and genomic data to uncover how cilia function in physiology and development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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GLOSSARY

Dynein-2
Molecular motor involved in the retrograde (tip-to-base) transport of the IFT machinery.

Kinesin-2
Heterotrimeric molecular motor required for the anterograde (base-to-tip) transport of the IFT machinery.

Centriolar satellites

Electron-dense puncta found at the periphery of centrosomes or basal bodies. May function as a temporary hub for several proteins that are required for the proper formation and function of cilia.

Exocyst complex
Protein complex involved in targeting Golgi-derived vesicles to the plasma membrane.

Mosaicism
Two or more cell populations with different genotypes in one single individual.

Mother centriole
Centriolar structure that is remodeled into a basal body prior to the onset of cilium formation.

Septins
Proteins which create barriers between different membrane compartments in several contexts, including possibly at the base of cilia.

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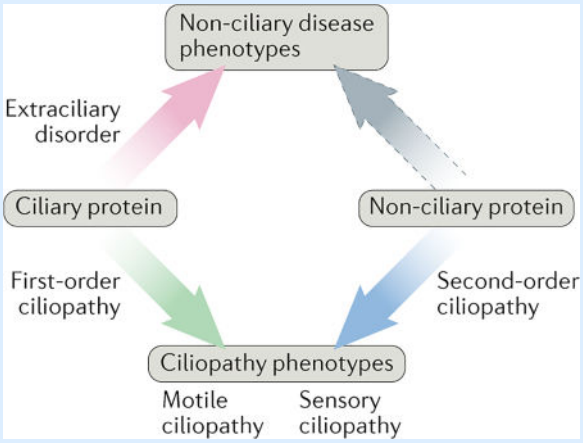
Box 1 | Proposed classification scheme for ciliopathies

The term ciliopathy, which was first used in 1984 (REF. 183) and popularized in the 21st century[184–186], describes human disorders that are caused by ciliary dysfunction. Dysfunction of basal body and ciliary proteins can affect both motile cilia and non-motile primary cilia, separately or together. Non-ciliary proteins can also contribute to ciliopathies, and ciliary proteins can have extraciliary functions that, when impaired, cause phenotypes that are unrelated to ciliopathies.

We propose a flexible classification system to describe the various ways in which ciliary and non-ciliary proteins relate to ciliopathies (see the figure).



First-order and second-order ciliopathies

- First-order ciliopathies: diseases that are caused by the dysfunction of a protein that principally localizes to, and functions within, the basal body and/or the ciliary compartment. For example, the disruption of intraflagellar transport (IFT) components, which are involved in protein transport to and within cilia, can result in the first-order ciliopathy Jeune asphyxiating thoracic dystrophy (JATD).
- Second-order ciliopathies: diseases that are caused by mutations in proteins that are not localized within cilia but that have a role in cilium formation or function. Examples include the cytoplasmic assembly factors for outer arm dyneins that are involved in primary ciliary dyskinesia (PCD). Ciliary defects that are not caused by mutations in protein-coding genes can also be classified as second-order ciliopathies. For example, multicilin (MCIDAS) is a transcription factor that regulates genes that are required for ciliary motility[63]. mlR-34-449, which is a micro RNA that regulates the levels of basal body proteins that are involved in motile ciliogenesis[187], also has a second-order ciliary function, although it has not yet been linked to a ciliopathy).

Motile and sensory ciliopathies

- Motile ciliopathies: disorders, such as PCD, which result from impairment of ciliary motility.

- Sensory ciliopathies: diseases that result from defects in the sensory and/or signalling functions of cilia. Examples include polycystic kidney disease (PKD) and Joubert syndrome (JBTS).

Using this classification scheme, first-order and second-order ciliopathies can be motile or sensory. For example, PCD that is caused by mutations in *DNAAF4* is a second-order motile ciliopathy, whereas PKD is a first-order sensory ciliopathy.

Ciliary proteins with non-ciliary functions that are relevant to disease

- Extraciliary disorder: disruption of a protein with both ciliary and non-ciliary functions causes phenotypes that are unrelated to ciliary function. For example, the role of IFT20 in collagen trafficking, which was discovered in a mouse model[166] of a craniofacial skeletal development disorder, may be ultimately linked to an extraciliary disorder.

Box 2 | The complexity of ciliopathies: multigenicity, allelism, cell type specificity, redundancy and modifiers

As the number of ciliopathy-associated genes grows and the range and overlap between ciliopathy phenotypes increase, it is clear that the relationship between a ciliary gene and a ciliopathy is often more complex than a deterministic, Mendelian one-gene-to-one-phenotype relationship.

For example, a gene can be implicated in multiple ciliopathies with no, or limited, phenotypic overlap. A single gene can be linked to multiple phenotypes if the alleles are of differing strength. For example, presumed nonsense mutations in *CC2D2A* may cause Meckel syndrome (MKS) (MKS6 subtype), whereas missense mutations in the same gene lead to Joubert syndrome (JBTS) (JBTS9 subtype)[188], suggesting that MKS and JBTS are caused by an allelic series that affects the same essential ciliary function. Similarly, different alleles of *TMEM231* are associated with MKS, orofaciodigital syndrome (OFD) and JBTS, even within one family[101,111,189].

Different missense mutations in the same gene can also result in ciliopathies that are associated with distinct ciliary functions. For example, hypomorphic mutations that affect the core IFT-B protein IFT172 result in a skeletal ciliopathy, whereas other mutations cause retinitis pigmentosa (RP) or Bardet–Biedl syndrome (BBS)[190]. An intriguing hypothesis to explain how IFT172 can give rise to disparate ciliopathies is that certain mutations do not impair core IFT-B functions but specifically disrupt the association of IFT172 with the BBSome and thus cause BBS. Mutations in *CEP290* provide another example, as they are associated with JBTS, BBS, Leber congenital amaurosis (LCA), MKS and Senior–Løken syndrome (SLSN)[186,191–193] (Supplementary information S1 (table)). As it is not clear whether these different ciliopathy-associated mutations form an allelic series, it is possible that they affect distinct functions of CEP290 at the transition zone[172,194] and centriolar satellites[88,195] (FIGS 4,5).

Another way in which different mutations in the same gene can result in distinct phenotypes is by affecting protein isoforms that have different functions. For example, disruption of the BBSome-associated protein ARL6 (also known as BBS3) causes typical BBS phenotypes, whereas a longer isoform (BBS3L) is specifically required for photoreceptor maintenance in mice and zebrafish[196].

Additionally, genetic modifiers influence the clinical manifestation of mutations in ciliopathy-associated genes. Such modifiers help to identify genes with overlapping or antagonistic functions. For example, mutations in *RPGRIP1L*, which encodes a transition zone component, are associated with MKS, JBTS and COACH (cerebellar vermis hypo/aplasia, oligophrenia (mental retardation), ataxia, ocular coloboma, and hepatic fibrosis) syndrome[197,198]. Mutations in a paralogue, *RPGRIP1*, cause isolated retinal phenotypes (cone–rod dystrophy (CRD) and LCA)[199,200]. *Caenorhabditis elegans* has only one orthologue of RPGRIP1L and RPGRIP1, which is crucial for transition zone assembly[10,99]. In mammals, *RPGRIP1* and *RPGRIP1L* might have overlapping functions, and non-pathogenic alleles may modify the phenotypes that are caused by the pathogenic alleles. Indeed, components of different complexes (transition zone and BBS)

have overlapping functions in cilium formation in *C. elegans* and mice[10,99,106]. These findings in model organisms indicate that the type of alleles, the modifiers present in different genetic backgrounds, overlapping protein functions and cell-type specificity can all influence the phenotypic outcome, suggesting that similar genetic complexities underlie human ciliopathies.

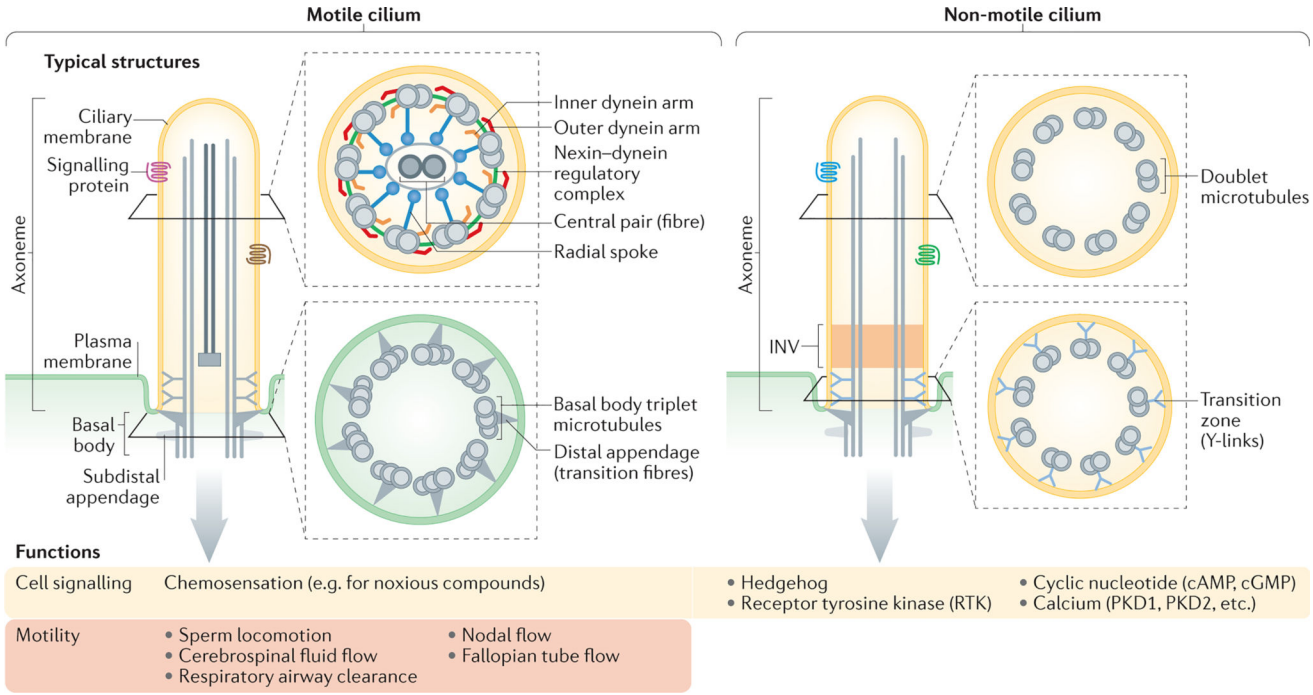


Figure 1. Structures and functions of motile and non-motile cilia

All cilia extend from a basal body that typically consists of triplet microtubules, and subdistal and distal appendages. Distal appendages (also known as transition fibres) tether the basal body to the base of the ciliary membrane. Immediately distal to the basal body is the transition zone, which contains doublet microtubules that are connected to the ciliary membrane via Y-shaped structures. Axonemes (the ciliary backbone) are composed of doublet microtubules. In motile cilia, axonemes usually contain associated structures and proteins (for example, the central pair and axonemal dyneins) that are required for ciliary motility. Nodal cilia are an exception as they are motile but lack a central pair of microtubules. Cilia may contain additional subdomains, including singlet microtubules at the distal end, and regions with specific protein compositions or functions (for example, the inversin domain (INV; involved in signalling). Key cell signalling functions and roles in motility are summarized. PKD, polycystin.

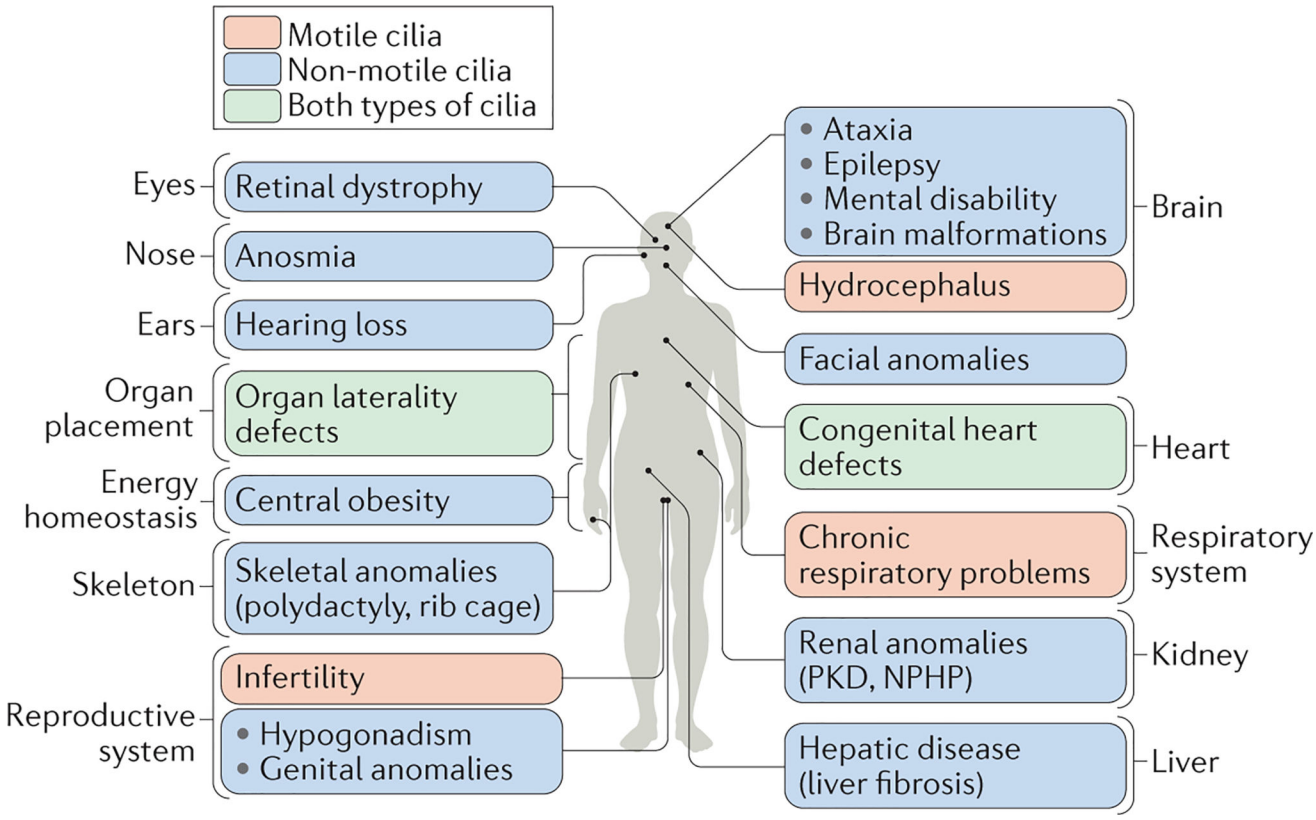


Figure 2. Dysfunctions in motile and/or non-motile cilia cause ciliopathies that encompass most human organ systems

The figure shows the different organ systems or tissues that are affected in diverse ciliopathies, and the principle phenotypic manifestations of the disease in each organ. Ciliopathies that are caused primarily by defects in motile cilia are shown in orange, those that result from defects in non-motile (primary) cilia are shown in blue and those associated with defects in both types of cilia are shown in green. NPHP, nephronophthisis; PKD, polycystic kidney disease.

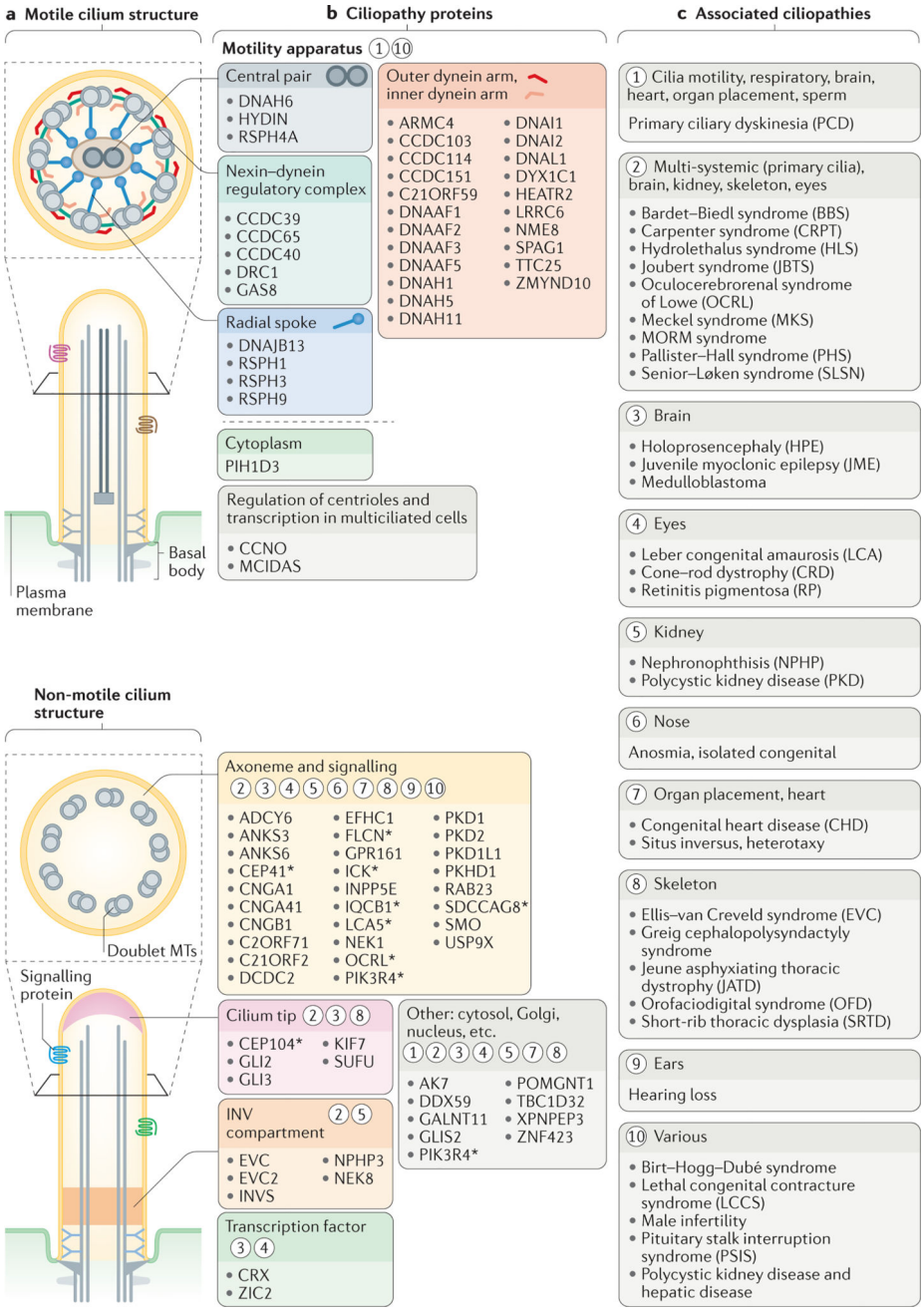


Figure 3. Structural and functional features of motile and sensory cilia are associated with ciliopathies

a | The major structures of motile and non-motile cilia (also see FIG. 1). **b** | Major sites of action for ciliopathy-associated proteins that are components of motile cilia (motility apparatus or transcription factors required for the generation of motile cilia) and sensory cilia (axonemal and signalling proteins, ciliary tip proteins or inversin (INV) compartment proteins). The asterisks indicate proteins that are also localized to other ciliary regions during ciliogenesis (shown in FIG. 4) or ciliary trafficking (shown in FIG. 5). Circled numbers indicate one or more ciliopathies that result from defects in the different ciliary

compartments and proteins. **c** | Ciliopathies grouped into major categories that are associated with the proteins and ciliary regions shown in part **b**.

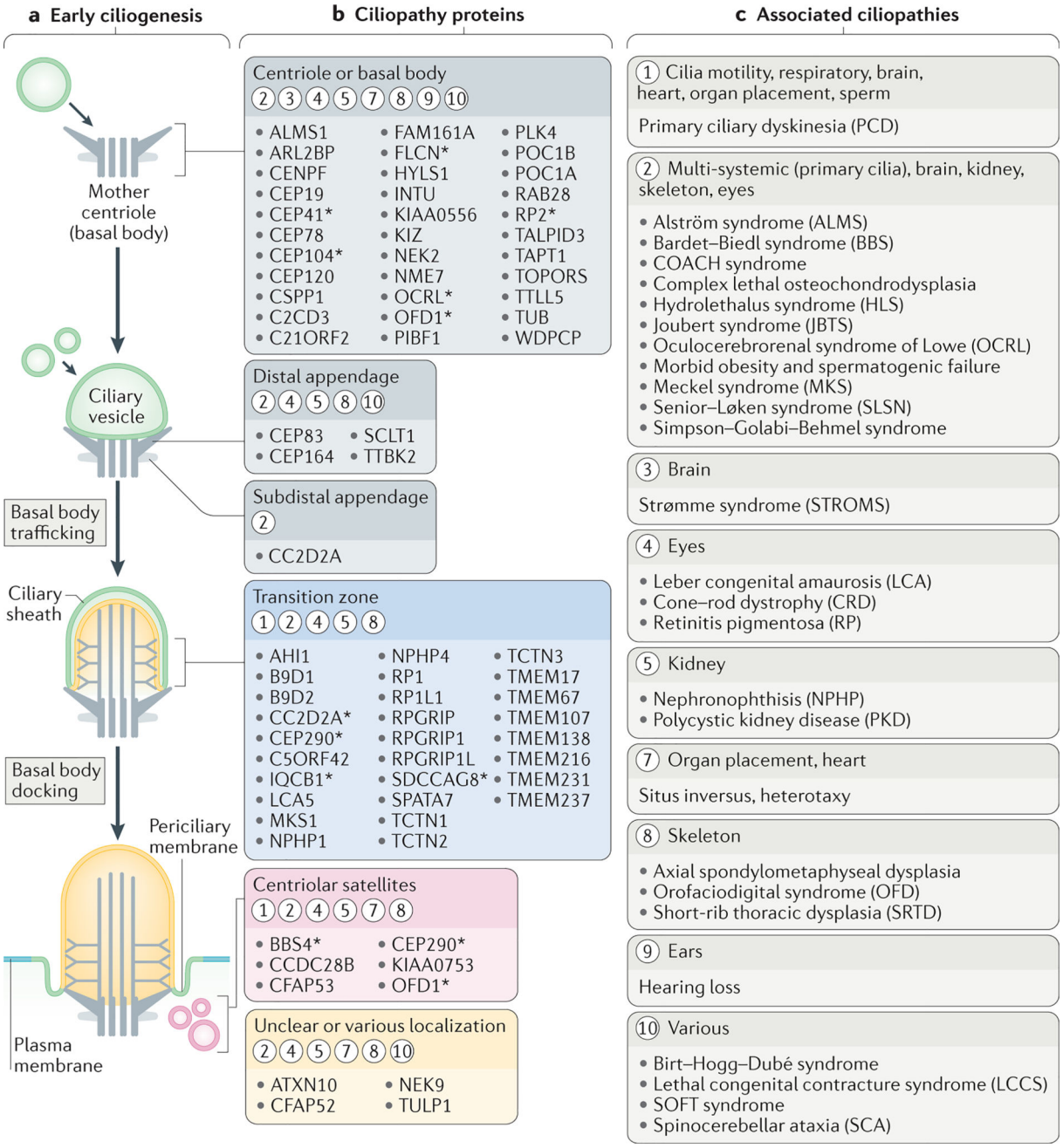


Figure 4. Ciliogenesis and ciliary compartmentalization are associated with ciliopathies

a | The early steps of ciliogenesis. A mother centriole matures into a basal body and migrates towards the plasma membrane. The basal body distal appendages interact either directly with the plasma membrane, or via an intermediary ciliary vesicle (as shown), and the basal body-associated membrane becomes the incipient ciliary membrane. The transition zone is the first ultrastructure of the cilium to form. Centriolar satellites have a role in ciliogenesis, potentially as an intermediate storage compartment for ciliogenic proteins. **b** | Ciliopathy proteins associated with different sub-compartments of the basal body, the centriolar satellites or the ciliary apparatus during and/or after ciliogenesis. Circled numbers

indicate which ciliopathies (listed in part **c**) result from defects in these sub-compartments, as well as the organs, tissues or physiological functions that are affected. The asterisks indicate proteins that are also localized to other ciliary regions during ciliogenesis or ciliary trafficking (shown in FIG. 5). **c** | Ciliopathies grouped into major categories that are associated with the proteins and ciliary compartments shown in part **b**.

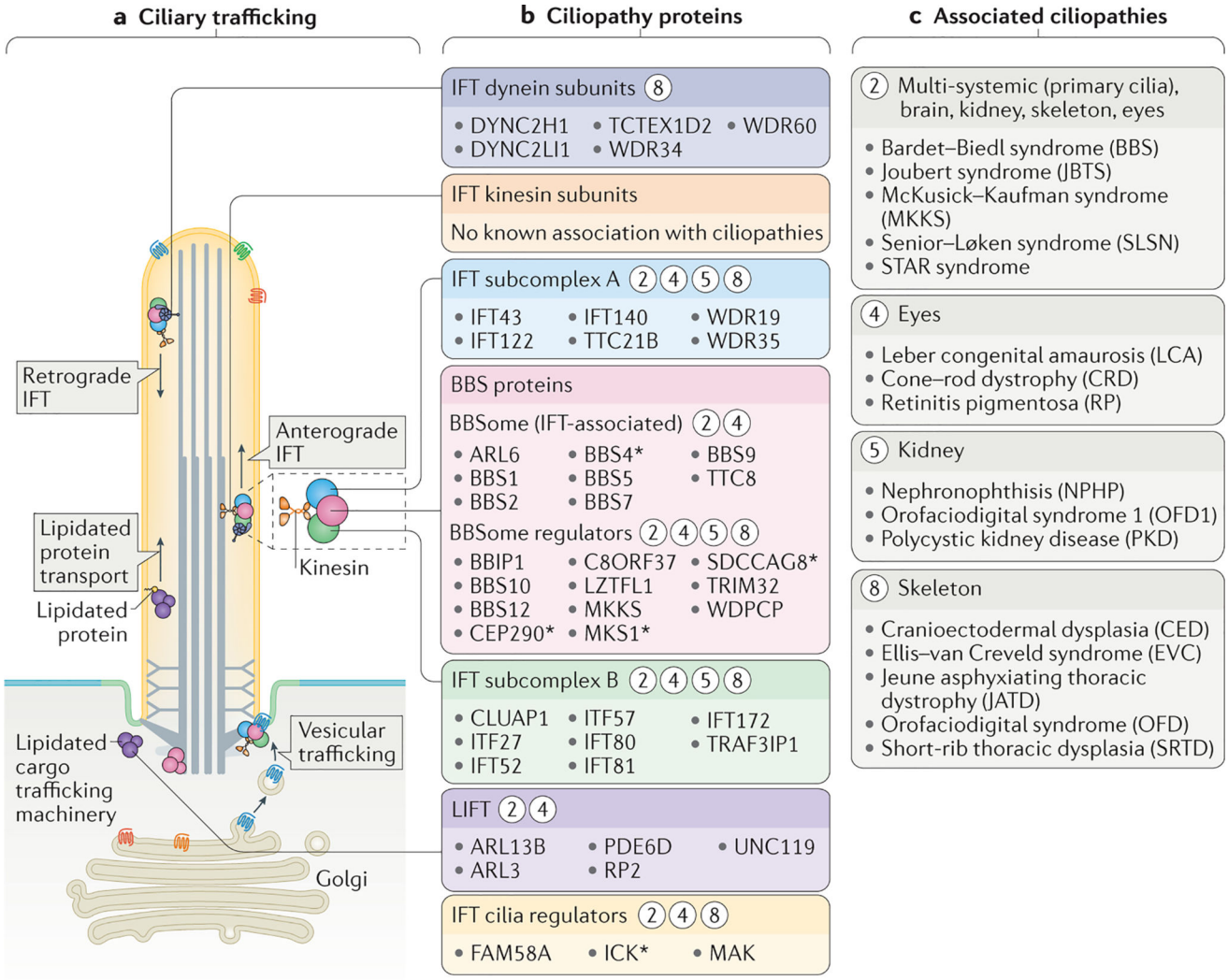


Figure 5. Links between ciliary trafficking and ciliopathies

a | The functional components of two ciliary trafficking pathways: intraflagellar transport (IFT) and lipidated protein intraflagellar targeting (LIFT). Ciliary proteins are trafficked from the Golgi or cytosol to the base of the cilium, after which they are transported into the ciliary compartment. IFT modules that mediate trafficking include anterograde (kinesin-2) and retrograde (dynein-2) motors, IFT subcomplexes A and B, and an accessory module that contains Bardet–Biedl syndrome (BBS) proteins (the BBSome). **b** | Ciliopathy proteins that constitute, or are regulators of, the IFT and LIFT trafficking systems. Circled numbers indicate which ciliopathies (listed in part **c**) result from defects in these ciliary trafficking components. The asterisks indicate proteins that are also localized to other ciliary regions during ciliogenesis (shown in FIG. 4) or ciliary trafficking. **c** | Ciliopathies that result from defects in ciliary trafficking grouped into categories according to the tissues affected.