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REVIEW

Motile cilia genetics and cell biology: big results from little mice

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Abstract

Our understanding of motile cilia and their role in disease has increased tremendously over the last two decades, with critical information and insight coming from the analysis of mouse models. Motile cilia form on specific epithelial cell types and typically beat in a coordinated, whip-like manner to facilitate the flow and clearance of fluids along the cell surface. Defects in formation and function of motile cilia result in primary ciliary dyskinesia (PCD), a genetically heterogeneous disorder with a well-characterized phenotype but no effective treatment. A number of model systems, ranging from unicellular eukaryotes to mammals, have provided information about the genetics, biochemistry, and structure of motile cilia. However, with remarkable resources available for genetic manipulation and developmental, pathological, and physiological analysis of phenotype, the mouse has risen to the forefront of understanding mammalian motile cilia and modeling PCD. This is evidenced by a large number of relevant mouse lines and an extensive body of genetic and phenotypic data. More recently, application of innovative cell biological techniques to these models has enabled substantial advancement in elucidating the molecular and cellular mechanisms underlying the biogenesis and function of mammalian motile cilia. In this article, we will review genetic and cell biological studies of motile cilia in mouse models and their contributions to our understanding of motile cilia and PCD pathogenesis.

Keywords Cilia · Motile cilia · Primary ciliary dyskinesia · PCD · Mouse · Mucociliary clearance

Introduction

For more than a century, the mouse has been an indispensable tool for studying basic biological processes and modeling human disease [1–5]. Compared to other mammalian models, the mouse is an economic system with a small body size, a relatively short gestation period, and relatively large litters. In addition, the mouse is a particularly powerful genetic model with an impressive toolkit. With fully sequenced inbred strains and highly effective technologies for genetic manipulation, there is a remarkable capability to generate and study a wide array of genetic alleles and modifiers of complex traits, including specific mutations identified in patients with human disease. Extensive repositories exist worldwide with a wide range of mouse models and tools available for biomedical research. Many of these models have already been employed for elucidating genetic, epigenetic, molecular, and cellular mechanisms underlying diverse human diseases and developmental processes. Mouse models have also been used successfully for pre-clinical testing of drug efficacy and safety, leading to clinical trials and FDA-approved disease therapeutics for several human disorders [6–9]. While the mouse is not without limitations as a model, it is a mammalian system that has been studied in depth and recapitulates many biological processes and human diseases well.

Dysfunction of motile cilia results in the rare disease primary ciliary dyskinesia (PCD) [10–15]. Multiple motile cilia form on the surface of epithelial cells lining the respiratory tract, oviduct, and ventricles of the brain, where they play a critical role in flow or clearance of fluids and particles through coordinated, whip-like beating. Single motile cilia form on nodal cells in the early embryo and regulate the directional flow of signals required for the establishment of

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left–right patterning, while the structurally related sperm flagella are responsible for cell motility. The highly conserved ciliary structure, or axoneme, is composed of nine microtubule doublets surrounding a central pair apparatus of two single microtubules in a "9 + 2" arrangement (Fig. 1) [13, 16]. The inner dynein arms (IDAs) and outer dynein arms (ODAs) are comprised of dynein motor proteins that enable the ciliary beating and are regulated by the nexin-dynein regulatory complex (N-DRC) and radial spokes. The axoneme is divided into a series of repeating 96 nm units along the length of the cilium, a consequence of the periodic organization of the main axonemal components. PCD is a genetically heterogeneous disorder that is typically inherited in an autosomal recessive manner, although rare cases of X-linked recessive and autosomal dominant inheritance have been described [11, 12, 17–20]. Defects in motile ciliary formation or function often result in chronic rhinosinusitis, otitis media, bronchiectasis, and neonatal respiratory distress. In addition, reduced fertility, heterotaxy and related congenital heart defects, and sporadic hydrocephalus are associated with PCD. There is currently no effective treatment for PCD. In contrast to the "9 + 2" motile cilia, many other cell types possess a primary cilium with a "9 + 0" microtubule ultrastructure and a mechanosensory function [15, 21–23]. Defects in primary cilia result in a class of related ciliopathies [15, 21–23], which will not be discussed here.

Current understanding of motile cilia comes from work performed in a variety of model systems, including mouse and other organisms [24]. Perhaps most notable among lower eukaryotes is the flagellated alga *Chlamydomonas reinhardtii*, a particularly tractable model system that has enabled detailed genetic, biochemical, and structural analysis of flagella [16, 25–28]. While non-mammalian organisms, such as *C. reinhardtii*, zebrafish, and frogs, remain essential resources, there is significant value in utilizing and further developing a readily available mammalian system. Mammalian motile cilia form on terminally differentiated cell types, including epithelial cells differentiating from airway progenitors, ependymal cells differentiating from radial glia, and nodal cells in the developing embryo [29–31]. Motile cilia are spatially oriented, and their motility is tightly coordinated within and between cells to effectively enable fluid clearance and flow [14, 31]. In addition, defects in ciliary biogenesis and motility result in organ dysfunction or developmental defects, including loss of host defense and lung function in the airway, tissue damage and hydrocephalus in the brain, and laterality defects in the developing embryo [10–15]. Finally, while the basic axonemal structure is well conserved across species, there are differences in structure, protein composition, and function between mammalian motile cilia and *C. reinhardtii* flagella [32–36]. Unlike lower vertebrate models, the mouse is a mammalian system whose anatomy and physiology are closer to human, and mouse models phenocopy PCD remarkably well. The only exception is the association of hydrocephalus, which is more common in mouse models than human patients, possibly due to anatomical differences in the brain ventricles and additional genetic modifiers that segregate in certain inbred mouse strains [37–39]. Understanding the mechanisms underlying mammalian motile cilia formation, regulation, and function, as well as the pathogenic process caused by defective cilia, will enable the development of specific PCD therapies. Further, as evidenced by studies of other diseases,

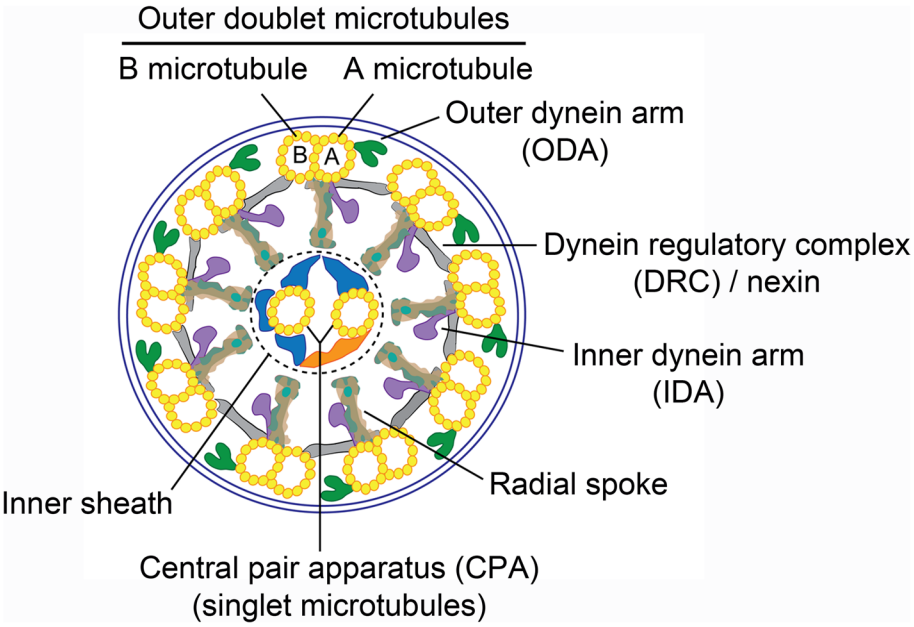

Fig. 1 Schematic diagram of the motile ciliary axoneme. Modified with permission from Bustamante-Marin and Ostrowski, 2017 [14]

mice can ultimately serve as a suitable pre-clinical model to test the efficacy of those therapeutics.

The mouse is not without its limitations as a model for studying motile cilia. Historically, *C. reinhardtii* and other ciliated or flagellated unicellular organisms have enabled advancements in ciliary structure and protein biochemistry that have been difficult to accomplish in a mammalian system [25, 40]. Zebrafish and frogs have been particularly useful for developmental studies, as these organisms develop *ex utero* and are transparent during embryonic development [24, 41]. In addition, mutagenesis and gene knockdown are typically more rapid in these non-mammalian species. There are also anatomical and physiological differences between mouse and human, most notably regarding the development of the brain ventricular system [37]. Finally, cell biological applications using mouse models typically rely on the culture of primary tracheal epithelial or brain ependymal cells, which necessitate highly specific growth conditions and require viral transduction for exogenous gene expression [42–44]. Despite these limitations, extensive genetic resources and an emerging cell biology toolkit are helping to overcome many of the historical challenges and making the mouse a particularly powerful model for understanding motile cilia and ciliary disease.

This review will discuss advancements in genetics and cell biology of motile cilia obtained using mouse models. Models of PCD have generated extensive information about multiciliated cell differentiation, ciliogenesis, ciliary function, and disease pathogenesis, as well as identified novel genes involved in ciliary mechanisms. Cell biological advancements are emerging in areas such as imaging, transcriptomics, and pharmacological modulation of motile cilia that can be applied to cultured ciliated cells or mouse tissues to answer new questions about the biology of cilia and multiciliated cells. When combined, genetic and cell biological applications in mice have the power to uncover the molecular and cellular mechanisms underlying mammalian motile cilia function and PCD pathogenesis.

Genetic models of cilia dysfunction and PCD

Over the last two decades, mouse models have provided an enormous amount of information about the genes, genetic mechanisms, and patterns of inheritance underlying PCD and motile ciliary function. Both forward and reverse genetic approaches have been used to study ciliary genetics, and a wide spectrum of genetic models has been reported, including spontaneous mutations, transgenic and gene-targeted knockout alleles, CRISPR/Cas9-edited alleles, gene-trapped alleles, and knock-in mice. In addition, large-scale N-ethyl-N-nitrosourea (ENU) mutagenesis screens have generated a large number of new models and unique alleles, particularly when screening for congenital heart defects and other laterality defects [45, 46]. As with human PCD patients, the mouse models display genetic and phenotypic heterogeneity, with most exhibiting a combination of some PCD-associated phenotypes, including mucus accumulation in the sinus cavity, otitis media, male infertility, female infertility, hydrocephalus, and heterotaxy or congenital heart defects. Each type of genetic model brings its own benefits to advance the field, which has enabled detailed histopathological analysis of phenotype and comprehensive evaluation of cilia structure and function in multiple cell types. These models have also validated PCD genes identified in human patients, identified new genes required for motile cilia function, and uncovered the effects of a large number of alleles on disease pathogenesis, multiciliated cell differentiation, and ciliary structure and function.

Dynein assembly and function

Ciliary beating is driven by the inner and outer dynein arms (Fig. 1), protein complexes comprised of heavy, intermediate, and light dynein chains that are tightly regulated to generate normal motility (reviewed in detail in King, 2016 and Viswanadha et al. 2017) [26, 47]. ODAs generate the motor force and regulate beat frequency, while the IDAs are believed to regulate ciliary bend. The dynein arm complexes undergo substantial cytoplasmic pre-assembly prior to transport into the cilium and docking with the doublet microtubules. A number of genes encoding dynein heavy and intermediate chains have been evaluated in mouse models, as well as axonemal and cytoplasmic proteins required for assembly of dynein arm components. Table 1 shows mouse models with mutations in IDA and ODA genes, demonstrating that mutations in each gene result in PCD-associated phenotypes and defects in ciliary motility or flow.

Mouse models have been reported with mutations in three ODA genes: *Dnah5*, *Dnaic1/Dnai1*, and *Dnah11*. *Dynein axonemal heavy chain 5* (*DNAH5*) is commonly mutated in PCD patients [11, 12, 48]. Two mouse models, one with a targeted null mutation and one with an ENU-induced allele, show an absence of ODAs by transmission electron microscopy (TEM) analysis [49–51]. Cilia-driven flow was assessed ex vivo via analysis of fluorescent particles over brain slices from the knockout mouse [50], an effective and highly employed high-speed video microscopic technique that has enabled analysis of ciliary physiology on multiple cell types and tissue samples. A reduction in ciliary beat frequency (CBF) and mucus transport were also assessed in a second ENU mutant, named *Dakshi*, through use of micro-optical coherence tomography (μOTC) analysis ex vivo [52]. Loss of ODAs is also associated with mutations in *Dynein axonemal intermediate chain 1* (*DNAI1*), the first PCD gene identified in human patients [53]. The phenotype

Table 1 Mouse models of dynein genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
DNAH1	KO	MI	Defects not detected	Reduced CBF	Neesen [64]
	ENU (*ferf1*)	MI	N/A	N/A	Hu [65]
DNAH5	KO	HC, HT, OM, sinus	No ODA	Decreased flow	Ibanez-Tallon [49, 50]
	ENU	HT	No ODA	N/A	Tan [51]
	ENU (*Dakshi*)	N/A	N/A	Reduced CBF, decreased flow	Solomon [52]
DNAH11	Sp (*iv*)	HT, OM, sinus	Defects not detected	Immotile	Layton [56], Supp [57], Lucas [60]
	ENU (*lrm3*)	HT	Defects not detected	Immotile	Ermakov [45], Lucas [60]
	ENU (*lrm5*)	HT	N/A	N/A	Ermakov [45]
	ENU (*avc4*)	HT	N/A	N/A	Burnicka-Turek [58]
	KO	HT	N/A	Immotile	Supp [59]
DNAI1	Inducible KO	Sinus	No ODA	Decreased flow	Ostrowski [55]

HC hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *OM* otitis media, *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis), *Sp* spontaneous

was confirmed through detailed airway analysis in an adult mouse, which was enabled by crossing a conditional targeted allele of the mouse homolog *Dnaic1* to a tamoxifen-inducible CreER line [54]. Importantly, infection of mutant mouse tracheal epithelial cells (mTECs), which are cultured and differentiated into multiciliated cells at an air–liquid interface, with a lentivirus expressing wild type *Dnaic1* in vitro rescues the ciliary phenotype [55], demonstrating the potential for gene therapy treatment of PCD. Mouse models with mutations in *Dynein axonemal heavy chain 11* (*Dnah11*), originally referred to as *left/right-dynein*, were initially identified by their prominent laterality phenotypes. The spontaneous, heritable mutant *inversus viscerum* (*iv*), a targeted knockout, and three models identified in ENU screens for heterotaxy and congenital heart defects (*lrm3, lrm5,* and *avc4*) all have laterality defects, with a full PCD phenotype reported for the *iv* mutant [45, 56–59]. Immotile cilia without structural defects detected by TEM are common in these models [59, 60], although TEM tomography detected subtle ODA defects in human PCD patients with *DNAH11* mutations [61–63]. In addition to the ODA genes, *Dynein axonemal heavy chain 1* (*DNAH1*) or mouse *Mdhc7*, is the only IDA gene reported in a mouse model to date. Its importance was first uncovered in a knockout mouse exhibiting male infertility and having tracheal epithelial cilia with a reduced CBF [64]. The infertility was confirmed in the ENU mutant *ferf1* [65]. *DNAH1* mutations have been reported in human patients diagnosed with PCD [66], as well as patients diagnosed only with multiple morphological abnormalities of the sperm flagella (MMAF) [67], a milder phenotype consistent with the mouse models.

Several genes encoding axonemal proteins involved in dynein assembly and docking have been studied in mouse models. Table 2 shows that mutations in four genes required for proper ODA attachment, *Armc4, Ccdc151, Mns1,* and *Ttc25*, result in PCD phenotypes with absent ODAs. The novel Armadillo repeat-containing 4 (ARMC4), which is not found in lower organisms like *C. reinhardtii*, has been

Table 2 Mouse models of dynein docking genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
ARMC4	ENU (*Aotea*)	HC, HT	No ODAs	N/A	Hjeij [68], Onoufriadis [69]
CCDC39	ENU	HC, HT	N/A	Reduced CBF, decreased flow	Solomon [52]
	ENU (*prh*)	HC	No IDAs	Reduced CBF, decreased flow	Abdelhamed [76]
CCDC40	ENU *(links*)	HT	N/A	N/A	Becker-Heck [79]
CCDC151	ENU (*snowball*)	HT	No ODAs	N/A	Hjeij [70]
	Inducible KO	HC, HT, MI	N/A	N/A	Chiani [71]
MNS1	KO	HC, HT, MI	No ODAs	N/A	Zhou [72]
TTC25	CRISPR	HT	No ODAs	N/A	Wallmeier [74]

HC hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *N/A* not reported

implicated in PCD in human patients and the ENU mouse model *Aotea* and is believed to play a role in the docking of ODA components [68, 69]. Mutations in *Coiled coil domain containing 151* (*CCDC151*) result in absence of ODAs in PCD patients [70]. The phenotype was confirmed in the ENU mutant *snowball* (*snbl*) and a tamoxifen-inducible targeted knockout, although only laterality defects were reported for *snbl* mutants [70, 71]. The knockout model replaces exons 2 and 3 with a *LacZ* reporter gene, which enabled microcomputed tomography (microCT) X-ray analysis of LacZ staining in heterozygous brains to detect *Ccdc151* expression throughout the ventricular system [71]. The importance of Meiosis-specific nuclear structural protein 1 (MNS1), which encodes a cytoplasmic and axonemal protein involved in the ODA docking complex assembly, was identified in a targeted knockout model and confirmed in patients with PCD phenotypes [72, 73]. Similarly, Tetratricopeptide repeat domain 25 (TTC25), which is also believed to play a role in ODA docking complex assembly, was evaluated in a null allele generated through CRISPR/Cas9 gene editing [74]. Mutants were only reported to have situs inversus, but the absence of ODAs was observed in cilia from mutant mice and PCD patients with *TTC25* mutations [74]. In addition, IDA and N-DRC docking are dependent on and determined by CCDC39 and CCDC40, which were shown in *C. reinhardtii* to interact to form the molecular ruler that determines the 96 nm axonemal repeat unit [75]. CCDC39 is required for proper IDA and N-DRC structure in human patients, and two ENU-induced mouse models exhibit PCD, reduced CBF, and perturbed ciliary clearance [52, 76–78]. Analysis of brains from the ENU mutant *progressive hydrocephalus (prh)* demonstrated increased gliosis and disrupted cerebrospinal fluid (CSF) flow in vivo [76]. Mutations in *CCDC40* have also been identified in human PCD patients and the ENU allele *lnks* [79]. Homozygous *lnks* mutants have shortened nodal cilia, indicating a defect in cilia assembly, and randomized expression of left–right patterning in the gastrulating embryo [79, 80].

Several additional proteins typically referred to as dynein axonemal assembly factors localize exclusively to the cytoplasm and function in pre-assembly of dynein arm components. Mouse models have been reported with PCD phenotypes and absence of inner and outer dynein arms due to mutations in genes encoding DNAAF1/LRRC50, DNAAF2/KTU, DNAAF4/DYX1C1, LRRC6, RUVBL1/PONTIN, DNAAF6/PIHD3, and DNAAF7/ZMYND10 (Table 3). Dynein axonemal assembly factor 1 (DNAAF1), also known as Leucine-rich repeat containing 50 (LRRC50), is required for dynein arm assembly in *C. reinhardtii* [81]. *DNAAF1* mutations were also identified in PCD patients, patients with congenital heart disease, and fetuses with neural tube defects and hydrocephalus [82–85], as well as an ENU mutant mouse with immunofluorescence (IF) analysis showing partial ODA assembly [86]. Mutations in *DNAAF2*, also known as *Kintoun* (*Ktu*), were identified in human PCD patients [87]. Ependymal cells in a traditional targeted knockout showed abnormal basal foot orientation and rotational polarity [88]. DNAAF2 expression was observed prior to ciliogenesis in mTECs, confirming an early role in cilia assembly. Interestingly, a second targeted allele reported by Cheong et al. has a severe developmental phenotype characterized by a morphologically abnormal node and left–right patterning defects during gastrulation [89]. Although both models removed exons at the 5′ end of the gene, the model reported by Cheong et al. is a congenic line on the C57BL/6NJ genetic background compared to the mixed genetic background reported for the earlier model by Matsuo et al., suggesting that genetic modifiers may influence the developmental phenotype.

Loss of *DNAAF4*, also known as *Dyslexia susceptibility candidate 1* (*DYX1C1*), results in PCD with dynein arm defects in human patients, a phenotype that was confirmed

Table 3 Mouse models of dynein pre-assembly genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
DNAAF1/LRRC50	ENU	HC, HT, sinus	N/A	N/A	Ha [86]
DNAAF2/KTU	KO	HC, HT	No IDAs, no ODAs	N/A	Matsuo [88]
	KO	HT	N/A	N/A	Cheong [89]
DNAAF4/DYX1C1	KO	HC, HT	No IDAs, no ODAs	N/A	Tarkar [90]
	ENU (*sharpei*)	HT	N/A	N/A	Tarkar [90]
LRRC6	KO	HC, HT	No ODAs	Reduced CBF	Inaba [93]
DNAAF6/PIH1D3	KO	MI	N/A	N/A	Dong [96]
RUVBL1/PONTIN	Conditional KO	HC, MI	No IDAs, no ODAs	N/A	Dafinger [95], Li [94]
DNAAF7/ZMYND10	KO	HC, sinus	No IDAs, no ODAs	N/A	Cho [99]
	CRISPR	HC, HT, sinus	No IDAs, no ODAs	Reduced CBF	Mali [100]

HC hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis)

in mice with a traditional targeted allele and the ENU allele *Sharpei* [90]. The role of the novel *Leucine-rich repeat containing 6* (*LRRC6*) in ODA assembly and cilia motility was identified in PCD patients and a traditional knockout mouse [91–93]. RuvB-like AAA ATPase (RUVBL1), also known as PONTIN, is an ATPase believed to function as a chaperone in cytoplasmic dynein arm pre-assembly [94]. Conditional knockout in cells assembling motile cilia using a tamoxifen-inducible *Foxj1-CreER* mouse line results in hydrocephalus and mislocalization of dynein proteins in ependymal cells [95], while conditional knockout in testis using a *Stra8-Cre* line results in immotile sperm with ODA defects [94]. A targeted mouse model lacking the cytoplasmic DNAAF6, also known as PIH1 domain-containing 3 (PIH1D3), was reported to have spermatogenesis defects with a loss of flagellar dynein arms, suggesting a role in pre-assembly of axonemal dynein arms [96]. *PIH1D3* mutations in human PCD patients represent rare cases of X-linked recessive inheritance of PCD [17, 18]. Finally, a role for the novel *DNAAF7,* also known as *Zinc finger MYND-type containing 10* (*ZMYND10*) was identified in PCD patients and confirmed in knockout mouse models generated by traditional gene targeting and CRISPR/Cas9 gene editing [97–100]. Co-immunoprecipitation (co-IP) in cultured mTECs and IP/mass spectrometry analysis in mouse testis showed that ZMYND10 interacts with several proteins involved in the cytoplasmic chaperone system required for dynein pre-assembly and maintenance of dynein stability [99, 100]. Taken together, models with mutations in genes encoding dynein arm proteins and assembly factors underscore the importance and complexity of the axonemal dynein system.

Central pair apparatus structure and assembly

The dynein motor force and ciliary waveform are believed to be regulated by the central pair apparatus (CPA) (Fig. 1), an intricate structure consisting of C1 and C2 singlet microtubules and an array of associated protein projections (C1a-f, C2a-e) (reviewed in detail in Loreng and Smith 2016 and Teves et al. 2016) [25, 101]. Several proteins associated with the CPA or required for CPA assembly have been studied in genetic mouse models of PCD, many of which have CPA structural defects and reduced CBF (Table 4). Because nodal cilia do not possess a CPA, laterality defects are never associated with the PCD phenotypes in these models. The spontaneous *hy3* mouse was first identified in the 1940s because of its severe hydrocephalus, although a nasal discharge was also observed in homozygous mutants [102–106]. The *hy3* phenotype was later found to result from a mutation in the gene encoding C2b projection protein Hydrocephalus-inducing (HYDIN), with mutant mouse cilia lacking the C2b projection and exhibiting abnormal ciliary bending and fluid flow [107–109]. Another hydrocephalic model, *ove459*, was

Table 4 Mouse models of central pair apparatus (CPA) structure and assembly genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
CFAP54	Gene trapped	HC, MI, sinus	CPA defects	Reduced CBF, decreased flow	McKenzie [136]
CFAP221/PCDP1	Sp (*nm1054*)	HC, MI, sinus	Defects not detected	Reduced CBF	Lee [134]
HYDIN	Sp (*hy3*)	HC, sinus	CPA defects	Reduced CBF, decreased flow	Berry [103], Gruneberg [102], Lawson [105], McLone [106], Raimondi [104], Davy [110], Lechtreck [108]
	Tg insertion (*ove459*)	HC	N/A	N/A	Davy [110]
SPAG6	KO	HC, MI, OM	CPA defects	Reduced CBF	Li [113], Sapiro [112], Teves [114]
SPAG16	KO (*SPAG16L*)	MI	N/A	N/A	Zhang [115]
SPAG6; SPAG16L	Double KO	HC, lung	Defects not detected	N/A	Zhang [118]
SPAG17	Conditional KO	HC, MI, lung, sinus	CPA defects	N/A	Teves [121], Kazarian [122]
SPEF2/KPL2	Sp (*bgh*)	HC, MI, sinus	Defects not detected	Reduced CBF	Sironen [127]
	Conditional KO	HC, MI	Defects not detected	N/A	Lehti [128, 129]
STK36/FUSED	KO	HC, MI, OM, sinus	N/A	N/A	Merchant [137]
	KO	HC, MI, OM, sinus	N/A	N/A	Vogel [138]
	KO	None	CPA defects	Reduced CBF	Chen [140], Nozawa [141], Wilson [254]

HC hydrocephalus, *KO* knockout, *Lung* lung abnormalities (including bronchitis and bronchiectasis), *MI* male infertility (including spermatogenesis and sperm motility defects), *OM* otitis media, *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis), *Sp* spontaneous, *Tg* transgene

reported to result from a transgene insertion that disrupted the *Hydin* gene [110]. *HYDIN* mutations were confirmed in human PCD patients with CPA defects [109].

A targeted mutation in *Sperm associated antigen 6* (*Spag6*), which encodes a C1 microtubule-associated protein required for C1 stability in *C. reinhardtii*, results in a PCD phenotype with CPA structural defects, disorganized basal feet, and abnormal ciliary polarity [111–114]. Scanning electron microscopy (SEM) revealed reduced numbers of motile cilia on tracheal epithelial and ependymal cells, suggesting a potential role for SPAG6 in ciliogenesis [114]. Targeted loss of the flagellar long form of SPAG16 (SPAG16L), which interacts with SPAG6 and localizes to the CPA microtubule bridge in *C. reinhardtii*, results in spermatogenesis defects [115–117]. Crossing the *Spag16l* and *Spag6* knockout lines result in a severe PCD phenotype in double mutants, demonstrating a genetic interaction between the two CPA genes [118].

SPAG17 localizes to the C1a projection in *C. reinhardtii* and is required for proper C1a assembly [119, 120]. Crossing a conditional *Spag17* knockout with a ubiquitous *CMV-Cre* line produces a PCD phenotype with CPA assembly defects [121], and crossing to a *Sox2-Cre* line to ensure loss in germ cells demonstrated severe defects in spermatogenesis [122]. These phenotypes were confirmed by identification of *SPAG17* mutations in PCD patients and patients with asthenozoospermia [123, 124]. Loss of *Sperm flagellar 2* (*Spef2*), also known as *Kpl2*, which encodes a C1b projection protein in *C. reinhardtii*, results in PCD phenotypes without ciliary axonemal defects detected by TEM in the spontaneous mouse line *big giant head* (*bgh*) and a targeted knockout [125–129]. Confirming its role, *SPEF2* mutations were recently identified in human PCD patients and MMAF patients without PCD [130–132]. Similarly, PCD without apparent axonemal defects in the spontaneous mouse model *nm1054* results from the deletion of *Cilia and flagella associated protein 221* (*Cfap221*), also known as *Primary ciliary dyskinesia protein 1* (*Pcdp1*), which encodes a C1d projection protein in *C. reinhardtii* [133, 134]. *CFAP221* mutations were also recently identified in human PCD patients [135]. Another member of the C1d projection complex, CFAP54, was studied in a mouse model with a gene-trapped allele that results in a similar phenotype but with a detected loss of the C1d projection by TEM [133, 136]. Finally, two separate targeted knockout mouse models lacking *Ser/Thr kinase 36* (*Stk36*), also known as *Fused*, showed a PCD phenotype [137, 138], and a third targeted allele was reported to have motile cilia lacking a CPA [139–141]. Although the precise localization of STK36 in the CPA is unknown, mouse models and PCD patients with *STK36* mutations demonstrate an important role in CPA assembly [142]. Taken together, mouse models with mutations in CPA genes highlight the striking complexity of this critical ciliary structure.

Radial spoke structure and assembly

The radial spokes connect the outer A microtubule to the CPA, with the stalk attached to the microtubule and the spoke head associating with the CPA (Fig. 1) (reviewed in detail in Zhu et al. 2017) [28]. Along with the CPA, the radial spokes play a pivotal role in regulating ciliary beat frequency and waveform. Mouse models with mutations in the radial spoke genes exhibit PCD phenotypes and axonemal assembly defects that often lead to a rotational ciliary waveform rather than planar beating (Table 5). Radial spoke head component 1 (RSPH1), a spoke head protein implicated in a relatively mild human PCD phenotype with CPA and spoke head defects, was studied in a traditional targeted mouse with CPA and waveform defects preventing proper mucociliary clearance [35, 143–146]. Similarly, traditional TEM and high-resolution cryo-electron tomographic analyses in a targeted knockout mouse lacking another radial spoke head gene, *Rsph4a*, showed spoke head defects, and CPA defects were observed in PCD patients with *RSPH4A* mutations

Table 5 Mouse models of radial spoke structure and assembly genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
CFAP206	KO	HC, MI, sinus	N/A	Increased CBF	Beckers [156]
DNAJB13	CRISPR	HC	N/A	N/A	Oji [153]
	Chimeric with biallelic mutations	MI	N/A	N/A	Oji [153]
NME5	KO	HC, MI, sinus	N/A	N/A	Vogel [138]
RSPH1	KO	HC, sinus	CPA defects	Rotational waveform, decreased flow	Yin [146]
RSPH4	KO	HC	RS defects	Rotational waveform	Shinohara [149], Yoke [150]

HC hydrocephalus, *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis)

[147–150]. Mutations in *Non-metastatic 5* (*Nme5*), which encodes a nucleoside diphosphate kinase that localizes to the radial spoke stalk in *C. reinhardtii*, result in a PCD phenotype in mice with a traditional targeted allele and human patients with radial spoke and CPA defects [138, 151, 152]. DnaJ heat shock protein family member B13 (DNAJB13), a heat shock protein 40 (HSP40) co-chaperone that localizes to the radial spoke, was studied in a CRISPR/Cas9 gene-edited mouse model that exhibits severe hydrocephalus and early mortality, while chimeric mice with bi-allelic mutations have male infertility due to spermatogenic defects [153, 154]. Human mutations result in PCD with CPA defects [155]. Finally, a traditional targeted allele of *Cfap206*, which is required for radial spoke and dynein docking in *Tetrahymena*, results in PCD with increased ciliary motility [156, 157]. These models support the hypothesis of an important functional link between the radial spokes and CPA, as well as demonstrate the critical role of both structures in regulating ciliary waveform.

The nexin-dynein regulatory complex

The N-DRC is a large complex associated with the outer microtubule doublets that regulates doublet alignment and sliding during axonemal bending, as well as other protein complexes within the 96 nm repeat unit (Fig. 1) [158, 159]. Genetic manipulation of two genes resulting in PCD has shed light on this structure (Table 6). The novel ciliary protein Growth arrest-specific 8 (GAS8), also known as Dynein regulatory complex 4 (DRC4), is required for N-DRC assembly and structural integrity in *C. reinhardtii*, and human mutations result in PCD with N-DRC assembly defects [160–163]. Mice with a gene-trapped allele have disorganized microtubule doublets resulting in abnormal ciliary waveform and reduced motility [163]. Crossing these null mice to a knock-in line with a missense mutation found in human patients resulted in compound heterozygotes displaying only mild hydrocephalus and lacking significant ciliary motility defects, suggesting that the compound heterozygous mouse is a hypomorph [163]. In addition, a PCD phenotype results from an ENU-induced missense mutation in *LRRC48*, also known as *DRC3*, which encodes an N-DRC protein that regulates *C. reinhardtii* flagella [86, 164]. Although it has been less studied in mouse models than other ciliary constituents, mutations affecting the N-DRC highlight its importance in regulating ciliary function.

Multiciliated cell differentiation

Differentiation of multiciliated cells from epithelial progenitors is a complex process initiated by inactivation of Notch signaling, which drives expression of a cascade of transcription factors that include GEMC1, E2F, MCIDAS, TAp73, and ultimately, the direct regulators of the ciliary transcriptome FOXJ1 and multiple RFX family transcription factors (reviewed in detail in Boutin and Kodjabachian 2019 and Spassky and Meunier 2017) [30, 31]. This pathway drives proper cell polarity, formation and apical localization of basal bodies derived from centrioles, and ciliogenesis on the apical surface of the cell. Formation of motile cilia is driven by the intraflagellar transport (IFT) machinery that carries ciliary cargo to the tip for assembly and recycles cargo for continued ciliary growth [165]. Table 7 shows that mouse models with mutations in genes driving multiciliated cell differentiation typically have PCD phenotypes and loss of motile cilia.

Forkhead box J1 (*Foxj1*), also known as *Hepatocyte nuclear factor 3 forkhead homolog 4* (*Hfh4*), encodes a winged-helix transcription factor expressed only in cells assembling motile cilia and, in 1998, was the first gene required for motile cilia formation or function to be studied in a knockout mouse model [166, 167]. Two separate targeted alleles showed an absence of motile cilia on the airway epithelial surface and loss of ependymal differentiation from radial glia in the brain [167–169]. While the presence of basal bodies in both models indicates a commitment to the ciliated cell fate upstream of FOXJ1, the basal bodies are disorganized and not properly localized to the apical surface [170, 171]. Gene expression studies demonstrated a direct role for FOXJ1 in driving the ciliary transcriptome. Microarray analysis of the rostral forebrain identified a number of ciliary genes that are down-regulated in knockout mice, identifying FOXJ1 as a master regulator of ciliogenesis [169]. *FOXJ1* mutations were recently identified in PCD

Table 6 Mouse models of nexin-dynein regulatory complex (N-DRC) genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
GAS8/DRC4	Gene trapped	HC, HT	Microtubule defects	Abnormal waveform, reduced CBF, decreased flow	Lewis [163]
	Gene trapped; CRISPR knock-in	HC	N/A	Normal	Lewis [163]
LRRC48/DRC3	ENU	HC, HT, sinus	N/A	N/A	Ha [86]

HC hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis)

Table 7 Mouse models of multiciliated cell differentiation genes

Gene	Allele	PCD phenotypes	Cell structure	Cilia function	References
CCNO	KO	HC, sinus	Fewer cilia, centriole defects	N/A	Funk [188]
	KO	HC, MI, FI	No cilia	N/A	Nunez-Olle [189]
FOXJ1/HFH4	KO	HC, HT	No cilia, basal body defects	N/A	Chen [167], Gomperts [171], You [170]
	KO	HC, HT	No cilia, basal body defects	N/A	Brody [168], Gomperts [171], You [170]
GEMC1	Conditional KO	HC	No cilia	N/A	Arbi [182], Lalioti [183]
	KO	HC, MI, FI	No cilia	N/A	Terre [184, 185]
MCIDAS	CRISPR	N/A	Fewer cilia, basal body defects	N/A	Lu [181]
MT1-MMP	KO	HC	Fewer cilia, shorter cilia, basal body defects	Decreased flow	Jiang [195]
RFX2	Gene trapped	MI	N/A	N/A	Shawlot [172]
	KO	MI	N/A	N/A	Kistler [173]
	KO	MI	N/A	N/A	Wu [174]
RFX3	KO	HC, HT	Fewer cilia, shorter cilia	Reduced CBF	Baas [176], Bonnafe [175], El Zein [177]
RFX4	Conditional KO	HC	Fewer cilia	N/A	Xu [178]
	ENU	None	N/A	N/A	Ashique [179]
SNX27	KO	HC	Fewer cilia	N/A	Wang [196]
TAp73	KO	HC, sinus	No cilia	N/A	Gonzalez-Cano [191], Nemajerova [192], Yang [190]
	KO	N/A	No cilia	N/A	Marshall [193]
	Conditional KO	HC	N/A	N/A	Fujitani [194]
	Knock-in	HC	N/A	N/A	Fujitani [194]
ULK4	KO	HC, sinus	N/A	N/A	Vogel [138]
	KO	HC	Microtubule defects	Decreased flow	Liu [200]
VPS35	Conditional KO	HC	Fewer cilia	N/A	Wu [199]

FI female infertility, *HC* hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis)

patients and, unlike the mouse models, represent the only known example of autosomal dominant PCD [20].

Several members of the Regulatory factor X (RFX) transcription factor family play varied roles in regulating multiciliated cell differentiation. *Rfx2*, which is expressed in both motile and primary ciliated cells, has been studied in two targeted knockout mouse models and one gene-trapped allele, all of which have spermatogenic defects but no other PCD phenotypes [172–174]. Quantitative RT PCR, RNA sequencing (RNA-seq), and ChIP-sequencing (ChIP-seq) analyses in testis from both knockout models identified a large number of ciliary genes that are regulated by RFX2 [173, 174]. Mice with a targeted allele of *Rfx3* have shortened cilia that are also fewer in number and have a reduced CBF [175–177]. Quantitative RT PCR and ChIP experiments showed regulation of ciliary genes, including *Foxj1*, in mutant ependymal cells [177]. Finally, family member *Rfx4* has been studied in a conditional targeted knockout and an ENU allele [178, 179]. Breeding the conditional knockout to a *Sox2-Cre* mouse line removed *Rfx4* during development and resulted in a severe phenotype of hydrocephalus and holoprosencephaly, accompanied by a reduced number of ependymal cilia [178]. The missense mutation in the ENU mutant prevents proper nuclear localization of RFX4 and similarly results in severe brain developmental defects and early post-natal mortality [179]. Gene expression studies in brains from wild type and the knockout mouse showed that RFX4 is also a direct regulator of *Foxj1* expression [178].

Mutations in the gene encoding transcription factor Multiciliate differentiation and DNA synthesis associated cell cycle protein (MCIDAS), also known as Multicilin, result in human PCD with a reduced number of cilia assembled on respiratory epithelial cells [180]. A mouse model lacking MCIDAS through CRISPR/Cas9 gene editing has airway epithelial cells with defects in basal body formation and ciliogenesis [181]. A role for transcription factor Geminin coiled-coil domain containing 1 (Gemc1) was uncovered in traditional and conditional knockout models lacking multiciliated cells [182–185]. Microarray analyses of mutant trachea and oviduct showed downregulation of several ciliary genes, including *Mcidas* and *Foxj1* [184], indicating that GEMC1 acts upstream of these factors to regulate differentiation. Experiments involving electroporation of *Gemc1* or *Mcidas* into E14.5 mouse

apical progenitor cells or radial glia showed that expression of either gene drives differentiation into ependymal cells [186]. A *GEMC1* mutation was identified in a patient with congenital hydrocephalus [183]. Cell cycle regulator Cyclin O (CCNO), which is regulated by the GEMC1 cascade, has been implicated in human PCD and studied in two targeted knockout models with loss of motile cilia [187–189]. Mutant mice have structurally abnormal centrioles, indicating that CCNO plays a role in centriole maturation [188]. Similarly, mutations in the gene encoding transcription factor Tumor-associated protein 73 (TAp73), also known as p73, have been reported in two traditional knockout mice and one conditional targeted allele crossed to an inducible *Foxj1-CreERT2* line, all of which lack multiciliated cells [190–194]. Applications of RNA-seq, ChIP, ChIP-seq, and luciferase experiments in mTECs showed direct regulation of several cilia-related genes, including *Foxj1, Rfx2,* and *Rfx3* [192, 193]. Consistent with the knockout lines, an additional *TAp73* knock-in mouse with the *TAp73* gene disrupted by insertion of a LacZ/neomycin cassette was also reported to have severe hydrocephalus [194].

A traditional targeted allele of *Membrane type 1 matrix metalloproteinase* (*MT1-MMP*) results in early mortality and hydrocephalus due to impaired ependymal cell differentiation and abnormal basal body polarity [195]. Mutant ependymal cells assemble fewer and shorter cilia with reduced motility, and treatment of brains with a Notch inhibitor ex vivo rescued the ciliogenesis defects, suggesting that MT1-MMP drives ependymal differentiation through inhibition of Notch signaling. A similar phenotype of hydrocephalus and perturbed ependymal differentiation was reported for a traditional knockout mouse lacking endosomal trafficking protein Sorting nexin 27 (SNX27) [196]. The hydrocephalus was ameliorated by injection of Notch signaling inhibitors into E14.5 embryos in utero, suggesting that SNX27 also regulates differentiation of ciliated ependymal cells through Notch inhibition. Vacuolar protein sorting-associated protein 35 (VPS35), a component of the retromer complex involved in transmembrane protein recycling, was initially implicated in late-onset Parkinson's Disease [197, 198]. However, crossing a conditional knockout mouse to the *Foxj1-CreER* and *GFAP-CreER* mouse lines to assess loss in ependyma resulted in hydrocephalus and defects in ciliated ependymal cell differentiation [199]. A role for serine/threonine kinase Unc-51 like kinase 4 (ULK4) was identified in two targeted knockout mice with PCD phenotypes [138, 200]. Ependymal cilia have microtubule defects that reduce CSF flow, and RNA-seq analysis showed that *Foxj1* expression is perturbed in the mutant cerebral cortex [200]. Overall, mouse models have been instrumental in understanding muticiliated cell differentiation and elucidating the molecular cascade driving these critical cellular changes.

Ciliogenesis and IFT

The IFT process responsible for the formation of both motile and primary cilia involves the assembly of IFT-A and IFT-B complexes into multi-unit IFT trains that carry cargo to the tip (anterograde transport) via the kinesin-2 motor protein and multi-unit IFT trains that recycle (retrograde transport) via the cytoplasmic dynein-2 motor protein (reviewed in detail in Ishikawa and Marshall 2017, Nakayama and Katoh 2020, and Prevo et al. 2017) [165, 201, 202]. A more detailed understanding of motile ciliogenesis has come from mouse models with mutations in genes involved in basal body formation, intracellular trafficking, and intraflagellar transport, as well as genes encoding several novel proteins. Table 8 shows that these models exhibit a wide range of PCD-related phenotypes but commonly display basal body defects and a reduced number of motile cilia. While additional mouse models lacking IFT proteins exhibit defects in primary cilia or sperm flagella, only those with aberrations in or a direct connection to motile cilia are discussed here.

Mice with a targeted allele of *Centrosomal protein 164* (*Cep164*) have a variety of developmental defects consistent with a primary ciliopathy, but conditional loss using a *Foxj1-Cre* mouse line shows a role in motile ciliogenesis with fewer cilia, defects in vesicle trafficking to the basal bodies, and abnormal localization of basal body proteins in mTECs [203]. The basal body protein CHIBBY (CBY) was revealed through a targeted mouse allele to play a pivotal role in ciliogenesis, with mutants having an altered ciliary structure, abnormally positioned basal bodies, a reduced number of ciliary vesicles, and defects in the recruitment of the CEP164 complex to the basal body [204–207]. A traditional knockout mouse lacking the Retinitis pigmentosa GTPase regulator (RPGR), which is thought to play a role in photoreceptor connecting cilium transport and localizes to the motile ciliary transition zone between the basal body and axoneme, showed an X-linked retinitis pigmentosa phenotype [208, 209]. A subsequent model with transgenic overexpression of *Rpgr* showed an absence of sperm flagella in the mutant testis [210], indicating that dysregulation of *Rpgr* perturbs spermatogenesis. A requirement in motile cilia was confirmed in human patients, where *RPGR* mutations result in X-linked PCD with associated retinitis pigmentosa, as well as defects in airway ciliary orientation and motility [19, 211]. The requirement of IFT-B complex member IFT88, also known as Polaris, in motile and primary ciliogenesis has been uncovered in a mouse line with a transgene insertion disrupting *Ift88* (*orpk*) and a conditional knockout [212–214]. Mutant motile cilia are fewer in number, abnormally shaped, and have a reduced CBF [212–214]. In addition, WDR69 was identified in *C. reinhardtii* as an adaptor protein involved in the transport of ODA components [215,

Table 8 Mouse models of ciliogenesis and IFT genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
CBY	KO	Sinus	Fewer cilia, microtubule and basal body defects	N/A	Love [205], Siller [206], Voronina [207]
CEP164	Conditional KO	HC	Fewer cilia, basal body trafficking defects	N/A	Siller [203]
CFAP157	KO	MI	N/A	N/A	Weidemann [218]
CYLD	KO	MI	Fewer cilia, shorter cilia, microtubule and basal body defects	N/A	Yang [217]
IFT88/Polaris	Tg insertion (*orpk*)	HC, HT	Fewer cilia, abnormal shape	Reduced CBF	Taulman [212], Banizs [213]
	Conditional KO	Lung	Fewer cilia	Reduced CBF	Gilley [214]
JHY	Enhancer trapped	HC	Fewer cilia, microtubule and basal foot defects	Decreased flow	Appelbe [222], Muniz-Talavera [223]
NHERF1	KO	HC	Fewer cilia, abnormal orientation	Decreased flow	Treat [224]
ODF2	Gene trapped	MI	N/A	N/A	Tarnasky [221]
	KO	HC, OM, sinus	Fewer cilia, basal body and basal foot defects	Abnormal waveform, decreased flow	Kunimoto [220]
RPGR	KO	N/A	N/A	N/A	Hong [208, 209]
	Tg over-expression	MI	N/A	N/A	Brunner [210]
WDR69	ENU	HC, HT	No ODAs	Dyskinetic cilia	Solomon [52]

HC hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *KO* knockout, *Lung* lung abnormalities (including bronchitis and bronchiectasis), *MI* male infertility (including spermatogenesis and sperm motility defects), *OM* otitis media, *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis), *Tg* transgene

216]. An ENU-induced allele of *Wdr69* was shown to have dyskinetic cilia using μOTC analysis [52].

Novel roles for CYLD, CFAP157, ODF2, JHY, and NHERF1 in ciliogenesis have also been revealed through mouse models. A traditional targeted allele of *CYLD lysine 63 deubiquitinase* (*Cyld*), which is thought to be required for de-ubiquitination and proper localization of centrosomal proteins, has defects in both motile and primary ciliogenesis, including abnormal axonemal structures and shortened motile cilia [217]. The novel CFAP157 localizes to the basal bodies of motile and primary ciliated cells, and male mice with a traditional targeted allele have reduced fertility due to flagellar axonemal defects [218]. A targeted knockout of the gene encoding centrosomal and basal body protein Outer dense fiber 2 (ODF2), also known as Cenexin 1, results in PCD with impaired ciliary waveform due to improper basal body polarization [219, 220]. High percentage chimeric male mice with a gene-trapped allele were also reported to have spermatogenic defects [221]. Loss of the novel Juvenile hydrocephalus (Jhy) in mice with an enhancer-trapped allele results in severe hydrocephalus with delayed differentiation of ependymal cells, microtubule defects and abnormal positioning of the basal feet in ependymal cilia, and abnormal directional flow [222, 223]. A severe hydrocephalus phenotype and abnormal ciliary orientation were also observed in mice with a traditional targeted allele of the gene encoding the cytoskeletal-associated Na + /H + exchanger regulatory factor 1 (NHERF1) [224]. Immunofluorescence analysis of mutant brain sections showed mislocalization of planar cell polarity (PCP) proteins, suggesting that NHERF1 regulation of ciliary orientation and biogenesis is dependent on the non-canonical Wnt signaling pathway.

One report has implicated microRNAs (miRNAs) in a mouse model with perturbed motile ciliogenesis. The *mir-34/449* miRNA family spans three genomic loci, and a triple knockout mouse has a PCD phenotype due to loss of multiciliated cells and mature sperm [225]. Tracheal epithelial cells showed a defect in basal body docking and decreased cilia-driven flow. Interestingly, mutant tracheae have an increase in expression of CEP110 [225], which plays a role in suppressing ciliogenesis [226], suggesting that the *mir-34/449* miRNAs promote ciliogenesis by inhibiting CEP110 expression. Although there is also an extensive body of information about ciliogenesis and IFT in primary cilia [23], these mouse models have effectively uncovered a number of important proteins and their role in motile cilia biogenesis.

Microtubule assembly and modification

The ciliary axoneme is highly dependent on the assembly of tubulin into the 9 + 2 microtubule structure. Post-translational modification of tubulin, including detyrosination, glutamylation, and glycylation, is critical for proper microtubule assembly and function in motile cilia (reviewed in

detail in Wloga et al. 2017) [227]. Several genes encoding proteins involved in post-translational modification and polymerization of microtubules have been studied in mouse models, with loss often producing a PCD phenotype due to a wide variety of ciliary defects (Table 9). Loss of heat shock transcription factor 1 (HSF1) in a traditional knockout results in axonemal microtubule defects, as well as reduced CBF and perturbed flow [228, 229]. Expression of several heat shock proteins is decreased in the mutant cilia, and ChIP analysis identified that HSF1 directly binds the promoter of HSP90, a chaperone involved in tubulin polymerization and pre-assembly of dynein arms [228–230], indicating that HSF1 is a regulator of heat shock protein-dependent axonemal assembly. TEKTIN-t, a member of the cytoskeletal tektin protein family that plays a role in stabilizing microtubules and regulating ciliary assembly, was studied in a mouse model with a gene-trapped allele that results in loss of IDAs in cilia and flagella, indicating a role in IDA stabilization [231, 232]. Similarly, traditional knockout and gene-trapped alleles of *tubulin tyrosine ligase-like 1* (*Ttll1*), which is responsible for tubulin glutamylation, produce a PCD phenotype, including a persistent cough detected in the knockout model [233–235]. Knockout cilia are abnormally shaped and exhibit an altered waveform, increased CBF, and decreased flow [235]. The kinesin motor protein Kinesin family member 19A (KIF19A) localizes to the ciliary tip and promotes microtubule depolymerization, and a traditional knockout has elongated cilia with an abnormal waveform [236, 237], indicating an important role for polymerization and depolymerization of microtubules in proper ciliary assembly. Loss of KIF27, another kinesin family member with a microtubule-binding domain, also produces a PCD phenotype in a mouse model with a gene-trapped allele [138]. A gene-trapped allele of *Calmodulin regulated spectrin associated protein 3* (*Camsap3*), a microtubule-binding protein involved in microtubule assembly, polarity, and stability in a variety of cell types, results in longer and abnormally curved motile cilia with CPA microtubule defects and reduced CBF, indicating an important role in axonemal microtubule assembly [238–240]. Loss of the novel galectin GAL3 in a traditional targeted knockout results in a reduced number of ependymal cilia and abnormally oriented tracheal epithelial cilia with microtubule defects and abnormal directional flow [241, 242]. Immunoelectron microscopy demonstrated that GAL3 localizes to the basal foot and ciliary rootlet, and EM tomography showed a disrupted association between the basal foot and the ciliary rootlet microtubules in knockout mice [241], indicating that GAL3 is required for proper microtubule assembly and interaction. Finally, mutations in *Growth arrest specific 2 like 2 (GAS2L2)*, which encodes a ciliary base protein involved in actin-microtubule interaction, were found to cause PCD in human patients with hyperkinetic cilia, and a conditional knockout mouse crossed to the tamoxifen-inducible *Foxj1-CreERT2::GFP* line for inducible loss in motile ciliated cells showed abnormal ciliary orientation, increased CBF, and reduced nasal clearance of radioactive particles in vivo [243, 244]. Mouse models disrupting microtubule assembly and modification underscore the important role for these varied proteins in proper ciliary assembly, integrity, and function.

Table 9 Mouse models of microtubule modification genes

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
CAMSAP3	Gene trapped	HC, FI, MI, sinus	Longer cilia, abnormal curvature, CPA microtubule defects	Reduced CBF	Robinson [238]
GAL3	KO	None	Abnormal orientation, microtubule and basal foot defects	Decreased flow	Clare [241]
GAS2L2	Conditional KO	HT, sinus	Abnormal orientation	Increased CBF, decreased flow	Bustamante-Marin [243]
HSF1	KO	HC, sinus	Microtubule defects	Reduced CBF, decreased flow	Takaki [228, 229]
KIF19A	KO	HC, FI	Longer cilia	Abnormal waveform	Niwa [237]
KIF27	Gene trapped	HC, OM, sinus	N/A	N/A	Vogel [138]
TEKTIN-t	Gene trapped	MI	No IDA	N/A	Tanaka [232]
TTLL1	KO	MI, sinus	Abnormal curvature	Increased CBF, abnormal waveform, decreased flow	Ikegami [235]
	Gene trapped	MI, sinus	N/A	N/A	Vogel [233]

FI female infertility, *HC* hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *OM* otitis media, *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis)

Genes with miscellaneous or unknown functions

A variety of mouse models have revealed previously unknown ciliary roles for proteins or implicated uncharacterized, novel genes in mammalian motile cilia. Although information about these genes is often limited, reported mouse models with a PCD phenotype and ciliary defects have uncovered their importance for motile cilia (Table 10). Protein disulfide isomerase Anterior gradient 3 (AGR3) localizes to the endoplasmic reticulum of motile ciliated cells, and a traditional targeted allele was reported to result in reduced CBF and abnormal cilia-driven flow despite an absence of PCD phenotypes or ciliary structural defects detected by TEM [245]. Two mouse models with mutations in the gene encoding adenylate kinase 7 (AK7), one gene-trapped allele and one with a transgene insertion disrupting the *Ak7* locus that results in cilia with microtubule abnormalities, have demonstrated a role for AK7 in motile cilia [138, 246, 247]. Consistent with these phenotypes, *AK7* mutations have been identified in human PCD patients and MMAF patients [247, 248]. Mice with a targeted allele of the gene encoding centrosomal protein ADP ribosylation factor like GTPase 2 binding protein (ARL2BP) were found to exhibit phenotypes associated with both primary and motile cilia, with tracheal epithelial cilia showing impaired motility, and *ARL2BP* mutations have been identified in patients with motile and primary ciliopathy phenotypes [249–251]. A gene-trapped allele of the microtubule-binding nucleoside diphosphate kinase gene *Nme7* results in a PCD phenotype [138, 252], and an *NME7* mutation was identified in a human family with heritable situs inversus but no detected defects in nasal epithelial cilia [253].

The spontaneous mutant mouse line *quaking*^{viable} uncovered a ciliary role for the *Parkin co-regulated gene* (*Pacrg*), which is regulated with the Parkinson's disease gene *parkin* through a bi-directional promoter and encodes a protein in *C. reinhardtii* that localizes to the inner junction of the outer doublet microtubules [254–258]. Deletion of the *Pacrg* gene in this model results in reduced ciliary motility [257]. A targeted allele of *EF-hand calcium binding domain 1* (*Efcab1*), also known as *Calaxin*, results in altered ciliary motility, waveform, and flow [259]. Situs inversus was reported in a human patient and a traditional targeted mouse model lacking axonemal calcium channel interacting protein Enkurin (ENKUR) [260]. Mutations in the gene encoding novel axonemal protein CFAP43 were identified in human MMAF patients and a family with normal-pressure hydrocephalus [261–264], and traditional and CRISPR/Cas9 gene-edited knockout mouse models were reported to have PCD phenotypes with increased CBF, decreased fluid flow, and CPA and radial spoke defects [263, 264]. A PCD phenotype with absent IDAs was reported for a mouse model with a traditional targeted allele of the gene encoding DNA polymerase λ (POLL) [265], although a subsequent report identified that the novel gene *Deleted in primary ciliary dyskinesia* (*Dpcd*) is transcribed in the opposite direction and is also likely disrupted by the mutation [266]. A mouse model with a gene-trapped allele disrupting both genes was also reported to have PCD [138, 252]. Unlike *Poll, Dpcd* expression is increased during differentiation of cultured ciliated human bronchial epithelial cells, and mice with a

Table 10 Mouse models of genes with miscellaneous or unknown function

Gene	Allele	PCD phenotypes	Cilia structure	Cilia function	References
AGR3	KO	None	Defects not detected	Reduced CBF, decreased flow	Bonser [245]
AK7	Tg insertion	HC, MI, sinus, lung	Microtubule defects	Reduced CBF	Fernandez-Gonzalez [246], Lores [247]
	Gene trapped	HC, MI, sinus	N/A	N/A	Vogel [138]
ARL2BP	KO	HC, HT, MI	N/A	Reduced CBF	Moye [249, 250]
CFAP43	CRISPR	HC, MI	Microtubule defects	N/A	Morimoto [264]
	KO	HC, MI, sinus	Defects not detected	Increased CBF, decreased flow	Rachev [263]
DPCD/POLL	KO	HC, HT, MI, sinus	No IDA	N/A	Kobayashi [265]
	Gene trapped	HC, HT, MI, sinus	N/A	N/A	Vogel [252]
EFCAB1/Calaxin	KO	HC, HT	Defects not detected	Reduced CBF, abnormal waveform, decreased flow	Sasaki [259]
ENKUR	KO	HT	N/A	Normal CBF	Sigg [260]
NME7	Gene trapped	HC, HT, sinus	N/A	N/A	Vogel [252], Vogel [138]
PACRG	Sp (*quaking viable*)	HC, MI	Defects not detected	Reduced CBF	Wilson [257], Li [258]
PK2	KO	None	Membrane bulges	Decreased flow	Tao [268]

HC hydrocephalus, *HT* heterotaxy (including situs inversus and congenital heart defects), *Lung* lung abnormalities (including bronchitis and bronchiectasis), *KO* knockout, *MI* male infertility (including spermatogenesis and sperm motility defects), *N/A* not reported, *Sinus* sinus abnormalities (including mucus accumulation and sinusitis), *Sp* spontaneous, *Tg* transgene

homozygous deletion of the *Poll* catalytic domain are viable and fertile, suggesting that disruption of *Dpcd* is responsible for the PCD phenotype [266, 267]. Finally, while the loss of Prickle planar cell polarity protein 2 (PK2) was initially shown to result in a seizure phenotype in a traditional targeted knockout and human patients [268], motile cilia from mutant mice have abnormal bulges protruding from the membrane and tip that perturb ciliary clearance [269, 270], although the connection between these defects and the seizure phenotype remains unclear. While the roles and phenotypes of these models are highly variable, they further underscore the complexity of mammalian motile cilia and the value of mouse models in understanding the effects of ciliary dysfunction on disease pathogenesis.

Novel genetic tools

In addition to the spectrum of mouse models with genetic mutations resulting in ciliary dysfunction and PCD, several mouse lines have been generated to serve as powerful tools for labeling, identifying, or manipulating ciliated cells. Recombination of a conditional allele in motile ciliated cells has been enabled by a *Foxj1-Cre* mouse line and a tamoxifen-inducible *Foxj1-CreERT2* line, each expressing a transgenic Cre recombinase under the *Foxj1* promoter [271, 272]. Alternatively, a knock-in line with a tamoxifen-inducible *CreERT2::GFP* in the *Foxj1* locus enables inducible Cre expression under the endogenous *Foxj1* promoter and GFP labeling in the same cells [273]. A mouse line expressing a *GFP* transgene under the *Foxj1* promoter allows GFP labeling of all motile ciliated cells [274]. In addition to enabling the study of conditional genetic knockouts, these mouse lines have been used effectively for lineage tracing experiments that monitor ciliated cell differentiation and turn-over in the airways and developing nervous system [272, 273, 275, 276]. In addition, the *CiliaGFP* transgenic line expressing the ciliary protein somatostatin receptor 3 (SSTR3) fused to GFP and a Cre-inducible line with ciliary protein ARL13B fused to RFP and knocked into the HPRT locus both enable labeling of motile and primary ciliated cell types [277, 278]. These novel genetic tools serve as a highly valuable resource that can be bred to PCD models to assess disease pathogenesis or used for downstream cell biological and biochemical analysis of ciliated cells.

Cell biological approaches to studying cilia in mouse models

In addition to the substantial amount of genetic information provided by mouse models, recent years have seen an increase in the application of cutting-edge cell biological techniques to mouse models and their tissues and primary cells. While cell biological advances in the motile cilia field have historically come from lower eukaryotes such as *C. reinhardtii*, recent progress has enabled the use of mouse models in innovative ways. This is resulting in a much deeper understanding of the molecular and cellular mechanisms regulating mammalian motile cilia and multiciliated cell differentiation. Advancements in the areas of genetic, pharmacological, and toxicological manipulation of ciliated cells; transcriptomic and large-scale gene expression techniques; and in vivo, ex vivo, and ciliary imaging are enabling substantial progress in the mammalian motile cilia field.

Genetic manipulation of ciliated cells

The primary culture of ciliated epithelia, particularly mTECs, at an air–liquid interface (ALI) has become a critical system for cell biological analysis of mammalian motile cilia [279]. Cells can be cultured from wild type or mutant mice and can be manipulated genetically through lentiviral transduction, enabling either exogenous over-expression or short hairpin RNA (shRNA) and small interfering RNA (siRNA) gene knockdown approaches to investigate cellular and biochemical processes. Notable examples include over-expression of transcription factors GEMC1, MCIDAS, and TAp73 and ChIP experiments in mTECs to demonstrate the molecular cascade required for differentiation into multiciliated cells and induction of ciliogenesis [182, 192, 193, 280], as well as knockdown experiments that shed light on the mechanisms underlying centriole assembly and basal body formation [281]. Over-expressing mutant forms of genes have been shown to alter mTEC differentiation. Expression of a dominant-negative *Mcidas* blocks differentiation and perturbs PCP signaling [280, 282], while a dominant-negative cyclin-dependent kinase *Cdk2* was shown to block cilia formation and identified a relationship between motile ciliogenesis and the cell cycle [283]. Expression of exogenous proteins also enables protein–protein interaction studies in ciliated mTECs, such as co-IP experiments demonstrating interactions between GST-tagged ZMYND10 and cytoplasmic dynein assembly factors [99].

Cultured mouse ependymal cells have also gained in prominence and have been used for similar applications, including several notable siRNA and shRNA gene knockdown studies. Knockdown of radial spoke gene *Rsph9*, which was implicated in human PCD, confirmed radial spoke and CPA defects and rotational ciliary beating [284]. Knocking down transcription factor genes *Gemc1* or *Mcidas* in cultured radial glial cells prevented differentiation into ependyma and *Foxj1* expression [186], validating their role in driving multiciliated cell differentiation. Systematic knockdown of several members of the diverse tubulin tyrosine ligase-like (TTLL) family identified which members were required for multiciliated cell differentiation and ciliary

motility [285]. Finally, knockdown of several novel genes, including *Cfap70, Wdr78*, and *Spef1* has resulted in perturbations of ciliary assembly, beat frequency, or waveform, thereby uncovering key roles for these genes in ependymal cilia [286–288].

In addition to culturing and differentiating progenitor epithelial cells from the trachea or the brain, cultured embryonic stem cells have also been differentiated into motile ciliated cells expressing *Foxj1* and other ciliated cell markers through the inhibition of BMP signaling [289]. Mouse induced pluripotent stem (iPS) cells have also been successfully driven toward an epithelial cell fate under serum-free and ALI conditions, exhibiting formation of motile cilia and expression of *Foxj1* but not markers of other airway epithelial cell types [290]. These cell types expand the platforms available for cell biological analysis using wild type or genetically modified mouse models.

Pharmacological manipulation of ciliated cells

In addition to genetic manipulation, mouse tissues and cultured cells have been used in recent years for pharmacological modulation of signaling pathways and cellular processes underlying ciliogenesis and cilia function. Notable examples include the treatment of cultured embryonic mouse lungs with a Notch inhibitor to drive differentiation of ciliated cells and reduce the number of non-ciliated Club cells [291], establishing the critical importance of Notch signaling in airway epithelial cell fate. Treatment of mouse tracheal rings or brain slices with adenosine A2B receptor agonists stimulates CBF and protein kinase A (PKA) activity [292, 293], demonstrating regulation of ciliary motility in a cAMP-dependent manner. Treatment of mouse tracheal rings with an inhibitor of heat shock protein HSP90 impaired ciliary assembly and motility [229], and treatment of dissected mouse fallopian tube with a progesterone agonist decreases CBF, while a progesterone antagonist has the opposite effect [294].

Similar to mouse tissues, cultured cells have also enabled pharmacological studies. For example, basal body alignment was disrupted in mTECs treated with the microtubule depolymerizing agent nocodazole, resembling defects observed in mTECs from *Odf2* knockout mice [295]. Cultured mTECs exposed to the endotoxin lipopolysaccharide (LPS) show an increase in intracellular calcium concentration that is blocked by treatment with an inhibitor of cation channel TRPV4 [296], uncovering an important role for TRPV4 channels in providing an extracellular calcium source to ciliated cells. Cultured cells have also been used effectively for small molecule screens. Cultured ependymal cells were used for a screen of small molecules that promote proper ependymal differentiation and *Foxj1* expression [297], and treatment of mTECs with small molecule inhibitors of cyclin-dependent kinases showed centriolar defects and impaired ciliogenesis, confirming the relationship to the cell cycle identified through over-expression of a dominant-negative *Cdk2* mutant [283]. Not only does pharmacological modulation of cultured ciliated cells enable elucidation of fundamental cellular and molecular processes, but cells cultured from the wide spectrum of PCD models or manipulated by the genetic approaches described above could also serve as a highly useful system for testing the effect of pharmacological PCD therapies on mammalian cilia function.

Toxicological manipulation of ciliated cells

Ciliated mouse cells have also served as a system for studying the effects of several environmental factors on ciliary function. Cultured mTECs have been used to study the effects of alcohol on ciliary motility and its regulatory pathways [298–300], as well as the effect of hypoxic conditions on gene transcription during multiciliated cell differentiation [301]. Protective response to bacteria was assessed in mTECs incubated with *E. coli* prior to culturing under ALI conditions [296], and effect of mechanical pressure on ciliary stimulation was assessed by treating ALI mTECs with an air puff at the apical surface [302]. These studies have utilized cells from wild type and genetically modified mouse models, which enables identifying the effect of genetic mutations on response to environmental stimuli and identifies the molecular mechanisms underlying those responses.

Transcriptomic and gene expression techniques

Cutting-edge gene expression and transcriptome analysis techniques applied to ciliated cells and tissues have uncovered key mechanisms underlying multiciliated cell differentiation. Microarray and RNA-seq have been used effectively to identify differentially expressed genes in cells from genetically modified mouse models or at different stages of ciliogenesis. Notable experiments include the use of microarray analysis of flow-sorted mTECs from the *Foxj1-GFP* mouse described above to identify novel genes expressed at different stages of cell differentiation and ciliogenesis [274, 303, 304]. A microarray approach has also been used to identify ciliary genes differentially expressed in mTECs from mice lacking matrix metalloproteinase 7 (MMP7) [305], identify differentially expressed genes in hydrocephalic brains from mice lacking CPA protein CFAP221 on distinct genetic backgrounds [39], and evaluate gene expression changes in cultured radial glial cells following a screen for small molecules that promote ependymal differentiation [297]. Applications of the next-generation sequencing-based approach RNA-seq have included identification of differentially expressed ciliary genes in *TAp73* knockout mTECs or in subventricular zone (SVZ) cells from the brains of mice lacking

transcription factor PRDM16 [192, 193, 306]. Single-cell RNA-seq was applied to labeled SVZ ependymal cells and neural stem cells to identify ciliary genes expressed only in the ependymal population [307]. ChIP-seq, an approach that combines traditional ChiP with next-generation sequencing to identify interactions between proteins and promoter sequences, has identified ciliary genes directly regulated by key transcription factors, including TAp73 and RFX2 [173, 193]. These studies highlight the ability of large-scale transcriptomic techniques to uncover a wealth of gene expression data underlying key molecular mechanisms.

In vivo and ex vivo animal imaging

Advances in in vivo and ex vivo applications of imaging have enabled a detailed assessment of ciliary physiology and fluid clearance. Examples of in vivo imaging applications in live mice include gamma scintigraphy to detect radioactive particle clearance in the nasal cavity [243, 308], two-photon microscopic analysis of fluorescent bead clearance in the trachea following oropharyngeal instillation delivery [309], synchrotron phase-contrast X-ray imaging to analyze the rate of lead dust particle clearance in trachea [310], and functional optical coherence tomography (fOCT) with high-resolution and tissue penetration depth to achieve detailed mapping of cilia length and beat frequency in the mouse oviduct [311]. In addition to the relatively common applications of high-speed video microscopy to analyze CBF and live imaging of fluorescent bead flow over ciliated cells, both of which were discussed above for analysis of genetic models, several advanced ex vivo imaging approaches have been described. High-resolution live imaging of ventricular walls from wild type mouse brains enabled the identification of three distinct classes of ependymal cells in the third ventricle based on distribution, ciliary angle, and beat frequency [312]. Micro-optical coherence tomography (μOTC) was used for high-resolution imaging of ciliary motility in tracheal explants from several PCD models [52]. Spectral-domain optical coherence phase microscopy (SD-OCPM), which combines the depth of OCT and resolution of confocal microscopy, was used for a high-resolution, high-speed analysis of mouse tracheal morphology and ciliary dynamics [313]. These imaging approaches have distinct capabilities and advantages, but taken together have a substantially advanced analysis of mouse models.

Cilia microscopy techniques

Innovative microscopy techniques have also been applied to cultured cells and have enabled a more detailed analysis of ciliary structure and function. Super-resolution microscopy applications have been employed for fine analysis of protein localization in mTECs, including mapping the region of CBY localization in the centriole [314]. When combined with ultra-high-voltage electron tomography, super-resolution microscopy has enabled analysis of the distribution of apical actin filaments, intermediate filaments, and microtubules in the mTEC basal bodies [295]. Super-resolution structural illumination microscopy (SIM) has been used for high-resolution analyses of deuterosome assembly and centriole amplification in mTECs [315, 316], as well as detailed protein localization in undifferentiated and differentiated cultured ependymal cells [288]. Three-dimensional, two-color stochastic optical reconstruction microscopy (STORM) has been used for detailed analysis of mTEC transition zone protein distribution [317]. Continued application of high-resolution imaging to cultured mouse cells will enable a much more detailed and high-resolution analysis of intraciliary mechanisms and dynamics.

Conclusions

Advancements in genetic manipulation and phenotypic analysis of mouse models, along with the application of innovative cell biological techniques, have led to considerable progress in understanding mammalian motile cilia and PCD pathogenesis. The wide spectrum of genetic models has validated the importance of many ciliary genes and uncovered new roles for numerous novel genes involved in cilia function. In addition, the emergence of cutting-edge cell biological techniques and their application to mouse models has enabled a much deeper investigation of the molecular and cellular mechanisms regulating multiciliated cell differentiation and mammalian motile cilia function. Because our understanding of these mechanisms is still far from complete, continued innovation in cell biological strategies and their application to the murine system is necessary. As advancements progress, mouse models can also serve as a powerful pre-clinical platform for identifying, developing, and testing the efficacy of potential PCD therapeutics.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflicts.

References

1. Justice MJ, Dhillon P (2016) Using the mouse to model human disease: increasing validity and reproducibility. Dis Model Mech 9(2):101–103. https://doi.org/10.1242/dmm.024547
2. Rosenthal N, Brown S (2007) The mouse ascending: perspectives for human-disease models. Nat Cell Biol 9(9):993–999. https://doi.org/10.1038/ncb437
3. Oliver PL, Bitoun E, Davies KE (2007) Comparative genetic analysis: the utility of mouse genetic systems for studying human monogenic disease. Mamm Genome 18(6–7):412–424. https://doi.org/10.1007/s00335-007-9014-8
4. Peters LL, Robledo RF, Bult CJ, Churchill GA, Paigen BJ, Svenson KL (2007) The mouse as a model for human biology: a resource guide for complex trait analysis. Nat Rev Genet 8(1):58–69. https://doi.org/10.1038/nrg2025
5. Gurumurthy CB, Lloyd KCK (2019) Generating mouse models for biomedical research: technological advances. Dis Model Mech 12:1. https://doi.org/10.1242/dmm.029462
6. Lutz C (2018) Mouse models of ALS: past, present and future. Brain Res 1693(Pt A):1–10. https://doi.org/10.1016/j.brainres.2018.03.024
7. Kosior N, Leavitt BR (2018) Murine models of huntington's disease for evaluating therapeutics. Methods Mol Biol 1780:179–207. https://doi.org/10.1007/978-1-4939-7825-0_10
8. Carias KV, Wevrick R (2019) Preclinical testing in translational animal models of prader-willi syndrome: overview and gap analysis. Mol Ther Methods Clin Dev 13:344–358. https://doi.org/10.1016/j.omtm.2019.03.001
9. Noble JN, Mishra A (2019) Development and significance of mouse models in lymphoma research. Curr Hematol Malig Rep 14(2):119–126. https://doi.org/10.1007/s11899-019-00504-0
10. Lee L (2011) Mechanisms of mammalian ciliary motility: insights from primary ciliary dyskinesia genetics. Gene 473(2):57–66. https://doi.org/10.1016/j.gene.2010.11.006**(S0378-1119(10)00432-4[pii])**
11. Knowles MR, Zariwala M, Leigh M (2016) Primary ciliary dyskinesia. Clin Chest Med 37(3):449–461. https://doi.org/10.1016/j.ccm.2016.04.008
12. Horani A, Ferkol TW, Dutcher SK, Brody SL (2016) Genetics and biology of primary ciliary dyskinesia. Paediatr Respir Rev 18:18–24. https://doi.org/10.1016/j.prrv.2015.09.001
13. Satir P, Christensen ST (2007) Overview of structure and function of mammalian cilia. Annu Rev Physiol 69:377–400
14. Bustamante-Marin XM, Ostrowski LE (2017) Cilia and mucociliary clearance. Cold Spring Harb Perspect Biol 9:4. https://doi.org/10.1101/cshperspect.a028241
15. Brown JM, Witman GB (2014) Cilia and diseases. Bioscience 64(12):1126–1137. https://doi.org/10.1093/biosci/biu174
16. Ishikawa T (2017) Axoneme structure from motile cilia. Cold Spring Harb Perspect Biol 9:1. https://doi.org/10.1101/cshperspect.a028076
17. Paff T, Loges NT, Aprea I, Wu K, Bakey Z, Haarman EG, Daniels JMA, Sistermans EA, Bogunovic N, Dougherty GW, Hoben IM, Grosse-Onnebrink J, Matter A, Olbrich H, Werner C, Pals G, Schmidts M, Omran H, Micha D (2017) Mutations in PIH1D3 cause X-linked primary ciliary dyskinesia with outer and inner dynein arm defects. Am J Hum Genet 100(1):160–168. https://doi.org/10.1016/j.ajhg.2016.11.019
18. Olcese C, Patel MP, Shoemark A, Kiviluoto S, Legendre M, Williams HJ, Vaughan CK, Hayward J, Goldenberg A, Emes RD, Munye MM, Dyer L, Cahill T, Bevillard J, Gehrig C, Guipponi M, Chantot S, Duquesnoy P, Thomas L, Jeanson L, Copin B, Tamalet A, Thauvin-Robinet C, Papon JF, Garin A, Pin I, Vera G, Aurora P, Fassad MR, Jenkins L, Boustred C, Cullup T, Dixon M, Onoufriadis A, Bush A, Chung EM, Antonarakis SE, Loebinger MR, Wilson R, Armengot M, Escudier E, Hogg C, Group UKR, Amselem S, Sun Z, Bartoloni L, Blouin JL, Mitchison HM (2017) X-linked primary ciliary dyskinesia due to mutations in the cytoplasmic axonemal dynein assembly factor PIH1D3. Nat Commun 8:14279. https://doi.org/10.1038/ncomms14279
19. Moore A, Escudier E, Roger G, Tamalet A, Pelosse B, Marlin S, Clement A, Geremek M, Delaisi B, Bridoux AM, Coste A, Witt M, Duriez B, Amselem S (2006) RPGR is mutated in patients with a complex X linked phenotype combining primary ciliary dyskinesia and retinitis pigmentosa. J Med Genet 43(4):326–333
20. Wallmeier J, Frank D, Shoemark A, Nothe-Menchen T, Cindric S, Olbrich H, Loges NT, Aprea I, Dougherty GW, Pennekamp P, Kaiser T, Mitchison HM, Hogg C, Carr SB, Zariwala MA, Ferkol T, Leigh MW, Davis SD, Atkinson J, Dutcher SK, Knowles MR, Thiele H, Altmuller J, Krenz H, Woste M, Brentrup A, Ahrens F, Vogelberg C, Morris-Rosendahl DJ, Omran H (2019) De novo mutations in FOXJ1 result in a motile ciliopathy with hydrocephalus and randomization of left/right body asymmetry. Am J Hum Genet 105(5):1030–1039. https://doi.org/10.1016/j.ajhg.2019.09.022
21. Berbari NF, O'Connor AK, Haycraft CJ, Yoder BK (2009) The primary cilium as a complex signaling center. Curr Biol 19(13):R526–535. https://doi.org/10.1016/j.cub.2009.05.025
22. Mitchison HM, Valente EM (2017) Motile and non-motile cilia in human pathology: from function to phenotypes. J Pathol 241(2):294–309. https://doi.org/10.1002/path.4843
23. Reiter JF, Leroux MR (2017) Genes and molecular pathways underpinning ciliopathies. Nat Rev Mol Cell Biol 18(9):533–547. https://doi.org/10.1038/nrm.2017.60
24. Poprzeczko M, Bicka M, Farahat H, Bazan R, Osinka A, Fabczak H, Joachimiak E, Wloga D (2019) Rare human diseases: model organisms in deciphering the molecular basis of primary ciliary dyskinesia. Cells 8:12. https://doi.org/10.3390/cells8121614
25. Loreng TD, Smith EF (2017) The central apparatus of cilia and eukaryotic flagella. Cold Spring Harb Perspect Biol 9:2. https://doi.org/10.1101/cshperspect.a028118
26. King SM (2016) Axonemal dynein arms. Cold Spring Harb Perspect Biol 8:11. https://doi.org/10.1101/cshperspect.a028100
27. Wirschell M, Yamamoto R, Alford L, Gokhale A, Gaillard A, Sale WS (2011) Regulation of ciliary motility: conserved protein kinases and phosphatases are targeted and anchored in the ciliary axoneme. Arch Biochem Biophys 510(2):93–100. https://doi.org/10.1016/j.abb.2011.04.003**(S0003-9861(11)00144-5[pii])**
28. Zhu X, Liu Y, Yang P (2017) Radial spokes-A snapshot of the motility regulation, assembly, and evolution of cilia and flagella. Cold Spring Harb Perspect Biol 9:5. https://doi.org/10.1101/cshperspect.a028126
29. Meunier A, Azimzadeh J (2016) Multiciliated cells in animals. Cold Spring Harb Perspect Biol 8:12. https://doi.org/10.1101/cshperspect.a028233
30. Spassky N, Meunier A (2017) The development and functions of multiciliated epithelia. Nat Rev Mol Cell Biol 18(7):423–436. https://doi.org/10.1038/nrm.2017.21
31. Boutin C, Kodjabachian L (2019) Biology of multiciliated cells. Curr Opin Genet Dev 56:1–7. https://doi.org/10.1016/j.gde.2019.04.006
32. Wingfield JL, Lechtreck KF (2018) Chlamydomonas basal bodies as flagella organizing centers. Cells 7:7. https://doi.org/10.3390/cells7070079
33. O'Toole ET, Giddings TH Jr, Porter ME, Ostrowski LE (2012) Computer-assisted image analysis of human cilia and Chlamydomonas flagella reveals both similarities and differences in axoneme structure. Cytoskeleton (Hoboken) 69(8):577–590. https://doi.org/10.1002/cm.21035

34. Burgoyne T, Dixon M, Luther P, Hogg C, Shoemark A (2012) Generation of a three-dimensional ultrastructural model of human respiratory cilia. Am J Respir Cell Mol Biol 47(6):800–806. https://doi.org/10.1165/rcmb.2011-0440OC
35. Lin J, Yin W, Smith MC, Song K, Leigh MW, Zariwala MA, Knowles MR, Ostrowski LE, Nicastro D (2014) Cryo-electron tomography reveals ciliary defects underlying human RSPH1 primary ciliary dyskinesia. Nat Commun 5:5727. https://doi.org/10.1038/ncomms6727
36. Zhao L, Hou Y, McNeill NA, Witman GB (2020) The unity and diversity of the ciliary central apparatus. Philos Trans R Soc Lond B Biol Sci 375(1792):20190164. https://doi.org/10.1098/rstb.2019.0164
37. Lee L (2013) Riding the wave of ependymal cilia: genetic susceptibility to hydrocephalus in primary ciliary dyskinesia. J Neurosci Res 91(9):1117–1132. https://doi.org/10.1002/jnr.23238
38. Finn R, Evans CC, Lee L (2014) Strain-dependent brain defects in mouse models of primary ciliary dyskinesia with mutations in Pcdp1 and Spef2. Neuroscience 277:552–567. https://doi.org/10.1016/j.neuroscience.2014.07.029
39. McKenzie CW, Preston CC, Finn R, Eyster KM, Faustino RS, Lee L (2018) Strain-specific differences in brain gene expression in a hydrocephalic mouse model with motile cilia dysfunction. Sci Rep 8(1):13370. https://doi.org/10.1038/s41598-018-31743-5
40. Kamiya R, Yagi T (2014) Functional diversity of axonemal dyneins as assessed by in vitro and in vivo motility assays of Chlamydomonas mutants. Zoolog Sci 31(10):633–644. https://doi.org/10.2108/zs140066
41. Blum M, Ott T (2018) Xenopus: an undervalued model organism to study and model human genetic disease. Cells Tissues Org 205(5–6):303–313. https://doi.org/10.1159/000490898
42. Vladar EK, Brody SL (2013) Analysis of ciliogenesis in primary culture mouse tracheal epithelial cells. Methods Enzymol 525:285–309. https://doi.org/10.1016/B978-0-12-397944-5.00014-6
43. You Y, Brody SL (2013) Culture and differentiation of mouse tracheal epithelial cells. Methods Mol Biol 945:123–143. https://doi.org/10.1007/978-1-62703-125-7_9
44. Delgehyr N, Meunier A, Faucourt M, Bosch Grau M, Strehl L, Janke C, Spassky N (2015) Ependymal cell differentiation, from monociliated to multiciliated cells. Methods Cell Biol 127:19–35. https://doi.org/10.1016/bs.mcb.2015.01.004
45. Ermakov A, Stevens JL, Whitehill E, Robson JE, Pieles G, Brooker D, Goggolidou P, Powles-Glover N, Hacker T, Young SR, Dear N, Hirst E, Tymowska-Lalanne Z, Briscoe J, Bhattacharya S, Norris DP (2009) Mouse mutagenesis identifies novel roles for left-right patterning genes in pulmonary, craniofacial, ocular, and limb development. Dev Dyn 238(3):581–594. https://doi.org/10.1002/dvdy.21874
46. Li Y, Klena NT, Gabriel GC, Liu X, Kim AJ, Lemke K, Chen Y, Chatterjee B, Devine W, Damerla RR, Chang C, Yagi H, San Agustin JT, Thahir M, Anderton S, Lawhead C, Vescovi A, Pratt H, Morgan J, Haynes L, Smith CL, Eppig JT, Reinholdt L, Francis R, Leatherbury L, Ganapathiraju MK, Tobita K, Pazour GJ, Lo CW (2015) Global genetic analysis in mice unveils central role for cilia in congenital heart disease. Nature 521(7553):520–524. https://doi.org/10.1038/nature14269
47. Viswanadha R, Sale WS, Porter ME (2017) Ciliary motility: regulation of axonemal dynein motors. Cold Spring Harb Perspect Biol 9:8. https://doi.org/10.1101/cshperspect.a018325
48. Olbrich H, Haffner K, Kispert A, Volkel A, Volz A, Sasmaz G, Reinhardt R, Hennig S, Lehrach H, Konietzko N, Zariwala M, Noone PG, Knowles M, Mitchison HM, Meeks M, Chung EM, Hildebrandt F, Sudbrak R, Omran H (2002) Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry. Nat Genet 30(2):143–144
49. Ibanez-Tallon I, Gorokhova S, Heintz N (2002) Loss of function of axonemal dynein Mdnah5 causes primary ciliary dyskinesia and hydrocephalus. Hum Mol Genet 11(6):715–721
50. Ibanez-Tallon I, Pagenstecher A, Fliegauf M, Olbrich H, Kispert A, Ketelsen UP, North A, Heintz N, Omran H (2004) Dysfunction of axonemal dynein heavy chain Mdnah5 inhibits ependymal flow and reveals a novel mechanism for hydrocephalus formation. Hum Mol Genet 13(18):2133–2141
51. Tan SY, Rosenthal J, Zhao XQ, Francis RJ, Chatterjee B, Sabol SL, Linask KL, Bracero L, Connelly PS, Daniels MP, Yu Q, Omran H, Leatherbury L, Lo CW (2007) Heterotaxy and complex structural heart defects in a mutant mouse model of primary ciliary dyskinesia. J Clin Invest 117(12):3742–3752. https://doi.org/10.1172/JCI33284
52. Solomon GM, Francis R, Chu KK, Birket SE, Gabriel G, Trombley JE, Lemke KL, Klena N, Turner B, Tearney GJ, Lo CW, Rowe SM (2017) Assessment of ciliary phenotype in primary ciliary dyskinesia by micro-optical coherence tomography. JCI Insight 2(5):e91702. https://doi.org/10.1172/jci.insight.91702
53. Pennarun G, Escudier E, Chapelin C, Bridoux AM, Cacheux V, Roger G, Clement A, Goossens M, Amselem S, Duriez B (1999) Loss-of-function mutations in a human gene related to chlamydomonas reinhardtii dynein IC78 result in primary ciliary dyskinesia. Am J Hum Genet 65(6):1508–1519
54. Ostrowski LE, Yin W, Rogers TD, Busalacchi KB, Chua M, O'Neal WK, Grubb BR (2010) Conditional deletion of dnaic1 in a murine model of primary ciliary dyskinesia causes chronic rhinosinusitis. Am J Respir Cell Mol Biol 43(1):55–63. https://doi.org/10.1165/rcmb.2009-0118OC**(2009-0118OC[pii])**
55. Ostrowski LE, Yin W, Patel M, Sechelski J, Rogers T, Burns K, Grubb BR, Olsen JC (2014) Restoring ciliary function to differentiated primary ciliary dyskinesia cells with a lentiviral vector. Gene Ther 21(3):253–261. https://doi.org/10.1038/gt.2013.79
56. Layton WM Jr (1976) Random determination of a developmental process: reversal of normal visceral asymmetry in the mouse. J Hered 67(6):336–338. https://doi.org/10.1093/oxfordjournals.jhered.a108749
57. Supp DM, Witte DP, Potter SS, Brueckner M (1997) Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. Nature 389(6654):963–966
58. Burnicka-Turek O, Steimle JD, Huang W, Felker L, Kamp A, Kweon J, Peterson M, Reeves RH, Maslen CL, Gruber PJ, Yang XH, Shendure J, Moskowitz IP (2016) Cilia gene mutations cause atrioventricular septal defects by multiple mechanisms. Hum Mol Genet 25(14):3011–3028. https://doi.org/10.1093/hmg/ddw155
59. Supp DM, Brueckner M, Kuehn MR, Witte DP, Lowe LA, McGrath J, Corrales J, Potter SS (1999) Targeted deletion of the ATP binding domain of left-right dynein confirms its role in specifying development of left-right asymmetries. Development 126(23):5495–5504
60. Lucas JS, Adam EC, Goggin PM, Jackson CL, Powles-Glover N, Patel SH, Humphreys J, Fray MD, Falconnet E, Blouin JL, Cheeseman MT, Bartoloni L, Norris DP, Lackie PM (2012) Static respiratory cilia associated with mutations in Dnahc11/DNAH11: a mouse model of PCD. Hum Mutat 33(3):495–503. https://doi.org/10.1002/humu.22001
61. Bartoloni L, Blouin JL, Pan Y, Gehrig C, Maiti AK, Scamuffa N, Rossier C, Jorissen M, Armengot M, Meeks M, Mitchison HM, Chung EM, Delozier-Blanchet CD, Craigen WJ, Antonarakis SE (2002) Mutations in the DNAH11 (axonemal heavy chain dynein type 11) gene cause one form of situs inversus totalis and most likely primary ciliary dyskinesia. Proc Natl Acad Sci USA 99(16):10282–10286

62. Schwabe GC, Hoffmann K, Loges NT, Birker D, Rossier C, de Santi MM, Olbrich H, Fliegauf M, Failly M, Liebers U, Collura M, Gaedicke G, Mundlos S, Wahn U, Blouin JL, Niggemann B, Omran H, Antonarakis SE, Bartoloni L (2008) Primary ciliary dyskinesia associated with normal axoneme ultrastructure is caused by DNAH11 mutations. Hum Mutat 29(2):289–298
63. Dougherty GW, Loges NT, Klinkenbusch JA, Olbrich H, Pennekamp P, Menchen T, Raidt J, Wallmeier J, Werner C, Westermann C, Ruckert C, Mirra V, Hjeij R, Memari Y, Durbin R, Kolb-Kokocinski A, Praveen K, Kashef MA, Kashef S, Eghtedari F, Haffner K, Valmari P, Baktai G, Aviram M, Bentur L, Amirav I, Davis EE, Katsanis N, Brueckner M, Shaposhnykov A, Pigino G, Dworniczak B, Omran H (2016) DNAH11 localization in the proximal region of respiratory cilia defines distinct outer dynein arm complexes. Am J Respir Cell Mol Biol 55(2):213–224. https://doi.org/10.1165/rcmb.2015-0353OC
64. Neesen J, Kirschner R, Ochs M, Schmiedl A, Habermann B, Mueller C, Holstein AF, Nuesslein T, Adham I, Engel W (2001) Disruption of an inner arm dynein heavy chain gene results in asthenozoospermia and reduced ciliary beat frequency. Hum Mol Genet 10(11):1117–1128
65. Hu J, Lessard C, Longstaff C, O'Brien M, Palmer K, Reinholdt L, Eppig J, Schimenti J, Handel MA (2019) ENU-induced mutant allele of Dnah1, ferf1, causes abnormal sperm behavior and fertilization failure in mice. Mol Reprod Dev 86(4):416–425. https://doi.org/10.1002/mrd.23120
66. Imtiaz F, Allam R, Ramzan K, Al-Sayed M (2015) Variation in DNAH1 may contribute to primary ciliary dyskinesia. BMC Med Genet 16:14. https://doi.org/10.1186/s12881-015-0162-5
67. Ben Khelifa M, Coutton C, Zouari R, Karaouzene T, Rendu J, Bidart M, Yassine S, Pierre V, Delaroche J, Hennebicq S, Grunwald D, Escalier D, Pernet-Gallay K, Jouk PS, Thierry-Mieg N, Toure A, Arnoult C, Ray PF (2014) Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella. Am J Hum Genet 94(1):95–104. https://doi.org/10.1016/j.ajhg.2013.11.017
68. Hjeij R, Lindstrand A, Francis R, Zariwala MA, Liu X, Li Y, Damerla R, Dougherty GW, Abouhamed M, Olbrich H, Loges NT, Pennekamp P, Davis EE, Carvalho CM, Pehlivan D, Werner C, Raidt J, Kohler G, Haffner K, Reyes-Mugica M, Lupski JR, Leigh MW, Rosenfeld M, Morgan LC, Knowles MR, Lo CW, Katsanis N, Omran H (2013) ARMC4 mutations cause primary ciliary dyskinesia with randomization of left/right body asymmetry. Am J Hum Genet 93(2):357–367. https://doi.org/10.1016/j.ajhg.2013.06.009
69. Onoufriadis A, Shoemark A, Munye MM, James CT, Schmidts M, Patel M, Rosser EM, Bacchelli C, Beales PL, Scambler PJ, Hart SL, Danke-Roelse JE, Sloper JJ, Hull S, Hogg C, Emes RD, Pals G, Moore AT, Chung EM, Mitchison HM (2014) Combined exome and whole-genome sequencing identifies mutations in ARMC4 as a cause of primary ciliary dyskinesia with defects in the outer dynein arm. J Med Genet 51(1):61–67. https://doi.org/10.1136/jmedgenet-2013-101938**(Uk10K)**
70. Hjeij R, Onoufriadis A, Watson CM, Slagle CE, Klena NT, Dougherty GW, Kurkowiak M, Loges NT, Diggle CP, Morante NF, Gabriel GC, Lemke KL, Li Y, Pennekamp P, Menchen T, Konert F, Marthin JK, Mans DA, Letteboer SJ, Werner C, Burgoyne T, Westermann C, Rutman A, Carr IM, O'Callaghan C, Moya E, Chung EM, Consortium UK, Sheridan E, Nielsen KG, Roepman R, Bartscherer K, Burdine RD, Lo CW, Omran H, Mitchison HM (2014) CCDC151 mutations cause primary ciliary dyskinesia by disruption of the outer dynein arm docking complex formation. Am J Hum Genet 95(3):257–274. https://doi.org/10.1016/j.ajhg.2014.08.005
71. Chiani F, Orsini T, Gambadoro A, Pasquini M, Putti S, Cirilli M, Ermakova O, Tocchini-Valentini GP (2019) Functional loss of Ccdc1 51 leads to hydrocephalus in a mouse model of primary ciliary dyskinesia. Dis Model Mech 12:8. https://doi.org/10.1242/dmm.038489
72. Zhou J, Yang F, Leu NA, Wang PJ (2012) MNS1 is essential for spermiogenesis and motile ciliary functions in mice. PLoS Genet 8(3):e1002516. https://doi.org/10.1371/journal.pgen.1002516**(PGENETICS-D-11-01820[pii])**
73. Ta-Shma A, Hjeij R, Perles Z, Dougherty GW, Abu Zahira I, Letteboer SJF, Antony D, Darwish A, Mans DA, Spittler S, Edelbusch C, Cindric S, Nothe-Menchen T, Olbrich H, Stuhlmann F, Aprea I, Pennekamp P, Loges NT, Breuer O, Shaag A, Rein A, Gulec EY, Gezdirici A, Abitbul R, Elias N, Amirav I, Schmidts M, Roepman R, Elpeleg O, Omran H (2018) Homozygous loss-of-function mutations in MNS1 cause laterality defects and likely male infertility. PLoS Genet 14(8):e1007602. https://doi.org/10.1371/journal.pgen.1007602
74. Wallmeier J, Shiratori H, Dougherty GW, Edelbusch C, Hjeij R, Loges NT, Menchen T, Olbrich H, Pennekamp P, Raidt J, Werner C, Minegishi K, Shinohara K, Asai Y, Takaoka K, Lee C, Griese M, Memari Y, Durbin R, Kolb-Kokocinski A, Sauer S, Wallingford JB, Hamada H, Omran H (2016) TTC25 deficiency results in defects of the outer dynein arm docking machinery and primary ciliary dyskinesia with left-right body asymmetry randomization. Am J Hum Genet 99(2):460–469. https://doi.org/10.1016/j.ajhg.2016.06.014
75. Oda T, Yanagisawa H, Kamiya R, Kikkawa M (2014) A molecular ruler determines the repeat length in eukaryotic cilia and flagella. Science 346(6211):857–860. https://doi.org/10.1126/science.1260214
76. Abdelhamed Z, Vuong SM, Hill L, Shula C, Timms A, Beier D, Campbell K, Mangano FT, Stottmann RW, Goto J (2018) A mutation in Ccdc39 causes neonatal hydrocephalus with abnormal motile cilia development in mice. Development 145:1. https://doi.org/10.1242/dev.154500
77. Antony D, Becker-Heck A, Zariwala MA, Schmidts M, Onoufriadis A, Forouhan M, Wilson R, Taylor-Cox T, Dewar A, Jackson C, Goggin P, Loges NT, Olbrich H, Jaspers M, Jorissen M, Leigh MW, Wolf WE, Daniels ML, Noone PG, Ferkol TW, Sagel SD, Rosenfeld M, Rutman A, Dixit A, O'Callaghan C, Lucas JS, Hogg C, Scambler PJ, EmesChung RDEM, Shoemark A, Knowles MR, Omran H, Mitchison HM (2013) Mutations in CCDC39 and CCDC40 are the major cause of primary ciliary dyskinesia with axonemal disorganization and absent inner dynein arms. Hum Mutat 34(3):462–472. https://doi.org/10.1002/humu.22261**(Uk10k)**
78. Merveille AC, Davis EE, Becker-Heck A, Legendre M, Amirav I, Bataille G, Belmont J, Beydon N, Billen F, Clement A, Clercx C, Coste A, Crosbie R, de Blic J, Deleuze S, Duquesnoy P, Escalier D, Escudier E, Fliegauf M, Horvath J, Hill K, Jorissen M, Just J, Kispert A, Lathrop M, Loges NT, Marthin JK, Momozawa Y, Montantin G, Nielsen KG, Olbrich H, Papon JF, Rayet I, Roger G, Schmidts M, Tenreiro H, Towbin JA, Zelenika D, Zentgraf H, Georges M, Lequarre AS, Katsanis N, Omran H, Amselem S (2011) CCDC39 is required for assembly of inner dynein arms and the dynein regulatory complex and for normal ciliary motility in humans and dogs. Nat Genet 43(1):72–78 **(ng.726[pii]10.1038/ng.726)**
79. Becker-Heck A, Zohn IE, Okabe N, Pollock A, Lenhart KB, Sullivan-Brown J, McSheene J, Loges NT, Olbrich H, Haeffner K, Fliegauf M, Horvath J, Reinhardt R, Nielsen KG, Marthin JK, Baktai G, Anderson KV, Geisler R, Niswander L, Omran H, Burdine RD (2011) The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation. Nat Genet 43(1):79–84 **(ng.727[pii]10.1038/ng.727)**

80. Sugrue KF, Zohn IE (2017) Mechanism for generation of left isomerism in Ccdc40 mutant embryos. PLoS ONE 12(2):e0171180. https://doi.org/10.1371/journal.pone.0171180
81. Freshour J, Yokoyama R, Mitchell DR (2007) Chlamydomonas flagellar outer row dynein assembly protein ODA7 interacts with both outer row and I1 inner row dyneins. J Biol Chem 282(8):5404–5412
82. Duquesnoy P, Escudier E, Vincensini L, Freshour J, Bridoux AM, Coste A, Deschildre A, de Blic J, Legendre M, Montantin G, Tenreiro H, Vojtek AM, Loussert C, Clement A, Escalier D, Bastin P, Mitchell DR, Amselem S (2009) Loss-of-function mutations in the human ortholog of Chlamydomonas reinhardtii ODA7 disrupt dynein arm assembly and cause primary ciliary dyskinesia. Am J Hum Genet 85(6):890–896
83. Loges NT, Olbrich H, Becker-Heck A, Haffner K, Heer A, Reinhard C, Schmidts M, Kispert A, Zariwala MA, Leigh MW, Knowles MR, Zentgraf H, Seithe H, Nurnberg G, Nurnberg P, Reinhardt R, Omran H (2009) Deletions and point mutations of LRRC50 cause primary ciliary dyskinesia due to dynein arm defects. Am J Hum Genet 85(6):883–889
84. Hartill VL, van de Hoek G, Patel MP, Little R, Watson CM, Berry IR, Shoemark A, Abdelmottaleb D, Parkes E, Bacchelli C, Szymanska K, Knoers NV, Scambler PJ, Ueffing M, Boldt K, Yates R, Winyard PJ, Adler B, Moya E, Hattingh L, Shenoy A, Hogg C, Sheridan E, Roepman R, Norris D, Mitchison HM, Giles RH, Johnson CA (2018) DNAAF1 links heart laterality with the AAA+ ATPase RUVBL1 and ciliary intraflagellar transport. Hum Mol Genet 27(3):529–545. https://doi.org/10.1093/hmg/ddx422
85. Miao C, Jiang Q, Li H, Zhang Q, Bai B, Bao Y, Zhang T (2016) Mutations in the motile cilia gene DNAAF1 are associated with neural tube defects in humans. G3 (Bethesda) 6(10):3307–3316. https://doi.org/10.1534/g3.116.033696
86. Ha S, Lindsay AM, Timms AE, Beier DR (2016) Mutations in Dnaaf1 and Lrrc48 cause hydrocephalus, laterality defects, and sinusitis in mice. G3 (Bethesda) 6(8):2479–2487. https://doi.org/10.1534/g3.116.030791
87. Omran H, Kobayashi D, Olbrich H, Tsukahara T, Loges NT, Hagiwara H, Zhang Q, Leblond G, O'Toole E, Hara C, Mizuno H, Kawano H, Fliegauf M, Yagi T, Koshida S, Miyawaki A, Zentgraf H, Seithe H, Reinhardt R, Watanabe Y, Kamiya R, Mitchell DR, Takeda H (2008) Ktu/PF13 is required for cytoplasmic preassembly of axonemal dyneins. Nature 456(7222):611–616
88. Matsuo M, Shimada A, Koshida S, Saga Y, Takeda H (2013) The establishment of rotational polarity in the airway and ependymal cilia: analysis with a novel cilium motility mutant mouse. Am J Physiol Lung Cell Mol Physiol 304(11):L736–745. https://doi.org/10.1152/ajplung.00425.2012
89. Cheong A, Degani R, Tremblay KD, Mager J (2019) A null allele of Dnaaf2 displays embryonic lethality and mimics human ciliary dyskinesia. Hum Mol Genet 28(16):2775–2784. https://doi.org/10.1093/hmg/ddz106
90. Tarkar A, Loges NT, Slagle CE, Francis R, Dougherty GW, Tamayo JV, Shook B, Cantino M, Schwartz D, Jahnke C, Olbrich H, Werner C, Raidt J, Pennekamp P, Abouhamed M, Hjeij R, Kohler G, Griese M, Li Y, Lemke K, Klena N, Liu X, Gabriel G, Tobita K, Jaspers M, Morgan LC, Shapiro AJ, Letteboer SJ, Mans DA, Carson JL, Leigh MW, Wolf WE, Chen S, Lucas JS, Onoufriadis A, Plagnol V, Schmidts M, Boldt K, Roepman R, Zariwala MA, Lo CW, Mitchison HM, Knowles MR, Burdine RD, Loturco JJ, Omran H (2013) DYX1C1 is required for axonemal dynein assembly and ciliary motility. Nat Genet 45(9):995–1003. https://doi.org/10.1038/ng.2707**(Uk10K)**
91. Kott E, Duquesnoy P, Copin B, Legendre M, Dastot-Le Moal F, Montantin G, Jeanson L, Tamalet A, Papon JF, Siffroi JP, Rives N, Mitchell V, de Blic J, Coste A, Clement A, Escalier D, Toure A, Escudier E, Amselem S (2012) Loss-of-function mutations in LRRC6, a gene essential for proper axonemal assembly of inner and outer dynein arms, cause primary ciliary dyskinesia. Am J Hum Genet 91(5):958–964. https://doi.org/10.1016/j.ajhg.2012.10.003**(S0002-9297(12)00518-6[pii])**
92. Horani A, Ferkol TW, Shoseyov D, Wasserman MG, Oren YS, Kerem B, Amirav I, Cohen-Cymberknoh M, Dutcher SK, Brody SL, Elpeleg O, Kerem E (2013) LRRC6 mutation causes primary ciliary dyskinesia with dynein arm defects. PLoS ONE 8(3):e59436. https://doi.org/10.1371/journal.pone.0059436
93. Inaba Y, Shinohara K, Botilde Y, Nabeshima R, Takaoka K, Ajima R, Lamri L, Takeda H, Saga Y, Nakamura T, Hamada H (2016) Transport of the outer dynein arm complex to cilia requires a cytoplasmic protein Lrrc6. Genes Cells 21(7):728–739. https://doi.org/10.1111/gtc.12380
94. Li Y, Zhao L, Yuan S, Zhang J, Sun Z (2017) Axonemal dynein assembly requires the R2TP complex component pontin. Development 144(24):4684–4693. https://doi.org/10.1242/dev.152314
95. Dafinger C, Rinschen MM, Borgal L, Ehrenberg C, Basten SG, Franke M, Hohne M, Rauh M, Gobel H, Bloch W, Wunderlich FT, Peters DJM, Tasche D, Mishra T, Habbig S, Dotsch J, Muller RU, Bruning JC, Persigehl T, Giles RH, Benzing T, Schermer B, Liebau MC (2018) Targeted deletion of the AAA-ATPase Ruvbl1 in mice disrupts ciliary integrity and causes renal disease and hydrocephalus. Exp Mol Med 50(6):75. https://doi.org/10.1038/s12276-018-0108-z
96. Dong F, Shinohara K, Botilde Y, Nabeshima R, Asai Y, Fukumoto A, Hasegawa T, Matsuo M, Takeda H, Shiratori H, Nakamura T, Hamada H (2014) Pih1d3 is required for cytoplasmic preassembly of axonemal dynein in mouse sperm. J Cell Biol 204(2):203–213. https://doi.org/10.1083/jcb.201304076
97. Zariwala MA, Gee HY, Kurkowiak M, Al-Mutairi DA, Leigh MW, Hurd TW, Hjeij R, Dell SD, Chaki M, Dougherty GW, Adan M, Spear PC, Esteve-Rudd J, Loges NT, Rosenfeld M, Diaz KA, Olbrich H, Wolf WE, Sheridan E, Batten TF, Halbritter J, Porath JD, Kohl S, Lovric S, Hwang DY, Pittman JE, Burns KA, Ferkol TW, Sagel SD, Olivier KN, Morgan LC, Werner C, Raidt J, Pennekamp P, Sun Z, Zhou W, Airik R, Natarajan S, Allen SJ, Amirav I, Wieczorek D, Landwehr K, Nielsen K, Schwerk N, Sertic J, Kohler G, Washburn J, Levy S, Fan S, Koerner-Rettberg C, Amselem S, Williams DS, Mitchell BJ, Drummond IA, Otto EA, Omran H, Knowles MR, Hildebrandt F (2013) ZMYND10 is mutated in primary ciliary dyskinesia and interacts with LRRC6. Am J Hum Genet 93(2):336–345. https://doi.org/10.1016/j.ajhg.2013.06.007
98. Moore DJ, Onoufriadis A, Shoemark A, Simpson MA, Zur Lage PI, de Castro SC, Bartoloni L, Gallone G, Petridi S, Woollard WJ, Antony D, Schmidts M, Didonna T, Makrythanasis P, Bevillard J, Mongan NP, Djakow J, Pals G, Lucas JS, Marthin JK, Nielsen KG, Santoni F, Guipponi M, Hogg C, Antonarakis SE, Emes RD, Chung EM, Greene ND, Blouin JL, Jarman AP, Mitchison HM (2013) Mutations in ZMYND10, a gene essential for proper axonemal assembly of inner and outer dynein arms in humans and flies, cause primary ciliary dyskinesia. Am J Hum Genet 93(2):346–356. https://doi.org/10.1016/j.ajhg.2013.07.009
99. Cho KJ, Noh SH, Han SM, Choi WI, Kim HY, Yu S, Lee JS, Rim JH, Lee MG, Hildebrandt F, Gee HY (2018) ZMYND10 stabilizes intermediate chain proteins in the cytoplasmic preassembly of dynein arms. PLoS Genet 14(3):e1007316. https://doi.org/10.1371/journal.pgen.1007316
100. Mali GR, Yeyati PL, Mizuno S, Dodd DO, Tennant PA, Keighren MA, Zur Lage P, Shoemark A, Garcia-Munoz A, Shimada A, Takeda H, Edlich F, Takahashi S, von Kreigsheim A, Jarman AP, Mill P (2018) ZMYND10 functions in a chaperone relay during

axonemal dynein assembly. Elife. https://doi.org/10.7554/eLife.34389
101. Teves ME, Nagarkatti-Gude DR, Zhang Z, Strauss JF 3rd (2016) Mammalian axoneme central pair complex proteins: broader roles revealed by gene knockout phenotypes. Cytoskelet (Hoboken) 73(1):3–22. https://doi.org/10.1002/cm.21271
102. Gruneberg H (1943) Two new mutant genes in the house mouse. J Genet 45:22–28
103. Berry RJ (1961) The inheritance and pathogenesis of hydrocephalus-3 in the mouse. J Path Bact 81(1):157–167
104. Raimondi AJ, Bailey OT, McLone DG, Lawson RF, Echeverry A (1973) The pathophysiology and morphology of murine hydrocephalus in Hy-3 and Ch mutants. Surg Neurol 1(1):50–55
105. Lawson RF, Raimondi AJ (1973) Hydrocephalus-3, a murine mutant: I. Alterations in fine structure of choroid plexus and ependyma. Surg Neurol 1(2):115–128
106. McLone DG, Bondareff W, Raimondi AJ (1973) Hydrocephalus-3, a murine mutant. II. Changes in the brain extracellular space. Surg Neurol 1(4):233–242
107. Lechtreck KF, Witman GB (2007) Chlamydomonas reinhardtii hydin is a central pair protein required for flagellar motility. J Cell Biol 176(4):473–482
108. Lechtreck KF, Delmotte P, Robinson ML, Sanderson MJ, Witman GB (2008) Mutations in Hydin impair ciliary motility in mice. J Cell Biol 180(3):633–643. https://doi.org/10.1083/jcb.200710162**(jcb.200710162[pii])**
109. Olbrich H, Schmidts M, Werner C, Onoufriadis A, Loges NT, Raidt J, Banki NF, Shoemark A, Burgoyne T, Al Turki S, Hurles ME, Kohler G, Schroeder J, Nurnberg G, Nurnberg P, Chung EM, Reinhardt R, Marthin JK, Nielsen KG, Mitchison HM, Omran H (2012) Recessive HYDIN mutations cause primary ciliary dyskinesia without randomization of left-right body asymmetry. Am J Hum Genet. https://doi.org/10.1016/j.ajhg.2012.08.016**(S0002-9297(12)00428-4[pii])**
110. Davy BE, Robinson ML (2003) Congenital hydrocephalus in hy3 mice is caused by a frameshift mutation in Hydin, a large novel gene. Hum Mol Genet 12(10):1163–1170
111. Smith EF, Lefebvre PA (1996) PF16 encodes a protein with armadillo repeats and localizes to a single microtubule of the central apparatus in Chlamydomonas flagella. J Cell Biol 132(3):359–370
112. Sapiro R, Kostetskii I, Olds-Clarke P, Gerton GL, Radice GL, Strauss IJ (2002) Male infertility, impaired sperm motility, and hydrocephalus in mice deficient in sperm-associated antigen 6. Mol Cell Biol 22(17):6298–6305
113. Li X, Xu L, Li J, Li B, Bai X, Strauss JF 3rd, Zhang Z, Wang H (2014) Otitis media in sperm-associated antigen 6 (Spag6)-deficient mice. PLoS ONE 9(11):e112879. https://doi.org/10.1371/journal.pone.0112879
114. Teves ME, Sears PR, Li W, Zhang Z, Tang W, van Reesema L, Costanzo RM, Davis CW, Knowles MR, Strauss JF 3rd, Zhang Z (2014) Sperm-associated antigen 6 (SPAG6) deficiency and defects in ciliogenesis and cilia function: polarity, density, and beat. PLoS ONE 9(10):e107271. https://doi.org/10.1371/journal.pone.0107271
115. Zhang Z, Kostetskii I, Tang W, Haig-Ladewig L, Sapiro R, Wei Z, Patel AM, Bennett J, Gerton GL, Moss SB, Radice GL, Strauss JF 3rd (2006) Deficiency of SPAG16L causes male infertility associated with impaired sperm motility. Biol Reprod 74(4):751–759
116. Smith EF, Lefebvre PA (1997) PF20 gene product contains WD repeats and localizes to the intermicrotubule bridges in chlamydomonas flagella. Mol Biol Cell 8(3):455–467
117. Zhang Z, Sapiro R, Kapfhamer D, Bucan M, Bray J, Chennathukuzhi V, McNamara P, Curtis A, Zhang M, Blanchette-Mackie EJ, Strauss JF 3rd (2002) A sperm-associated WD repeat protein orthologous to chlamydomonas PF20 associates with Spag6, the mammalian orthologue of chlamydomonas PF16. Mol Cell Biol 22(22):7993–8004
118. Zhang Z, Tang W, Zhou R, Shen X, Wei Z, Patel AM, Povlishock JT, Bennett J, Strauss JF 3rd (2007) Accelerated mortality from hydrocephalus and pneumonia in mice with a combined deficiency of SPAG6 and SPAG16L reveals a functional interrelationship between the two central apparatus proteins. Cell Motil Cytoskeleton 64(5):360–376
119. Rupp G, O'Toole E, Porter ME (2001) The chlamydomonas PF6 locus encodes a large alanine/proline-rich polypeptide that is required for assembly of a central pair projection and regulates flagellar motility. Mol Biol Cell 12(3):739–751
120. Wargo MJ, Dymek EE, Smith EF (2005) Calmodulin and PF6 are components of a complex that localizes to the C1 microtubule of the flagellar central apparatus. J Cell Sci 118(Pt 20):4655–4665. https://doi.org/10.1242/jcs.02585**(jcs.02585[pii])**
121. Teves ME, Zhang Z, Costanzo RM, Henderson SC, Corwin FD, Zweit J, Sundaresan G, Subler M, Salloum FN, Rubin BK, Strauss JF 3rd (2013) Sperm-associated antigen-17 gene is essential for motile cilia function and neonatal survival. Am J Respir Cell Mol Biol 48(6):765–772. https://doi.org/10.1165/rcmb.2012-0362OC**(rcmb.2012-0362OC[pii])**
122. Kazarian E, Son H, Sapao P, Li W, Zhang Z, Strauss JF, Teves ME (2018) SPAG17 is required for male germ cell differentiation and fertility. Int J Mol Sci 19:4. https://doi.org/10.3390/ijms19041252
123. Xu X, Sha YW, Mei LB, Ji ZY, Qiu PP, Ji H, Li P, Wang T, Li L (2018) A familial study of twins with severe asthenozoospermia identified a homozygous SPAG17 mutation by whole-exome sequencing. Clin Genet 93(2):345–349. https://doi.org/10.1111/cge.13059
124. Andjelkovic M, Minic P, Vreca M, Stojiljkovic M, Skakic A, Sovtic A, Rodic M, Skodric-Trifunovic V, Maric N, Visekruna J, Spasovski V, Pavlovic S (2018) Genomic profiling supports the diagnosis of primary ciliary dyskinesia and reveals novel candidate genes and genetic variants. PLoS ONE 13(10):e0205422. https://doi.org/10.1371/journal.pone.0205422
125. Ostrowski LE, Andrews K, Potdar P, Matsuura H, Jetten A, Nettesheim P (1999) Cloning and characterization of KPL2, a novel gene induced during ciliogenesis of tracheal epithelial cells. Am J Respir Cell Mol Biol 20(4):675–683. https://doi.org/10.1165/ajrcmb.20.4.3496
126. Zhang H, Mitchell DR (2004) Cpc1, a chlamydomonas central pair protein with an adenylate kinase domain. J Cell Sci 117(Pt 18):4179–4188
127. Sironen A, Kotaja N, Mulhern H, Wyatt TA, Sisson JH, Pavlik JA, Miiluniemi M, Fleming MD, Lee L (2011) Loss of SPEF2 function in mice results in spermatogenesis defects and primary ciliary dyskinesia. Biol Reprod 85(4):690–701. https://doi.org/10.1095/biolreprod.111.091132**(biolreprod.111.091132[pii])**
128. Lehti MS, Zhang FP, Kotaja N, Sironen A (2017) SPEF2 functions in microtubule-mediated transport in elongating spermatids to ensure proper male germ cell differentiation. Development 144(14):2683–2693. https://doi.org/10.1242/dev.152108
129. Lehti MS, Henriksson H, Rummukainen P, Wang F, Uusitalo-Kylmala L, Kiviranta R, Heino TJ, Kotaja N, Sironen A (2018) Cilia-related protein SPEF2 regulates osteoblast differentiation. Sci Rep 8(1):859. https://doi.org/10.1038/s41598-018-19204-5
130. Tu C, Nie H, Meng L, Wang W, Li H, Yuan S, Cheng D, He W, Liu G, Du J, Gong F, Lu G, Lin G, Zhang Q, Tan YQ (2020) Novel mutations in SPEF2 causing different defects between flagella and cilia bridge: the phenotypic link between MMAF and PCD. Hum Genet 139(2):257–271. https://doi.org/10.1007/s00439-020-02110-0

131. Cindric S, Dougherty GW, Olbrich H, Hjeij R, Loges NT, Amirav I, Philipsen MC, Marthin JK, Nielsen KG, Sutharsan S, Raidt J, Werner C, Pennekamp P, Dworniczak B, Omran H (2020) SPEF2- and HYDIN-mutant cilia lack the central pair-associated protein SPEF2, aiding primary ciliary dyskinesia diagnostics. Am J Respir Cell Mol Biol 62(3):382–396. https://doi.org/10.1165/rcmb.2019-0086OC
132. Liu W, Sha Y, Li Y, Mei L, Lin S, Huang X, Lu J, Ding L, Kong S, Lu Z (2019) Loss-of-function mutations in SPEF2 cause multiple morphological abnormalities of the sperm flagella (MMAF). J Med Genet 56(10):678–684. https://doi.org/10.1136/jmedgenet-2018-105952
133. DiPetrillo CG, Smith EF (2010) Pcdp1 is a central apparatus protein that binds Ca(2+)-calmodulin and regulates ciliary motility. J Cell Biol 189(3):601–612. https://doi.org/10.1083/jcb.200912009**(jcb.200912009[pii])**
134. Lee L, Campagna DR, Pinkus JL, Mulhern H, Wyatt TA, Sisson JH, Pavlik JA, Pinkus GS, Fleming MD (2008) Primary ciliary dyskinesia in mice lacking the novel ciliary protein Pcdp1. Mol Cell Biol 28(3):949–957. https://doi.org/10.1128/MCB.00354-07**(MCB.00354-07[pii])**
135. Bustamante-Marin XM, Shapiro A, Sears PR, Charng WL, Conrad DF, Leigh MW, Knowles MR, Ostrowski LE, Zariwala MA (2020) Identification of genetic variants in CFAP221 as a cause of primary ciliary dyskinesia. J Hum Genet 65(2):175–180. https://doi.org/10.1038/s10038-019-0686-1
136. McKenzie CW, Craige B, Kroeger TV, Finn R, Wyatt TA, Sisson JH, Pavlik JA, Strittmatter L, Hendricks GM, Witman GB, Lee L (2015) CFAP54 is required for proper ciliary motility and assembly of the central pair apparatus in mice. Mol Biol Cell 26(18):3140–3149. https://doi.org/10.1091/mbc.E15-02-0121
137. Merchant M, Evangelista M, Luoh SM, Frantz GD, Chalasani S, Carano RA, van Hoy M, Ramirez J, Ogasawara AK, McFarland LM, Filvaroff EH, French DM, de Sauvage FJ (2005) Loss of the serine/threonine kinase fused results in postnatal growth defects and lethality due to progressive hydrocephalus. Mol Cell Biol 25(16):7054–7068. https://doi.org/10.1128/MCB.25.16.7054-7068.2005**(25/16/7054[pii])**
138. Vogel P, Read RW, Hansen GM, Payne BJ, Small D, Sands AT, Zambrowicz BP (2012) Congenital hydrocephalus in genetically engineered mice. Vet Pathol 49(1):166–181. https://doi.org/10.1177/0300985811415708**(0300985811415708[pii])**
139. Wilson CW, Nguyen CT, Chen MH, Yang JH, Gacayan R, Huang J, Chen JN, Chuang PT (2009) Fused has evolved divergent roles in vertebrate Hedgehog signalling and motile ciliogenesis. Nature 459(7243):98–102. https://doi.org/10.1038/nature07883**(nature07883[pii])**
140. Chen MH, Gao N, Kawakami T, Chuang PT (2005) Mice deficient in the fused homolog do not exhibit phenotypes indicative of perturbed hedgehog signaling during embryonic development. Mol Cell Biol 25(16):7042–7053. https://doi.org/10.1128/MCB.25.16.7042-7053.2005
141. Nozawa YI, Yao E, Lin C, Yang JH, Wilson CW, Gacayan R, Chuang PT (2013) Fused (Stk36) is a ciliary protein required for central pair assembly and motile cilia orientation in the mammalian oviduct. Dev Dyn 242(11):1307–1319. https://doi.org/10.1002/dvdy.24024
142. Edelbusch C, Cindric S, Dougherty GW, Loges NT, Olbrich H, Rivlin J, Wallmeier J, Pennekamp P, Amirav I, Omran H (2017) Mutation of serine/threonine protein kinase 36 (STK36) causes primary ciliary dyskinesia with a central pair defect. Hum Mutat 38(8):964–969. https://doi.org/10.1002/humu.23261
143. Kott E, Legendre M, Copin B, Papon JF, Dastot-Le Moal F, Montantin G, Duquesnoy P, Piterboth W, Amram D, Bassinet L, Beucher J, Beydon N, Deneuville E, Houdouin V, Journel H, Just J, Nathan N, Tamalet A, Collot N, Jeanson L, Le Gouez M, Vallette B, Vojtek AM, Epaud R, Coste A, Clement A, Housset B, Louis B, Escudier E, Amselem S (2013) Loss-of-function mutations in RSPH1 cause primary ciliary dyskinesia with central-complex and radial-spoke defects. Am J Hum Genet 93(3):561–570. https://doi.org/10.1016/j.ajhg.2013.07.013
144. Knowles MR, Ostrowski LE, Leigh MW, Sears PR, Davis SD, Wolf WE, Hazucha MJ, Carson JL, Olivier KN, Sagel SD, Rosenfeld M, Ferkol TW, Dell SD, Milla CE, Randell SH, Yin W, Sannuti A, Metjian HM, Noone PG, Noone PJ, Olson CA, Patrone MV, Dang H, Lee HS, Hurd TW, Gee HY, Otto EA, Halbritter J, Kohl S, Kircher M, Krischer J, Bamshad MJ, Nickerson DA, Hildebrandt F, Shendure J, Zariwala MA (2014) Mutations in RSPH1 cause primary ciliary dyskinesia with a unique clinical and ciliary phenotype. Am J Respir Crit Care Med 189(6):707–717. https://doi.org/10.1164/rccm.201311-2047OC
145. Onoufriadis A, Shoemark A, Schmidts M, Patel M, Jimenez G, Liu H, Thomas B, Dixon M, Hirst RA, Rutman A, Burgoyne T, Williams C, Scully J, Bolard F, Lafitte JJ, Beales PL, Hogg C, Yang P, Chung EM, Emes RD, O'CallaghanBouvagnet CP, Mitchison HM (2014) Targeted NGS gene panel identifies mutations in RSPH1 causing primary ciliary dyskinesia and a common mechanism for ciliary central pair agenesis due to radial spoke defects. Hum Mol Genet 23(13):3362–3374. https://doi.org/10.1093/hmg/ddu046**(Uk10K)**
146. Yin W, Livraghi-Butrico A, Sears PR, Rogers TD, Burns KA, Grubb BR, Ostrowski LE (2019) Mice with a deletion of Rsph1 exhibit a low level of mucociliary clearance and develop a primary ciliary dyskinesia phenotype. Am J Respir Cell Mol Biol 61(3):312–321. https://doi.org/10.1165/rcmb.2017-0387OC
147. Castleman VH, Romio L, Chodhari R, Hirst RA, de Castro SC, Parker KA, Ybot-Gonzalez P, Emes RD, Wilson SW, Wallis C, Johnson CA, Herrera RJ, Rutman A, Dixon M, Shoemark A, Bush A, Hogg C, Gardiner RM, Reish O, Greene ND, O'Callaghan C, Purton S, Chung EM, Mitchison HM (2009) Mutations in radial spoke head protein genes RSPH9 and RSPH4A cause primary ciliary dyskinesia with central-microtubular-pair abnormalities. Am J Hum Genet 84(2):197–209
148. Burgoyne T, Lewis A, Dewar A, Luther P, Hogg C, Shoemark A, Dixon M (2014) Characterizing the ultrastructure of primary ciliary dyskinesia transposition defect using electron tomography. Cytoskelet (Hoboken) 71(5):294–301. https://doi.org/10.1002/cm.21171
149. Shinohara K, Chen D, Nishida T, Misaki K, Yonemura S, Hamada H (2015) Absence of radial spokes in mouse node cilia is required for rotational movement but confers ultrastructural instability as a trade-off. Dev Cell 35(2):236–246. https://doi.org/10.1016/j.devcel.2015.10.001
150. Yoke H, Ueno H, Narita A, Sakai T, Horiuchi K, Shingyoji C, Hamada H, Shinohara K (2020) Rsph4a is essential for the triplet radial spoke head assembly of the mouse motile cilia. PLoS Genet 16(3):e1008664. https://doi.org/10.1371/journal.pgen.1008664
151. Patel-King RS, Gorbatyuk O, Takebe S, King SM (2004) Flagellar radial spokes contain a Ca2+-stimulated nucleoside diphosphate kinase. Mol Biol Cell 15(8):3891–3902. https://doi.org/10.1091/mbc.E04-04-0352E04-04-0352**([pii])**
152. Cho EH, Huh HJ, Jeong I, Lee NY, Koh WJ, Park HC, Ki CS (2020) A nonsense variant in NME5 causes human primary ciliary dyskinesia with radial spoke defects. Clin Genet 98(1):64–68. https://doi.org/10.1111/cge.13742
153. Oji A, Noda T, Fujihara Y, Miyata H, Kim YJ, Muto M, Nozawa K, Matsumura T, Isotani A, Ikawa M (2016) CRISPR/Cas9 mediated genome editing in ES cells and its application for chimeric analysis in mice. Sci Rep 6:31666. https://doi.org/10.1038/srep31666

154. Guan J, Ekwurtzel E, Kvist U, Hultenby K, Yuan L (2010) DNAJB13 is a radial spoke protein of mouse '9+2' axoneme. Reprod Domest Anim 45(6):992–996. https://doi.org/10.1111/j.1439-0531.2009.01473.x
155. El Khouri E, Thomas L, Jeanson L, Bequignon E, Vallette B, Duquesnoy P, Montantin G, Copin B, Dastot-Le Moal F, Blanchon S, Papon JF, Lores P, Yuan L, Collot N, Tissier S, Faucon C, Gacon G, Patrat C, Wolf JP, Dulioust E, Crestani B, Escudier E, Coste A, Legendre M, Toure A, Amselem S (2016) Mutations in DNAJB13, encoding an HSP40 family member, cause primary ciliary dyskinesia and male infertility. Am J Hum Genet 99(2):489–500. https://doi.org/10.1016/j.ajhg.2016.06.022
156. Beckers A, Adis C, Schuster-Gossler K, Tveriakhina L, Ott T, Fuhl F, Hegermann J, Boldt K, Serth K, Rachev E, Alten L, Kremmer E, Ueffing M, Blum M, Gossler A (2020) The FOXJ1 target Cfap206 is required for sperm motility, mucociliary clearance of the airways and brain development. Development 147:21. https://doi.org/10.1242/dev.188052
157. Vasudevan KK, Song K, Alford LM, Sale WS, Dymek EE, Smith EF, Hennessey T, Joachimiak E, Urbanska P, Wloga D, Dentler W, Nicastro D, Gaertig J (2015) FAP206 is a microtubule-docking adapter for ciliary radial spoke 2 and dynein c. Mol Biol Cell 26(4):696–710. https://doi.org/10.1091/mbc.E14-11-1506
158. Heuser T, Raytchev M, Krell J, Porter ME, Nicastro D (2009) The dynein regulatory complex is the nexin link and a major regulatory node in cilia and flagella. J Cell Biol 187(6):921–933. https://doi.org/10.1083/jcb.200908067**(jcb.200908067[pii])**
159. Osinka A, Poprzeczko M, Zielinska MM, Fabczak H, Joachimiak E, Wloga D (2019) Ciliary proteins: filling the gaps. Recent advances in deciphering the protein composition of motile ciliary complexes. Cells 8:7. https://doi.org/10.3390/cells8070730
160. Olbrich H, Cremers C, Loges NT, Werner C, Nielsen KG, Marthin JK, Philipsen M, Wallmeier J, Pennekamp P, Menchen T, Edelbusch C, Dougherty GW, Schwartz O, Thiele H, Altmuller J, Rommelmann F, Omran H (2015) Loss-of-function GAS8 mutations cause primary ciliary dyskinesia and disrupt the nexin-dynein regulatory complex. Am J Hum Genet 97(4):546–554. https://doi.org/10.1016/j.ajhg.2015.08.012
161. Rupp G, Porter ME (2003) A subunit of the dynein regulatory complex in chlamydomonas is a homologue of a growth arrest-specific gene product. J Cell Biol 162(1):47–57. https://doi.org/10.1083/jcb.200303019
162. Bower R, Tritschler D, Vanderwaal K, Perrone CA, Mueller J, Fox L, Sale WS, Porter ME (2013) The N-DRC forms a conserved biochemical complex that maintains outer doublet alignment and limits microtubule sliding in motile axonemes. Mol Biol Cell 24(8):1134–1152. https://doi.org/10.1091/mbc.E12-11-0801
163. Lewis WR, Malarkey EB, Tritschler D, Bower R, Pasek RC, Porath JD, Birket SE, Saunier S, Antignac C, Knowles MR, Leigh MW, Zariwala MA, Challa AK, Kesterson RA, Rowe SM, Drummond IA, Parant JM, Hildebrandt F, Porter ME, Yoder BK, Berbari NF (2016) Mutation of growth arrest specific 8 reveals a role in motile cilia function and human disease. PLoS Genet 12(7):e1006220. https://doi.org/10.1371/journal.pgen.1006220
164. Awata J, Song K, Lin J, King SM, Sanderson MJ, Nicastro D, Witman GB (2015) DRC3 connects the N-DRC to dynein g to regulate flagellar waveform. Mol Biol Cell 26(15):2788–2800. https://doi.org/10.1091/mbc.E15-01-0018
165. Ishikawa H, Marshall WF (2017) Intraflagellar transport and ciliary dynamics. Cold Spring Harb Perspect Biol 9:3. https://doi.org/10.1101/cshperspect.a021998
166. Blatt EN, Yan XH, Wuerffel MK, Hamilos DL, Brody SL (1999) Forkhead transcription factor HFH-4 expression is temporally related to ciliogenesis. Am J Respir Cell Mol Biol 21(2):168–176
167. Chen J, Knowles HJ, Hebert JL, Hackett BP (1998) Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. J Clin Invest 102(6):1077–1082
168. Brody SL, Yan XH, Wuerffel MK, Song SK, Shapiro SD (2000) Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. Am J Respir Cell Mol Biol 23(1):45–51
169. Jacquet BV, Salinas-Mondragon R, Liang H, Therit B, Buie JD, Dykstra M, Campbell K, Ostrowski LE, Brody SL, Ghashghaei HT (2009) FoxJ1-dependent gene expression is required for differentiation of radial glia into ependymal cells and a subset of astrocytes in the postnatal brain. Development 136(23):4021–4031. https://doi.org/10.1242/dev.041129
170. You Y, Huang T, Richer EJ, Schmidt JE, Zabner J, Borok Z, Brody SL (2004) Role of f-box factor foxj1 in differentiation of ciliated airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 286(4):L650–657. https://doi.org/10.1152/ajplung.00170.2003
171. Gomperts BN, Gong-Cooper X, Hackett BP (2004) Foxj1 regulates basal body anchoring to the cytoskeleton of ciliated pulmonary epithelial cells. J Cell Sci 117(Pt 8):1329–1337
172. Shawlot W, Vazquez-Chantada M, Wallingford JB, Finnell RH (2015) Rfx2 is required for spermatogenesis in the mouse. Genesis 53(9):604–611. https://doi.org/10.1002/dvg.22880
173. Kistler WS, Baas D, Lemeille S, Paschaki M, Seguin-Estevez Q, Barras E, Ma W, Duteyrat JL, Morle L, Durand B, Reith W (2015) RFX2 Is a Major Transcriptional Regulator of Spermiogenesis. PLoS Genet 11(7):e1005368. https://doi.org/10.1371/journal.pgen.1005368
174. Wu Y, Hu X, Li Z, Wang M, Li S, Wang X, Lin X, Liao S, Zhang Z, Feng X, Wang S, Cui X, Wang Y, Gao F, Hess RA, Han C (2016) Transcription factor RFX2 is a key regulator of mouse spermiogenesis. Sci Rep 6:20435. https://doi.org/10.1038/srep20435
175. Bonnafe E, Touka M, AitLounis A, Baas D, Barras E, Ucla C, Moreau A, Flamant F, Dubruille R, Couble P, Collignon J, Durand B, Reith W (2004) The transcription factor RFX3 directs nodal cilium development and left-right asymmetry specification. Mol Cell Biol 24(10):4417–4427
176. Baas D, Meiniel A, Benadiba C, Bonnafe E, Meiniel O, Reith W, Durand B (2006) A deficiency in RFX3 causes hydrocephalus associated with abnormal differentiation of ependymal cells. Eur J Neurosci 24(4):1020–1030
177. El Zein L, Ait-Lounis A, Morle L, Thomas J, Chhin B, Spassky N, Reith W, Durand B (2009) RFX3 governs growth and beating efficiency of motile cilia in mouse and controls the expression of genes involved in human ciliopathies. J Cell Sci 122(Pt 17):3180–3189. https://doi.org/10.1242/jcs.048348
178. Xu P, Morrison JP, Foley JF, Stumpo DJ, Ward T, Zeldin DC, Blackshear PJ (2018) Conditional ablation of the RFX4 isoform 1 transcription factor: Allele dosage effects on brain phenotype. PLoS ONE 13(1):e0190561. https://doi.org/10.1371/journal.pone.0190561
179. Ashique AM, Choe Y, Karlen M, May SR, Phamluong K, Solloway MJ, Ericson J, Peterson AS (2009) The Rfx4 transcription factor modulates Shh signaling by regional control of ciliogenesis. Sci Signal 2(95):ra70. https://doi.org/10.1126/scisignal.2000602
180. Boon M, Wallmeier J, Ma L, Loges NT, Jaspers M, Olbrich H, Dougherty GW, Raidt J, Werner C, Amirav I, Hevroni A, Abitbul R, Avital A, Soferman R, Wessels M, O'Callaghan C, Chung EM, Rutman A, Hirst RA, Moya E, Mitchison HM, Van Daele S, De Boeck K, Jorissen M, Kintner C, Cuppens H, Omran H (2014) MCIDAS mutations result in a mucociliary clearance disorder with reduced generation of multiple motile cilia. Nat Commun 5:4418. https://doi.org/10.1038/ncomms5418

181. Lu H, Anujan P, Zhou F, Zhang Y, Chong YL, Bingle CD, Roy S (2019) Mcidas mutant mice reveal a two-step process for the specification and differentiation of multiciliated cells in mammals. Development 146:6. https://doi.org/10.1242/dev.172643
182. Arbi M, Pefani DE, Kyrousi C, Lalioti ME, Kalogeropoulou A, Papanastasiou AD, Taraviras S, Lygerou Z (2016) GemC1 controls multiciliogenesis in the airway epithelium. EMBO Rep 17(3):400–413. https://doi.org/10.15252/embr.201540882
183. Lalioti ME, Kaplani K, Lokka G, Georgomanolis T, Kyrousi C, Dong W, Dunbar A, Parlapani E, Damianidou E, Spassky N, Kahle KT, Papantonis A, Lygerou Z, Taraviras S (2019) GemC1 is a critical switch for neural stem cell generation in the postnatal brain. Glia 67(12):2360–2373. https://doi.org/10.1002/glia.23690
184. Terre B, Piergiovanni G, Segura-Bayona S, Gil-Gomez G, Youssef SA, Attolini CS, Wilsch-Brauninger M, Jung C, Rojas AM, Marjanovic M, Knobel PA, Palenzuela L, Lopez-Rovira T, Forrow S, Huttner WB, Valverde MA, de Bruin A, Costanzo V, Stracker TH (2016) GEMC1 is a critical regulator of multiciliated cell differentiation. EMBO J 35(9):942–960. https://doi.org/10.15252/embj.201592821
185. Terre B, Lewis M, Gil-Gomez G, Han Z, Lu H, Aguilera M, Prats N, Roy S, Zhao H, Stracker TH (2019) Defects in efferent duct multiciliogenesis underlie male infertility in GEMC1- MCIDAS- or CCNO-deficient mice. Development 146:8. https://doi.org/10.1242/dev.162628
186. Kyrousi C, Arbi M, Pilz GA, Pefani DE, Lalioti ME, Ninkovic J, Gotz M, Lygerou Z, Taraviras S (2015) Mcidas and GemC1 are key regulators for the generation of multiciliated ependymal cells in the adult neurogenic niche. Development 142(21):3661–3674. https://doi.org/10.1242/dev.126342
187. Wallmeier J, Al-Mutairi DA, Chen CT, Loges NT, Pennekamp P, Menchen T, Ma L, Shamseldin HE, Olbrich H, Dougherty GW, Werner C, Alsabah BH, Kohler G, Jaspers M, Boon M, Griese M, Schmitt-Grohe S, Zimmermann T, Koerner-Rettberg C, Horak E, Kintner C, Alkuraya FS, Omran H (2014) Mutations in CCNO result in congenital mucociliary clearance disorder with reduced generation of multiple motile cilia. Nat Genet 46(6):646–651. https://doi.org/10.1038/ng.2961
188. Funk MC, Bera AN, Menchen T, Kuales G, Thriene K, Lienkamp SS, Dengjel J, Omran H, Frank M, Arnold SJ (2015) Cyclin O (Ccno) functions during deuterosome-mediated centriole amplification of multiciliated cells. EMBO J 34(8):1078–1089. https://doi.org/10.15252/embj.201490805
189. Nunez-Olle M, Jung C, Terre B, Balsiger NA, Plata C, Roset R, Pardo-Pastor C, Garrido M, Rojas S, Alameda F, Lloreta J, Martin-Caballero J, Flores JM, Stracker TH, Valverde MA, Munoz FJ, Gil-Gomez G (2017) Constitutive cyclin O deficiency results in penetrant hydrocephalus, impaired growth and infertility. Oncotarget 8(59):99261–99273. https://doi.org/10.18632/oncotarget.21818
190. Yang A, Walker N, Bronson R, Kaghad M, Oosterwegel M, Bonnin J, Vagner C, Bonnet H, Dikkes P, Sharpe A, McKeon F, Caput D (2000) p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. Nature 404(6773):99–103. https://doi.org/10.1038/35003607
191. Gonzalez-Cano L, Fuertes-Alvarez S, Robledinos-Anton N, Bizy A, Villena-Cortes A, Farinas I, Marques MM, Marin MC (2016) p73 is required for ependymal cell maturation and neurogenic SVZ cytoarchitecture. Dev Neurobiol 76(7):730–747. https://doi.org/10.1002/dneu.22356
192. Nemajerova A, Kramer D, Siller SS, Herr C, Shomroni O, Pena T, Gallinas Suazo C, Glaser K, Wildung M, Steffen H, Sriraman A, Oberle F, Wienken M, Hennion M, Vidal R, Royen B, Alevra M, Schild D, Bals R, Donitz J, Riedel D, Bonn S, Takemaru K, Moll UM, Lize M (2016) TAp73 is a central transcriptional regulator of airway multiciliogenesis. Genes Dev 30(11):1300–1312. https://doi.org/10.1101/gad.279836.116
193. Marshall CB, Mays DJ, Beeler JS, Rosenbluth JM, Boyd KL, Santos Guasch GL, Shaver TM, Tang LJ, Liu Q, Shyr Y, Venters BJ, Magnuson MA, Pietenpol JA (2016) p73 is required for multiciliogenesis and regulates the Foxj1-associated gene network. Cell Rep 14(10):2289–2300. https://doi.org/10.1016/j.celrep.2016.02.035
194. Fujitani M, Sato R, Yamashita T (2017) Loss of p73 in ependymal cells during the perinatal period leads to aqueductal stenosis. Sci Rep 7(1):12007. https://doi.org/10.1038/s41598-017-12105-z
195. Jiang Z, Zhou J, Qin X, Zheng H, Gao B, Liu X, Jin G, Zhou Z (2020) MT1-MMP deficiency leads to defective ependymal cell maturation, impaired ciliogenesis, and hydrocephalus. JCI Insight 5:9. https://doi.org/10.1172/jci.insight.132782
196. Wang X, Zhou Y, Wang J, Tseng IC, Huang T, Zhao Y, Zheng Q, Gao Y, Luo H, Zhang X, Bu G, Hong W, Xu H (2016) SNX27 deletion causes hydrocephalus by impairing ependymal cell differentiation and ciliogenesis. J Neurosci 36(50):12586–12597. https://doi.org/10.1523/JNEUROSCI.1620-16.2016
197. Seaman MN (2012) The retromer complex: endosomal protein recycling and beyond. J Cell Sci 125(Pt 20):4693–4702. https://doi.org/10.1242/jcs.103440
198. Zimprich A, Benet-Pages A, Struhal W, Graf E, Eck SH, Offman MN, Haubenberger D, Spielberger S, Schulte EC, Lichtner P, Rossle SC, Klopp N, Wolf E, Seppi K, Pirker W, Presslauer S, Mollenhauer B, Katzenschlager R, Foki T, Hotzy C, Reinthaler E, Harutyunyan A, Kralovics R, Peters A, Zimprich F, Brucke T, Poewe W, Auff E, Trenkwalder C, Rost B, Ransmayr G, Winkelmann J, Meitinger T, Strom TM (2011) A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. Am J Hum Genet 89(1):168–175. https://doi.org/10.1016/j.ajhg.2011.06.008
199. Wu KY, Tang FL, Lee D, Zhao Y, Song H, Zhu XJ, Mei L, Xiong WC (2020) Ependymal Vps35 promotes ependymal cell differentiation and survival, suppresses microglial activation, and prevents neonatal hydrocephalus. J Neurosci 40(19):3862–3879. https://doi.org/10.1523/JNEUROSCI.1520-19.2020
200. Liu M, Guan Z, Shen Q, Lalor P, Fitzgerald U, O'Brien T, Dockery P, Shen S (2016) Ulk4 is essential for ciliogenesis and CSF flow. J Neurosci 36(29):7589–7600. https://doi.org/10.1523/JNEUROSCI.0621-16.2016
201. Nakayama K, Katoh Y (2020) Architecture of the IFT ciliary trafficking machinery and interplay between its components. Crit Rev Biochem Mol Biol 55(2):179–196. https://doi.org/10.1080/10409238.2020.1768206
202. Prevo B, Scholey JM, Peterman EJG (2017) Intraflagellar transport: mechanisms of motor action, cooperation, and cargo delivery. FEBS J 284(18):2905–2931. https://doi.org/10.1111/febs.14068
203. Siller SS, Sharma H, Li S, Yang J, Zhang Y, Holtzman MJ, Winuthayanon W, Colognato H, Holdener BC, Li FQ, Takemaru KI (2017) Conditional knockout mice for the distal appendage protein CEP164 reveal its essential roles in airway multiciliated cell differentiation. PLoS Genet 13(12):e1007128. https://doi.org/10.1371/journal.pgen.1007128
204. Burke MC, Li FQ, Cyge B, Arashiro T, Brechbuhl HM, Chen X, Siller SS, Weiss MA, O'Connell CB, Love D, Westlake CJ, Reynolds SD, Kuriyama R, Takemaru K (2014) Chibby promotes ciliary vesicle formation and basal body docking during airway cell differentiation. J Cell Biol 207(1):123–137. https://doi.org/10.1083/jcb.201406140
205. Love D, Li FQ, Burke MC, Cyge B, Ohmitsu M, Cabello J, Larson JE, Brody SL, Cohen JC, Takemaru K (2010) Altered lung morphogenesis, epithelial cell differentiation and mechanics

in mice deficient in the Wnt/beta-catenin antagonist Chibby. PLoS ONE 5(10):e13600. https://doi.org/10.1371/journal.pone.0013600
206. Siller SS, Burke MC, Li FQ, Takemaru K (2015) Chibby functions to preserve normal ciliary morphology through the regulation of intraflagellar transport in airway ciliated cells. Cell Cycle 14(19):3163–3172. https://doi.org/10.1080/15384101.2015.1080396
207. Voronina VA, Takemaru K, Treuting P, Love D, Grubb BR, Hajjar AM, Adams A, Li FQ, Moon RT (2009) Inactivation of chibby affects function of motile airway cilia. J Cell Biol 185(2):225–233
208. Hong DH, Pawlyk BS, Shang J, Sandberg MA, Berson EL, Li T (2000) A retinitis pigmentosa GTPase regulator (RPGR)-deficient mouse model for X-linked retinitis pigmentosa (RP3). Proc Natl Acad Sci USA 97(7):3649–3654. https://doi.org/10.1073/pnas.060037497
209. Hong DH, Pawlyk B, Sokolov M, Strissel KJ, Yang J, Tulloch B, Wright AF, Arshavsky VY, Li T (2003) RPGR isoforms in photoreceptor connecting cilia and the transitional zone of motile cilia. Invest Ophthalmol Vis Sci 44(6):2413–2421
210. Brunner S, Colman D, Travis AJ, Luhmann UF, Shi W, Feil S, Imsand C, Nelson J, Grimm C, Rulicke T, Fundele R, Neidhardt J, Berger W (2008) Overexpression of RPGR leads to male infertility in mice due to defects in flagellar assembly. Biol Reprod 79(4):608–617
211. Bukowy-Bieryllo Z, Zietkiewicz E, Loges NT, Wittmer M, Geremek M, Olbrich H, Fliegauf M, Voelkel K, Rutkiewicz E, Rutland J, Morgan L, Pogorzelski A, Martin J, Haan E, Berger W, Omran H, Witt M (2013) RPGR mutations might cause reduced orientation of respiratory cilia. Pediatr Pulmonol 48(4):352–363. https://doi.org/10.1002/ppul.22632
212. Taulman PD, Haycraft CJ, Balkovetz DF, Yoder BK (2001) Polaris, a protein involved in left-right axis patterning, localizes to basal bodies and cilia. Mol Biol Cell 12(3):589–599
213. Banizs B, Pike MM, Millican CL, Ferguson WB, Komlosi P, Sheetz J, Bell PD, Schwiebert EM, Yoder BK (2005) Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. Development 132(23):5329–5339
214. Gilley SK, Stenbit AE, Pasek RC, Sas KM, Steele SL, Amria M, Bunni MA, Estell KP, Schwiebert LM, Flume P, Gooz M, Haycraft CJ, Yoder BK, Miller C, Pavlik JA, Turner GA, Sisson JH, Bell PD (2014) Deletion of airway cilia results in non-inflammatory bronchiectasis and hyperreactive airways. Am J Physiol Lung Cell Mol Physiol 306(2):L162–169. https://doi.org/10.1152/ajplung.00095.2013
215. Ahmed NT, Gao C, Lucker BF, Cole DG, Mitchell DR (2008) ODA16 aids axonemal outer row dynein assembly through an interaction with the intraflagellar transport machinery. J Cell Biol 183(2):313–322. https://doi.org/10.1083/jcb.200802025
216. Taschner M, Mourao A, Awasthi M, Basquin J, Lorentzen E (2017) Structural basis of outer dynein arm intraflagellar transport by the transport adaptor protein ODA16 and the intraflagellar transport protein IFT46. J Biol Chem 292(18):7462–7473. https://doi.org/10.1074/jbc.M117.780155
217. Yang Y, Ran J, Liu M, Li D, Li Y, Shi X, Meng D, Pan J, Ou G, Aneja R, Sun SC, Zhou J (2014) CYLD mediates ciliogenesis in multiple organs by deubiquitinating Cep70 and inactivating HDAC6. Cell Res 24(11):1342–1353. https://doi.org/10.1038/cr.2014.136
218. Weidemann M, Schuster-Gossler K, Stauber M, Wrede C, Hegermann J, Ott T, Boldt K, Beyer T, Serth K, Kremmer E, Blum M, Ueffing M, Gossler A (2016) CFAP157 is a murine downstream effector of FOXJ1 that is specifically required for flagellum morphogenesis and sperm motility. Development 143(24):4736–4748. https://doi.org/10.1242/dev.139626
219. Schweizer S, Hoyer-Fender S (2009) Mouse Odf2 localizes to centrosomes and basal bodies in adult tissues and to the photoreceptor primary cilium. Cell Tissue Res 338(2):295–301. https://doi.org/10.1007/s00441-009-0861-3
220. Kunimoto K, Yamazaki Y, Nishida T, Shinohara K, Ishikawa H, Hasegawa T, Okanoue T, Hamada H, Noda T, Tamura A, Tsukita S (2012) Coordinated ciliary beating requires Odf2-mediated polarization of basal bodies via basal feet. Cell 148(1–2):189–200. https://doi.org/10.1016/j.cell.2011.10.052**(S0092-8674(11)01366-3[pii])**
221. Tarnasky H, Cheng M, Ou Y, Thundathil JC, Oko R, van der Hoorn FA (2010) Gene trap mutation of murine outer dense fiber protein-2 gene can result in sperm tail abnormalities in mice with high percentage chimaerism. BMC Dev Biol 10:67. https://doi.org/10.1186/1471-213X-10-67
222. Appelbe OK, Bollman B, Attarwala A, Triebes LA, Muniz-Talavera H, Curry DJ, Schmidt JV (2013) Disruption of the mouse Jhy gene causes abnormal ciliary microtubule patterning and juvenile hydrocephalus. Dev Biol 382(1):172–185. https://doi.org/10.1016/j.ydbio.2013.07.003
223. Muniz-Talavera H, Schmidt JV (2017) The mouse Jhy gene regulates ependymal cell differentiation and ciliogenesis. PLoS ONE 12(12):e0184957. https://doi.org/10.1371/journal.pone.0184957
224. Treat AC, Wheeler DS, Stolz DB, Tsang M, Friedman PA, Romero G (2016) The PDZ Protein Na+/H+ exchanger regulatory factor-1 (NHERF1) regulates planar cell polarity and motile cilia organization. PLoS ONE 11(4):e0153144. https://doi.org/10.1371/journal.pone.0153144
225. Song R, Walentek P, Sponer N, Klimke A, Lee JS, Dixon G, Harland R, Wan Y, Lishko P, Lize M, Kessel M, He L (2014) miR-34/449 miRNAs are required for motile ciliogenesis by repressing cp110. Nature 510(7503):115–120. https://doi.org/10.1038/nature13413
226. Spektor A, Tsang WY, Khoo D, Dynlacht BD (2007) Cep97 and CP110 suppress a cilia assembly program. Cell 130(4):678–690. https://doi.org/10.1016/j.cell.2007.06.027
227. Wloga D, Joachimiak E, Louka P, Gaertig J (2017) Posttranslational modifications of tubulin and cilia. Cold Spring Harb Perspect Biol 9:6. https://doi.org/10.1101/cshperspect.a028159
228. Takaki E, Fujimoto M, Sugahara K, Nakahari T, Yonemura S, Tanaka Y, Hayashida N, Inouye S, Takemoto T, Yamashita H, Nakai A (2006) Maintenance of olfactory neurogenesis requires HSF1, a major heat shock transcription factor in mice. J Biol Chem 281(8):4931–4937. https://doi.org/10.1074/jbc.M506911200**(M506911200[pii])**
229. Takaki E, Fujimoto M, Nakahari T, Yonemura S, Miyata Y, Hayashida N, Yamamoto K, Vallee RB, Mikuriya T, Sugahara K, Yamashita H, Inouye S, Nakai A (2007) Heat shock transcription factor 1 is required for maintenance of ciliary beating in mice. J Biol Chem 282(51):37285–37292
230. Fabczak H, Osinka A (2019) Role of the novel Hsp90 co-chaperones in dynein arms' preassembly. Int J Mol Sci 20:24. https://doi.org/10.3390/ijms20246174
231. Amos LA (2008) The tektin family of microtubule-stabilizing proteins. Genome Biol 9(7):229. https://doi.org/10.1186/gb-2008-9-7-229
232. Tanaka H, Iguchi N, Toyama Y, Kitamura K, Takahashi T, Kaseda K, Maekawa M, Nishimune Y (2004) Mice deficient in the axonemal protein Tektin-t exhibit male infertility and immotile-cilium syndrome due to impaired inner arm dynein function. Mol Cell Biol 24(18):7958–7964
233. Vogel P, Hansen G, Fontenot G, Read R (2010) Tubulin tyrosine ligase-like 1 deficiency results in chronic rhinosinusitis and abnormal development of spermatid flagella in mice. Vet

Pathol 47(4):703–712. https://doi.org/10.1177/0300985810363485**(0300985810363485[pii])**
234. Konno A, Setou M, Ikegami K (2012) Ciliary and flagellar structure and function–their regulations by posttranslational modifications of axonemal tubulin. Int Rev Cell Mol Biol 294:133–170. https://doi.org/10.1016/B978-0-12-394305-7.00003-3
235. Ikegami K, Sato S, Nakamura K, Ostrowski LE, Setou M (2010) Tubulin polyglutamylation is essential for airway ciliary function through the regulation of beating asymmetry. Proc Natl Acad Sci USA 107(23):10490–10495. https://doi.org/10.1073/pnas.1002128107**(1002128107[pii])**
236. Wang D, Nitta R, Morikawa M, Yajima H, Inoue S, Shigematsu H, Kikkawa M, Hirokawa N (2016) Motility and microtubule depolymerization mechanisms of the Kinesin-8 motor, KIF19A. Elife. https://doi.org/10.7554/eLife.18101
237. Niwa S, Nakajima K, Miki H, Minato Y, Wang D, Hirokawa N (2012) KIF19A is a microtubule-depolymerizing kinesin for ciliary length control. Dev Cell 23(6):1167–1175. https://doi.org/10.1016/j.devcel.2012.10.016**(S1534-5807(12)00479-0[pii])**
238. Robinson AM, Takahashi S, Brotslaw EJ, Ahmad A, Ferrer E, Procissi D, Richter CP, Cheatham MA, Mitchell BJ, Zheng J (2020) CAMSAP3 facilitates basal body polarity and the formation of the central pair of microtubules in motile cilia. Proc Natl Acad Sci USA 117(24):13571–13579. https://doi.org/10.1073/pnas.1907335117
239. Pongrakhananon V, Saito H, Hiver S, Abe T, Shioi G, Meng W, Takeichi M (2018) CAMSAP3 maintains neuronal polarity through regulation of microtubule stability. Proc Natl Acad Sci USA 115(39):9750–9755. https://doi.org/10.1073/pnas.1803875115
240. Toya M, Kobayashi S, Kawasaki M, Shioi G, Kaneko M, Ishiuchi T, Misaki K, Meng W, Takeichi M (2016) CAMSAP3 orients the apical-to-basal polarity of microtubule arrays in epithelial cells. Proc Natl Acad Sci USA 113(2):332–337. https://doi.org/10.1073/pnas.1520638113
241. Clare DK, Magescas J, Piolot T, Dumoux M, Vesque C, Pichard E, Dang T, Duvauchelle B, Poirier F, Delacour D (2014) Basal foot MTOC organizes pillar MTs required for coordination of beating cilia. Nat Commun 5:4888. https://doi.org/10.1038/ncomms5888
242. Comte I, Kim Y, Young CC, van der Harg JM, Hockberger P, Bolam PJ, Poirier F, Szele FG (2011) Galectin-3 maintains cell motility from the subventricular zone to the olfactory bulb. J Cell Sci 124(Pt 14):2438–2447. https://doi.org/10.1242/jcs.079954
243. Bustamante-Marin XM, Yin WN, Sears PR, Werner ME, Brotslaw EJ, Mitchell BJ, Jania CM, Zeman KL, Rogers TD, Herring LE, Refabert L, Thomas L, Amselem S, Escudier E, Legendre M, Grubb BR, Knowles MR, Zariwala MA, Ostrowski LE (2019) Lack of GAS2L2 Causes PCD by impairing cilia orientation and mucociliary clearance. Am J Hum Genet 104(2):229–245. https://doi.org/10.1016/j.ajhg.2018.12.009
244. Stroud MJ, Nazgiewicz A, McKenzie EA, Wang Y, Kammerer RA, Ballestrem C (2014) GAS2-like proteins mediate communication between microtubules and actin through interactions with end-binding proteins. J Cell Sci 127(Pt 12):2672–2682. https://doi.org/10.1242/jcs.140558
245. Bonser LR, Schroeder BW, Ostrin LA, Baumlin N, Olson JL, Salathe M, Erle DJ (2015) The endoplasmic reticulum resident protein AGR3. Required for regulation of ciliary beat frequency in the airway. Am J Respir Cell Mol Biol 53(4):536–543. https://doi.org/10.1165/rcmb.2014-0318OC
246. Fernandez-Gonzalez A, Kourembanas S, Wyatt TA, Mitsialis SA (2009) Mutation of murine adenylate kinase 7 underlies a primary ciliary dyskinesia phenotype. Am J Respir Cell Mol Biol 40(3):305–313
247. Lores P, Coutton C, El Khouri E, Stouvenel L, Givelet M, Thomas L, Rode B, Schmitt A, Louis B, Sakheli Z, Chaudhry M, Fernandez-Gonzales A, Mitsialis A, Dacheux D, Wolf JP, Papon JF, Gacon G, Escudier E, Arnoult C, Bonhivers M, Savinov SN, Amselem S, Ray PF, Dulioust E, Toure A (2018) Homozygous missense mutation L673P in adenylate kinase 7 (AK7) leads to primary male infertility and multiple morphological anomalies of the flagella but not to primary ciliary dyskinesia. Hum Mol Genet 27(7):1196–1211. https://doi.org/10.1093/hmg/ddy034
248. Mata M, Lluch-Estelles J, Armengot M, Sarrion I, Carda C, Cortijo J (2012) New adenylate kinase 7 (AK7) mutation in primary ciliary dyskinesia. Am J Rhinol Allergy 26(4):260–264. https://doi.org/10.2500/ajra.2012.26.3784
249. Moye AR, Singh R, Kimler VA, Dilan TL, Munezero D, Saravanan T, Goldberg AFX, Ramamurthy V (2018) ARL2BP, a protein linked to retinitis pigmentosa, is needed for normal photoreceptor cilia doublets and outer segment structure. Mol Biol Cell 29(13):1590–1598. https://doi.org/10.1091/mbc.E18-01-0040
250. Moye AR, Bedoni N, Cunningham JG, Sanzhaeva U, Tucker ES, Mathers P, Peter VG, Quinodoz M, Paris LP, Coutinho-Santos L, Camacho P, Purcell MG, Winkelmann AC, Foster JA, Pugacheva EN, Rivolta C, Ramamurthy V (2019) Mutations in ARL2BP, a protein required for ciliary microtubule structure, cause syndromic male infertility in humans and mice. PLoS Genet 15(8):e1008315. https://doi.org/10.1371/journal.pgen.1008315
251. Davidson AE, Schwarz N, Zelinger L, Stern-Schneider G, Shoemark A, Spitzbarth B, Gross M, Laxer U, Sosna J, Sergouniotis PI, Waseem NH, Wilson R, Kahn RA, Plagnol V, Wolfrum U, Banin E, Hardcastle AJ, Cheetham ME, Sharon D, Webster AR (2013) Mutations in ARL2BP, encoding ADP-ribosylation-factor-like 2 binding protein, cause autosomal-recessive retinitis pigmentosa. Am J Hum Genet 93(2):321–329. https://doi.org/10.1016/j.ajhg.2013.06.003
252. Vogel P, Read R, Hansen GM, Freay LC, Zambrowicz BP, Sands AT (2010) Situs inversus in Dpcd/Poll-/-, Nme7-/-, and Pkd1l1-/- mice. Vet Pathol 47(1):120–131. https://doi.org/10.1177/0300985809353553**(47/1/120[pii])**
253. Reish O, Aspit L, Zouella A, Roth Y, Polak-Charcon S, Baboushkin T, Benyamini L, Scheetz TE, Mussaffi H, Sheffield VC, Parvari R (2016) A Homozygous Nme7 mutation is associated with situs inversus totalis. Hum Mutat 37(8):727–731. https://doi.org/10.1002/humu.22998
254. Wilson GR, Tan JT, Brody KM, Taylor JM, Delatycki MB, Lockhart PJ (2009) Expression and localization of the Parkin co-regulated gene in mouse CNS suggests a role in ependymal cilia function. Neurosci Lett 460(1):97–101. https://doi.org/10.1016/j.neulet.2009.05.043
255. Dymek EE, Lin J, Fu G, Porter ME, Nicastro D, Smith EF (2019) PACRG and FAP20 form the inner junction of axonemal doublet microtubules and regulate ciliary motility. Mol Biol Cell 30(15):1805–1816. https://doi.org/10.1091/mbc.E19-01-0063
256. West AB, Lockhart PJ, O'Farell C, Farrer MJ (2003) Identification of a novel gene linked to parkin via a bi-directional promoter. J Mol Biol 326(1):11–19. https://doi.org/10.1016/s0022-2836(02)01376-1
257. Wilson GR, Wang HX, Egan GF, Robinson PJ, Delatycki MB, O'Bryan MK, Lockhart PJ (2010) Deletion of the parkin co-regulated gene causes defects in ependymal ciliary motility and hydrocephalus in the quakingviable mutant mouse. Hum Mol Genet 19(8):1593–1602. https://doi.org/10.1093/hmg/ddq031**(ddq031[pii])**
258. Li W, Tang W, Teves ME, Zhang Z, Zhang L, Li H, Archer KJ, Peterson DL, Williams DC Jr, Strauss JF 3rd, Zhang Z (2015) A MEIG1/PACRG complex in the manchette is essential for building the sperm flagella. Development 142(5):921–930. https://doi.org/10.1242/dev.119834

259. Sasaki K, Shiba K, Nakamura A, Kawano N, Satouh Y, Yamaguchi H, Morikawa M, Shibata D, Yanase R, Jokura K, Nomura M, Miyado M, Takada S, Ueno H, Nonaka S, Baba T, Ikawa M, Kikkawa M, Miyado K, Inaba K (2019) Calaxin is required for cilia-driven determination of vertebrate laterality. Commun Biol 2:226. https://doi.org/10.1038/s42003-019-0462-y
260. Sigg MA, Menchen T, Lee C, Johnson J, Jungnickel MK, Choksi SP, Garcia G 3rd, Busengdal H, Dougherty GW, Pennekamp P, Werner C, Rentzsch F, Florman HM, Krogan N, Wallingford JB, Omran H, Reiter JF (2017) Evolutionary proteomics uncovers ancient associations of cilia with signaling pathways. Dev Cell 43(6):744–762. https://doi.org/10.1016/j.devcel.2017.11.014**(e711)**
261. Tang S, Wang X, Li W, Yang X, Li Z, Liu W, Li C, Zhu Z, Wang L, Wang J, Zhang L, Sun X, Zhi E, Wang H, Li H, Jin L, Luo Y, Wang J, Yang S, Zhang F (2017) Biallelic mutations in CFAP43 and CFAP44 cause male infertility with multiple morphological abnormalities of the sperm flagella. Am J Hum Genet 100(6):854–864. https://doi.org/10.1016/j.ajhg.2017.04.012
262. Coutton C, Vargas AS, Amiri-Yekta A, Kherraf ZE, Ben Mustapha SF, Le Tanno P, Wambergue-Legrand C, Karaouzene T, Martinez G, Crouzy S, Daneshipour A, Hosseini SH, Mitchell V, Halouani L, Marrakchi O, Makni M, Latrous H, Kharouf M, Deleuze JF, Boland A, Hennebicq S, Satre V, Jouk PS, Thierry-Mieg N, Conne B, Dacheux D, Landrein N, Schmitt A, Stouvenel L, Lores P, El Khouri E, Bottari SP, Faure J, Wolf JP, Pernet-Gallay K, Escoffier J, Gourabi H, Robinson DR, Nef S, Dulioust E, Zouari R, Bonhivers M, Toure A, Arnoult C, Ray PF (2018) Mutations in CFAP43 and CFAP44 cause male infertility and flagellum defects in Trypanosoma and human. Nat Commun 9(1):686. https://doi.org/10.1038/s41467-017-02792-7
263. Rachev E, Schuster-Gossler K, Fuhl F, Ott T, Tveriakhina L, Beckers A, Hegermann J, Boldt K, Mai M, Kremmer E, Ueffing M, Blum M, Gossler A (2020) CFAP43 modulates ciliary beating in mouse and Xenopus. Dev Biol 459(2):109–125. https://doi.org/10.1016/j.ydbio.2019.12.010
264. Morimoto Y, Yoshida S, Kinoshita A, Satoh C, Mishima H, Yamaguchi N, Matsuda K, Sakaguchi M, Tanaka T, Komohara Y, Imamura A, Ozawa H, Nakashima M, Kurotaki N, Kishino T, Yoshiura KI, Ono S (2019) Nonsense mutation in CFAP43 causes normal-pressure hydrocephalus with ciliary abnormalities. Neurology 92(20):e2364–e2374. https://doi.org/10.1212/WNL.0000000000007505
265. Kobayashi Y, Watanabe M, Okada Y, Sawa H, Takai H, Nakanishi M, Kawase Y, Suzuki H, Nagashima K, Ikeda K, Motoyama N (2002) Hydrocephalus, situs inversus, chronic sinusitis, and male infertility in DNA polymerase lambda-deficient mice: possible implication for the pathogenesis of immotile cilia syndrome. Mol Cell Biol 22(8):2769–2776
266. Zariwala M, O'Neal WK, Noone PG, Leigh MW, Knowles MR, Ostrowski LE (2004) Investigation of the possible role of a novel gene, DPCD, in primary ciliary dyskinesia. Am J Respir Cell Mol Biol 30(4):428–434. https://doi.org/10.1165/rcmb.2003-0338RC2003-0338RC**([pii])**
267. Bertocci B, De Smet A, Flatter E, Dahan A, Bories JC, Landreau C, Weill JC, Reynaud CA (2002) Cutting edge: DNA polymerases mu and lambda are dispensable for Ig gene hypermutation. J Immunol 168(8):3702–3706
268. Tao H, Manak JR, Sowers L, Mei X, Kiyonari H, Abe T, Dahdaleh NS, Yang T, Wu S, Chen S, Fox MH, Gurnett C, Montine T, Bird T, Shaffer LG, Rosenfeld JA, McConnell J, Madan-Khetarpal S, Berry-Kravis E, Griesbach H, Saneto RP, Scott MP, Antic D, Reed J, Boland R, Ehaideb SN, El-Shanti H, Mahajan VB, Ferguson PJ, Axelrod JD, Lehesjoki AE, Fritzsch B, Slusarski DC, Wemmie J, Ueno N, Bassuk AG (2011) Mutations in prickle orthologs cause seizures in flies, mice, and humans. Am J Hum Genet 88(2):138–149. https://doi.org/10.1016/j.ajhg.2010.12.012
269. Tao H, Inoue K, Kiyonari H, Bassuk AG, Axelrod JD, Sasaki H, Aizawa S, Ueno N (2012) Nuclear localization of Prickle2 is required to establish cell polarity during early mouse embryogenesis. Dev Biol 364(2):138–148. https://doi.org/10.1016/j.ydbio.2012.01.025
270. Sowers LP, Yin T, Mahajan VB, Bassuk AG (2014) Defective motile cilia in Prickle2-deficient mice. J Neurogenet 28(1–2):146–152. https://doi.org/10.3109/01677063.2014.885966
271. Zhang Y, Huang G, Shornick LP, Roswit WT, Shipley JM, Brody SL, Holtzman MJ (2007) A transgenic FOXJ1-Cre system for gene inactivation in ciliated epithelial cells. Am J Respir Cell Mol Biol 36(5):515–519. https://doi.org/10.1165/rcmb.2006-0475RC
272. Rawlins EL, Ostrowski LE, Randell SH, Hogan BL (2007) Lung development and repair: contribution of the ciliated lineage. Proc Natl Acad Sci USA 104(2):410–417. https://doi.org/10.1073/pnas.0610770104
273. Muthusamy N, Vijayakumar A, Cheng G Jr, Ghashghaei HT (2014) A knock-in Foxj1(CreERT2:GFP) mouse for recombination in epithelial cells with motile cilia. Genesis 52(4):350–358. https://doi.org/10.1002/dvg.22753
274. Ostrowski LE, Hutchins JR, Zakel K, O'Neal WK (2003) Targeting expression of a transgene to the airway surface epithelium using a ciliated cell-specific promoter. Mol Ther 8(4):637–645. https://doi.org/10.1016/s1525-0016(03)00221-1
275. Li X, Floriddia EM, Toskas K, Chalfouh C, Honore A, Aumont A, Vallieres N, Lacroix S, Fernandes KJL, Guerout N, Barnabe-Heider F (2018) FoxJ1 regulates spinal cord development and is required for the maintenance of spinal cord stem cell potential. Exp Cell Res 368(1):84–100. https://doi.org/10.1016/j.yexcr.2018.04.017
276. Rawlins EL, Hogan BL (2008) Ciliated epithelial cell lifespan in the mouse trachea and lung. Am J Physiol Lung Cell Mol Physiol 295(1):L231–234. https://doi.org/10.1152/ajplung.90209.2008
277. O'Connor AK, Malarkey EB, Berbari NF, Croyle MJ, Haycraft CJ, Bell PD, Hohenstein P, Kesterson RA, Yoder BK (2013) An inducible CiliaGFP mouse model for in vivo visualization and analysis of cilia in live tissue. Cilia 2(1):8. https://doi.org/10.1186/2046-2530-2-8
278. Schmitz F, Burtscher I, Stauber M, Gossler A, Lickert H (2017) A novel Cre-inducible knock-in ARL13B-tRFP fusion cilium reporter. Genesis 55:11. https://doi.org/10.1002/dvg.23073
279. You Y, Richer EJ, Huang T, Brody SL (2002) Growth and differentiation of mouse tracheal epithelial cells: selection of a proliferative population. Am J Physiol Lung Cell Mol Physiol 283(6):L1315–1321. https://doi.org/10.1152/ajplung.00169.2002
280. Stubbs JL, Vladar EK, Axelrod JD, Kintner C (2012) Multicilin promotes centriole assembly and ciliogenesis during multiciliate cell differentiation. Nat Cell Biol 14(2):140–147. https://doi.org/10.1038/ncb2406
281. Vladar EK, Stearns T (2007) Molecular characterization of centriole assembly in ciliated epithelial cells. J Cell Biol 178(1):31–42. https://doi.org/10.1083/jcb.200703064
282. Vladar EK, Nayak JV, Milla CE, Axelrod JD (2016) Airway epithelial homeostasis and planar cell polarity signaling depend on multiciliated cell differentiation. JCI Insight 1:13. https://doi.org/10.1172/jci.insight.88027
283. Vladar EK, Stratton MB, Saal ML, Salazar-De Simone G, Wang X, Wolgemuth D, Stearns T, Axelrod JD (2018) Cyclin-dependent kinase control of motile ciliogenesis. Elife. https://doi.org/10.7554/eLife.36375
284. Zhu L, Liu H, Chen Y, Yan, X, Zhu X (2019) Rsph9 is critical for ciliary radial spoke assembly and central pair microtubule

stability. Biol Cell 111(2):29–38. https://doi.org/10.1111/boc.201800060
285. Bosch Grau M, Gonzalez Curto G, Rocha C, Magiera MM, Marques Sousa P, Giordano T, Spassky N, Janke C (2013) Tubulin glycylases and glutamylases have distinct functions in stabilization and motility of ependymal cilia. J Cell Biol 202(3):441–451. https://doi.org/10.1083/jcb.201305041
286. Shamoto N, Narita K, Kubo T, Oda T, Takeda S (2018) CFAP70 is a novel axoneme-binding protein that localizes at the base of the outer dynein arm and regulates ciliary motility. Cells 7:9. https://doi.org/10.3390/cells7090124
287. Zhang Y, Chen Y, Zheng J, Wang J, Duan S, Zhang W, Yan X, Zhu X (2019) Vertebrate dynein-f depends on Wdr78 for axonemal localization and is essential for ciliary beat. J Mol Cell Biol 11(5):383–394. https://doi.org/10.1093/jmcb/mjy043
288. Zheng J, Liu H, Zhu L, Chen Y, Zhao H, Zhang W, Li F, Xie L, Yan X, Zhu X (2019) Microtubule-bundling protein Spef1 enables mammalian ciliary central apparatus formation. J Mol Cell Biol 11(1):67–77. https://doi.org/10.1093/jmcb/mjy014
289. Nishimura Y, Kurisaki A, Nakanishi M, Ohnuma K, Ninomiya N, Komazaki S, Ishiura S, Asashima M (2010) Inhibitory smad proteins promote the differentiation of mouse embryonic stem cells into ependymal-like ciliated cells. Biochem Biophys Res Commun 401(1):1–6. https://doi.org/10.1016/j.bbrc.2010.08.099**(S0006-291X(10)01620-7[pii])**
290. Yoshie S, Imaizumi M, Nakamura R, Otsuki K, Ikeda M, Nomoto Y, Wada I, Omori K (2016) Generation of airway epithelial cells with native characteristics from mouse induced pluripotent stem cells. Cell Tissue Res 364(2):319–330. https://doi.org/10.1007/s00441-015-2304-7
291. Tsao PN, Vasconcelos M, Izvolsky KI, Qian J, Lu J, Cardoso WV (2009) Notch signaling controls the balance of ciliated and secretory cell fates in developing airways. Development 136(13):2297–2307. https://doi.org/10.1242/dev.034884
292. Genzen JR, Yang D, Ravid K, Bordey A (2009) Activation of adenosine A2B receptors enhances ciliary beat frequency in mouse lateral ventricle ependymal cells. Cerebrospinal Fluid Res 6:15. https://doi.org/10.1186/1743-8454-6-15**(1743-8454-6-15[pii])**
293. Allen-Gipson DS, Blackburn MR, Schneider DJ, Zhang H, Bluitt DL, Jarrell JC, Yanov D, Sisson JH, Wyatt TA (2011) Adenosine activation of A(2B) receptor(s) is essential for stimulated epithelial ciliary motility and clearance. Am J Physiol Lung Cell Mol Physiol 301(2):L171–180. https://doi.org/10.1152/ajplung.00203.2010**(ajplung.00203.2010[pii])**
294. Bylander A, Lind K, Goksor M, Billig H, Larsson DG (2013) The classical progesterone receptor mediates the rapid reduction of fallopian tube ciliary beat frequency by progesterone. Reprod Biol Endocrinol 11:33. https://doi.org/10.1186/1477-7827-11-33
295. Herawati E, Taniguchi D, Kanoh H, Tateishi K, Ishihara S, Tsukita S (2016) Multiciliated cell basal bodies align in stereotypical patterns coordinated by the apical cytoskeleton. J Cell Biol 214(5):571–586. https://doi.org/10.1083/jcb.201601023
296. Alpizar YA, Boonen B, Sanchez A, Jung C, Lopez-Requena A, Naert R, Steelant B, Luyts K, Plata C, De Vooght V, Vanoirbeek JAJ, Meseguer VM, Voets T, Alvarez JL, Hellings PW, Hoet PHM, Nemery B, Valverde MA, Talavera K (2017) TRPV4 activation triggers protective responses to bacterial lipopolysaccharides in airway epithelial cells. Nat Commun 8(1):1059. https://doi.org/10.1038/s41467-017-01201-3
297. Abdi K, Lai CH, Paez-Gonzalez P, Lay M, Pyun J, Kuo CT (2018) Uncovering inherent cellular plasticity of multiciliated ependyma leading to ventricular wall transformation and hydrocephalus. Nat Commun 9(1):1655. https://doi.org/10.1038/s41467-018-03812-w
298. Price ME, Pavlik JA, Sisson JH, Wyatt TA (2015) Inhibition of protein phosphatase 1 reverses alcohol-induced ciliary dysfunction. Am J Physiol Lung Cell Mol Physiol 308(6):L577–585. https://doi.org/10.1152/ajplung.00336.2014
299. Wyatt TA, Wells SM, Alsaidi ZA, DeVasure JM, Klein EB, Bailey KL, Sisson JH (2013) Asymmetric dimethylarginine blocks nitric oxide-mediated alcohol-stimulated cilia beating. Mediators Inflamm 2013:592892. https://doi.org/10.1155/2013/592892
300. Wyatt TA, Bailey KL, Simet SM, Warren KJ, Sweeter JM, DeVasure JM, Pavlik JA, Sisson JH (2019) Alcohol potentiates RSV-mediated injury to ciliated airway epithelium. Alcohol 80:17–24. https://doi.org/10.1016/j.alcohol.2018.07.010
301. Villa M, Crotta S, Dingwell KS, Hirst EM, Gialitakis M, Ahlfors H, Smith JC, Stockinger B, Wack A (2016) The aryl hydrocarbon receptor controls cyclin O to promote epithelial multiciliogenesis. Nat Commun 7:12652. https://doi.org/10.1038/ncomms12652
302. Workman AD, Carey RM, Chen B, Saunders CJ, Marambaud P, Mitchell CH, Tordoff MG, Lee RJ, Cohen NA (2017) CALHM1-mediated ATP release and ciliary beat frequency modulation in nasal epithelial cells. Sci Rep 7(1):6687. https://doi.org/10.1038/s41598-017-07221-9
303. Hoh RA, Stowe TR, Turk E, Stearns T (2012) Transcriptional program of ciliated epithelial cells reveals new cilium and centrosome components and links to human disease. PLoS ONE 7(12):e52166. https://doi.org/10.1371/journal.pone.0052166PONE-D-12-33835**([pii])**
304. Johnson JA, Watson JK, Nikolic MZ, Rawlins EL (2018) Fank1 and Jazf1 promote multiciliated cell differentiation in the mouse airway epithelium. Biol Open 7:4. https://doi.org/10.1242/bio.033944
305. Gharib SA, Altemeier WA, Van Winkle LS, Plopper CG, Schlesinger SY, Buell CA, Brauer R, Lee V, Parks WC, Chen P (2013) Matrix metalloproteinase-7 coordinates airway epithelial injury response and differentiation of ciliated cells. Am J Respir Cell Mol Biol 48(3):390–396. https://doi.org/10.1165/rcmb.2012-0083OC
306. Shimada IS, Acar M, Burgess RJ, Zhao Z, Morrison SJ (2017) Prdm16 is required for the maintenance of neural stem cells in the postnatal forebrain and their differentiation into ependymal cells. Genes Dev 31(11):1134–1146. https://doi.org/10.1101/gad.291773.116
307. Shah PT, Stratton JA, Stykel MG, Abbasi S, Sharma S, Mayr KA, Koblinger K, Whelan PJ, Biernaskie J (2018) Single-cell transcriptomics and fate mapping of ependymal cells reveals an absence of neural stem cell function. Cell 173(4):1045–1057. https://doi.org/10.1016/j.cell.2018.03.063**(e1049)**
308. Hua X, Zeman KL, Zhou B, Hua Q, Senior BA, Tilley SL (1985) Bennett WD (2010) Noninvasive real-time measurement of nasal mucociliary clearance in mice by pinhole gamma scintigraphy. J Appl Physiol 108(1):189–196. https://doi.org/10.1152/japplphysiol.00669.2009
309. Veres TZ, Kopcsanyi T, Tirri M, Braun A, Miyasaka M, Germain RN, Jalkanen S, Salmi M (2017) Intubation-free in vivo imaging of the tracheal mucosa using two-photon microscopy. Sci Rep 7(1):694. https://doi.org/10.1038/s41598-017-00769-6
310. Donnelley M, Morgan KS, Siu KK, Fouras A, Farrow NR, Carnibella RP, Parsons DW (2014) Tracking extended mucociliary transport activity of individual deposited particles: longitudinal synchrotron X-ray imaging in live mice. J Synchrotron Radiat 21(Pt 4):768–773. https://doi.org/10.1107/S160057751400856X
311. Wang S, Burton JC, Behringer RR, Larina IV (2015) In vivo micro-scale tomography of ciliary behavior in the mammalian oviduct. Sci Rep 5:13216. https://doi.org/10.1038/srep13216
312. Liu T, Jin X, Prasad RM, Sari Y, Nauli SM (2014) Three types of ependymal cells with intracellular calcium oscillation are

characterized by distinct cilia beating properties. J Neurosci Res 92(9):1199–1204. https://doi.org/10.1002/jnr.23405
313. Ansari R, Buj C, Pieper M, Konig P, Schweikard A, Huttmann G (2015) Micro-anatomical and functional assessment of ciliated epithelium in mouse trachea using optical coherence phase microscopy. Opt Express 23(18):23217–23224. https://doi.org/10.1364/OE.23.023217
314. Lee YL, Sante J, Comerci CJ, Cyge B, Menezes LF, Li FQ, Germino GG, Moerner WE, Takemaru K, Stearns T (2014) Cby1 promotes Ahi1 recruitment to a ring-shaped domain at the centriole-cilium interface and facilitates proper cilium formation and function. Mol Biol Cell 25(19):2919–2933. https://doi.org/10.1091/mbc.E14-02-0735
315. Mori M, Hazan R, Danielian PS, Mahoney JE, Li H, Lu J, Miller ES, Zhu X, Lees JA, Cardoso WV (2017) Cytoplasmic E2f4 forms organizing centres for initiation of centriole amplification during multiciliogenesis. Nat Commun 8:15857. https://doi.org/10.1038/ncomms15857
316. Nanjundappa R, Kong D, Shim K, Stearns T, Brody SL, Loncarek J, Mahjoub MR (2019) Regulation of cilia abundance in multiciliated cells. Elife. https://doi.org/10.7554/eLife.44039
317. Shi X, Garcia G 3rd, Van De Weghe JC, McGorty R, Pazour GJ, Doherty D, Huang B, Reiter JF (2017) Super-resolution microscopy reveals that disruption of ciliary transition-zone architecture causes Joubert syndrome. Nat Cell Biol 19(10):1178–1188. https://doi.org/10.1038/ncb3599

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