The ciliary transition zone: From Morphology and Molecules to Medicine

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Abstract

Researchers from various disciplines, including cell and developmental biology, genetics and molecular medicine have revealed an exceptional diversity of cellular functions that are mediated by cilia-dependent mechanisms. Recent studies have directed our attention to proteins that localize to the ciliary transition zone (TZ), a small evolutionarily conserved subcompartment that is situated between the basal body and the more distal ciliary axoneme. These reports shed light on the roles of TZ proteins in ciliogenesis, ciliary protein homeostasis and specification of ciliary signaling, and pave the way for understanding their contribution to human ciliopathies. In the present review, we describe the interplay of multimeric protein complexes at the TZ, integrating morphological, genetic and proteomic data towards an account of TZ function in ciliary physiology.

Cilia: From architecture to function

From their discovery at the end of the nineteenth century, cilia (and flagella) have been recognized to be present in many branches of the eukaryotic tree and on virtually all mammalian cells [1]. The earliest studies focused on the role of cilia in motility as seen in many single-celled organisms and specialized vertebrate tissues (e.g. respiratory epithelia or cells of the reproductive tract). In the past ten years, interest in cilia has experienced a renaissance. Driven by modern developments in genetics, a rapidly growing body of experimental evidence has established the role of cilia-dependent mechanisms in cellular signaling processes, tissue homeostasis, development and disease pathogenesis.

A significant part of our existing knowledge of ciliary structure emerged with the progress in electron microscopy techniques from the 1950's to the 1970's (reviewed in [2]). At that time, careful and systematic ultrastructural analysis of cilia and flagella in many organisms provided a detailed and astonishingly conserved morphological map. From this map, the cilium can be dissected into distinct substructural zones with consensus anatomic features: the basal body (BB), the transition zone (TZ), the doublet zone and the singlet zone with the ciliary tip complex (Box 1). Of these zones, the TZ houses a set of remarkable protein

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assemblies that are thought to mediate linkage to the ciliary membrane, establishment of a barrier to membrane diffusion and a gate for proteins destined for the ciliary compartment. Moreover, in human diseases of ciliary dysfunction, the so-called "ciliopathies", a significant number of implicated proteins localize to the TZ. Presented with this complex architecture and myriad protein constituents, it has become the task of the research community to connect the disease genes to the architecture and the architecture to function. In the present review, we will discuss TZ anatomy and function in the context of novel insights from genetic, proteomic and systems biology approaches.

The ciliary transition zone and the ciliary necklace

The ciliary transition zone constitutes the proximal portion of the cilium with the following defining features (Figure 1): 1. The TZ originates at the distal end of the BB. It encompasses a propeller-like array of so-called transitional fibers, which project from the B-tubule at the level of termination of the C-tubule and insert into the periciliary plasma membrane. As a result, the TZ backbone is composed of nine outer microtubule doublets with a nine-bladed propeller structure at its base [3]. 2. The main body of the TZ is characterized by the presence of multiple rows of Y-shaped linkers projecting out from the outer doublets and attaching to the ciliary membrane [4]. These linkers coincide with a characteristic circumferential arrangement of membrane particle insertions that can be observed by freeze fracture electron microscopy preparations, referred to as the ciliary necklace (Figure 1B) [5]. 3. The TZ terminates distally with the last row of Y-linkers. In 9+2 cilia, this coincides with the level of the basal plate and the proximal end of the central microtubule pair (Figure 1C).

There exist a number of key differences in transition zone features between organisms (Figure 1D). For example, motile flagella have the hallmark 9+2 configuration not seen in 9+0 primary cilia, along with the characteristic dynein arms and radial spokes. In addition, the internal cylinders within the TZ lumen are unique to the motile cilium, as seen in *Chlamydomonas* (Figure 1D, left). The basal body in *Caenorhabditis* is poorly discerned by electron microscopy but these sensory cilia do have robust transitional fibres and this arrangement may represent a minimal structure for nucleation of the transition zone [6] (Figure 1D, middle). *Caenorhabditis* sensory cilia also have singlet microtubules present in the lumen of the axoneme and transition zone that are unrelated to the central pair in motile cilia. Even with these differences, the high degree of morphological similarity between TZs from diverse cell types implies a set of conserved constituents and common pathways for assembly.

Many human ciliopathy syndrome proteins localize to the transition zone

Ciliopathies are defined as a group of genetic disorders whose gene products localize to the cilium. Many ciliopathies are complex syndromes that include renal cysts as one of many organ manifestations. Moreover, a large number of ciliopathy genes have been identified by genetic analyses of cystic kidney disease syndrome families. These findings have led to the ciliary hypothesis of polycystic kidney disease, which suggests that protein products of polycystic kidney disease genes localize to and exert their function at or near the primary cilium [7]. For example, PKD1 and PKD2, the gene products responsible for >95% of all cases of autosomal dominant polycystic kidney disease (ADPKD) are both found in the primary cilia of kidney epithelial cells [8].

A clinical continuum of autosomal recessive cystic kidney syndromes begins with nephronophthisis (NPHP), a form of congenital cystic renal dysplasia with progressive loss of kidney function (Box 2). As an isolated entity, NPHP is the most common cause of end-stage renal disease in the first three decades of life [9]. However, it may also be found as a component of more complex disorders. In Senior-Løken-syndrome (SLSN), NPHP is

associated with retinitis pigmentosa, resulting in degeneration of retinal photoreceptor cells and consequent vision loss. In Joubert syndrome (JBTS), NPHP is accompanied by retinal degeneration, as well as central nervous system (CNS) patterning defects primarily affecting the cerebellum. In Meckel-Gruber syndrome (MKS), the most severe disease in the continuum, NPHP-like renal pathology is associated with fibrocystic changes of liver and biliary system, retinal defects, polydactyly, severe CNS malformations, such as occipital encephalocele, and perinatal lethality.

Although phenotypic overlap between human NPHP-associated ciliopathies is common, it is still remarkable that mutations in some 30 different genes manifest in a small set of well-defined clinical syndromes (Table 1 and Box 3). This is in large part due to the localization of ciliopathy genes to a common structure, the TZ of the primary cilium. This places the TZ at the center of the ciliary hypothesis; linking ultrastructural assemblies to a pleiotropy of defects in ciliated tissues. How these gene products interact to assemble the TZ and function to specify the ciliary compartment has been a central focus of recent studies. The emerging model postulates an assembly hierarchy of NPHP/JBTS/MKS gene products, acting as multimeric protein complexes to form the TZ that in turn determines the signaling repertoire of the cilium.

Assembling the transition zone from protein complexes

How is the intricate structure of the transition zone built and maintained? Genetic studies of TZ proteins employing mutations, knockout and RNAi knockdown strategies have revealed dependency patterns of TZ localization and, in some cases, ultrastructural correlations with specific TZ assemblies [10–14]. These studies have been complemented by proteomic analyses of affinity purified protein complexes, using various TZ proteins as "bait" [11, 15, 16] or via the direct enrichment of ciliary material [17–19]. Systematic analysis and correlation of localization data, genetic analyses and proteomic interaction patterns has revealed a preliminary map of the TZ protein network. This map involves many known and a few novel ciliopathy proteins and reveals an organization into at least four communicating multi-protein complexes: NPHP1-4-8, MKS/B9, NPHP5–6 and the Inversin compartment (Figure 2).

The Rpgrip1L/NPHP8 protein organizes both the 1-4-8 module and the MKS module at the TZ. Evidence for this complex emerges from two lines of work. In cultured cells, the systematic proetomic analysis using tandem affinity purification followed by mass spectrometry-based protein identification (TAP/MS) showed clear reciprocal interactions between Rpgrip1L, Nphp1 and Nphp4 [15]. The complex was well-conserved in both epithelial (IMCD) and mesenchymal (3T3) cells demonstrating a basic core consensus of the complex across cell types. The second line of evidence came from a systematic genetic approach in *Caenorhabditis,* where RPGRIP1L was shown to be at the top of an assembly hierarchy controlling the localization of Nphp1 and Nphp4 and the entire nematode MKS complex [14]. Mutation of NPHP1 or NPHP4 had no effect on the MKS complex members. Interestingly, when mutated together (i.e. one mutation from 1-4-8 and one from MKS), the transition zone showed complete loss of Y-linkers and membrane attachment, defective ciliogenesis and deranged cilium protein trafficking. The multitude of associations of Rpgrip1L and its role in supporting large domains of the TZ may explain its involvement in ciliopathies across the entire severity spectrum (Table 1) and conversely, why NPHP1 and NPHP4 may be associated with less severe phenotypes.

In contrast to Rpgrip1L or NPHP1/4, nearly all of the proteins in the MKS/B9 module have been implicated in the most severe disease of the NPHP continuum, Meckel-Gruber Syndrome (MKS). This module was found through proteomic methods in multiple

independent studies [11, 15, 16]. Both studies used TAP/MS methods but different baits, strengthening the evidence for a distinct MKS module. Genetic evidence in mice and *Caenorhabditis*, along with interactomic analyses reveal the MKS module as a multiprotein complex rich in membrane-targeting B9 and C2 domains, as well as a number of membrane proteins (Table 1). As described above any combination of MKS and NPHP1-4-8 mutations produces significant disruptions of TZ ultrastructure in nematodes [14]. Unlike mutations in *Caenorhabditis*, correlative ultrastructural data on cilia and TZ architecture of human MKS cases or mouse models is lacking. A recent publication describes the identification of a novel Joubert syndrome related protein, TMEM237/JBTS15, that has been localized to the TZ. Its deletion exacerbates ciliary membrane defects in an NPHP4 negative background, as described for other MKS module proteins. Although interactomic data is not yet available, it will likely be identified as an MKS complex component [22].

Comparing MKS components of *Caenorhabditis* with interactomic data from mammalian cells, obvious homologues for the tectonic (Tctn) family of proteins are absent from the nematode genome. Another difference between nematode and mammalian MKS proteins is their effect on ciliogenesis. While there were only few defects in isolated MKS mutations in *Caenorhabditis*, MKS proteins had more significant effects on ciliogenesis in mammalian cells. For example, RNAi-mediated depletion of the Mks1 protein in cell culture was reported to result in reduced ciliogenesis and defective basal body migration [20]. Genetic deletion of MKS1 in a mouse model of MKS showed tissue specific loss of cilia – with reduced ciliation in the developing neural tube but largely normal cilia in the renal tubule [21]. Similar tissue specific defects were seen in mouse models of genetic deletions of the MKS module proteins Tctn1, Tctn2 and Cc2d2a [11], but were not reported in TMEM231 and B9D1 deleted mice. One important aspect of development in worms is that all cilia reside in neurons of the sensory system and may follow a common ciliogenic program [6]. In mammals, cilia from cells of epithelial versus mesenchymal origin can have different morphological appearances and signaling roles, perhaps requiring different ciliogenic proteins. Such tissue-specific effects are likely to be reflected in varied composition or differential requirement of TZ protein modules for both, ciliogenesis and signaling.

The "NPHP5-6" or Cep290 module emerges primarily from proteomic studies in mammalian cell lines. Tandem affinity approaches show a strong interaction of Cep290 with the MKS [11], while others postulate a distinct module grouped around a Cep290-NPHP5 cluster [15], highlighting a previously described functional interaction [23]. In *Chlamydomonas*, immunogold electron microscopy maps Cep290 to the Y-linkers of the TZ, and its mutation results in a substantial, but not complete, loss of Y-linkers [10], indicating Cep290 contributes to Y-linker formation. Interestingly, an obvious CEP290 orthologue is lacking from the *Caenorhabditis* genome, where morphologically intact Y-linkers are present underscoring the role of other proteins (or a functional worm homologue of Cep290) in defining these substructures. With such a striking phenotype in *Chlamydomonas* and the involvement of CEP290 throughout the entire severity spectrum of human ciliopathies, it is surprising that a spontaneous in-frame deletion mutant in mice displays only retinal degeneration (rd16 mutation, [24]). Moreover, a genetic deletion has a mild JBTS-like phenotype (retinal degeneration and cerebellar patterning defects) [25] but no cystic kidney disease or defects of early development, such as we might expect. How the many recessive mutations in CEP290 result in defects more severe than the genetic deletion will require more detailed investigation.

A special role is attributed to Inversin, which interacts and colocalizes with Nphp3 and Nek8, but is not restricted to the BB or TZ. This broad localization may belie a bridging function between multiple modules within the network. Indeed, interactions were independently established with NPHP1, NPHP5 and the MKS module via the JBTS protein

Ahi1 [15]. From all available evidence to date, the Inversin compartment appears dynamic, including the basal body and a variable distal extension along the ciliary shaft. Mutations in Inversin, NPHP3 or Nek8 have not been correlated with disruptions of the TZ structure itself, as yet [26].

Where can we go with such a map? Sang and colleagues were able to use the proteins in their complexes to identify the new ciliopathy genes Tectonic2 and Ataxin-10 which cause JBTS and NPHP, respectively [15]. With the possibility of identifying new ciliopathy genes a variety of proteomic, bioinformatic [27] and next generation sequencing [28] approaches, even in non-mammalian organisms, are likely to add significantly to the list of transition zone proteins and their interactions. Even more than a set of experimental approaches, these studies highlight the necessity of systematic studies. The cilium is an organelle of systems biology. Like any machine that has many moving parts, it is the assembly and coordinated effort of these parts that is necessary for proper functioning. Loss of one protein may give a dramatic phenotype, but to even hypothesize a mechanism we will need to know how that loss has affected the other components.

With the exception of Cep290, none of the described TZ proteins has been mapped to a defined TZ substructure. The next step in the characterization of NPHP and MKS protein complexes will be to localize individual proteins to specific TZ constituents. This will add a morphological dimension to the TZ protein map that is otherwise only based on interactomic and genetic data. Moreover, detailed protein-protein interactions and ultrastructural analysis will be necessary not only in the wild-type setting, but also under genetic perturbation. While immunofluorescence and GFP-tagging is possible in almost all the model ciliary systems, light microscopy does not yield the resolution to define TZ architecture. There is potential for new technologies that promise super-resolution beyond the conventional ~200 nm limit to illuminate our understanding of the TZ [29]. However, even with such technologies, the high resolution ultrastructural methods of visualizing the TZ available to models such as *Chlamydomonas* and *Caenorhabditis* must find their way to vertebrate systems (e.g [30]) to observe the more intricate elements of the TZ and their dependency on the TZ modules.

Assembling signaling pathways through the TZ trafficking gate

How are proteins trafficking through the complex assembly of structures at the ciliary base, and what role does the TZ play? It has long been postulated that a selective barrier at the ciliary base acts to recognize proteins and membrane vesicles that are intended to translocate into the ciliary compartment, while prohibiting access to others. The presence of transitional fibers, Y-linkers and necklace structures constitutes an obvious physical boundary and is suggestive of a gating mechanism. Furthermore, a variety of cytoskeletal substructures occupy a large amount of space within and around the ciliary lumen at the ciliary base. In the region of the transitional fibers, the space between two neighboring sheets is estimated not to be larger than 60 nm, thus making vesicular transport to the ciliary membrane unlikely through an axial route [31]. These considerations are further complicated by findings demonstrating that a distinct ciliary membrane domain extends into an about 1.8 µm diameter apical membrane patch around the ciliary base [32, 33]. This extraciliary region is characterized by a specific lipid and protein composition that is distinct from that of the surrounding apical membrane. This region has been proposed to be a docking site for vesicles with ciliary cargo components that would then travel laterally across the transition zone into the cilium [32, 33]. There is also evidence that entry into the cilium can be prevented by proteins tethering them to the surrounding cytoskeleton [32]. This larger region may serve as a docking site for cilium-destined cargos, but is itself far from the TZ.

Structures more proximal to the TZ have been proposed to carry out the local gating function. A barrier function has been ascribed to a septin network (Sept2) at the base of the cilium that is required for ciliogenesis and enrichment of proteins within the compartment [34]. Recent evidence suggest that Sept2 exerts this barrier fucntion through the localization of MKS/B9 protein complex members Tmem231, B9D1 and Cc2d2a [16]. This barrier appears to be passive, preventing mixing between the apical and ciliary membranes, but not "smart" enough to distinguish between ciliary and non-ciliary cargos. Disruption of TZ Y-linkers, as in *Chlamydomonas* CEP290 mutants or in *Caenorhabditis* NPHP/MKS module double mutants, resulted in the inappropriate localization of non-ciliary proteins into the cilium [10, 14]. In *Chlamydomonas*, flagellar proteomes from wild-type and CEP290 mutants were profiled. In addition to the relative loss of otherwise abundant flagellar proteins, some proteins were identified that are usually not found in flagella [10]. Similarly, in *Caenorhabditis* mutants, GFP-tagged ciliary and non-ciliary proteins were assessed for cilium localization [14]. The membrane-associated X-linked retinitis pigmentosa gene product RP2, was absent from cilia in wildtype, MKS1, TMEM67 and NPHP1 mutant worms, but present in the cilium of MKS1/TMEM67 double mutants and single mutants of B9D1, B9D2, RPGRIP1L, CC2D2A and NPHP4. The transmembrane protein TRAM-1a showed almost identical behavior. Importantly, in these genetic backgrounds, axoneme-membrane interactions and Y-linkers were ultrastructurally intact, indicating that a subtle molecular selectivity determinant has been lost. Altogether the data supports a smart barrier model of TZ function, with ciliary proteins permitted access by determinants within the TZ and non-ciliary proteins excluded. When the TZ is disrupted, ciliary proteins are either unable to enter or accumulate and non-ciliary proteins enter inappropriately. Alternatively, it has been proposed that gating may occur in a bidirectional manner, based on live observations of flagellar trafficking in *Chalmydomonas* [35]. As such, the inappropriate ciliary accumulation of proteins may imply an active removal pathway that is defective when the TZ is disrupted. Such a bidirectional gating is reminiscent of the nuclear pore complex, whose trafficking determinants, the Ran-Importin pathway, have been implicated in some aspects of ciliary trafficking [36].

Restrictive signals can keep proteins out of the ciliary compartment, but gatekeeping into the cilium is also dependent on permissive signal recognition [31]. The somatostatin receptor SSTR3 localizes to cilia in many genetic backgrounds. However, the adenylylate cyclase III transmembrane (ACIII) enzyme was totally absent from cilia in TCTN1, TCTN2, CC2D2A or TMEM67 null MEF cells [11]. One of the ADPKD gene products, Pkd2, was also absent from cilia in TCTN1, TCTN2 or CC2D2A null MEFs, but showed unimpaired ciliary localization in TMEM67 null cells. The Hh signaling pathway has been intimately linked to cilia through a number of studies of developmental defects in mice [37]. Activation of the hedgehog signaling pathway involves the ciliary translocation of Smoothened, a process that was shown to be dependent on Tctn1, Tctn2 and Cc2d2a, but independent of Tmem67. Similarly, Arl13b, a lipid modified and membrane associated ciliary protein, was markedly reduced in cilia of TCTN1, TCTN2 or CC2D2A null cells, while the reduction in TMEM67 null cells was modest. TCTN1/2 and CC2D2A null mice all exhibited dramatic developmental defects with situs inversus, tissue-specific ciliogenesis defects and embryonic lethality, whereas TMEM67 null mice survive until birth, have kidney cysts during embryogenesis and reduced cilia in kidney tubules. Together, this indicates that simply making a cilium is not sufficient; equipping it with the right components is just as crucial. Understanding the relationship between disruption in the TZ assembly hierarchy and the change in cilium protein localization could provide rules for protein trafficking to the cilium. Moreover, we may be able to identify those TZ proteins that recognize specific permissive signals on a cargo and the nature of these signals themselves.

From a signaling perspective, complete pathways must be assembled component by component within the cilium. The inability to target a component of a signaling pathway would result in the disruption of the cascade itself. For example, in the CEP290 mutant mouse (rd16), odorant receptors, a type of G-protein coupled receptor (GPCR), and the corresponding adenylate cyclase isoform (ACIII) were properly localized to olfactory cilia, however, the heterotrimeric G protein (G_{olf}) that acts between the GPCR and ACIII did not [38]. As a result, these mice and patients with CEP290 mutations have poor olfaction. Ciliary signaling pathways such as G-protein coupled receptors (e.g. somatostatin or melanocortin [39]), receptor tyrosine kinases (e.g. PDGF [40]) and calcium channels [8] are all localized to and transduced through the primary cilium. Each protein in the cascade could be trafficked to the cilium independently or pre-formed signaling centers could be transported en masse. Testing how entire signaling pathways are transported and assembled within the primary cilium will require the integrative and systems biology approaches described here. Understanding these mechanisms would help to elucidate the mechanisms by which cell-specific specification of the ciliary compartment is achieved through TZ-mediated ciliary protein gating. Ultimately, we would be able to assess the types of cellular defects that translate into different severity states of ciliopathy phenotypes: the partial loss of a signaling pathway would perhaps underlie a mild phenotype, while a complete loss of cilium specification could result in more dramatic tissue dysfunction as seen in the most severely affected patients.

In summary, the complex phenotypes seen in TZ dysfunction all point to the concept of a smart gate that helps regulate ciliary protein homeostasis. Proteins that arrive at the TZ, presumably through a set of trafficking signals, are asked for their "documents" to permit them entry into the ciliary compartment. Moreover, those proteins may be queried within the ciliary compartment repeatedly and trafficked out of the cilium when no longer required. How such a gate is built from the unique ultrastructural features and protein modules reviewed here will be the central area of investigation for the future. Alongside how the transition zone itself is assembled, we will need to understand how the TZ assembles signaling pathways by documenting ciliary proteins under normal and perturbative conditions. By integrating our knowledge of molecular complexes, ultrastructural assemblies, cellular functions and organismal phenotypes, we can begin to elucidate how this small organelle exerts its control in development and homeostasis, and how its disruptions produce human disease phenotypes.

Box 1: General Aspects of Ciliary Structure

The ciliary transition zone and axoneme arise from the basal body. The structural core of the BB consists of a nonamer of microtubule (MT) triplets, in which the A-tubule is defined as the complete MT, associated with incomplete B- and C- tubules (Box I). The BB terminates with the distal end of the C-tubule. From there on, the ciliary axoneme is composed of a scaffold of nine outer A–B-microtubule doublets. In vertebrates, variations of the common ciliary theme are limited to immotile primary cilia and motile cilia, with a few exceptions. Immotile cilia share a common 9+0 (outer doublets, no central MTs) microtubular axonemal architecture and can be found in the form of primary cilia of epithelial and mesenchymal cells, or as highly specialized sensory cilia in olfactory or retinal photoreceptor cells. Motile cilia are characterized by a 9+2 constellation (outer doublets + central MTs) and are found on multi-ciliated cells of the respiratory tract or fallopian tube, as well as in the form of sperm flagella. One notable exception are the motile 9+0 cilia found at the embryonic node which harbor dynein arms but no central pair. Toward the ciliary tip, B-tubules terminate in various organism- and cell-specific patterns, leaving behind distal A-tubule singlets. Those ultimately terminate in a ciliary tip substructure that also carries a variety of cell type specific characteristics.

Not shown here are the molecules that support the dynamic process of intraflagellar transport (IFT) (reviewed in [43]). IFT is the primary mechanism by which proteins are transported within the ciliary compartment and as such plays a central role in cilium assembly and maintenance.

Box 2: Clinical and Pathological Features of Human Ciliopathies

Human ciliopathies entail a large number of genetic defects that give rise to a wide spectrum of clinico-pathologic entities [9]. Disorders of the motile cilium, like *Kartagener's* syndrome were the first discovered [44]. The large group of disorders of the primary cilium includes cystic malformation of the kidneys as one of the most consistent findings. In autosomal dominant polycystic kidney disease (ADPKD), cysts grow throughout the renal parenchyma, resulting in massively enlarged kidneys (Figure II**A**, reprinted with permission from [45], mm ruler).

The Nephronophthisis (NPHP) spectrum of syndromes follows an autosomal recessive pattern of inheritance. In Nephronophthisis, cysts emerge in one distinct zone of the kidney parenchyma, the cortico-medullary junction, and lead to tubular atrophy and parenchymal fibrosis. Unlike ADPKD, however, kidneys are not significantly enlarged (Figure II**C**, reprinted with permission from [46], cm ruler).

Senior-Løken-syndrome (SLSN) represents an association of NPHP with retinitis pigmentosa, resulting in degeneration of the retinal pigment epithelium and consecutive vision loss.

In Joubert syndrome (JBTS), variable degrees of NPHP are accompanied by retinal degeneration and CNS malformations. The characteristic cerebellar vermis hypoplasia manifests clinically in hypotonia, ataxia, abnormal eye and tongue movements, breathing pattern disturbances and psychomotor retardation. The structural equivalent is the "molar tooth sign" observed on brain imaging (Figure II**B**; normal MRI on the left, molar tooth sign on the right, reprinted with permission from [47]). The phenotypic spectrum of JBTS may include further distinctive organ manifestations with variable penetrance, leading to subclasses within the cluster of Joubert syndrome related disorders (JSRD): Syndromes with predominant cerebellar, ocular and renal pathology are sometimes referred to as CORS. Syndromes including congenital liver fibrosis are also known as COACH syndrome (coloboma, oligophrenia, ataxia, cerebellar anomalies, hepatic fibrosis).

Meckel-Gruber syndrome (MKS) is characterized by renal cystic dysplasia, biliary cysts and hepatic fibrosis. CNS malformations are very severe and often involve occipital encephalocele with posterior extrusion of the brain outside the skull (Figure II**D**, reprinted with permission from [48]). Associated defects include Leber congenital amaurosis (LCA) and postaxial polydactyly (Figure II**E**, reprinted with permission from [49]). MKS leads to fetal or perinatal death.

LCA is an isolated developmental defect of the retinal photoreceptor cell. In contrast to retinitis pigmentosa, there is no pattern of progressive vision loss, but severe vision loss from birth on. Other ciliopathies such as Bardet-Biedl syndrome (BBS), Alstrom syndrome, orofacial digital syndrome and Jeune syndrome exhibit symptoms clinically distinct from the NPHP spectrum and are reviewed elsewhere [9].

Box 3 : Complex Genetics of Human Ciliopathies

NPHP syndromes are characterized by oligogenicity and significant phenotypic pleiotropy, and a lack of predictable genotype-phenotype correlations [50, 51] (Table 1). For example, in MKS, the constellation of pathologic manifestations is quite complex and the penetrance of individual single organ phenotypes is variable from patient to patient. Despite the characterization of many mutations, it is difficult to predict whether an affected individual carries homozygous mutations in MKS1, in TMEM67, or in another one of the ten identified MKS genes, or in a yet unidentified gene. Moreover, certain single genes can produce the full spectrum of NPHP-related disorders, depending on the nature of the mutation. More than 100 unique human mutations were identified in the CEP290 ciliopathy gene (also referred to as NPHP6, JBTS5 or MKS4), giving rise to a range of phenotypes including isolated NPHP, LCA, characteristic SLSN, JBTS, Bardet-Biedl syndrome (BBS) or the lethal MKS [52]. We also find that some genes are frequently associated with the less severe manifestations of the NPHP continuum, whereas others are found in the context of more severe ciliopathies. Ideally, systematic genetic screening would be a routine element in the clinico-pathologic diagnosis of ciliopathy cases, but complete sequencing data is often unavailable. As our understanding evolves, we may indeed find a linkage of specific genes to more or less severe disease phenotypes. Alternatively, we may find that most ciliopathy genes, like CEP290, produce the full pathologic spectrum of ciliopathy phenotypes.

Acknowledgments

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Glossary

ADPKD	autosomal dominant polycystic kidney disease
ANK	ankyrin repeat
BB	basal body

BBS	Bardet-Biedl syndrome
CC	coiled coil domain
IFT	intraflagellar transport
JBTS	Joubert syndrome
LCA	Leber congenital amaurosis
MEF	murine embryonic fibroblasts
MKS	Meckel-Gruber syndrome
MT	Microtubule
NPHP	Nephronophthisis
RCC1	"regulator of chromosome condensation-1" domain
RPGRIP1L	X-linked Retinitis pigmentosa GTPase regulator interacting protein-1-like
SH3	Src-homology-3 domain
SLSN	Senior-Løken syndrome
TZ	ciliary transition zone

Gene names are written in capital letters (*TMEM67*); proteins in capital and small letters (*Tmem67*)
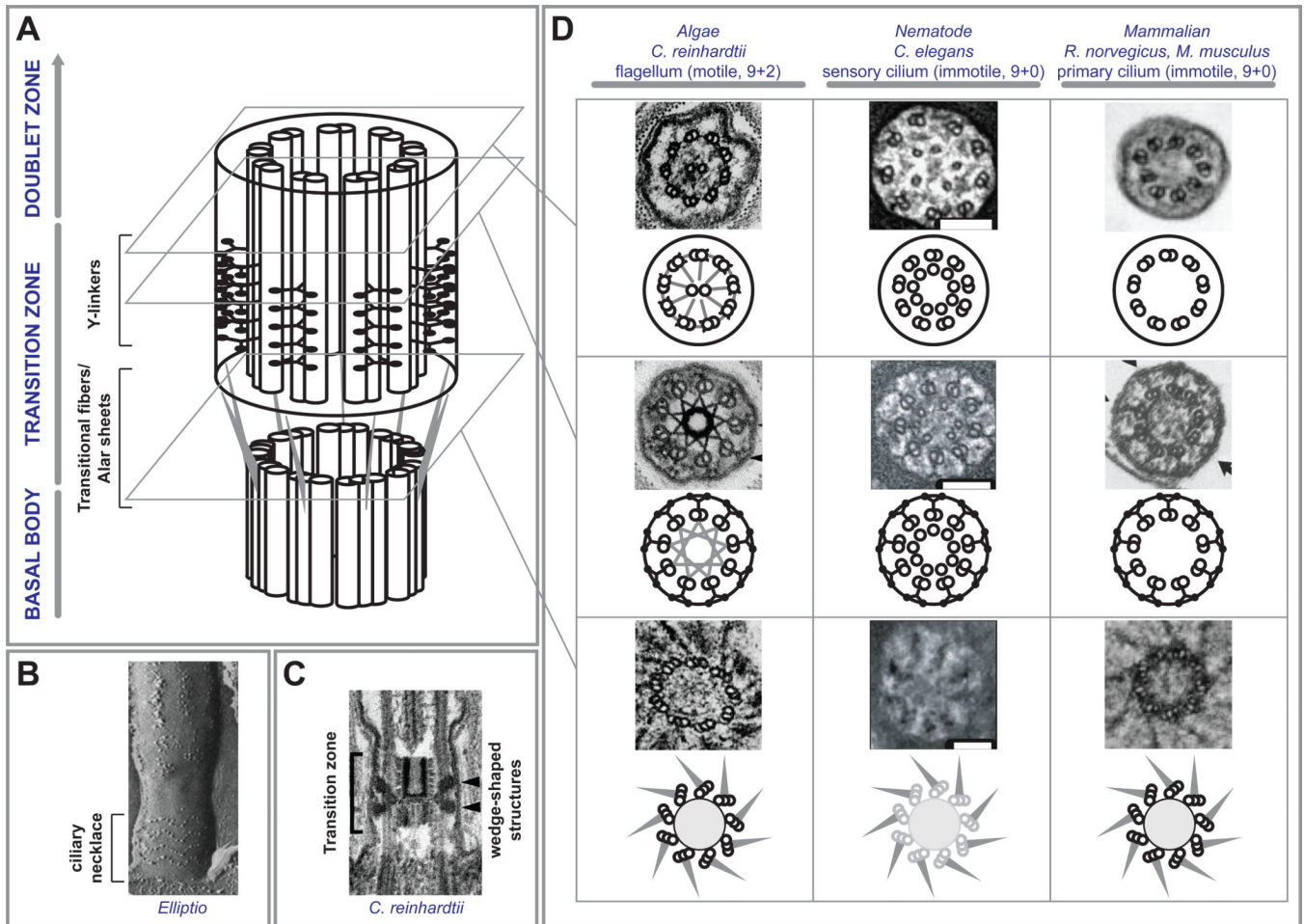
Figure 1. The Ciliary Transition Zone

A – A schematic representation of the ciliary transition zone shows its location between the basal body compartment and the doublet zone of the axoneme. At the distal end of the basal body, terminal triplet MT architecture and transitional fibers are seen. The A–B tubules of the triplet MTs are continuous with the doublets (not shown here for clarity) within the transition zone proper where characteristic Y-linkers span from the doublets to the surrounding membrane. It is thought that these interactions produce characteristic ciliary membrane imprints referred to as the ciliary necklace (**B**, reproduced with permission from [5]). In the transition zone of *Chlamydomonas* flagella, there is an additional central density with a barrel-shaped body within the space defined by the MT doublets and a wedge shaped density projecting towards the membrane from the outer MTs (**C**, reproduced with permission from [10]). **D** - Although remarkably conserved, subtle structural differences exist between model organisms. At the start of the transition zone, transitional fibers project from the B-tubule to the ciliary membrane at its junction with the apical cell membrane. The appearance of transitional fibers indicate the distal end of the basal body, since at that point, C-tubules terminate and the transition zone and axoneme consist only of outer MT doublets. Transitional fibers, also called alar sheets, form a propeller-like assembly leaving spaces of about 60 nm between neighboring sheets. The BB structures (grayed MT triplets) are only poorly discerned in *Caenorhabditis* sensory cilia, but transitional fibers can be observed. Within the transition zone, all cilia have the characteristic Y-linkers connecting the outer

doublet MTs to the ciliary membrane. In addition, *Chlamydomonas* has a stellate fiber array within the outer doublet array connected to all A-tubules that appears near the basal plate. Within the axoneme of motile cilia, such as in *Chlamydomonas*, an arrangement of radial spoke proteins connect the inner central pair of MTs to the outer doublets, and nexin fibers and dynein arms connect the outer doublets to each other. The sensory cilia of *Caenorhabditis* and the primary cilium of mammalian cells both lack the inner central pair and the basal plate from which they originate. In addition, they lack radial spokes, nexin fibers and dynein arms (reproduced with permission from [10, 11, 30, 41, 42]). Scale bars, 100 nm.
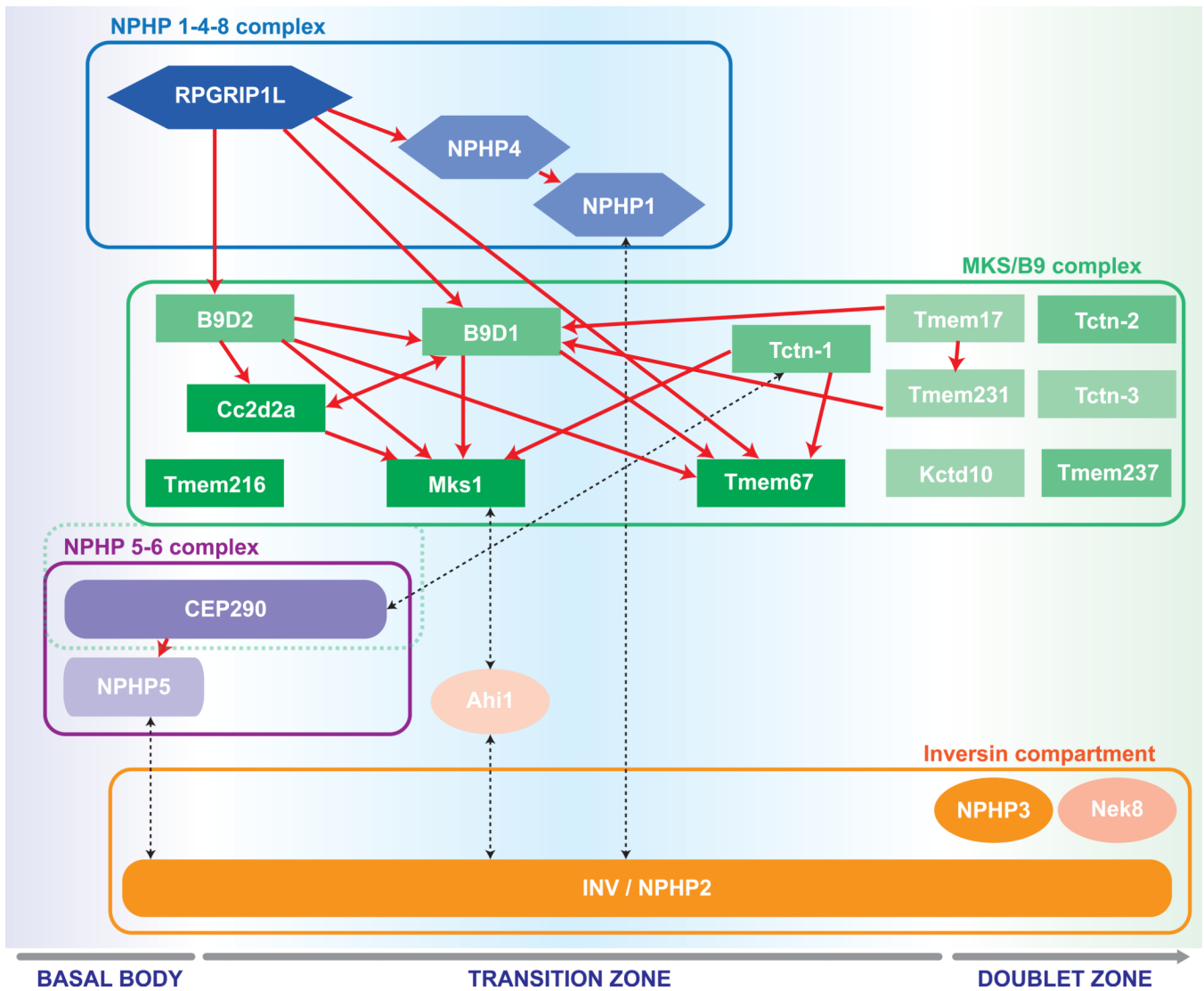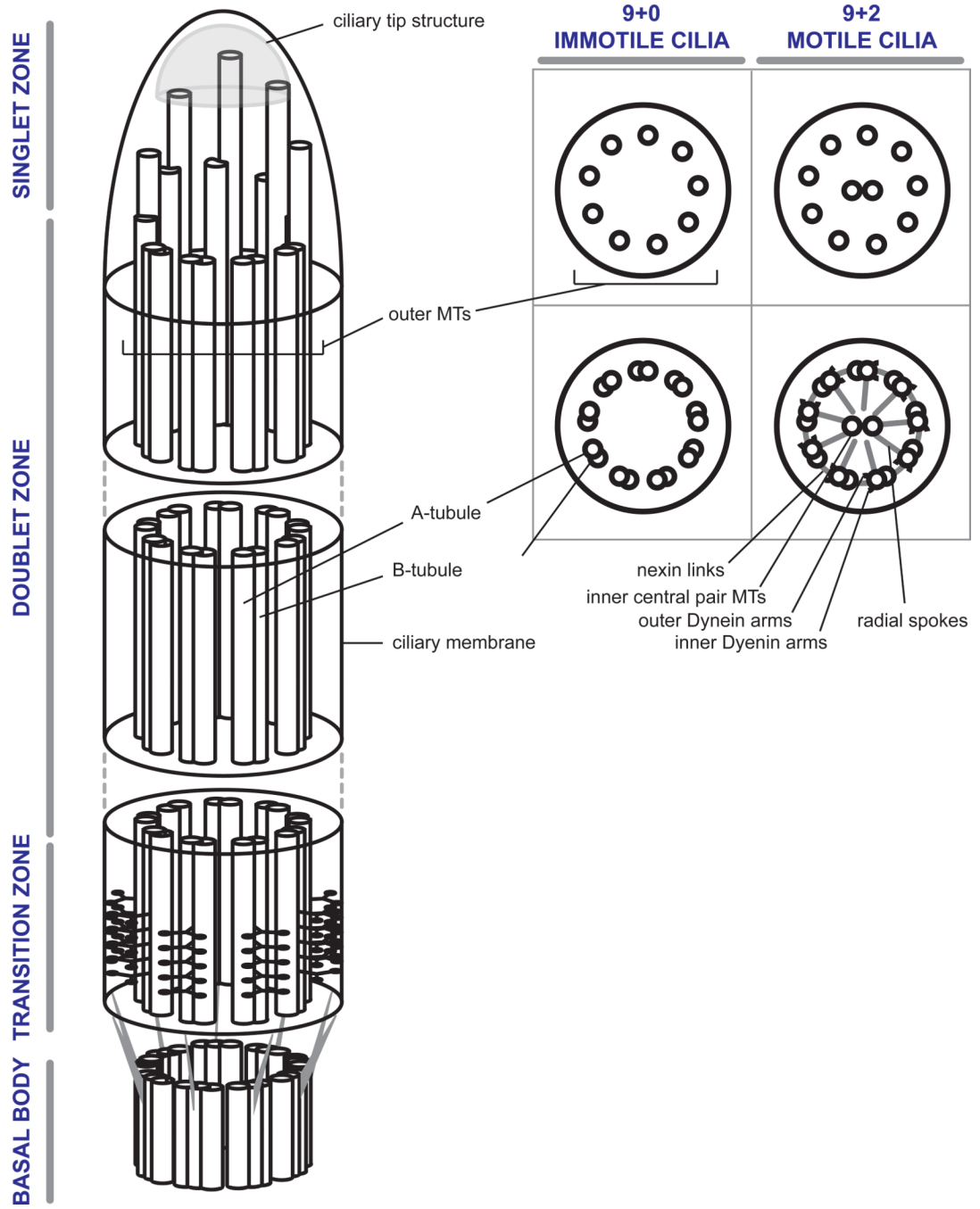
Figure 2. Organizational chart of transition zone functional modules
Rpgrip1L/NPHP8 localizes independently and influences proper TZ localization of the
NPHP1-4 and MKS/B9 complexes. The localization may be direct or through a cascade of
consecutive localization and complex organization events. Within the MKS/B9 module,
Mks1, Tmem216 and Tmem67, which are consistently associated with severe pathologies in
humans, appear at the bottom of the localization hierarchy within the module. Cep290 was
considered the core of its own NPHP5-6 network in one study [15], but part of an MKS-like
complex in another [11]. The inversin compartment is highly dynamic and spans a long
stretch of ciliary axoneme from the basal body into the distal cilium proper. Through its
localization and its interactions with members of all protein modules, it may serve as a
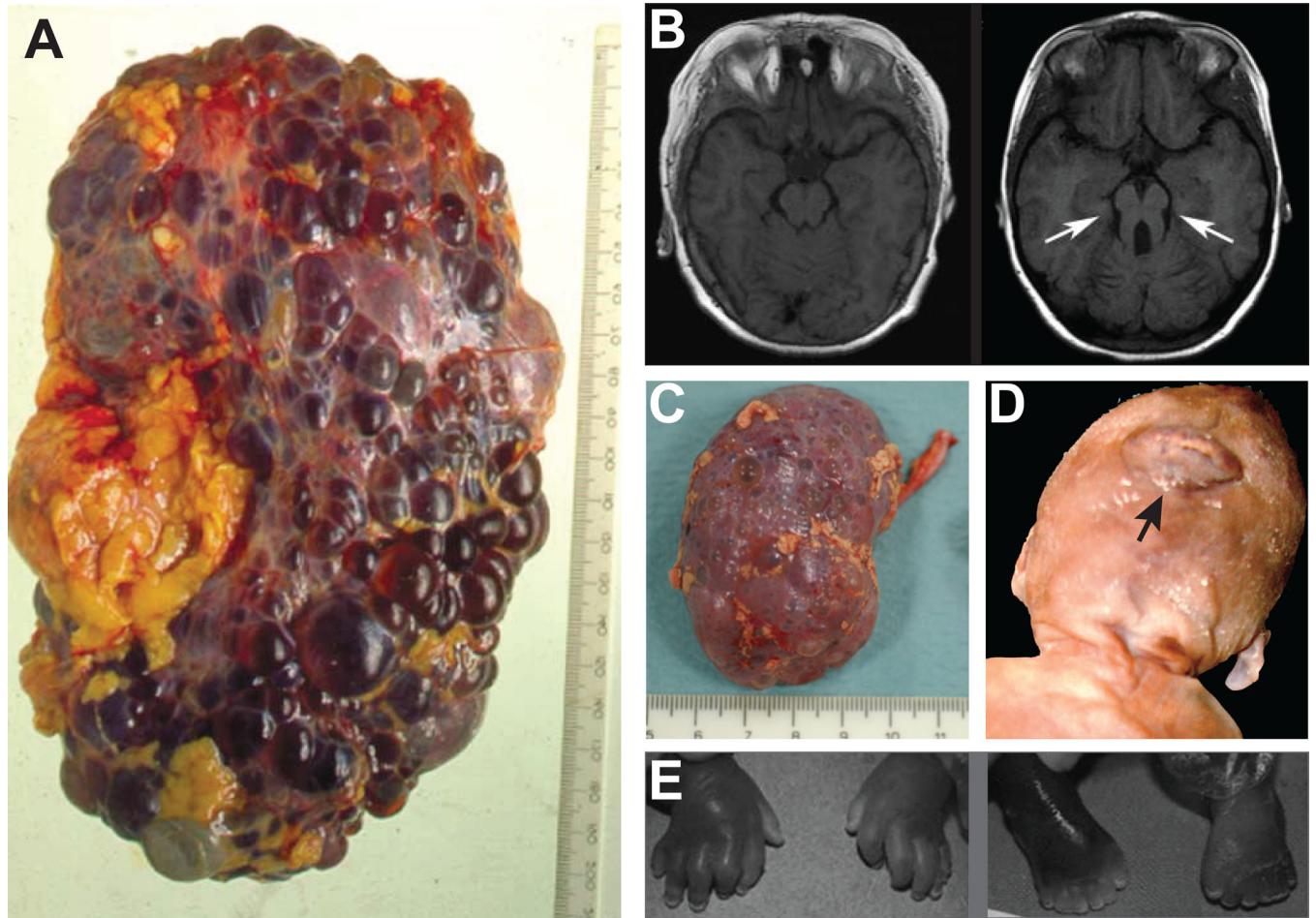bridge and contribute to multiple distinct signaling processes.
Proteins within one module are highlighted by the same shape and color. Brighter color
indicates more frequent association with severe disease. Higher position within one group
and red arrows indicate intra-complex localization hierarchy. Black dotted arrows indicate
physical interactions across modules.
Note that not all depicted proteins are exclusively located in the TZ, but may also be found
at the basal body (Rpgrip1L, B9D1, Cc2d2a, Tmem216, Cep290, NPHP5, inversin), or in

the proximal doublet zone of the cilium proper (inversin, Nphp3, Nek8, Tmem67, Tctn-2, Tctn-3).

ciliary tip structure

9+0
IMMOTILE CILIA

9+2
MOTILE CILIA

SINGLET ZONE

outer MTs

DOUBLET ZONE

A-tubule

B-tubule

ciliary membrane

nexin links
inner central pair MTs
outer Dynein arms
inner Dyenin arms
radial spokes

TRANSITION ZONE

BASAL BODY

Box 1.

Box 2.

Table 1

An overview of ciliopathy genes and their products

Protein Complex	Gene Name	Alternate name by syndrome			Localization[a]	Protein domains[b]	Selected References
		NPHP	MKS	JBTS			
NPHP 1-4-8	RPGRIP1L	NPHP8	MKS5	JBTS7	TZ, BB	C2, CC	[53, 54]
	NPHP4	NPHP4			TZ		[13, 55, 56]
	NPHP1	NPHP1		JBTS4	TZ	SH3, CC	[13, 57, 58]
MKS	B9D1		MKS9		TZ, BB	B9	[59, 60]
	B9D2		MKS10		TZ	B9	[60, 61]
	TCTN1			JBTS13	TZ		[11, 62]
	TCTN2		MKS8		TZ, AX	TM	[11, 15, 63]
	TCTN3				TZ, AX	TM	[62]
	CC2D2A		MKS6	JBTS9	TZ	C2, CC	[11, 12, 64]
	TMEM216		MKS2	JBTS2	TZ, BB	TM	[65]
	TMEM67	NPHP11	MKS3	JBTS6	TZ, AX	TM	[20, 66, 67]
	MKS1		MKS1		TZ	B9	[20, 21, 60, 67, 68]
	TMEM237			JBTS14	TZ	TM	[22]
NPHP 5-6	Cep290	NPHP6	MKS4	JBTS5	TZ, BB	CC	[23, 25, 38, 69]
	IQCB1	NPHP5			BB	IQ, CC	[23, 70]
Inversin compartment	Inversin	NPHP2			BB, TZ, AX	ANK, IQ, CC	[26, 71–73]
	NPHP3	NPHP3	MKS7		AX	TPR, CC	[46, 74, 75]
	Nek8	NPHP9			AX	Serine/Threonine kinase, RCC1	[75–78]
Other	Glis2	NPHP7				C2H2	[79, 80]
	SDCCAG8	NPHP10				CC	[28]
	TTC21B/IFT139	NPHP12		JBTS11	AX	TPR	[81, 82]
	INPP5E			JBTS1	AX	Inositol phosphatase	[83, 84]
	AHI1/Jouberin			JBTS3		SH3, WD40	[25, 85]
	OFD1			JBTS10	BB	CC	[86, 87]
	KIF7			JBTS12	AX	Kinesin motor, CC	[88, 89]

Protein Complex	Gene Name	Alternate name by syndrome			Localization[a]	Protein domains[b]	Selected References
		NPHP	MKS	JBTS			
	ARL13b			JBTS8	AX	GTPase, CC	[90, 91]
	XPNPEP3				Mitochondria	Aminopeptidase	[92]
	CEP41			JBTS15	BB, AX	Rhod	[93]

[a]BB – basal body, TZ – transition zone, AX – axonemal compartment

[b]TM – transmembrane, SH3 – Src homology 3, ANK – ankyrin repeat, TPR – tetratricopeptide repeat, WD40 - WD40- or β-transducin domain, RCC1 – regulator of chromosome condensation 1-like, C2, B9 – membrane association domains, C2H2 – zinc finger-like domain, IQ – IQ calmodulin-binding domain.