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Ciliary kinesins beyond IFT: cilium length, disassembly, cargo transport and signaling

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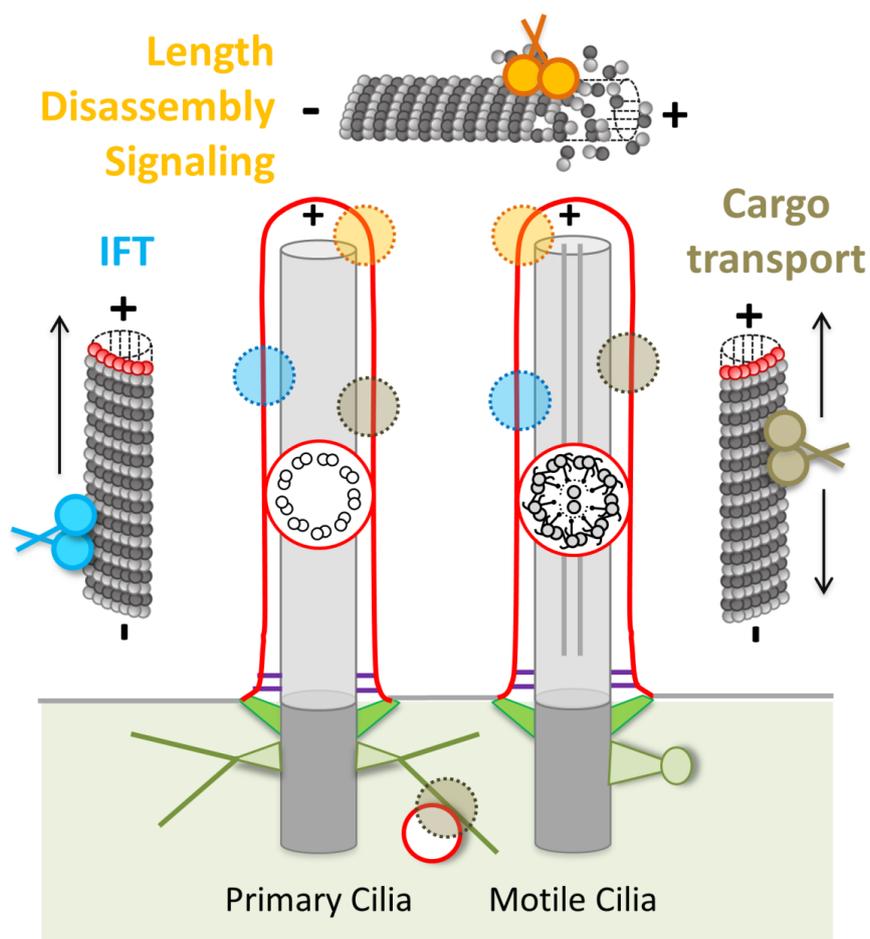
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Ciliary Kinesins Beyond IFT



Cilia are highly conserved, microtubule-based organelles subdivided into motile and non-motile/primary cilia, both playing key roles during development and tissue homeostasis. This review summarizes the ciliary functions of the Kinesin superfamily (KIFs) of microtubule-based motors. Initially implicated in ciliary assembly, KIFs were later shown to be involved in many other key ciliary functions, including cargo transport, signaling (Hedgehog) and the regulation of cilia length and disassembly.

Abstract:

Cilia and flagella are microtubule-based antenna which are highly conserved among eukaryotes. In vertebrates, primary and motile cilia have evolved to exert several key functions during development and tissue homeostasis. Ciliary dysfunction in humans causes a highly heterogeneous group of diseases called ciliopathies, a class of genetic multisystemic disorders primarily affecting kidney, skeleton, retina, lung and the central nervous system. Among key ciliary proteins, kinesin family members (KIF) are microtubule-interacting proteins involved in many diverse cellular functions, including transport of cargo (organelles, proteins, lipids) along microtubules and regulating the dynamics of cytoplasmic and spindle microtubules through their depolymerizing activity. Many KIFs are also involved in diverse ciliary functions including assembly/disassembly, motility and signaling. We here review these ciliary kinesins in vertebrates and focus on their involvement in ciliopathy-related disorders.

Abbreviations

BB: basal body

BBS: Bardet Biedl Syndrome

CSF: cerebrospinal fluid

IFT: intraflagellar transport

KIF: Kinesin family member

PC: primary cilia

PCD: Primary ciliary dyskinesia

SHh: Sonic Hedgehog

Kinesins: microtubule based motors and beyond

Kinesin superfamily (KIF) members are highly conserved proteins characterized by the presence of a motor domain which binds microtubules in an ATP-dependent manner and can be involved in various functions. The motor domain is present at either the N- or C-terminus or in the middle of the protein sequence (Figure 1), defining three structurally and functionally different kinesin families (N-, C- and M-KIFs). Motor domains are usually followed (in the case of N- and M- KIFs) or preceded (in C-KIFs) by both coiled-coil regions, allowing homo- or heterodimerisation, and diverse interacting motifs, promoting binding to very different sets of cargo (microtubules, proteins, lipids). Some Kinesins also interact with accessory, or light chains, which can be involved in cargo binding and or activation of the motor domain (Hirokawa *et al.*, 2009). Kinesins are frequently maintained in an inactive state and require either cargo or light/accessory chain binding or phosphorylation events to release the constitutive inhibition of the motor domain (Verhey and Hammond, 2009).

In the classical motor Kinesins (Kinesins-1/2/3), with an N-terminal motor domain, ATP hydrolysis allows the movement of the kinesin toward the growing plus (+) ends of microtubules in a “walking” like process. C-Kinesins (Kinesin-14) function similarly except that they move towards the minus-end of microtubules, reminiscent of dyneins. Finally, M-Kinesins (Kinesin-13) are not motile and possess microtubule depolymerizing activity, again mediated by the motor domain (Hirokawa *et al.*, 2009). However, there are exceptions to this general classification, such as KIF7 (Kinesin-4), an immotile N-KIF which binds microtubule + ends directly and promotes their disassembly (He *et al.*, 2014; Yue *et al.*, 2018). Additionally, KIF19A (Kinesin-8) presents both plus-end directed motility and depolymerizing activity, both of which are mediated by the motor domain (Niwa *et al.*, 2012; Wang *et al.*, 2016).

These differing functions allow Kinesins to play key roles in various microtubule-dependent processes, including vesicular trafficking and organelle transport (Hirokawa *et al.*, 2009), as well as the organization and dynamics of the mitotic spindle (Cross and McAinsh, 2014). A number of Kinesin family members also have cilia-specific activities. In addition to the well-established role of Kinesin-2 in ciliogenesis (Scholey, 2013), recent works have highlighted the role of many other KIFs in diverse cilia-related functions, including vesicular trafficking, control of cilia length and ciliary disassembly.

Biogenesis of Primary and Motile cilia.

Cilia and flagella are highly conserved cellular structures present in most eukaryotes. They are all assembled from basal bodies (BB), which in vertebrates correspond to the mother centriole of the centrosome, in the case of primary cilia, and to amplified centrioles or BBs, in the case of the motile cilia of multiciliated cells. During ciliogenesis (Figure 2), centrioles dock onto cellular membranes

through their distal appendages (transition fibers) and their A/B tubules extend to form the axoneme, the shared architecture/skeleton of all cilia, composed of 9 peripheral doublets of microtubules. A major structural difference observed between cilia, and frequently associated with the number of cilia present per cell, is the presence of an additional central pair of microtubules. This structural difference, often associated with motility, is easy to observe in transverse sections following electron microscopy. Cilia have subsequently been classified as '9+0' and '9+2' based upon the absence or presence, respectively, of the central pair of microtubules (Satir and Christensen, 2007; Ishikawa and Marshall, 2011).

In vertebrates, or at least in mammalian cells, ciliogenesis occurs primarily in quiescent or post-mitotic terminally differentiated cells. The initial phase of this highly conserved process is the anchoring of the BB to cellular membranes through their distal appendages, which are then called the transition fibers. In most cases, the first membranes onto which BB dock are intracellular vesicles (Sorokin, 1968; Park *et al.*, 2008; Wu *et al.*, 2018). These incoming vesicles (primary ciliary vesicles) will fuse to form the ciliary vesicle and cap the distal part of the BB (Bernabé-Rubio and Alonso, 2017). This step will lead to the removal of CP110, a key regulator of ciliogenesis, allowing the elongation of the axoneme (Sánchez and Dynlacht, 2016). Following the formation of the transition zone, ciliary proteins are actively transported into the ciliary compartment through a highly conserved process, intraflagellar transport (IFT), initially identified in *Chlamydomonas reinhardtii*. During this complex transport, ciliary components (cargo) are captured in the cytoplasmic compartment by IFT complexes which dock onto transition fibers, cross the transition zone and move along doublets of microtubules of the forming axoneme. The subsequent plus-end directed, or anterograde, transport requires Kinesin-2 motor proteins (Scholey, 2013). The BBSome, a complex formed by products of Bardet-Biedl Syndrome (BBS) genes (see below), also participates in the transport of ciliary membrane proteins through interactions with IFT trains. After remodeling at the distal tip of cilia and the dissociation of anterograde cargo, IFT/BBS complexes undergo retrograde IFT transport, requiring cytoplasmic dynein 2 which mediates the recycling of IFT/BBS complexes in the cytoplasmic compartment (Lechtreck, 2015; Nakayama and Katoh, 2018) and the exit of specific cargo (GPCRs, signaling proteins) from the ciliary compartment (Nachury, 2018). These processes are highly conserved among eukaryotes and are responsible for the biogenesis of the great majority of cilia. In addition to IFT and BBSome, the CPLANE complex, which shares some elements with components of the PCP pathway, was recently identified in vertebrates as a regulator of retrograde IFT components required for ciliogenesis (Adler and Wallingford, 2017).

Ciliogenesis is governed by the expression of specific 'ciliary' genes (IFT, BBS) which are controlled by transcription factors from the RFX family, as well as FOXJ1, a master regulator of motile cilia biogenesis (Thomas *et al.*, 2010; Meunier and Azimzadeh, 2016). In addition to the basic

ciliogenesis machinery, an additional set of genes must be activated in the case of centriole biogenesis to allow amplification of centrioles (deuterosome) and the formation of axonemal structures required for motility (dynein arms, radial spokes, central pair).

Ciliary disassembly in vertebrates mainly concerns primary cilia (see below), which can be formed in cells retaining the potential to proliferate (stem cells, neuron progenitors and fibroblasts). When ciliated G₀ cells are stimulated, through serum addition *in vitro*, and re-enter the cell cycle, specific sets of proteins are activated. The activation of these proteins, including kinesin family members, will lead to the depolymerization of axonemal microtubules. This step is required to allow the duplicating centrosome to detach from the plasma membrane, which is then free to participate in the assembly of the mitotic spindle (Sánchez and Dynlacht, 2016).

Cilia/ciliopathies subtypes:

Primary cilia (PC) are sensory cilia with key roles during development in vertebrates. They were called 'primary' by Sorokin as they are the first to appear during development, even in cells which will assemble multiple motile cilia (Sorokin, 1968). They are characterized by a '9+0' axoneme and a BB composed of two centrioles, corresponding to the unique centrosome of the cell. They can be divided in two groups depending upon the presence of a ciliary pocket at their base (Ghossoub *et al.*, 2011). However, the specific functional consequences associated with the presence or absence of the ciliary pocket remains to be fully determined (Pedersen *et al.*, 2016).

Sensory cilia were first characterized in photoreceptors. The photoreceptor outer segment corresponds to a modified cilium which is connected to the inner segment through the connecting cilium (DE ROBERTIS, 1956). Based on these observations, it was suggested that similarly organized cilia found in other tissues may play sensory or signaling functions (BARNES, 1961). Support for these suggested roles came from the establishment of the link between dysfunctions of '9+0' cilia and complex human genetic diseases, subsequently termed 'ciliopathies' (Badano *et al.*, 2006b). Studies in the last 25 years have since provided strong evidence that PC control numerous key signaling pathways during development and tissue homeostasis (Wheway *et al.*, 2018). In addition to the sensation of urine flow in renal epithelial cells, the archetype of these cilia-dependent pathways is Sonic Hedgehog (SHh). While most tissues in *Drosophila* are unciliated and thus Hh signaling is independent of cilia, this is not the case in vertebrates. Here, the complex in and out trafficking events of SHh signaling components control the downstream activation or downregulation of the pathway. This control is effected by the activation or generation of repressor forms of the GLI2 and GLI3 transcription factors (Bangs and Anderson, 2017).

Ciliopathies, linked to dysfunctions of primary/sensory cilia, were initially identified in the context of polycystic kidney disease (Barr and Sternberg, 1999; Pazour *et al.*, 2000). Cystic kidney

was, therefore, one of the first manifestations identified as a cilia-related phenotype. Many phenotypes have subsequently been associated with PC ciliopathies (see below) and the broad spectrum of affected organs reflects the presence of a PC in almost all vertebrate cell types and their key functions during development and/or tissue homeostasis. The notion of a “ciliopathic” disorder was first attributed to Bardet–Biedl syndrome (MIM #209900, BBS), a condition characterized by retinal dystrophy, cystic kidneys, polydactyly and obesity. Central nervous system defects are also observed in ciliopathies highlighting the important role of cilia during brain development (Gomez-Gamboa *et al.*, 2014; Thomas, Reilly and Benmerah, manuscript in preparation). Most if not all the genes mutated in these PC-Ciliopathies encode ciliary proteins, which can be classified in functional subdomains. Meckel and Joubert syndromes are largely caused by mutations in genes encoding transition zone proteins, while BBS results from mutations in genes encoding BBSome components, and skeletal ciliopathies from mutations in genes encoding retrograde IFT and cytoplasmic dynein 2 subunits (Huber and Cormier-Daire, 2012; Braun and Hildebrandt, 2017; Reiter and Leroux, 2017).

Motile cilia are present at the apical surface of epithelial cells in several tissues, including the upper airways (bronchus, nasal epithelium), fallopian tubes and lateral ventricles (ependymal cells). Their coordinated beating is based on planar cell polarity signaling and is required for the movement of fluids present at the apical surface of these multiciliated cells (Meunier and Azimzadeh, 2016). Motile cilia are usually characterized by a ‘9+2’ axoneme and the presence of additional structures required for motility, including dynein arms (inner and outer), radial spokes and nexin links, as well as by intra-doublet structures such as tektin filaments (Figure 2). There are, however, non-motile ‘9+2’ cilia (olfactory neurons, hair cells) which lack accessory structures, and motile ‘9+0’ cilia (the node in mammals, Kupffer’s vesicle in zebrafish) which present dynein arms. When involved in the motility of the cell upon which they are present, motile cilia are called flagella (sperm cells). Flagella of sperm cells present a modified BB along with additional structures along the axoneme involved in motility (Satir and Christensen, 2007).

Primary ciliary dyskinesia (PCD) is linked to motile cilia dysfunction, including perturbed motility and/or a reduction in the number of motile cilia at the surface of ciliated cells. PCD comprises chronic airway diseases, otitis media and sinusitis, all of which are linked to motile cilia defects in the airways, and male infertility. In Kartagener syndrome, these manifestations are associated with *situs inversus*, which is caused by defects of node cilia and can lead to congenital heart disease. PCD results from mutations in genes encoding proteins required for motile cilia function or biogenesis, including dynein arms, radial spoke components and transcription factors, as well as cytoplasmic chaperones required for the assembly of dynein arms (Mitchison and Valente, 2017; Reiter and Leroux, 2017).

The identification of the cellular machineries required for ciliary assembly and regulation were made in several models, including cultured mammalian cell lines and two key model organisms, the biflagellated green algae *Chlamydomonas reinhardtii* and the worm *Caenorhabditis elegans*. Many of the actors involved in motile and sensory cilia assembly and regulation were initially described in these two organisms, respectively. In addition, both mouse and zebrafish have been used to generate models of ciliopathies and to further characterize the role of cilia subtypes and ciliary proteins in vertebrates (Vincensini *et al.*, 2011). Although the phenotypes in mouse models are frequently reminiscent of those found in human ciliopathies, the use of zebrafish larvae has been crucial to characterize the function of a number of ciliary genes (Song *et al.*, 2016). In addition to various experimental advantages, zebrafish have recently revealed the role of motile cilia in development of the spine, implicating, therefore, motile cilia dysfunction in scoliosis (Grimes *et al.*, 2016). Additionally, a ventrally curved body axis, known to be a ciliopathy-associated phenotype in zebrafish but for which the etiology was not clearly understood, has recently been linked to the crucial role of motile cilia in the organization of the Reissner fibre. The Reissner fibre is a component of the cerebrospinal fluid in vertebrates, including mammals, and is essential for the morphogenesis of a straight posterior body axis (Cantaut-Belarif *et al.*, 2018).

The key role of Kinesins at cilia was first identified through the observation of the requirement of Kinesin 2 family members for IFT in *Chlamydomonas* (Kozminski *et al.*, 1995). Additional Kinesin family members were subsequently shown to be involved in regulating cilia length, ciliary disassembly and to have motile cilia-specific functions. Here, we have focused on studies performed using vertebrates and mammals in relation to human ciliopathies and diseases (Table 1).

IFT transport:

The role of Kinesin-2 in anterograde IFT was comprehensively reviewed elsewhere (Scholey, 2013; Lehtreck, 2015) and will be described very briefly here. While both PC and motile cilia assembly require functional IFT, and therefore the KIF3A/B anterograde motor, the role of KIF17 in vertebrates remains to be clearly determined.

KIF3A/KIF3B/KAP3 (Kinesin-2):

Heterotrimeric Kinesin-2, composed of homo- or heterodimers of KIF3A and KIF3B associated with the accessory subunit KAP3, mediates anterograde IFT through interactions with IFT-B subunits (Scholey, 2013; Nakayama and Katoh, 2018). Initially shown in *Chlamydomonas*, the essential role of kinesin-2 in anterograde IFT and ciliogenesis was also later demonstrated in many different

organisms, including mouse and zebrafish. KIF3C, which is able to dimerize with both KIF3A and KIF3B, is not required for ciliogenesis. Members of this subfamily have also been implicated in vesicular trafficking (Hirokawa *et al.*, 2010; Scholey, 2013). However, despite contributing to the transport of post-Golgi vesicles and endosomes, the potential role of Kinesin-2 in the trafficking of the primary ciliary vesicles has not yet been investigated.

KIF17 (kinesin-2):

Kif17 functions as a homodimer and was shown to mediate anterograde IFT along the distal singlets of microtubules within the sensory cilia of the worm, and to be required for the assembly of this singlet only region (Snow *et al.*, 2004). However, the ciliary function of KIF17 in vertebrates remains puzzling (Scholey, 2013). While the morpholino-mediated KD of *kif17* in zebrafish was shown to lead to retinal degeneration (Insinna *et al.*, 2008), the invalidation of *KIF17* in both zebrafish (Zhao *et al.*, 2012) and mouse (Yin *et al.*, 2011; Jiang *et al.*, 2015) did not lead to any significant ciliary phenotypes besides a small delay in outer segment development in Zebrafish (Lewis *et al.*, 2017). Recent works also indicate that the activity of KIF17 controls outer segment turnover (Lewis *et al.*, 2018). Although singlet microtubule regions were identified in the distal segments of sensory cilia in mammals, including olfactory neurons and in the outer segment of photoreceptors (Falk *et al.*, 2015), the singlet specific transport function of KIF17 does not appear to be conserved in vertebrates. KIF17, along with heterodimeric kinesin-2, has been shown to participate in IFT along the entire axoneme of olfactory cilia (Williams *et al.*, 2014). KIF17 was additionally implicated in the transport to the cilium of several membrane proteins with important functions in signaling (Jenkins *et al.*, 2006; Leaf and Von Zastrow, 2015). Kif17, along with KIF3A/3B, plays a well-documented role in vesicular transport in the dendrites of neurons, which has been shown to be involved in memory (Yin *et al.*, 2011; Scholey, 2013). Again, a link between these vesicular transport-related functions and its roles at cilia is still missing.

PC-specific Kinesins

Axonemal microtubule dynamics:

In addition to their role in IFT, several KIFs have more recently been shown to function in regulating the length of the axoneme or in the disassembly of the axoneme during cell cycle re-entry. While such roles could be expected for members of the Kinesin-13 subfamily, known to have microtubule depolymerizing activities, these functions were only demonstrated more recently for the Kinesin-4 subfamily. Described below are KIFs which appear to play a role at PC. In addition to these PC-specific depolymerizing KIFs, another set of KIFs appear to regulate the length of motile cilia and are described thereafter.

KIF7 (Kinesin-4):

The ciliary and SHh-related roles of KIF7 have recently been reviewed elsewhere (Bangs and Anderson, 2017). Briefly, KIF7 is one of the two Costal2 orthologues in mammals (with KIF27, see below) and is a key actor of PC-dependent SHh signaling in vertebrates (Cheung *et al.*, 2009; Endoh-Yamagami *et al.*, 2009; Liem *et al.*, 2009; Wilson *et al.*, 2009). Mutations in *KIF7* were identified in ciliopathies characterized by developmental brain anomalies linked to impaired SHh signaling (Dafinger *et al.*, 2011; Putoux *et al.*, 2011, 2012). Initially thought to be involved in the intraciliary transport of SHh signaling components (GLI2, SuFu), KIF7 was recently shown to lack motor activity. Instead, KIF7 possesses microtubule depolymerizing activity and, therefore, negatively controls the length of axonemal microtubules in order to organize a SHh signaling platform at the homogenously-sized distal ends of ciliary microtubules (He *et al.*, 2014).

KIF2A (Kinesin-13):

KIF2A is the prototype of the Kinesin-13B subfamily which plays various roles in mitotic spindle dynamics/organization (Walczak *et al.*, 2013). KIF2A was shown to be involved in the disassembly of cilia upon cell cycle entry through a PLK1-mediated activation of its depolymerizing activity (Miyamoto *et al.*, 2015). Loss of *Kif2A* in mice leads to severe brain defects and dominant *KIF2A* mutations in humans lead to microcephaly (Poirier *et al.*, 2013), due, at least partially, to the cell cycle delay in neuron progenitors resulting from cilia disassembly defects (Broix *et al.*, 2018). Intriguingly, besides brain anomalies, loss of *Kif2A* in mouse does not lead to obvious ciliopathy phenotypes, suggesting that its role in ciliary disassembly might be brain specific. It may also reflect a specific requirement for ciliary disassembly in neuron progenitors which are among the very rare ciliated cells which are also highly proliferative, requiring fast ciliary assembly/disassembly cycles (Thomas, Reilly and Benmerah, manuscript in preparation).

KIF24 (Kinesin-13):

KIF24 is the unique member of Kinesin-13A subfamily. It is a centriolar protein which was initially identified as a partner of CP110. KIF24 is required for the recruitment/stabilization of CP110 at the distal end of the mother centriole and therefore negatively regulates the early steps of ciliogenesis (Kobayashi *et al.*, 2011). KIF24 was also shown to possess microtubule depolymerizing activity, specifically targeting centriolar microtubules (Kobayashi *et al.*, 2011). This depolymerizing activity is in fact triggered during cell cycle reentry via phosphorylation by NEK2 and contributes to cilia disassembly (Kim *et al.*, 2015). Of note and surprisingly, KIF24 is not conserved in zebrafish. The effects of the *in vivo* invalidation of KIF24 have not yet been investigated. Interestingly, loss of CP110

in mouse causes severe developmental defects reminiscent of skeletal ciliopathies (Yadav *et al.*, 2016), making both *CP110* and *KIF24* good candidate genes for skeletal ciliopathies in humans. Finally, *KIF24* presents a SAM domain in the N-terminal region. The role of this domain within *KIF24* has not yet been investigated but could potentially establish functional links with other SAM domain-containing proteins involved in ciliopathy-related disorders, including *BICC1*, *ANKS6* and *ANKS3* (Kraus *et al.*, 2012; Hoff *et al.*, 2013; Shamseldin *et al.*, 2016).

Recently identified PC-specific kinesins.

In addition to IFT and depolymerizing kinesins involved in PC assembly/disassembly cycles, recent research has identified several new Kinesin family members involved in diverse functions at PC.

KIF5B (Kinesin-1):

Kinesin-1 family members are the prototypes of dimeric motor kinesins and have been shown to be responsible for the movement of many different cargos (endosomes, lysosomes, nuclei) along cytoplasmic microtubules (Hirokawa *et al.*, 2010). While *KIF5B* is ubiquitously expressed, *KIF5A* and *KIF5C* are brain specific (see below). The role of *KIF5B* at cilia was identified based on an interaction with the product of *MGC1203/CCDC28* (Novas *et al.*, 2018), a modifier of BBS (Badano *et al.*, 2006a). *CCDC28* interacts with BBSome subunits (Badano *et al.*, 2006a) and with *SIN1* (Cardenas-Rodriguez *et al.*, 2013), an mTORC2 subunit, to positively regulate cilium length. *KIF5B* and its associated light chain (*KLC1*) appear to negatively regulate *CCDC28*, and, therefore, their downregulation result in longer cilia. *KIF5* and *KLC1* were both shown to localize to the BB regions and could not be detected in the cilium (Novas *et al.*, 2018). It is currently unclear whether they present an intraciliary IFT motor-like role, or if they are involved in the cytoplasmic transport of *CCDC28* and partners. Invalidation of *KIF5B* in the mouse is embryonic lethal, although whether the mutant embryos presented with ciliopathy-related phenotypes was not described (Tanaka *et al.*, 1998). In Zebrafish, *KIF5B* isoforms were involved in cartilage remodeling and maintenance during craniofacial morphogenesis, but without an obvious connection to cilia-related function (Santos-Ledo *et al.*, 2017). Loss of *KLC1* in the mouse leads to brain specific defects which were not related to cilia (Rahman *et al.*, 1999).

KIFC1/HSET (Kinesin-14):

KIFC1 is a member of the Kinesin-14 family and was shown to play an important role during mitotic spindle assembly through microtubule bundling and sliding activities (Cross and McAinsh, 2014). A recent study has shown that *KIFC1* is required for the early steps of ciliogenesis, before the removal of *CP110* from the mother centriole (Lee *et al.*, 2018). In addition, this study also showed that *KIFC1*

interacts with ASAP1, implicated in the transport of membrane proteins from the Golgi apparatus to the outer segment in photoreceptors (Mazelova *et al.*, 2009). Taken together these data indicate that KIFC1 may participate in the delivery of ciliary vesicles to the mother centriole during the early steps of ciliogenesis and suggest a general role in Golgi to PC trafficking. KIFC2 and KIFC3, which were previously implicated in vesicular transport and Golgi organization (Hirokawa *et al.*, 2010), do not seem to play similar ciliary functions (Lee *et al.*, 2018). Interestingly, KIFC1 was also involved in the transport of endosomes through an interaction with KIF5B (Nath *et al.*, 2007) suggesting that they might functionally interact during ciliogenesis. To our knowledge, there is currently no published study on the impact of the invalidation of *KIFC1* on cilia *in vivo*, however invalidation in the mouse does not appear to lead to an obvious phenotype (Hirokawa *et al.*, 2010). Similarly, no phenotype is evident upon invalidation of *KIFC2* or *KIFC3* (Yang *et al.*, 2001b, 2001a; Xu *et al.*, 2002), suggesting a possible compensation between Kinesin-14 family members *in vivo*.

KIF13B/GAKIN (kinesin-3):

KIF13B/Gakin is a member of the large Kinesin-3 family which was previously involved in endosomal transport (Hirokawa *et al.*, 2010) as well as in caveolae-mediated trafficking (Kanai *et al.*, 2014). A role at PC was identified based on the high expression in serum-starved fibroblasts and was recently extensively reviewed elsewhere (Morthorst *et al.*, 2018). Briefly, KIF13B localizes to the BB and axoneme and has been shown to interact with NPHP4, a key component of the transition zone. KIF13B organizes a caveolin-1 domain at the base of cilia which appears to be required for efficient ciliary trafficking of smoothed and downstream SHh signaling (Schou *et al.*, 2017). Invalidation of *Kif13b* in the mouse does not lead to an obvious phenotype (Kanai *et al.*, 2014). Interestingly, KIF13B is close to the mammalian orthologue of Klp-6, which was identified in the worm as a cilia subtype-specific kinesin involved in IFT regulation and targeting of PKD2 to cilia and extra-cellular vesicles (Peden and Barr, 2005; Wang *et al.*, 2014). The rat KLP6 was involved in the transport of mitochondria (Tanaka *et al.*, 2011), although a putative ciliary function was not investigated. The closest orthologue of Klp-6 in human, *KIF28*, is considered a pseudo gene.

Potential additional PC-specific KIFs?

KIF11/EG5 (Kinesin-5):

KIF11/Eg5 is a key mitotic kinesin required for the assembly of a bipolar mitotic spindle (Sawin *et al.*, 1992). The crucial role of KIF11/Eg5 during development was first identified in *Drosophila* where the loss of KLP61F results in strong defects in proliferative tissues, including in the larval brain (Heck *et al.*, 1993). Invalidation of *Kif11* in the mouse results in an early block in embryogenesis, prior to the

implantation stage (Castillo and Justice, 2007; Chauvière *et al.*, 2008), whereas in zebrafish, it leads to a severe delay in brain development (Amsterdam *et al.*, 2004) linked to the mitotic block (monopolar spindle) and apoptosis of progenitors (Johnson *et al.*, 2014). The latter results in zebrafish suggest a shared and important role during brain development in vertebrates. In humans, autosomal dominant mutations in *KIF11* were identified in individuals presenting with microcephaly with or without chorioretinopathy, lymphoedema, or mental retardation (MCLMR; MIM #152950; (Ostergaard *et al.*, 2012)). Despite recent evidence for non-mitotic functions related to vesicular trafficking (Wakana *et al.*, 2013), there was no strong evidence for a potential role of *KIF11* at cilia and its key role in spindle assembly was sufficient to explain the delay in the proliferation of neuron progenitors and subsequent microcephaly. However, it was recently shown that the spectrum of *KIF11*-associated retinopathy includes a new form of retinal ciliopathy and that *KIF11* localizes to the connecting cilium of photoreceptors suggesting a potential function at sensory/primary cilia and that *KIF11*-related diseases represent syndromic ciliopathies (Birtel *et al.*, 2017). The potential ciliary function for *KIF11* in neuron progenitors and its involvement in the *KIF11*-associated microcephaly remains an open question.

KIF14 (Kinesin-3):

KIF14 is a member of the kinesin-3 subfamily and was classified as an N-Kinesin despite the presence of an N-terminal extension which binds PRC1 (Gruneberg *et al.*, 2006), a key component of the central spindle. *KIF14* is expressed in mitotic cells and is required for cytokinesis (Carleton *et al.*, 2006; Gruneberg *et al.*, 2006). Invalidation of *KIF14* in rodents leads to severe microcephaly and growth retardation but no ciliopathy-like phenotypes (Fujikura *et al.*, 2013). In humans, biallelic recessive mutations in *KIF14* (Moawia *et al.*, 2017; Makrythanasis *et al.*, 2018) were recently identified in individuals with mild to very severe microcephaly. The pathogenic effects of the mutations were thought to be linked to the major role of *KIF14* at the midbody and to impaired cytokinesis. However and interestingly, loss of function mutations in *KIF14* in humans cause a syndromic microcephaly associated with cystic renal hypodysplasia (Filges *et al.*, 2013; Heidet *et al.*, 2017). In addition, as the presence of cystic kidneys and developmental brain defects in the affected fetuses were reminiscent of Meckel-Grüber syndrome, *KIF14* was therefore termed *MKS12*. These observations suggested a potential link between *KIF14* and PC, however, our recent works failed to establish such a functional link (Reilly *et al.*, 2018).

Motile cilia Kinesins

In addition to the shared function of Kinesin-2 family members in the IFT-dependent formation of cilia, other Kinesins appear to play specific functions in motile cilia. Interestingly, the roles of most of

these KIFs in motile cilia in vertebrates were identified or confirmed in screens aiming to identify targets of FOXJ1.

KIF27 (Kinesin-4):

KIF27 is a mammalian-specific paralogue of KIF7, the two mammalian orthologues of Costal2 (Wilson *et al.*, 2009). KIF27 was, therefore, expected to play a major role in SHh signaling in vertebrates, resembling KIF7 (see above). Surprisingly, KIF27 is not conserved in zebrafish (Wilson *et al.*, 2009), and invalidation in the mouse did not lead to the expected SHh phenotypes (Chen *et al.*, 2005), indicating that it is unlikely to play a conserved role in this pathway. Interestingly, the unique Costal2 orthologue in planarians is not involved in SHh signaling, but is required for ciliogenesis of motile cilia (Rink *et al.*, 2009). Similarly, loss of *Kif27* in the mouse leads to PCD-like phenotypes, including sinusitis, otitis media and hydrocephalus (Vogel *et al.*, 2012), suggesting a motile cilia-specific role in mammals. KIF27 localizes to BB in tracheal epithelial cells (Wilson *et al.*, 2009) and oviduct epithelial cells (Nozawa *et al.*, 2013), but a precise function in motile cilia was not further investigated. Interestingly, and reminiscent of Costal2 in *Drosophila*, KIF27 interacts with Fused/Stk36, which, again, is not involved in SHh signaling in vertebrates. Fused is required for the assembly of the central pair of microtubules in motile cilia (Wilson *et al.*, 2009) and KIF27 may participate in this Fused-mediated function. *In vitro* studies have shown that KIF27 presents slow processivity (Yue *et al.*, 2018), although the dynamics within motile cilia and the *in vivo* consequences of this processive activity remain to be investigated. Intriguingly, KIF27 was found at the tip of PC when ectopically expressed in mesenchymal cells (Nozawa *et al.*, 2013), and while the impact of this finding remains to be investigated, this localization is suggestive of a + end directed motion.

KIF9 (Kinesin-9):

KIF9, and KIF6 (see below), are similar to KLP1 which was identified in *Chlamydomonas* as a component of the central pair (Bernstein *et al.*, 1994), where it is required for motility, likely through the regulation of dynein activity (Yokoyama *et al.*, 2004). KLP1 also interacts with Hydin (Lechtreck and Witman, 2007) which has been implicated in PCD in humans (Olbrich *et al.*, 2012). The orthologue of KLP1 in trypanosomes, termed KIF9A, localizes along the axoneme and is also required for motility (Demonchy *et al.*, 2009). KIF9 is highly expressed in sperm cells in both mammals (Chauvin *et al.*, 2012) and *Ciona intestinalis* (Konno *et al.*, 2015). In addition, *KIF9* was identified in a transcriptomic analysis during mucociliary differentiation in human airway epithelial cells (Ross *et al.*, 2007) and as a target of FOXJ1 in murine ependymal cells (Jacquet *et al.*, 2009). The ciliary function of KIF9 remains to be investigated in vertebrates, where it has been implicated in the classical

microtubule-based transport of various cargoes in non-ciliated cells (Cornfine *et al.*, 2011; Gache *et al.*, 2017).

KIF6 (Kinesin-9):

KIF6 is a paralogue of KIF9, termed KIF9B in trypanosomes, where it localizes to both the BB and axoneme and is involved in the assembly of the paraflagellar rod, an extra-axonemal structure required for motility (Demonchy *et al.*, 2009). *KIF6* was identified as a target of FOXJ1 in both mouse (Jacquet *et al.*, 2009) and zebrafish, where it localizes to the BB region of motile cilia (Choksi *et al.*, 2014). However, knockdown of *kif6* did not lead to classical cilia-related phenotypes in Zebrafish larvae, aside from a curved body axis (Buchan *et al.*, 2014), which could potentially be indicative of a role in motile cilia in ependymal cells (Grimes *et al.*, 2016) and/or in Reissner fiber biogenesis (Cantaut-Belarif *et al.*, 2018). Interestingly, mutations in *kif6* in zebrafish lead to scoliosis (Buchan *et al.*, 2014), which has recently been linked to abnormalities in CSF flow caused by defects in motile cilia in the spine and brain ventricles. The motile cilia-related function of KIF6 was expected to be conserved in mammals based on its expression in ciliated tissues (Buchan *et al.*, 2014). *Kif6* invalidated mice did not appear to present typical PCD-like phenotypes (Hameed *et al.*, 2013). However and interestingly, a recent report indicates that KIF6 plays a crucial and specific role in ciliogenesis in ependymal cells and that its loss in both species leads to enlargement of brain ventricles (Konjikusic *et al.*, 2018). It is not currently known whether this role is conserved in humans although this study also characterizes biallelic *KIF6* mutations in a case of macroencephaly with intellectual disabilities.

KIF18B (kinesin-8):

KIF18b is a member of the Kinesin-8 family and was previously characterized as a negative regulator of the microtubule dynamics of the mitotic spindle (Stout *et al.*, 2011; Tanenbaum *et al.*, 2011), by promoting microtubule catastrophe (McHugh *et al.*, 2018). In parallel, it was identified as a target of foxj1 in Zebrafish. *Kif18b* is present at the BB region of motile cilia and typical ciliopathy-related phenotypes were observed in morpholino-treated larvae, supporting its key role in motile cilia biogenesis/function in this organism (Choksi *et al.*, 2014). Surprisingly, the loss of *kif18b* results in shorter cilia in the pronephros (Choksi *et al.*, 2014), which was unexpected given the known function of KIF18B at the spindle in mammals. This result suggests either that the function at ciliary microtubules is different compared to cytoplasmic microtubules or that *kif18b* acquired a different function in Zebrafish. Interestingly, KIF18B was also shown to interact with EB1 (Stout *et al.*, 2011), a positive regulator of microtubule growth and ciliogenesis (Schrøder *et al.*, 2011), which may provide

a clue to understanding the ciliary functions of KIF18B. To our knowledge, there is no mouse model for *Kif18B* and its ciliary function *in vivo* remains to be investigated in mammals.

KIF19A (kinesin-8):

KIF19A was an uncharacterized Kinesin-8 whose function was directly investigated via knockout in mouse, which led to strong motile cilia-related phenotypes, including hydrocephalus and female infertility (Niwa *et al.*, 2012). KIF19A possesses a '+' end directed motor activity and localizes to the distal end of the axoneme where it negatively controls ciliary length through its microtubule depolymerizing activity (Niwa *et al.*, 2012; Wang *et al.*, 2016). The loss of KIF19A causes elongation of motile cilia in all tissues examined thus far, impaired beating and subsequently inefficient fluid flow (Niwa *et al.*, 2012). A potential role in the regulation of primary cilia length was not investigated, however, the lack of PC-related phenotypes in the invalidated mouse model suggests that the loss of Kif19A does not severely impact upon PC-dependent signaling pathways.

Different cilia, different set of kinesins?

It appears that a specific set of kinesins were selected during evolution to exert motile cilia-specific functions in vertebrates and particularly in mammals. Indeed, Kinesin-8 family members, including KIF18B which was initially characterized as a mitotic kinesin, play unanticipated roles in cilium length regulation, confirming the current use of mitotic proteins in ciliary functions and vice versa (Hua and Ferland, 2018). Interestingly, it was recently shown that the CDK1 cell cycle signaling axis is partially reactivated during the differentiation of ependymal cells (Al Jord *et al.*, 2017). This result may also explain how some proteins mainly expressed/activated in mitosis, including kinesins, could additionally possess ciliary functions in these cells. Another striking example of the acquisition of motile cilia-specific functions is KIF27, which is specific to mammals and has evolved together with its partner Fused to play a key role at motile cilia. This may highlight the need to tightly couple ciliogenesis and the length of motile cilia at steady-state in different ciliated tissues, where they are required to generate the efficient flow of fluids of different composition/viscosities.

Intriguingly, it seems that different sets of Kinesins were selected to control ciliary length in primary versus motile cilia. While *Kif19A* invalidated mice do not show evidence of PC-associated phenotypes (Niwa *et al.*, 2012), *Kif2A* invalidated mice do not show PCD-like phenotype (Homma *et al.*, 2003). Although CP110 plays key roles in both primary and motile cilia ciliogenesis (Song *et al.*, 2014; Walentek *et al.*, 2016), the function of KIF24 has not yet been investigated in the context of motile cilia. Notably, both KIF2A and KIF24 are involved in PC disassembly during cell cycle re-entry, a process which has not been described and is not expected to occur in post-mitotic, multiciliated cells. In contrast, motile cilia disassembly does occur prior to cell division in *Chlamydomonas*, as well as

other unicellular eukaryotes, or in response to various stresses, and Kinesin-13 orthologues have been implicated in these processes (Hu *et al.*, 2015). The potential role of KIF7 at motile cilia has not been investigated directly. Interestingly, the loss of *kif7* in zebrafish causes *situs inversus* (Wilson *et al.*, 2009), a phenotype not observed in the context of *KIF7* mutations in humans, suggesting a role at motile cilia in the laterality organ of this organism. Additionally, hydrocephalus is often present in individuals harboring *KIF7* mutations (Putoux *et al.*, 2011), suggesting a specific role at motile cilia in ependymal cells. However, SHh signaling plays a critical role in ependymal cell specification/differentiation (Yu *et al.*, 2013), possibly indicating that hydrocephalus in the absence of *KIF7* can be a consequence of impaired SHh signaling. Finally, *KIF7* mutations do not lead to classical PCD-like phenotypes, suggesting that it is unlikely to play a general role at motile cilia.

General conclusion

Numerous Kinesins appear to play important and specialized roles at primary and/or motile cilia, including assembly through IFT, cell cycle-regulated disassembly, axonemal length control and signaling (SHh). Most kinesins represent good candidate genes for ciliopathies but only a few have so far been reported. An outstanding question remains whether all ciliary KIFs are now known? Redundancy among KIF family members, their involvement in crucial cytoplasmic functions, and/or restricted function in the transport of specific sets of cargoes to cilia might have delayed their identification. In addition to the proteomic and transcriptomic data already available, the improvement of proximity labeling approaches and single cell transcriptomic analysis of ciliated tissues may provide new and interesting data in order to identify the expression of specific KIFs in cells where cilia are known to have crucial functions.

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Conflict of interest:

None to declare.

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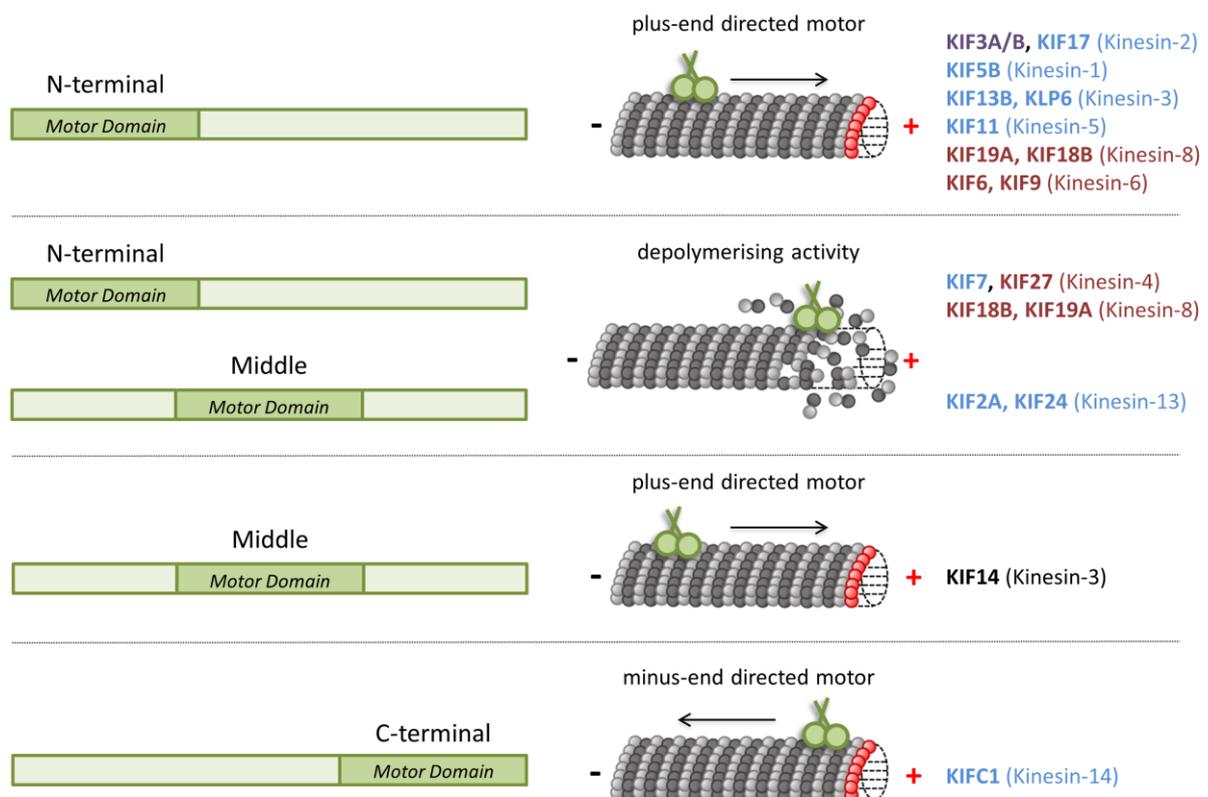


Figure 1: Functional Kinesin families.

Kinesin family members (KIFs) can be classified based on the localization of their motor domain (N-terminal, middle, C-terminal) and their activity (motor or depolymerizing). Kinesins involved in ciliary functions are indicated on the right depending on this classification. Kinesin involved in PC-specific functions are indicated in blue, those in motile cilia functions are indicated in red and those involved in both types of cilia in purple.

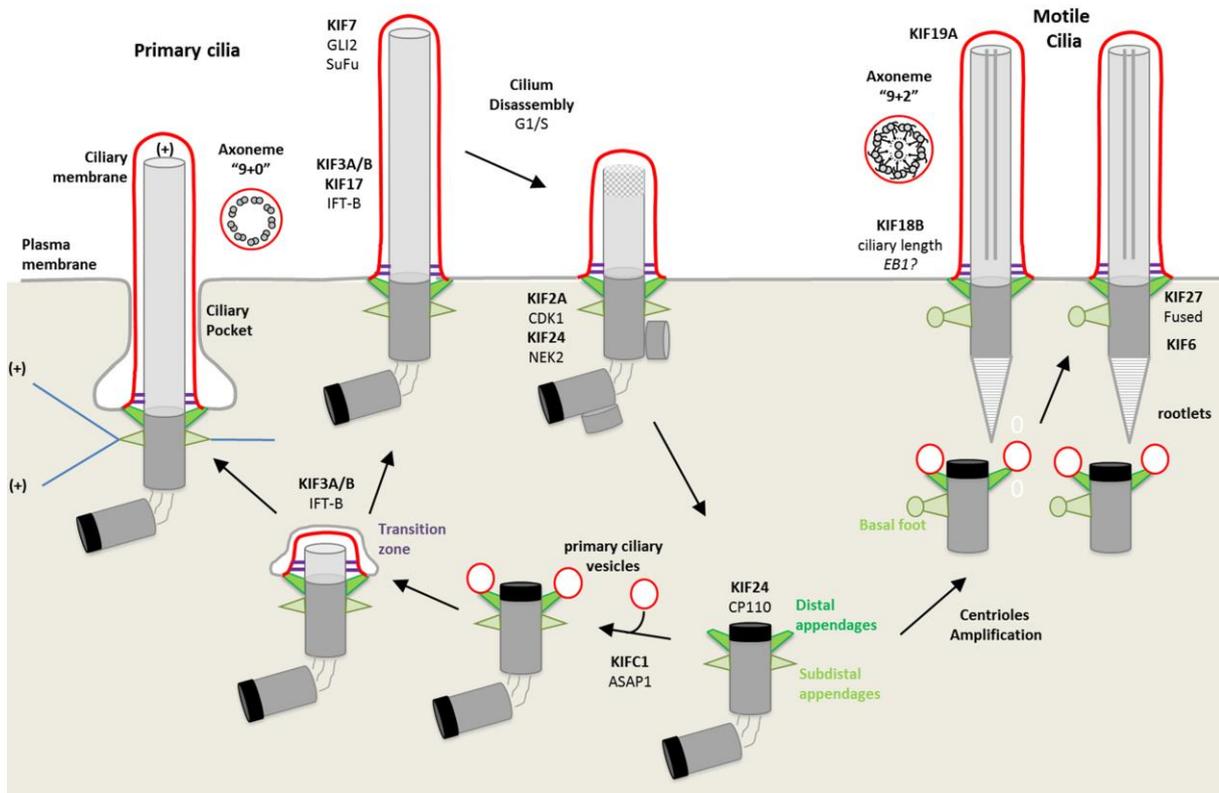


Figure 2: Role of kinesins at primary and motile cilia.

The scheme represents ciliogenesis pathways for primary (left) and motile (right) cilia. Ciliary KIFs are indicated in the scheme together with their known functional partners where they are known to act.

	Ciliary kinesins	Localization, function	Phenotypes in vertebrates	Diseases
Primary cilia	KIF3A,B (Kinesin-2)	IFT, ciliogenesis	embryonic lethal (Ms), ciliopathy (Zb)	
	KIF17 (Kinesin-2)	IFT, cargo transport	no phenotype (Mo), <i>retina</i> (Zb)	
	KIF7 (Kinesin 4)	tip, ciliary length + SHh signaling	development (Ms), laterality (Zb)	Cerebral ciliopathies
	KIF2A (Kinesin 13)	BB, cilia disassembly	brain development (Ms)	microcephaly
	KIF24 (Kinesin 13)	BB, ciliogenesis/disassembly	nd	
	KIF13B (Kinesin 3)	BB + Ax, SHh signaling	no phenotype (Ms)	
	KIF5B (Kinesin 1)	BB, ciliary length	early development (Ms), cartilage (Zb)	
	KIFC1/HSET (Kinesin 14)	Golgi, ciliogenesis	no phenotype (Ms)	
	KLP6 (Kinesin-3)	Ax, PKD2 transport (Ce)	nd	
	KIF11/Eg5 (Kinesin-5)	connecting cilium (Hu)	early development (Ms), Brain (Zb)	microcephaly + retina
Motile cilia	KIF27 (Kinesin 4)	BB, <i>motility?</i>	PCD-like (Ms)	
	KIF6 (Kinesin-9)	BB (Zb), ciliogenesis (Zb)	scoliosis (Zb), hydrocephaly (Ms+Zb)	Intellectual disability
	KIF9 (Kinesin 9)	BB + Ax, ciliary transport (Tb)	nd	
	KIF19A (Kinesin 8)	tip, ciliary length	hydrocephalus, female infertility (Ms)	
	KIF18B (kinesin-8)	BB, ciliary length (Zb)	ciliopathy phenotypes (Zb)	

Table 1: Ciliary kinesins.

Localization, and in vitro and in vivo functions of ciliary kinesins are indicated in the table. Different colors are used to stress KIFs involved in either PC (blue), motile cilia (pink) or both (purple). Indicated in vivo phenotypes are for mouse models except when indicated. Zb: Zebrafish; Ce *Caenorhabditis elegans*; Tb: *Trypanosoma brucei*; Ax: Axoneme; BB: Basal body; IFT: Intraflagellar transport; SHh: Sonic Hedgehog.