**Motile ciliopathies**

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**Abstract**

Motile cilia are highly complex hair-like organelles of epithelial cells lining the surface of various organ systems. Genetic mutations (usually with autosomal recessive inheritance) that impair ciliary beating cause a variety of motile ciliopathies, a heterogeneous group of rare disorders. The pathogenetic mechanisms, clinical symptoms and severity of the disease depend on the specific affected genes and the tissues in which they are expressed. Defects in the ependymal cilia can result in hydrocephalus, defects in the cilia in the fallopian tubes or in sperm flagella can cause female and male subfertility, respectively, and Malfunctional motile monocilia of the left–right organizer during early embryonic development can lead to laterality defects such as situs inversus and heterotaxy. If muco-ciliary clearance in the respiratory epithelium is severely impaired, the disorder is referred to as primary ciliary dyskinesia (PCD), the most common motile ciliopathy. No single test can confirm a diagnosis of motile ciliopathy, which is based on a combination of tests including nasal nitric oxide measurement; TEM, immunofluorescence and genetic analyses; and high-speed video microscopy. With the exception of azithromycin, there is no evidence-based treatment for PCD; therapies aim at relieving symptoms and reduce the effects of reduced ciliary motility.

**[H1] Introduction**

### Cilia are highly complex hair-like organelles protruding from the surface of almost all human cells and contain a tubulin-based axoneme (the backbone of the cilium). Most human cells exhibit a solitary non-motile cilium, also referred to as primary cilium, which acts as mechanosensor or chemosensor and has a crucial role for signal transduction1. By contrast, only a few cell types develop during differentiation highly specialized cilia that can move utilizing large biological motor machineries attached to the microtubules. The respiratory epithelium, the ependyma (a layer of glial cells covering the brain ventricles and the central canal of the spinal cord) and the epithelium of the fallopian ducts are covered with multiple motile cilia, beating in a coordinated fashion to propel mucus or fluid along their surfaces. The sperm cells move along the female reproductive system using a flagellum that shares most of the axonemal structure of a motile cilium. Motile monocilia (one cilium per cell) at the node (also known as the left–right organizer), a transient structure that forms during early embryogenesis, have a crucial functional role for determination of left–right body asymmetry.

Inborn defects of primary cilia, also referred to as primary ciliopathies or non-motile ciliopathies, cause a wide spectrum of hereditary disorders affecting the central nervous system, the eyes, kidneys, liver, heart and various other organs2. Inborn dysfunctions of motile cilia, referred to as motile ciliopathies, are also responsible for disease manifestations in various organ systems1. Dyskinetic motile cilia lining the airways can impair mucociliary clearance and result in chronic destructive disease of the upper and lower airways. Motility of nodal monocilia is essential for correct determination of left–right body asymmetry.3,4 Whereas regular asymmetrical organogenesis results in the typical arrangement of the visceral organs (a situation called situs solitus), failure to establish this anatomy manifests in laterality defects, such as completely reversed arrangement of organs, a condition called situs inversus totalis. In addition, the term situs ambiguus or heterotaxy summarizes all other kinds of laterality defects, such as left-isomerism, right -isomerism or situs inversus of just one body cavity (situs inversus thoracalis, when the involved organs are in the thorax, or situs inversus abdominalis, when the organs in the abdomen are affected), and is also often associated with congenital heart defects (Figure 1).In accordance with previous publications, here we refer to any defect other that situs inversus totalis as situs ambiguus5. Mutations in genes encoding components essential for nodal cilia motility during early embryogenesis result in situs inversus or situs ambiguus in approximately half of the individuals owing to randomization of left-right body asymmetry6. Dysfunction of cilia lining the fallopian tubes (which have a role in the transport of the egg through the human female reproductive system) or dyskinetic sperm flagella can reduce fertility in women and men, respectively7. Dysmotility of ependymal cilia lining the brain ventricles can contribute to the formation of hydrocephalus (that is, accumulation of cerebrospinal fluid in the brain, which results in distension of brain ventricles). All these clinical features of motile ciliopathies can occur alone or in various combinations. So far mutations in 57 genes are known to cause motile ciliopathies or overlap syndromes affecting both non-motile and motile cilia. 8.

The spectrum of ciliopathies is rapidly expanding. Motile ciliopathies can range from isolated laterality defects or male subfertility to multisystem disorders such as primary ciliary dyskinesia (PCD, MIM244400), the most common motile ciliopathy, in which mucociliary clearance is impaired and chronic destructive airway disease is present. Most mutations associated with PCD disease traits follow autosomal recessive or, less frequently, X-chromosomal recessive9–12 inheritance. Recently, *de novo* autosomal dominant mutations in *FOXJ1* have been described in sporadic cases of PCD8. To diagnose PCD, awareness of the typical clinical symptoms such as chronic infections of the upper and lower airways, laterality defects, subfertility and, very rarely, hydrocephalus is essential. Owing to the variability of the underlying disease mechanisms, the diagnosis of PCD cannot be confirmed or ruled out by a single gold standard diagnostic test: determination of the nasal nitric oxide (NO) production rate, high speed video microscopy (HSVM) analysis, transmission electron microscopy (TEM) and immunofluorescence analysis of respiratory cells are currently used13. Owing to successful gene discovery, genetic testing is now increasingly used and now part of European and North American (US and Canada) diagnostic guidelines13,14. However, current genetic tests can only identify genetic defects in ~70% of definitive PCD cases15.

Currently there is no definitive cure for motile ciliopathies, and in most contexts the treatment is empirically based on therapies for clinically similar diseases (for example, cystic fibrosis and bronchiectasis (the abnormal dilatation of the bronchi), among others) and the experiences of clinicians. However, a recent multinational randomized controlled trial on antibiotic maintenance therapy in PCD has been successfully performed and paves the way for the first evidence-based treatment recommendations16. In addition, precision medicine approaches such as gene and mRNA transcript therapy are studied to correct the underlying disease mechanisms.

This Primer focuses on data obtained in humans; data obtained in model organisms is only presented in a very limited manner. The article addresses the broad phenotypical spectrum of motile ciliopathies and focuses on PCD, discussing epidemiology, pathophysiology, clinical picture, diagnosis, management and quality of life aspects.

[H1] Epidemiology

Available data on the epidemiology of PCD is scarce. There are no reliable data on prevalence, only estimates, as only a proportion of patients with PCD are correctly diagnosed, and among these, only few are included in national or regional registries. Routine medical statistics, such as hospital episode statistics or mortality statistics, are non-informative, because the International Classification of Diseases, Version 10 and earlier versions have no specific code for PCD. Thus, these patients remain invisible in official statistics.

Early studies estimated prevalence by extrapolating data from x-ray surveys. For instance, a Norwegian study from the 1940s based on X-rays from a third of the population found a prevalence of situs inversus of 1:8,000 and bronchiectasis in 10% of those with situs inversus17. Assuming that 50% of patients with PCD have situs inversus, this observation results in an estimated prevalence of 1:40,000. However, this prevalence is an underestimate, because standard chest x-rays are not very sensitive to detect bronchiectasis, and young patients or those with mild disease might not have developed bronchiectasis. Also, several causative mutations for PCD are not associated with situs inversus, which suggests that the proportion of patients with situs inversus is slightly below 50%. Another study using chest radiographs from survivors of the atomic bombs in Hiroshima and Nagasaki found 4 patients with PCD among the 16,566 adults investigated, corresponding to an estimated prevalence of 1:4,100 (Ref18). Differences between estimates could be explained by different prevalences of genetic mutations in different populations or simply differences in the quality of x-rays or their interpretation.

More recent estimates based on surveys among PCD centres and registries found that prevalence seems to vary widely between countries, but it is unclear whether this variability reflects variations in diagnostic practices or true differences in disease frequency19. A 2008 international survey found the prevalence to range from 1:10,000 (Cyprus) and 1:20,000 (Denmark and Switzerland, and consistent with a prevalence of 1:22,000 for Sweden20) to much lower frequencies in many other countries. 21 . Thus, the global prevalence is probably at least 1:10,000. Of note, in highly consanguineous populations, such as British citizens of South Asian origin22 or inhabitants of Volendam, a fishing village in North Holland isolated since the 15th century, 23 very high prevalences (1:2,265 and 1:400, respectively) have been found. Males and females are equally affected24, but the distribution of causative genes varies between ethnic groups and regions. For instance, a study from the UK found that 52% of white European families carried *DNAH5* or *DNAH11* mutations, 42% of Arab families carried *CCDC39* or *CCDC40* mutations and 36% of South Asian families carried single *LRRC6* or *CCDC103* mutations. This observation suggests a strong genetic stratification due to founder mutations according to the population of origin25.

As diagnostic practices have not substantially changed since the 2010s in most countries, recent data remain unreliable 24. This uncertainty is particularly true for low income and middle income countries, where the highly specialised and costly PCD diagnostic facilities are often not available, and bronchiectasis, most commonly caused by tuberculosis and other severe infections, is frequent even in young people, 26,27. Diagnosis can be further delayed if affected people, especially young females, attempt to hide their symptoms because of the social stigma that can accompany chronic cough and the diagnosis of a chronic or genetic illness in some communities, particularly if such disease is associated with subfertility28. To date, most diagnosed patients are children and young adults, and PCD in people of >40 years of age remains largely undiagnosed. For instance, the largest available research dataset, the international PCD cohort (iPCD cohort) includes >3,800 patients, of whom 17% are of 0-9 years of age, 38% of 10-19 years, 18% of 20–29 years, 11% of 30-39 years, 7% of40-49 years, 5% of 50-59 years and 4% of ≥60 years.29 These proportions are largely due to underdiagnosis in adults and could be explained if patients who received a wrong (for example, atypical chronic obstructive pulmonary disease (COPD)) or incomplete (for example, bronchiectasis of unknown origin) diagnosis in their youth, when PCD diagnostics were less developed, never had their diagnosis revisited later in life30. Another explanation might be that children with chronic respiratory symptoms are usually cared for in University Paediatric Pulmonology clinics that have a focus on rare diseases, whereas adults with similar symptoms are often treated by Pulmonologists in private practice, who are less aware of rare diseases because they might encounter only one or two such cases in their career.

**[H2] Long-term outcomes and risk factors**

We know little about natural history and life expectancy in PCD. Long-term course was previously considered benign, but scarce data from clinical studies in adults suggest that most have bronchiectasis, some progress to severe lung disease and a minority requires oxygen supplementation and lung transplantation31–33. Early studies suggested that timely diagnosis and initiation of treatment might positively influence long-term course, but this observation has not been consistently replicated32,34–36. It is possible that more severely affected patients are diagnosed earlier than patients with mild disease, so that they fare worse despite earlier start of treatment. Life expectancy is probably shortened, but quantitative data of good quality are lacking32.

Although PCD is a genetic disease, environmental exposures, such as air pollution, smoking and malnutrition, and host-related risk factors, such as lack of physical activity, are likely to influence disease severity and long-term course. However, there is little research on this topic. Many patients with PCD have a lower height and body mass index (BMI) than international and national reference values; 37 a small British study reported also lower Vitamin D levels than those in healthy children38. Results are not easy to compare between studies, because the BMI and height z-scores depend on the reference values used, which were sometimes out of date, or derived from different populations, so did not apply to the population studied 37. The question is whether there is an intrinsic or genetic defect leading to malnutrition or whether malnutrition is secondary to late diagnosis or suboptimal management of PCD. As several studies suggest that normal nutritional status can be maintained37,39–41,

the benchmark should be to obtain normal, stable nutrition, and if this goal is not being achieved, then a quality improvement initiative, such as effectively pioneered for cystic fibrosis, should be tested and instituted42. This issue is particularly important as several studies found that height and BMI are associated with lung function in PCD, with poor lung function values in patients with low BMI 36,38,43. Internationally standardised instruments for clinical follow-up in patients with PCD might help to monitor and improve nutrition, growth and lung function44. However, although nutritional advice with increased caloric intake and possibly vitamin supplements38 could delay disease progression and lung function impairment, evidence from intervention studies is lacking.

[H1] Mechanisms/pathophysiology

**[H2] Cilia structure and subtypes**

Cilia are based on a highly ordered structure of nine peripheral microtubule doublets, with or without a central pair (CP), which consists of two single microtubules and associated multiprotein complexes and acts as the stabilizing network of the outer doublets. Four ciliary subtypes have been reported in humans: motile 9+2 cilia (9 peripheral microtubule doublets and the CP, for example, respiratory cilia), motile 9+0 cilia (9 peripheral microtubule doublets and no CP, for example,. nodal cilia), non-motile 9+2 cilia (for example, kinocilia in the inner ear) and non-motile 9+0 cilia (for example, renal monocilia and photoreceptor connecting cilia). The vestibular stereocilia are actin based and, therefore, not considered as cilia. Of note, motile cilia can also exhibit sensory function45.

The structure and function of ciliary motility related proteins are evolutionary conserved and well-studied in various model organisms such as the unicellular algae *Chlamydomonas* spp*., planaria* (flatworms)*,* xenopus, zebrafish or mouse. The architecture of the axoneme, the core structure of the cilium, is very complex and contains large multiprotein complexes attached in a highly organized manner to the 9 outer doublets of microtubules (the A-tubule and the B-tubule, each composed of α-tubulin and β-tubulin heterodimers), with or without the CP (Figure 2). Outer dynein arms (ODAs) are composed of dynein heavy chains (400-500 kDa), intermediate chains (45-140 kDa) and light chains (8-28 kDa), contain the biological motors responsible for ciliary beat generation and are spaced every 24 nm along the length of the axoneme46. ODAs are attached to the A-tubule by distinct ODA docking complexes (ODA-DCs). The regulation of the ciliary beating is complex and involves the function of several distinct machineries, such as the inner dynein arms (IDAs) and the nexin–dynein regulatory complexes (N-DRCs, which connect the outer doublets to each other47), that are all connected to the outer doublets via the coiled-coil domain containing proteins CCDC39 and CCDC40; these two proteins form a ruler complex that determines the length of the 96 nm repeat, the basic structural unit that is repeated every 96 nm along the microtubules of the axoneme of cilia and flagella48.The motor protein-complexes generate the power driving the ciliary beat by converting the chemical energy of ATP binding and hydrolysis into mechanical force49. These ATP-dependent conformational changes result in transient binding and coordinated sliding of adjacent microtubules by conformational changes of the dynein heavy chains. Whereas the ODAs generate the majority of the beating force, the IDAs are thought to regulate the waveform50. Immunofluorescence microscopy data indicate that in human respiratory epithelial cells ODAs contain two axonemal dynein heavy chains, and at least two types of ODAs exist: ODA type-1 (containing the dynein heavy chains DNAH11 and DNAH5 and located in the proximal part of the axoneme) and ODA type-2 (containing DNAH9 and DNAH5 and distal to the axoneme) (Figure 2)51–53. Interestingly, sperm flagella contain a third type of ODA (ODA type-3), containing the axonemal dynein heavy chains DNAH17 and DNAH854.

In 9+2 cilia, the radial spokes (RSs) and the CP apparatus interconnect the outer doublets with the CP. The RS is a T-shaped macromolecular complex comprising a stalk, neck and head compartment. In human respiratory cilia, three distinct RS types have been described within the 96 nm repeat55. The N-DRC, the RSs and the CP apparatus modulate the ciliary movement and regulate the dynein arms activity. Inhibition of the sliding forces by the N-DRC converts the sliding forces into bending of the axoneme (Figure 2)56. The N-DRC, CP and RS system also acts as a mechano- chemical transducer57–59. Finally, a recent report identified a novel group of proteins located within the microtubules, the microtubule inner proteins (MIPs), that project to other axonemal components such as the ODA-DCs and, therefore, seem to stabilize the axonemal structure and regulate ciliary beat60.

Cilia have diversified into different subtypes in vertebrates1. Some specialized epithelial cells are densely covered with multiple motile cilia (200–300 per cell), generating a directed fluid flow along their surface. Whereas the human respiratory cilia covering the airways beat with a frequency of about 4–12 Hz at 25°C (a temperature close to the typical temperature in the nasal cavity) 61 and 11.5–12.9 Hz (7.7Hz–18.1Hz (5th and 95th percentile, respectively)) at 37°C62 to move mucus, inhaled particles and microbes towards the throat, the cilia of the female fallopian tubes beat with a frequency of ~3.5 Hz at 25°C 63. Beating of the ependymal cilia generates flow of the cerebrospinal fluid within the brain cavities and aids to maintain patency of the ventricular system, including narrow sites of passage such as the aqueduct connecting the third and the fourth brain ventricles.64,65. In rat, ependymal cilia beat with a frequency of ~28 Hz at 30°C66 Motile monocilia in the node generate a leftward flow during early embryogenesis3.The flagellum of the spermatozoon acts as an intrinsic motor to move the sperm within the female fallopian tube towards the oocyte, beating with a frequency of ~20 Hz67

**[H2] Defects in Ciliary structural components**

Several mutations in genes encoding structural cilia proteins have been identified and associated with motile ciliopathies (Table 1, Figure 3). Mutations in *DRC1*68, *CCDC65*69 and *GAS8*70 encoding N-DRC components result in isolated N-DRC defects and PCD. Mutations in *CCDC39*71 and *CCDC40*72,73 in individuals with PCD result in severe tubular disorganization due to the loss of the 96 nm repeat structure. In addition, loss of DNALI1-containing IDA complexes and N-DRC proteins from the ciliary axonemes has been noted, indicating that the CCDC39–CCDC40 complex display attachment sites for IDAs and the N-DRC71,72. Recessive mutations in genes encoding 6 of the 12 RS proteins known in humans have been described, affecting the function and composition of the RS complexes74–80. Mutations in *HYDIN*81, *SPEF2*82, *STK36*83 and *CFAP221* 84 encoding proteins of the CP complex are known to cause PCD. Recently a group of MIPs was described in *Chlamydomonas* spp.85. A cryoelectron tomography study in *Chlamydomonas reinhardtii* localized the *Chlamydomonas* orthologues of the proteins ENKURIN86, CCDC11 (Ref87), WDR16 (Ref88), MNS1 (Ref89) and NME7 (Ref90), which, in humans, are associated with motile ciliopathies60. The localization of these proteins within the microtubule in *C. reinhardtii* indicates that the human orthologues probably share an evolutionary conserved function for motile cilia and preserved their function as microtubule inner binding proteins. The GAS2-like protein 2 (encoded by *GAS2L2*) localizes to the basal body (a protein structure at the base of the cilium), basal feet, rootlets and actin filaments. GAS2L2 deficiency has been shown to cause defects in ciliary orientation91 similar to *RPGR* defects.92

Defects in the ODAs53,93–95 and intermediate chains96–99 were the first to be identified in PCD and are the most common cause for PCD. (Ref96–98*)* *Ref*100*)* Interestingly, sperm flagella contain a third type of ODA (ODA type-3), containing the axonemal dynein heavy chains DNAH1754 and DNAH8101 (Ref54). Mutations in the sperm specific ODA DNAH8101 and DNAH1754 cause isolated male subfertility due to asthenozoospermia (reduced sperm motility).

Defects in five ODA-DC components affecting both ODA type-1 and ODA type-2 have been identified and result in PCD4,102–105. CCDC103 does not seem to be a classical ODA docking protein but an axonemal attachement factor, which acts independently of the ODA-DC machinery4. Of note, data indicate that MNS1 recently identified as a MIP can modulate ODA docking60,89.

**[H2] Cytoplasmic dynein axonemal assembly factors**

ODA components are assembled in large multiprotein complexes within the cytoplasm in a step-wise fashion by cytoplasmic dynein axonemal assembly factors (DNAAFs). The various DNAAFs interact with each other and co-localize in distinct cytoplasmic compartments referred to as dynein axonemal particles106. Mutations in genes encoding DNAAFs are responsible for combined defects of the ODAs and IDAs (Figure 4). Whereas *DNAAF2 (Ref*107) or *DNAAF4 (Ref*108) mutations lead to loss of ODA type2 and absence of the IDA light chain DNALI1 from the ciliary axonemes, mutations in the genes encoding other DNAAFs (109,110, 111, 112, 113, 114, 115, 129 ) predominantly result in the loss of both ODA types and DNALI1. Autosomal recessive mutations in *TTC12*116(encoding a DNAAF) cause a defect in the IDA in respiratory epithelial cells, whereas in sperm flagella ODAs and IDAs are affected116, indicating distinct dynein assembly mechanisms in different cell types. Additional genetic defects will probably be identified.

The ODA-DC is also first assembled in the cytoplasm and then transported independently of the ODAs; multiprotein complexes such as ODAs and ODA-DCs are transported via a mechanism referred to as intraflagellar transport (IFT) towards their destined sites of attachment within the ciliary axonemes. Defects in ODA8 (an orthologue of the IFT associated protein LRRC56) have been associated with subtle defects of distal ODAs in a study in trypanosomes.117.

**[H2] Reduced generation of multiple motile cilia**

During the short period of differentiation in multiciliated cells, hundreds of centrioles are generated to serve as basal bodies required as nucleation points for cilia growth. In multiciliogenesis of airway cells, NOTCH1 functions as a repressor of multicilin (encoded by *MCIDAS*), a transcription regulator that regulates cyclin O (encoded by *CCNO*) 118,119 and the transcription factor FOXJ1118,120,121. As a result of the inhibition of multicilin, the developing multiciliated cell exits the cell cycle, and new centrioles start to form. Cyclin O mediates centriole amplification and migration to the apical cell surface, and FOXJ1 mediates docking at the apical cell. Each docked centriole (now called a basal body) nucleates a motile 9+2 ciliary axoneme in a multiciliated cell (Figure 4).

Defects of ciliogenesis in multiciliated cells are referred to as reduced generation of multiple motile cilia (RGMC). Mutations in *MCIDAS* and *CCNO* result in a centriole amplification defect in the deuterosome (one of the protein structures that generate multiple centrioles)-dependent pathway, which is specific for multiciliated cells, leading to a severely reduced number of basal bodies122,123 and, therefore, of cilia per cell. By contrast, in *FOXJ1* mutant respiratory epithelial cells, the number of basal bodies is normal, but basal bodies are mislocalized within the cytoplasm; thus, fewer cilia are correctly assembled, and whereas some respiratory epithelial cells exhibit a normal number of cilia, the number of cilia is reduced in most8. *FOXJ1* haploinsufficiency is also associated with male subfertility, consistent with findings in *Foxj1* mutant mice showing defects in sperm development124 Female subfertility and hydrocephalus indicate that also the female fallopian tube cilia and the ependymal cilia are affected in patients with RGMC. Recently a ciliogenesis defect that has been not connected to the NOTCH1 pathway has been reported. The Serine/threonine-protein kinase Nek10 (also known as Nima-related protein kinase 10), encoded by *NEK10*, acts as a ciliated-cell specific kinase actively regulating the motile ciliary proteome125 to promote ciliary growth and transport. NEK10 deficient multiple motile cilia are shorter than controls, but their number, radial structure or beat are not affected126.

**[H2] Central nervous system defects**

The distribution of cerebro-spinal fluid within and across brain ventricles depends on ciliary motility127, and abnormalities in cerebro-spinal fluid physiology can result in hydrocephalus. Hydrocephalus can occur for various reasons; hydrocephalus without any obvious extrinsic cause (for example, tumor, bleeding or infection) is usually referred to as congenital hydrocephalus and accounts for ~10% of all cases127.

Motile ciliopathies, especially those due to RGMC, can result in hydrocephalus (Table 1, Figure 1), owing to defective cerebrospinal fluid flow during brain formation in rodents64,65. However, whereas so far all mouse models with aberrant ependymal cilia beating exhibit a hydrocephalus phenotype, idiopathic hydrocephalus is less frequent in humans than in mice, probably because the human brain ascertained a much bigger size than rodent brain during evolution 65. Furthermore, studies in mice have indicated that cilia have an important role in ion transport as well as cerebrospinal fluid production.128 Interestingly, a study of a cohort of patients with congenital heart defects and abnormal ciliary beating reported an increased incidence of subtle brain malformation 129. Further studies are needed to clarify whether these findings occured incidentally or are caused by the same underlying molecular defect affecting cilia function in both organ systems.

**[H2] Nodal cilia and left–right body asymmetry**

Motility of nodal monocilia resulting in a nodal flow during early embryogenesis is essential for determination of left–right body asymmetry130. However, how the nodal flow contributes to this determination is still a matter of debate. Two models are still discussed, the nodal vesicular parcel model and the two cilia model. In the nodal vesicular parcel model, vesicles filled with morphogens (for example, sonic hedgehog) are secreted from the right side of the embryonic node and transported to the left side by nodal flow, where they release their cargo. The morphogens bind to transmembrane receptors in the axonemal membrane of cilia on the left side and trigger Ca2+ release, which induces downstream signalling events to determine laterality131. In the two cilia model, motile nodal monocilia promote a leftward nodal flow in the center of the node to create a flow that is sensed through passive bending by non-motile sensory cilia in the periphery of the node. The ciliary bending on the left side is supposed to cause the Ca2+ release that initiates the establishment of body asymmetry132,133. Thus, in both models the left-sided asymmetric Ca2+ release initiates down-stream signalling events such as the Nodal signalling cascade. The involvement of non-motile cilia in the determination of left–right body asymmetry explains also why many individuals with genetic defects resulting in a non-motile ciliopathy also exhibit laterality defects.

Interestingly, the current nodal flow hypotheses cannot sufficiently explain the complex laterality defects that are observed in humans with motile ciliopathies. Although most patients with genetic defects resulting in a motile ciliopathy with abnormal nodal cilia function exhibit *situs solitus* or *situs inversus totalis* (Figure 1), a small proportion show partial laterality defects such as *situs inversus abdominalis* and *situs inversus thoracalis*5. These observations indicate that the reversal of left–right body asymmetry can independently occur along the anterior–posterior axis The site of the future diaphragm separates the two body cavities in which determination of laterality independently occurs1 (corresponding to the upper and lower part of the human body) and is controlled by nodal cilia motility. The site of the future diaphragm seems to be the border of this determination.

Because *DNAH5* mutations represent the most frequent cause of PCD and randomization of left–right body asymmetry, the disease mechanism has been recently studied in *Dnah5* deficient mice. In *Dnah5* deficient mice, monocilia at the embryonic node lack ODAs, resulting in immotile cilia, impaired flow at the node and randomization of Nodal signaling with normal, reversed or bilateral expression of key molecules6.

**[H2] Male and female subfertility**

Male subfertility is a multifactorial disease affecting ~7% of the male population globally134. Despite several diagnostic efforts, in ~40% of cases the cause for the subfertility cannot be identified. Asthenozoospermia, due to defective sperm flagellar function, predominantly contributes to these idiopathic cases135. Sperm flagella contain a 9+2 axoneme which is evolutionarily conserved and also present in eukaryotic motile cilia. Dynein based multimeric protein complexes (ODAs) are essential for the generation of the sperm flagellar beat by converting chemical energy released upon ATP binding and hydrolysis into mechanical force for microtubule sliding136. However, between the axoneme and the plasma membrane mammalian sperm possess accessory structures, such as mitochondria, outer dense fibers and the fibrous sheath137. In addition to these differences, axonemal proteins such as ODA components have cell type-specific spatial localization along the length of the ciliary and flagellar axonemes72. This similar but not identical composition of sperm tails and respiratory cilia might explain why sperm flagella dyskinesia is often, but not necessarily, associated with PCD and vice versa. In addition, the assembly processes of human cilia and sperm flagella are not completely identical116. Defects in genes coding for sperm associated proteins are known to cause subfertility137–143. Some males with subfertility have multiple morphological abnormalities of the sperm flagella, a disease characterized by teratozoospermia (abnormal morphology of sperm) and sperm flagella anomalies that can result in short, angulated or absent flagella or flagella of irregular caliber.137,144

Fallopian tubes connect the ovaries to the uterine cavity and are composed of (from lateral to medial) the fimbria, infundibulum, ampulla and isthmus63. Ciliated and secretory cells represent the main mucosal cell types. Ciliated cells are mostly found at the apex of the mucosal folds 145. Coordinated ciliary beating, in concert with muscle contractions, has a role in human reproduction by creating a laminar flow that directs the oocyte through the fallopian tubes to the uterus145; thus, dysmotility of fallopian tube cilia probably impairs transport of the oocyte to the uterine cavity.

**[H2] Defects of non-motile and motile cilia**

Mutations in genes encoding proteins important for the ciliary base such as the transition zone (a specialized domain at the base of the cilium) or basal bodies (for example, *OFD1*, *INVS*, *CEP290* and *RPGR*) or proteins important for intraflagellar transport (for example, IFT proteins and cytoplasmic dynein heavy chains) can result in a pleiotropic range of disease manifestations affecting multiple organ systems (Table 1, Figure 3)owing to defects of non-motile and motile cilia.

Mutations in *RPGR* cause a non-motile ciliopathy referred to as retinitis pigmentosa, which is caused by abnormal protein transport through the connecting cilia of the photoreceptors (rods and cones) in the eye. Although the RPGR protein is mainly localized to the connecting cilium between the inner and outer segment of photoreceptors, it has been also found in the transition zone at the ciliary base of respiratory cilia10,92.146146 Interestingly, individuals with *RPGR* mutations with typical PCD symptoms have been reported92. Consistently, i*n vitro* ciliogenesis experiments have demonstrated that defects in RPGR can cause reduced orientation of respiratory cilia resulting in abnormal clearance of the airways 92. Owing to impaired IFT function at the photoreceptor connecting cilia, mutations in *CEP290* cause Leber congenital amaurosis (amaurosis is complete or partial blindness without any visible defect of the eye). Furthermore the protein CEP290 is localized at the Y-shaped linkers at the ciliary base, thereby also affecting cilia function in various other organ systems147; for example, a study . noted various structural defects in respiratory cilia as well as a variable defect in the ciliary beating pattern, possibly explaining respiratory phenotypes in individuals with Leber congenital amaurosis.148

Other non-motile ciliopathies have been shown to affect respiratory cilia. *OFD1* inactivation has been associated with defective sonic hedgehog and canonical Wnt signaling pathways in non-motile cilia in various organ systems149. In addition, *OFD1* mutant respiratory epithelial cells have been reported to exhibit mislocalized basal bodies, reduced numbers and length of cilia and a dyskinetic ciliary beating pattern with a lack of cilia orientation. 150. 151,152

Defects of the BBsome, a protein complex which is present at the basal bodies and is involved in trafficking cargos to the cilium, or its chaperone complex are known to cause Bardet–Biedl syndrome 153. Assessement of respiratory epithelium in individuals this syndrome revealed cellular damage, substantial ciliary depletion and goblet cell hyperplasia154 , even though ciliary structure and function were essentially normal154.

Mutations in the *ALMS1* gene encoding a ciliary base protein have been shown to cause the rare autosomal recessive ciliopathy Alström syndrome155. A knowck-down of *Alms1* in murine kidney epithelial cells *in vitro* has been shown to cause shortened cilia and seems to abrogate calcium influx in response to mechanical stimuli 156.

The ankyrin repeat protein inversin (encoded by *INVS*) is a member of the nephronophthisis (NPHP) associated protein family that localizes in the proximal segment of cilia; inversin may have a role as gate keeper for protein inport and export at the ciliary base and functions as a molecular switch between Wnt signaling pathways157,158. *INVS* deficient respiratory cilia have been shown by TEM to miss CPs in individuals with chronic lung disease159 160

Mutations in *DYNC2H1*, which encodes the heavy chain 1 of cytoplasmic dynein 2, a component of the IFT motor complex regulating the ciliary retrograde intraflagellar protein transport, result in Jeune syndrome (also known as asphyxiating thoracic dystrophy, a skeletal dysplasia) 161. In individuals with *DYNC2H1* mutations chronic infections of the airways and abnormalities of the respiratory cilia have also been reported162, which might be explained by abnormal IFT in the respiratory motile cilia.Mutations in genes encoding the IFT more commonly cause chondrodysplasias with obligate renal involvement, such as Mainzer–Saldino syndrome and Sensenbrenner syndrome)156.

**[H1] Diagnosis, screening and prevention**

***[H2] Upper and lower airway and ear disease***

***[H3] Clinical presentation***

Cilia power mucus transport from the middle ear, the bronchial tree and the paranasal sinuses towards the pharynx and help to clear the upper and lower airway system. Dysfunction of motile cilia can cause chronic infections of the upper respiratory tract. Chronic infections of the lower airways often progress to bronchiectasis and lung destruction. The earliest manifestations of PCD occur in the neonatal period, as up to >80%163 of full-term neonates with PCD might present with respiratory distress, including tachypnoea (rapid breathing) and dyspnoea (increased work of breathing) caused by atelectasis (collapse of lung tissue) and inhomogenous ventilation of the chest. Affected babies might require oxygen supplementation for a few hours up to weeks164. Productive (wet) cough is the major clinical sign for PCD, starting soon after birth165. In a meta-analysis, nearly all (mean 89%) individuals with PCD have year-round daily wet cough, and infections such as pneumonia were described in a mean of 72% of individuals 163. Chronic rhinitis (inflammation of the mucosa of the nose), rhinorrhoea (runny nose), nasal congestion and sinusitis (inflammation of the sinuses, the air cavities in the nose) are common findings. A meta-analysis reported that on average 75% of patients of any age had chronic rhinitis and 69% sinusitis, but there was much variation between the studies. Nasal polyps have been described in 19%-33% of affected individuals163,166.

These recurrent infections result in reduced lung function and obstructive lung disease, deterioration of lung structure and development of bronchiectasis167 (Figure 1). Bronchiectasis (documented by high resolution CT) was reported in 56% of children and 100% ofadults in a cohort of 45 individuals 163,168–170. The exact prevalence of bronchiectasis in each age group is not known and needs further investigation. Radiographic studies using high-resolution chest CT show that lung disease in PCD, including subsegmental atelectasis, mucus plugging, air trapping, ground-glass opacity and peribronchial thickening and bronchiectasis, begins in early childhood 170,171. Middle and lingual lobes are predominantly affected, followed by the lower lobes32. In contrast to cystic fibrosis, upper lobes are usually only affected at late disease stages170. Lung function is already decreased in schoolchildren and declines with age34. Whereas the forced expiratory volume in 1 second (FEV1, the amount of air a person can forcefully exhale in 1 second in a forced expiratory volume test) in young adults with PCD was better than in the FEV1 in young adults with CF, FEV1 in children with PCD was similarly or even more severely reduced comparison with that of children with CF in the iPCD cohort36 and the English National172 cohort, respectively. Because only few adults were included in the study, data on FEV1 progression with age is still scarce. 41,173. 41,173. Pulmonary function testing using multiple-breath nitrogen washout and its various derived parameters including lung clearance index (LCI), which measures heterogeneities in lung ventilation, has shown considerable abnormality in the majority of patients with PCD, even when FEV1 is normal174,175 . Only very few studies have reported on the evolution of LCI in PCD, with slightly conflicting results showing either stable values over 5 years in a subset (n=12) of a cohort (n=29) of patients with PCD with median age at first visit and LCI measurement of 14 years (range 3-53) 174or a slight, but statistically significant, increase in LCI (which corresponds to high heterogeneity of ventilation and therefore reduced pulmonary function) over 1 year in 42 children and young adults (median age 15.4 years (range 6.5−29.7) with PCD 176. Similarly as in other lung diseases, a higher body mass index than international reference values is associated with better lung function in children and adults37. Chronic lung failure can progress to end-stage lung disease in adulthood 33.

Middle ear effusions progressing to otitis media with effusions (also known as glue ear) with conductive hearing loss (due to interrupted propagation of sound waves) have been frequently reported. Some sensorineural hearing deficits (due to defects of the inner ear) have been also reported166,177. Spontaneous resolution with conservative treatment of chronic otitis media may occur before adulthood178. In adults with PCD, otitis media with effusion was diagnosed in only <25%166. Otologic disease tends to spontaneously improve with age179,178,180. Recurrent acute otitis media is present in about 50-80% adult patients with PCD179,181,182 Otitis usually presents at 2 years of age and sometimes continues even into adulthood178,179,181

***[H3] Genotype–phenotype correlations*.**

Data suggest that lung disease is heterogeneous across all genotypes and ultrastructural defects but is worse in individuals with *CCDC39* and *CCDC40* mutations40,41. Furthermore, individuals with RGMC due to mutations in *CCNO* and *MCIDAS* seem to have more-severe lung disease and rapid progression of chronic lung failure120,121,183. By contrast, individuals with mutations in *DNAH9* or genes encoding MIPs (*MNS1*89, *CCDC11*87*, WDR16*88 and *ENKUR*86*)* exhibit only subtle or no respiratory symptoms that do not necessarily meet the diagnostic criteria of classical PCD (Table 1, Figure 3).

***[H2] Laterality defects.***

***[H3] Clinical presentation.***

In some motile ciliopathies, half of the affected individuals exhibit *situs inversus* totalis a complete mirror image of the body composition (Figure 1, Table1, Figure 3), which might already be noticed prenatally by ultrasonography at 20th week of gestation.163. Heterotaxy has a prevalence of 6.3% -14%3,5,184. Laterality defects can be associated with polysplenia (that is, multiple small spleens)(for example, in left isomerism), asplenia (for example, in right isomerism) or complex congenital heart defects in 2.3% of individuals with PCD5,185. A study in a cohort of 389 UK patients with PCD reported congenital heart defects with or without situs anomalies in 17% of patients184.

***[H3] Genotype-phenotype correlations.***

Individuals with defects in the cytoplasmic dynein axonemal assembly factors, ODAs, proteins involved intubular organisation and MIPs result in randomization of left–right body asymmetry (Table 1). Interestingly, in individuals with isolated defects of the N-DRC, the CP associated apparatus or the radial spokes, no laterality defects have been reported68,69,81,83,120,121. Whereas individuals with multiciliogenesis defects due to mutations in *CCNO* or *MCIDAS* also do not exhibit *situs inversus totalis* (Table 1, Figure 3)120,121,183, *FOXJ1* mutations result in randomization of left–right body asymmetry, indicating that this determination is independent of the NOTCH1 dependent pathway of multiciliogenesis8.

***[H2] Subfertility.***

***[H3] Clinical presentation***

Motile ciliopathies can result in impaired male and female fertility (Table1). As sperm flagella resemble the structure of 9+2 multiple motile cilia in the airways, molecular defects of respiratory cilia can also result in impaired sperm motility (for example, most *HYDIN*-mutant sperm cells are immotile and only few show some residual motility81), and the majority of affected male individuals have subfertility (75.5%)186. However, because respiratory cilia and sperm flagella do not share all proteins, some male individuals with PCD can father children without assisted reproduction (for example, individuals with mutations in *CCDC114*)102. In addition, several gene defects cause isolated motile ciliopathies of the sperm flagellum, resulting in oligozoospermia (low sperm count), asthenozoospermia and/or multiple morphological abnormalities of sperm flagella (Figure 1).

Female individuals with PCD have been reported to have subfertility145,187,188. Multiple motile cilia lining the oviduct contain motility related proteins and have a beating pattern similar to those of respiratory cilia 63. As some female individuals with PCD and molecular defects that are also supposed to disrupt fallopian tube cilia beating can become pregnant without assisted reproduction, transport of the ovum towards the uterus might depend less on cilia beating and more on muscle contractions of the fallopian tubes 63. Thus, fallopian tubes are possibly less likely to obstruct and obliterate than the bronchial airway system. Consistently, in 38.9% of women with genetically proven PCD a spontaneous pregnancy was reported186, and despite anecdotal reports, the study did not report any ectopic pregnancies186. In women with genetic defects affecting ciliogenesis, the rates of subfertility and ectopic pregnancy might be higher than in individuals with structural defects.120,121 (Figure 1). However, further studies are needed to address this question.

***[H3] Genotype-phenotype correlation.***

Some genetic defects (for example, *DNAH17* mutations) result only in sperm cell dysmotility, because the defective protein solely has a role in sperm flagella and not in respiratory cilia, whereas other genetic defects cause a clinical phenotype characterized by perturbed sperm tail motility associated with various other organ manifestations such as respiratory disease and/or laterality defects. Interestingly, the multiple morphological abnormalities of sperm flagella phenotype was originally reported in males with subfertility as a distinct disease entity. However, recent observations have shown that this phenotype can occur in classical PCD.137–139,189,190

***[H3] Hydrocephalus.***

Motile ciliopathies are associated with hydrocephalus (Figure 1, Figure 3)191. Hydrocephalus can be diagnosed prenatally via ultrasonography or can develop throughout life. Affected children can present with headache, nausea and vomiting and failure to thrive. In addition, owing to increased intracranial pressure, papilledema (swelling of the optic nerve) can occur and can progress to optic nerve atrophy associated with loss of vision127. In most individuals with hydrocephalus intracranial pressure is not increased120,121,183; however, some patients (for example, those with heterozygous *FOXJ1* loss of function mutations8) require surgical shunt insertion to relieve raised intracranial pressure due to obstructive hydrocephalus caused by stenosis of the cerebral aqueduct and/or the foramina of Magendie and Luschka8.

***[H3]Hydrocephalus.***

In individuals with severely impaired cilia beating owing to altered composition of axonemal motor proteins in multiciliated cells, the prevalence of hydrocephalus is only slightly increased compared with the healthy population (~ 1 in 75; 1.3%)192(Table1). Individuals with bi-allelic mutations in *MCIDAS*121 or *CCNO*183 develop hydrocephalus much more often (10%)183. In all reported individuals with *de novo* mutations in *FOXJ1,* severe obstructive hydrocephalus was present 8.

***[H3] Other non-motile cilia related disease manifestations.***

Genetic defects affecting both non-motile and motile cilia are associated with clinical symptoms beyond those of motile ciliopathies, affecting multiple organ systems (for example, brain, eye, ear, kidney, liver and skeleton) (Figure 3).2 Mutations in some genes result in defects in limited organ systems, such as mutations in *RPGR* associated with retinitis pigmentosa, which has X-chromosomal recessive inheritance and is the most common cause of inherited blindness in males156.

Autosomal recessive mutations in, for example, *INVS* or *CEP290* result in nephronophthisis, which means “vanishing nephrons” or “vanishing kidneys”. This clinically and genetically heterogenous group of tubulointerstitial cystic renal disorders represents the most frequent genetic cause of end-stage renal disease in children and young adults and is often associated with anomalies in other organ systems,193 including Senior–Løken syndrome, Joubert syndrome and Meckel–Gruber syndrome. In Senior-Løken syndrome, nephronophthisis is associated with retinal degeneration. The most severe variant of retinal disease, Leber congenital amaurosis, manifests in infancy and is accompanied by profound visual loss, nystagmus (involuntary eye movements) and poor pupillary reflexes193. Joubert syndrome is clinically characterized by muscular hypotonia, cerebellar ataxia, abnormal eye movements (congenital oculomotor apraxia, Cogan type (defect in horizontal gaze coordination)), abnormal breathing pattern in infancy and variable degrees of cognitive impairment;194 dysmorphic features such as hypertelorism (increased distance between the eyes), broad forehead and unilateral or bilateral ptosis (drooping of the upper eyelid) have also been described. Although there is a high phenotypic variability, the unifying pathognomonic radiographical finding is the so called “molar tooth sign”, which is visible on the axial brain MRI, reflecting complex malformation of the midbrain and hindbrain.193 A correlation between the type of *CEP290* mutations and disease severity has been demonstrated: whereas two missense mutations lead to a mild late-onset phenotype with limited organ involvement, two truncating mutations cause a severe early onset disorder, such as observed in Meckel-Gruber syndrome156, an often neonatally lethal dysmorphic disorder. Typical clinical features comprise occipital encephalocele (sac-like protrusion of the brain due to a neural tube defect), bilateral cystic kidney dysplasia, hepatobiliary ductal plate malformation and postaxial polydactyly (duplication of the fifth finger or toe). 156

Mutations in genes encoding components of the BBsome or its chaperone complex result in Bardet–Biedl syndrome, which is characterized by polydactyly, developmental delay, obesity, retinal degeneration, cystic renal disease and hypogenitalism. The Bardet–Biedl syndrome is a genetically heterogenous disease with phenotypic overlap with other non-motile ciliopathies, such as nephronophthisis-related ciliopathies. 156 In people with this syndrome, an increased incidence of respiratory distress at birth (12%), otitis media (33%) and rhinitis (36%) was reported.155

Mutations in genes encoding components of the ciliary IFT cause chondrodysplasias (skeletal disorders) with variable extraskeletal involvement. *DYNC2H1* mutations mostly cause the Jeune syndrome, which is characterized by narrow thorax with short ribs, trident acetabulum (small bony projections in the acetabulum, the socket of the hip bone), cone shaped epiphyses (the ends of long bones) and polydactyly. Extraskeletal symptoms such as kidney disease or retinal degeneration are usually not present in patients with *DYNC2H1* mutations156. Mainzer-Saldino syndrome and Sensenbrenner syndrome are chondrodysplasias associated with renal involvement. Mainzer-Saldino syndrome is referred to as cono-renal syndrome as the retina and kidneys can also be affected. Patients with Sensenbrenner syndrome furthermore present with ectodermal defects such as dysplastic fingernails and toenails, sparse and slow growing hair and teeth abnormalities referred to as cranio ectodermal dysplasia156

The Alström syndrome is characterized by retinal degeneration, sensorineural hearing loss, obesity, insulin-resistant diabetes, hypertriglyceridemia, cardiomyopathy, hepatorenal disease and recurrent respiratory infections. Recurrent otitis media is common in individuals with Alström syndrome155. Chronic bronchitis and sinusitis were reported in 61% and 50% of patients, respectively155.

*OFD1* mutations can also cause a broad clinical phenotype that can include skeletal defects (such as polydactyly, syndactyly (the fusion of two fingers or toes) and orofacial malformations), conductive hearing, congenital heart defects and brain malformations, such as agenesis of the corpus callosum or cerebellar atrophy156.

**[H2] PCD Diagnostics**

Individuals with PCD often experience a long delay before a correct diagnosis is achieved.The reasons for under-diagnosis are multiple. General practitioners and even pulmonologists typically encounter few cases of PCD during their career and their awareness of the condition is limited. An international survey found that 37% of patients with PCD had >40 medical visits for PCD-related symptoms before being referred for diagnostic testing30. PCD symptoms are non-specific, and only patients with *situs inversus* totalis are diagnosed early, at 3.5 years of age compared with 5.8 years in children without situs inversus totalis in a paediatric study21, and at 6 years of age compared with 16 years in a study analysing adults31.

There is no gold standard diagnostic test for PCD. Access to a combination of tests, such as measurement of nasal nitric oxide (nNO) production rate, high-speed video microscopy (HSVM; Supplementary videos), immunofluorescence microscopy, genetic analyses, and TEM to characterize the ciliary defect, is required for diagnosis. In Europe and North America, guidelines and recommendations about the use of one method or a combination of methods have been established based on international consensus and literature (Table 2); of note, the diagnostic algorithms are distinct owing in part to differences in the availability of equipment and insurance coverage. 13,195,196 The European Respiratory Society (ERS) guidelines13 recommend measurement of nNO production rate, TEM , HSVM and genetic analyses as diagnostic tests of choice for PCD. By contrast, the guidelines from the American Thoracic Society (ATS)14 recommend the use of TEM, measurement of nNO production rate and genetic testing. However, there are not sufficient data to strongly support either recommendation. Thus, the choice of PCD tests in many countries also largely depends on availability of equipment and experienced laboratories196,197.

The ERS Task force on PCD recommends referral for diagnostic testing if the individual presents several symptoms in addition to persistent wet cough, such as laterality defects, congenital cardiac defects, persistent rhinitis, chronic middle ear disease with or without hearing loss and, in term infants, a history of neonatal upper and lower respiratory symptoms or admission to neonatal intensive care or a high score in a specific clinical diagnostic predicting tool such as modified PICADAR.192 In the PICADAR score, the presence of seven different clinical factors (term birth, chest symptoms during the neonatal period, admission to a neonatal intensive care unit, laterality defects, congenital heart defects, rhinitis and chronic ear or hearing symptoms) are taken into account to estimate the probability of PCD. Combined with low nNO values, PICADAR has been tested as a suitable screening test for PCD in adults with bronchiectasis198 The ATS guidelines suggest referral for diagnostic testing for individuals presenting with chronic respiratory symptoms, particularly if they have at least one of the following four key clinical features in PCD: unexplained neonatal respiratory distress in term infants, year-round daily cough beginning before 6 month of age, year-round daily nasal congestion beginning before 6 month of age and laterality defects.14

***[H3] Nasal Nitric Oxide measurement.***

Nitric oxide (NO), a small diffusible gas molecule, is involved in multiple functions throughout the body, including regulation of ciliary motility199,200 and antimicrobial activity201 within the airways. Multiple studies have demonstrated that nNO production is very low in PCD (approximately a tenth of values in healthy controls), suggesting that nNO measurement may be a useful diagnostic test 33,202–205 . nNO measurement is non-invasive, as aspirated nasal air is directed to a NO analyzer. As low nNO levels have been reported in cystic fibrosis33,203,206–208 and individuals with nasal polyps209, these disorders should be excluded, and because acute respiratory infections also affect nNO values, the measurement should be performed during infection free periods210.

Recent efforts have focused on standardization of nNO measurement to define cut-off values that discriminate PCD from healthy controls and people with other respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) with adequate diagnostic accuracy for PCD204,211. Systematic reviews and meta-analyses have concluded that nNO testing is a sensitive (75-100%) and specific (87-100%) test for PCD211–213. According to ERS guidelines, additional tests for PCD are needed for a firm diagnosis; however, ATS guidelines accept use of nNO measurement alone if repeated measures are low (nNO <77 nl per min14,205) in individuals with a strong PCD clinical phenotype (≥2 of the 4 key PCD clinical features), when other diseases such as cystic fibrosis and primary or secondary immunodeficiency are ruled out14. Further studies in large populations are needed to define age-specific cut-off values in infants and young children and to define diagnostic utility of portable electrochemical analyzers, 214,215. Of note, in some individuals with mutations in *DNAH9, GAS8*, *STK36*, *CCDC103*, *RSPH1*, *GAS2L2*, *NEK10* or *FOXJ1*, nNO levels have been recorded within the normal range4,8,70,83,91,126 (Table1, Figure 3); thus, nNO measurement alone could be misleading and further diagnostics should be performed in individuals with typical PCD symptoms.

***[H3] High-speed video microscopy.***

Evaluation of the ciliary beat via high-speed video microscopy (HSVM) can be used as a diagnostic tool for PCD (Supplementary videos). Respiratory epithelial cells can be obtained from the inferior turbinate of the nose (turbinates are bony structures, covered by mucosa, that form long and narrow sinuses to help clear and humidify the air) or from the lower respiratory tract during bronchoscopy, and ciliary beat pattern and frequency can be recorded216. A normal beat cycle is characterized by a strong beating strike followed by a recovery stroke61. Some ultrastructural defects causing PCD may be associated with specific patterns of ciliary beating61,216,whereas others can result in very subtle ciliary beating abnormalities that are hardly or not detectable by HSVM.53,61,68–70,81,83,94 (Table1, Figure 3)

Analysis of ciliary beat frequency and pattern via HSVM is an accurate test for PCD when performed by an experienced observer, but it is not sufficient to rule out PCD217,218. Ciliary beat frequency does not adequately differentiate PCD from other conditions unless combined with beat pattern assessment. Because ciliary beat can be hampered owing to secondary alterations such as infections, the ERS guidelines recommend to demonstrate identical beating abnormalities in three independent biopsy samples, a requirement difficult to achieve in a normal clinical setting. Alternatively, HSVM of cultured respiratory cells may contribute to improve the accuracy, in particular to rule out false positives due to chronic infection140. However, whereas ciliary beat frequency can be measured objectively, ciliary beat pattern analysis in respiratory cells is a purely a descriptive method, and objective parameters are missing.

***[H3] Transmission Electron Microscopy.***

Transmission Electron Microscopy (TEM) had traditionally been considered as diagnostic “gold standard” for PCD confirmation, well before the first identification of PCD-causing gene mutations (Figure 5, Figure 6). However, as ~16-30% of PCD-causing ciliary defects are not easily detected by TEM, this technique cannot be used as a sole diagnostic method13,219,220. The number of defects that cannot be detected with TEM will probably increase, as current diagnostic techniques enable to identify individuals with PCD with normal ciliary ultrastructure (Figure 3).

Three ultrastructural phenotypes are diagnostic for PCD (Figure 6). The hallmark defects are ODA defects in ~50% of cases221(>50% of cilia cross sections222), microtubular disorganisation and IDA defects (~ 15%221) (>25% of cilia cross-sections222), and ODA–IDA defects (26%221) (>50% of cilia cross sections222) (Table1, Figure 3). The ODA defects are usually easily identified, because ODAs are much more abundant (every 24 nm in the 96 nm repeat). However, specific defects caused by mutations in the genes encoding the axonemal dynein heavy chains DNAH9 and DNAH11 are not readily identified by TEM. *DNAH11* mutations were identified as first cause of ultrastructural normal PCD95, whereas *DNAH9* mutations cause absence of ODAs from the distal ciliary axonemes53,94. In case of *DNAH11* mutations two scenarios are possible, and in each case at least parts of the proximal ODAs still remain present and, therefore, TEM can appear normal in both cases. In DNAH11 deficient cells, DNAH11 becomes substituted by paralogous DNAH952. In cells without complete loss-of-function *DNAH11* mutations, mutant DNAH11 proteins and other ODA components can still become assembled in the proximal ciliary axoneme.

The IDA structure is not easily detected, because most IDA types only contain one heavy chain and each IDA type is only once present within the 96 nm interval. Defects of the 96 nm ruler proteins are caused by *CCDC39* or *CCDC40* mutations and present in TEM with characteristic changes involving microtubular disorganization (eccentric CPs, abnormal alignment of outer doublets and occasional displacement of outer doublets) 61,71,72. Other defects involving the IDAs are not easily identified.

Other defects such defects of multiciliogenesis (mislocalisation of basal bodies with few or no cilia) and central pair defects (8+1 cilia composed of 8 outer doublets surrounding one single central tubule) are not considered pathognomonic, because they can also occur in healthy individuals, for example, after infection222. In RGMC, the number of cilia per cell is markedly reduced120,121,183. Furthermore, mislocalised basal bodies and rootlets can be identified in the cytoplasm of *CCNO* deficient cells120,121. In *FOXJ1* mutant cells, the number of basal bodies is not affected, but basal bodies are mislocalised within the cytoplasm8. Defects in N-DRC, MIP, radial spoke or CP cannot be reliably diagnosed by TEM for various reasons. N–DRC defects lead to subtle changes in TEM, such as occasional misplacement of peripheral doublets or the presence of supernumerary single tubules within the ciliary axoneme, which are not specific for PCD and can be observed only in a few sections222 and, therefore, are frequently missed by TEM analyses68,115. TEM analyses of radial spoke defective respiratory cilia revealed some ciliary transposition defects with 8+1 microtubule configurations, and some cilia lacked the CP (9+0). As defects in tubule composition are only present in some cilia, most cross sections are apparently normal 223**.** CP defects are also difficult to detect by TEM, because most cross-sections appear normal82.

TEM tomography enables visualisation of ciliary structures in three dimensions, showing that *HYDIN* mutant cells lack of one of the CP projections (C2b)81. Furthermore, abnormalities of the ODA composition within proximal ciliary axonemes in *DNAH11* mutant respiratory epithelial cells were reported 224. However, this method has not been evaluated for routine diagnostics and, owing to the time required for analysis, is best placed as research tool, although it might be useful in individuals with mutations of dubious significance.

***[H3] Immunofluorescence microscopy analysis.***

Immunofluorescence may also help to determine the PCD diagnosis225,226. In most motile ciliopathies, the molecular defects result in the inability to assemble specific large multiprotein complexes in the axoneme of respiratory cilia. Immunofluorescence microscopy using antibodies targeting specific components of these protein complexes can demonstrate the absence of those components and contribute to the diagnosis (Figure 3, Figure 6, Table 1). In ODA defects due to, for example, DNAH5 mutations, ODAs of the proximal and distal portions of the ciliary axoneme are defective, which can be demonstrated by using antibodies against the outer dynein arm heavy chain DNAH5 or the intermediate chains DNAI1 and DNAI2. In case of *DNAH11* loss-of-function mutations,51 immunofluorescence can demonstrate absence of DNAH11 from the proximal ciliary axonemes52. In respiratory cells of individuals with *DNAH9* mutations, the distally located ODAs are defective, which can be demonstrated by absence of the ODA proteins DNAH9, DNAH5, DNAI1 and DNAI2 from this compartment of the axoneme in immunofluorescence analyses. In cells with mutations in genes encoding cytoplasmic dynein axonemal assembly factors, immunofluorescence can demonstrate absence of axonemal ODA components such as DNAH5, DNAI1 and DNAI2, as well as the inner dynein arm chain DNALI1 (Ref227). The ruler proteins display the anchor sites of IDA and N–DRC proteins. Thus, the absence of N–DRC proteins such as GAS8 and the IDA light chain DNALI1 from the ciliary axonemes in immunofluorescence analysis indicates that the genetic defect could be in *CCDC39* or *CCDC40.*73 If the IDA light chain DNALI1 is still present, this is an indication that the genetic defect is probably related to the N–DRC68. In defects of the radial spoke heads caused by *RSPH1*, *RSPH4A* and *RSPH9* mutations, various defects of the radial spoke head structure can be demonstrated by immunofluorescence utilizing antibodies targeting the respective proteins; of note, the absence of RSPH9 does not necessarily mean that the genetic defect is in *RSPH9*, as it could be in genes encoding other radial spoke heads228. Mutations in genes encoding the N-DRC proteins GAS8, CCDC65 and CCDC164 result in isolated N-DRC defects. In each case, the N-DRC protein GAS8 cannot assemble in the ciliary axonemes, and, therefore, is not detectable by immunofluorescence; the absence of GAS8 indicates that the mutation could be in *GAS8*, but also in the other N-DRC genes. Furthermore, in a recent study of 189 individuals with PCD, antibodies against the central pair component SPEF2 enabled to detect *HYDIN* mutant cells, which cannot be diagnosed by routine TEM82.

In a cohort of 386 individuals, immunofluorescence studies with at least three antibodies were inconclusive or insufficient in 30% of cases. However, similar failure rates were reported for TEM[210].Since this study, further immunofluorescence studies became available, increasing the detection rate of immunofluorescence microscopy for motile ciliopathies 52,82. A study demonstrated that a combined analyses with anti-DNAH5, anti-DNALI1, anti-GAS8, anti-RSPH9 antibodies225 can identified the absence of one or more ciliary proteins in 88% of individuals with suspected PCD. Thus, immunofluorescence misses 12% of PCD cases and, therefore, is not suitable for use as a stand-alone test. However, this technique is less expensive and has faster turnaround times than TEM225. Of note, immunofluorescence can also be used to assist diagnosis of motile ciliopathies affecting the sperm flagella, such in case of *DNAH17* and *DNAH1* mutations54,189. By contrast, detection of axonemal abnormalities in fallopian tube and ependymal cilia is precluded, because of the inability to sample these cells for routine diagnostics.

***[H3] Genetics.***

Molecular genetic testing is an easy and quick option, especially when HSVM, TEM and immunofluorescence present with equivocal results such as normal ciliary ultrastructure in families harboring *DNAH11* mutations229. Next generation sequencing platforms enable querying multiple exons simultaneously, and many laboratories offer clinical molecular genetic testing for PCD using gene panels (Figure 3). Nevertheless, locus heterogeneity and large gene sizes complicate molecular testing. For comprehensive genetic diagnostics analysis of all known PCD genes is the recommended procedure; however, even when all genes are tested, a causative mutation is found in only ~70% of individuals with clinical manifestations of PCD .230

The caveats for interpretation of molecular testing include negative results due to mutations in a new gene or in regions of the known genes that are not included in the technology used and equivocal results due to the presence of variants of uncertain significance. The diagnosis with pathogenetic *HYDIN* mutations is further complicated by a nearly identical 360 kb paralogous segment (*HYDIN2*) in chromosomal region 1q21.1 (Ref81). By contrast, the interpretation of genetic results is helped by the fact that the majority are loss-of-function mutations (nonsense, frameshift, splice-site, and few large deletions), most of which occurring in five genes (*DNAH5*, *DNAH11*, *DNAI1*, *CCDC39* and *CCDC40*)165, as shown by large studies published from the US and Europe, in which the majority of patients were of European descent230. These observations were corroborated when 4 of these 5 genes (with *DNAI1* being the exception) were reaffirmed as the most frequently mutated genes25. Majority of the reported mutations tend to be private (found only in a single family); however, certain mutations recur in outbred as well as in inbred populations230.

In genetic results of unknown significance, HSVM, TEM and immunofluorescence can help to secure diagnosis; for example, immunofluorescence helped to confirm pathogenicity relevance of a missense variant of uncertain significance in *RSPH4A* and *RSPH9 (Ref*228). In addition, ancestry information is useful, as founder mutations have been observed in patients from certain regions or ethnic background, and this information can help to select the correct gene panel and to interpret the significance of the variant found 230. For example, one splice-site mutation in *RSPH4A*231, and one nonsense mutation in *HYDIN*81 have been associated with patients from Puerto Rico and Faroe Island, respectively, and *DNAH5* founder mutations have been reported in the white individuals of European descent230,232,233. Next generation DNA sequencing technologies such as whole exome sequencing will enable the accumulation of data from patients who tested negative for the known motile ciliopathy genes that will be useful for further research in identification of novel candidate genes or mutations.

**[H2] Screening and prevention**

Laterality defects can be observed via ultrasonography from 20th week of gestation and could be the first indication of a motile ciliopathy. For inconclusive cases, pre-natal genetic diagnosis is possible via DNA obtained by amniocentesis or circulating fetal cell-free DNA obtained from the maternal blood by non-invasive technique. Currently, neonatal screening for motile ciliopathies is not available. Most motile ciliopathies have autosomal recessive inheritance; thus, detailed family history should be collected by the physician or genetic counselor, as usually individuals who are heterozygous for a causative mutation do not show disease manifestation, and appropriate testing and counseling should be offered to the families Similarly, the probability of having a child who is affected and the carrier status for the X-linked and autosomal dominant forms should be discussed with the families230.

Parents of a child with a motile ciliopathy may seek prenatal diagnosis or preimplantation genetic diagnosis of an embryo for subsequent pregnancies, but these diagnostic modalities should be offered only if genetic testing of the affected child has already confirmed the molecular underpinnings. In addition, coordination between the medical specialists, genetic counselors and families is required, together with the careful review of medical and ethical implications. Additionally, since laterality defects are observed in 50% of the cases in many motile ciliopathies, prenatal ultrasonography can be a helpful indicator in subsequent pregnancies. Preventative treatment for motile ciliopathies is not available; however, early diagnosis can help disease management.

**[H1] Management**

With the exception of the randomized controlled trial on azithromycin in PCD (see below), there is no evidence-based treatment for PCD, and, therefore, the empirical treatments are extrapolated from other lung diseases, such as asthma and cystic fibrosis, but also from diseases with primary nasal focus such as chronic rhino-sinusitis. The repertoire of drugs used empirically is, therefore, quite extensive, and the rationale for using several of these drugs seems well founded, whereas others, such as antibiotics in the case of an obvious acute or chronic local infection, are evidently indispensable. The evaluation of evidence-based treatment is under way, but such treatments are only symptom relievers, as they target the secondary effects of mucociliary impairment and mucostasis or the immediate environment of the malfunctioning cilia (that is, they aim to change mucus properties,for example using inhaled hypertonic saline to ease clearance).

The current recommendations of clinical management are outlined in a few reviews74,195,234. It is currently considered best practice that both children and adults with PCD attend routine clinical visits every 3 to 4 months, regardless of the clinical presentation or severity of disease. At each visit, the patients are assessed by interview, symptom-directed physical examination, lung function measurement, and sampling of mucus (obtained by expectoration or, for example, laryngeal suction in infants, young children or individuals unable to expectorate) for microbiological analysis. Chest imaging by radiography or high-resolution CT is performed at baseline following diagnosis and when clinically indicated, in some centres more regularly. Whereas management should focus on lung disease throughout life, certain treatments will often need to be adjusted according to the age-specific symptom presentation (for example, hearing problems in childhood) and patients' needs (for example, subfertility).

**[H2] Sino-nasal disease**

The treatment principles for sinus disease, nasal secretion, congestion and polyps are similar to those of chronic rhinosinusitis and aim to relieve symptoms, by sinonasal irrigation with saline or nasal steroids or systemic antibiotics. Sinonasal irrigation with isotonic or hypertonic saline removes stagnant mucus and thereby promotes upper airway clearance in PCD235. Topical steroids have a well-documented positive effect in patients with chronic rhinosinusitis235 by reducing symptoms, nasal blockage and size of polyps, and can partly prevent polyps recurrence236. Thus, individuals with PCD might theoretically benefit from the use of topical steroids237, which are currently used frequently or occasionally in 80% of children and routinely in <10% of children 238. As therapy with macrolides is effective in treating chronic rhinosinusitis without nasal polyps in the general population235, its potential effect on PCD-associated chronic rhinosinusitis was further investigated in a recently conducted study, although it was not different from placebo16.

Functional endoscopic sinus surgery is a surgical treatment of recurrent, acute and chronic sinus problems to restore drainage and clearance of the paranasal sinuses and ventilation of the nasal cavity. It is a well-established treatment for chronic rhinosinusitis, when medical therapy has failed235. In PCD, subjective benefit of the procedure has been demonstrated237. Studies suggest that the sinuses in PCD may function as a bacterial reservoir causing repeated lower airway infection and, therefore, indicate that endoscopic sinus surgery, although it cannot completely restore normal sinus functionality, may be an important part of the eradication strategy for pathogenic bacteria in sinuses and protect the lower airways237,239,240.

**[H2] Lung disease**

The principles of the treatment of PCD lung disease primarily focus on stimulation of mucus clearance, attempting to mobilize mucus by all possible types of physiotherapy, enhancing hydration of mucus and amplifying cough clearance.Changes in mucus properties can also improve mucociliary clearance and cough clearance. However, these measures cannot compensate for the missing natural, constantly ongoing mucociliary clearance; thus, treatment of the secondary effects of mucostasis such as bacterial infection, inflammation, airway constriction and destruction is crucial.

**[H3] Airway clearance.**

Airway clearance techniques are well-established and used routinely in patients with PCD in >80% of European centres238. Because the use of physiotherapy is based solely on evidence extrapolated from the management of cystic fibrosis, as there are no well designed trials on physiotherapy in PCD, a systematic review covering a wide range of available airway clearance techniques241 questioned the lack of evidence for the current practice of physiotherapy in PCD242. Nonetheless, exercise was shown to be a more potent stimulus for bronchodilation than inhaled β2 adrenergic receptor agonists (bronchodilators), producing a statistically significant greater increase in peak expiratory flow in patients with PCD 243.

Whereas nebulized human recombinant DNase and N-acetylcysteine (NAC) may change mucus properties, hypertonic saline and inhaled dry powder mannitol change mucosal hydration. Hypertonic saline inhalation is frequently used in patients with PCD. Interestingly, the first randomized controlled trial in PCD exploring the effect of hypertonic saline on the quality of life (QOL) in adults244,245 found no statistically significant effect of hypertonic saline compared with isotonic saline in the primary outcome, probably because the study was small and not sufficiently powered245. Nevertheless, as QOL measure devised for bronchiectasis in general did improve statistically significantly with hypertonic saline, sufficiently powered studies using PCD specific QOL measures are advisable246–248.

Inhaled dry powder mannitol functions as an osmotic mucoactive agent able to increase mucociliary clearance by causing water to enter the airway lumen, hydrating airway secretions, lowering the viscosity of secretions and stimulating cough249. A study of children with cystic fibrosis showed statistically significant improvements in lung function and sputum weight; mannitol was well tolerated and was associated with a reduced incidence of pulmonary exacerbation249, and, therefore, it is considered a worthy candidate for future trials in patients with PCD.

Few case reports suggest possible benefit of the use of human recombinant DNAse in patients with PCD when used for both short-term and long-term periods250–252. Similar to PCD, in cystic fibosis, inflammatory cells, especially polymorphonuclear neutrophils, produce high levels of extracellular, polyanionic DNA, that is, neutrophil extracellular traps (NETs). NETs may be beneficial as part of innate immunity in general but in cystic fibrosis are detrimental, as they increase mucus clogging and lung injury. Nebulized human recombinant DNase is known to reduce the abnormal viscoelastic sputum in patients with CF by breaking these long extracellular DNA molecules into smaller fragments. Whereas the use of DNase in cystic fibrosis is evidence-based253, trials in adults with non-cystic fibrosis bronchiectasis showed either no benefits254 or, on the contrary, an increased frequency of respiratory exacerbations and worsened lung function255. This finding could in theory be explained by DNase causing reduction of NETs. Despite these disappointing results and speculations on the specific mechanisms by which DNAse might be detrimental, trials studying the effectiveness of DNase in PCD are definitely required.

The effect of oral NAC, which exerts its mucolytic action by reducing the disulfide bonds in the mucus matrix, thereby lowering the mucus viscosity, was tested in patients with PCD. In a double-blind, placebo-controlled, cross-over trial over two periods of three months followed by a three month follow-up, no effect was observed in patients with PCD (n=13), whereas improved lung function was shown during periods of lower airway infections in patients with CF (n=43)256 Despite this negative and probably underpowered trial, NAC should be further explored in patients with PCD.

Finally, bronchodilators are medication with solid evidence for treatment of asthma but are often also used in other chronic lung diseases and PCD. Bronchodilators are routinely or sometimes prescribed to patients with PCD in ~90% of European centres238, although at present there is no evidence supporting use of bronchodilators in individuals with PCD238,257,258. As individuals with PCD often exhibit an alarming level of airway obstruction shown in lung function assessments, further studies are necessary.

***[H3] Treatment of pulmonary infections.***

Systemic antibiotics are used for the majority of acute or mild, slowly evolving respiratory exacerbations with changes in cough, sputum production, respiratory rate or work of breathing (that is, energy used by the muscles for respiration, defined as the product of pressure and volume of each breath), with or without concurrent fever. Broad-spectrum oral antibiotics for 14 days (for example, amoxicillin with clavulanic acid or equivalent) to target the common respiratory pathogens are widely used195, and severe exacerbations may require intravenous antibiotics. In children with PCD, *Haemophilus influenzae*, *Staphylococcus aureus, Moraxella catarrhalis* and *Streptococcus pneumoniae* are the most common respiratory pathogens168. Many individuals present more than one type of bacteria in sputum samples. Nontuberculous mycobacteria are present in ~15% of adults with PCD195. *Pseudomonas aeruginosa* colonization is intermittently present in children, whereas adults are either intermittently or chronically infected33,168,259.Pulmonary infections with *Pseudomonas aeruginosa* can establish persistent lung infections exhibiting exactly the same pattern of slowly deterioration in lung function as in cystic fibrosis 260.

There are consensus statement and expert reviews261,262 with recommendations for the management of Gram negative infections (including *Pseudomonas aeruginosa* infection) in PCD. However, a new consensus statement on surveillance, segregation and treatment is under consideration. These statements will probably reflect in part position statements from the European Bronchiectasis Network (EMBARC)263 and in part practices in cystic fibrosis 264, enabling modifications to the dose of antibiotics (K.G.N. and H.O., unpublished observations). Furthermore, if there were evidence to support that infections in PCD are more easily eradicated than in cystic fibrosis 259, a less aggressive approach to infection prevention and control could be warranted. Epidemiological studies and randomized clinical trials are needed to investigate this topic further. Whereas antibiotic treatment in case of clinical signs of pulmonary infection and positive sputum culture is indispensable in PCD, eradication strategies in individuals with positive sputum culture but no clinical signs or disease progression are uncertain34,195,261. Maintenance therapy with macrolide antibioticshas been previously investigated in cystic fibrosis-associated 265,266 and non-cystic fibrosis bronchiectasis267,268, showing a positive effect. Similarly, a multicentre, double-blind, randomised, placebo-controlled phase 3 trial on efficacy and safety of azithromycin maintenance therapy over 6 months in PCD conducted by the BESTCILIA consortium demonstrated high tolerability and halved the rate of respiratory exacerbations in the azithromycin group16.

***[H3] Anti-inflammatory treatments.***

Inhaled corticosteroids are medications with primary indication for the treatment of asthma but are often also used in other chronic lung diseases and prescribed in ~80% of European PCD centres238, sometimes because treatment started when the patient was initially mis-diagnosed with asthma and was never stopped. However, as polymorphonuclear neutrophils, which are vastly unresponsive to glucocorticosteroids, represent the predominant fraction269 of the inflammatory cells in sputum from patients with PCD, the probability that corticosteroids have an effect is poor. Furthermore, these drugs may be topically immunosuppressive270 and, at least in adults, increase the risk of tuberculosis271, pneumonia272,273 and nontuberculous mycobacterial infection274. Using the iPCD Cohort it may be possible to search for patients currently on treatment and conduct a withdrawal trial as previously performed in patients with cystic fibrosis275.

α-1 antitrypsin treatment, possibly administered by inhalation, was suggested as a future option for a range of lung conditions involving neutrophilic inflammation and proteolytic tissue injury related to neutrophil elastase, including PCD-associated bronchiectasis. However, there is no evidence on this treatment for PCD, and large phase 3 studies in carefully selected patient populations will be needed276.

Hyaluronan (a glycosaminoglycan) and its degradation products may have an important role in airway diseases with a predominant inflammatory component, such as chronic rhino-sinusitis, asthma, COPD, cystic fibrosis and PCD. Aerosolized hyaluronan may have beneficial activity against airway inflammation, bronchial hyperreactivity and remodelling, and it also disrupts the biofilm associated with chronic infection. However, there is no evidence of the effects of this treatment in patients with PCD, andrandomized clinical trials in large patient populations must be performed before a clear role is identified277.

***[H3] Lung surgery.***

Approximately 40% of European centres declare to sometimes perform lung surgery in patients with PCD238 withlung segments or lobes that are atelectatic (collapsed) or show bronchiectasis and increasing fibrosis without any residual function, although the outcomes of resection of bronchiectasis are conflicting278. However, surgical intervention ought to be considered only in cases in which suppurative pulmonary disease cannot be controlled despite maximum medical intervention and the affected lung segment causes recurrent uncontrollable infections (especially *Pseudomonas aeruginosa* infection), including repeated pleural pain reactions. The decision to perform lobectomy or segmental resection is based on a multidisciplinary discussion on the condition of the patient. Serial measurements of lung function indicated statistically significantly lower estimated level at age 18 years in lobectomised than in the adult non-lobectomised patients; thus, lobectomy seems to be a poor prognostic factor for PCD in adulthood279,280. A comprehensive retrospective study on this subject was conducted using data from 2896 patients in the iPCD cohort, 5.6% of whom received lung resection predominantly either before diagnosis of PCD or after very late diagnosis; the study demonstrated that resection resulted in no benefit at all, only reduced and declining lung function.36,280

Lung transplantation has been performed in an unknown but certainly limited number of patients with PCD281–283and has to be considered as the last resort. Patients with situs inversus totalis, heterotaxy or the asymmetric arrangement of the thoracic vascular structures constitute a surgical challenge from the anatomic standpoint. Following transplantation, the postoperative challenge concerns the unchanged lack of mucociliary clearance in the upper and central airways of the recipient and, therefore, residual troublesome infections. However, available data demonstrate that long-term lung transplantation outcomes for PCD are similar to those in the population of lung transplantation recipients.283

**[H2] Middle ear disease and hearing impairment**

Treatment for recurrent and acute otitis media includes courses of antibiotics182. Whether long-term treatment (for example, 6 months with macrolides) affects otitis media with effusion and the hearing in patients with PCD was indirectly evaluated by inclusion of PCD health related (HR)-QOL, hearing and tympanometry (a test that assesses the condition of the middle ear and mobility of the eardrum and of the conduction bones) as secondary endpoints in a recently completed randomized controlled trial, although without showing any positive tendencies.16

Chronic otitis media with effusion, which is often associated with conductive hearing loss and speech delay in young children, is a dilemma for the PCD specialist and the oto-rhino-laryngologists because it remains unclear whether insertion of ventilation tubes (tympanostomy tubes, also known as grommets) to drain the fluid in the middle ear is more damaging than beneficial to the child with PCD and to what extent there are favorable alternatives284,285. Indeed, insertion of ventilation tubes definitely improves hearing179,285 but often at the expense of a severe and unpleasant otorrhoea (mucopurulent ear discharge) encountered in a large proportion of patients compared with the general paediatric population178,179. Indeed, all patients with grommets developed at least one episode of otorrhea, and 40 to 66% of patients experienced >2 episodes, depending on their age-group178. Currently, ERS PCD guidelines recommend against placement of ventilation tubes and in favour of awaiting spontaneous normalization of the hearing, which is achieved in almost 100% of children after 12 years of age180,261. Hearing aid technology is now so advanced, with a range of devices available for all ages286, that the use of hearing aids should be considered as a better temporary solution for most children.Randomized trials investigating effect and preference of insertion of ventilation tubes versus conservative expectation and hearing aid are clearly warranted. Autoinflation is a technique (which may include the use of a device) to open the Eustachian tubes by increasing the intranasal pressure; autoinflation for hearing loss associated with otitis media with effusion might be another interesting treatment approach for future research287–289.

**[H2] Subfertility**

The success of assisted reproductive technologies for male patients with subfertility, including in vitro fertilization or intracytoplasmic sperm injection, resulting in healthy offspring has been reported in several publications290. In vitro fertilization may be an option for patients whose sperm motility is retained, but intracytoplasmic sperm injection is currently the only treatment option for most male patients with dysmotile sperm cells. Despite the possibility of assisted fertilization, the residual fertility of male patients with a motile ciliopathy can still not be compared with that of the healthy population290. By contrast, women with dysmotile fallopian tube cilia may have only slightly decreased fertility, which underlines the necessity to advice on birth control measures if the patient has no wish to have children63.

**[H2] Neurological manifestations**

Monitoring head circumference is an important part of clinical checks in infancy. Abnormally increased head circumference or other signs of raised intracranial pressure (such as headache, nausea, ataxia (impaired coordination) and vomiting) should lead to brain imaging. Hydrocephalus is only rarely reported but in case of increased intracranial pressure will require the insertion of a ventriculoperitoneal shunt. In case of epilepsy, anticonvulsive pharmacotherapy should be considered291.

**[H2] Left–right body asymmetry**

As congenital heart disease and/or heterotaxy occur more frequently in individuals with motile ciliopathies than in the general population, mapping of the position of the heart and other organs is a mandatory part of the initial investigation after the diagnosis of PCD. In case of congenital heart defects, specialized treatments by cardiac surgeons and cardiologists might be necessary. Situs inversus thoracalis and situs inversus abdominalis should also be accurately mapped, and patients with these anomalies should be provided with a personal health card reporting their condition, which patients should always carry. This precaution is important, for example, in case of hospitalization with acute abdomen (for example, due to appendicitis) or trauma (for example, splenic rupture). In addition, it is important to rule out right isomerism associated with asplenia, because these individuals carry a high risk for pneumococcal and meningococcal infections and need vaccinations against these bacteria, or lifelong antibiotic prophylactic treatment. The US Centers for disease control issued recommendations for meningococcal vaccination (<https://www.cdc.gov/vaccines/vpd/mening/hcp/recommendations.html>) and pneumococcal vaccination (<https://www.cdc.gov/pneumococcal/vaccination.html>).

[H1] Quality of life

Health related quality of life (QOL) is an index of wellbeing from the individual’s perspective, including physical, psychological, spiritual and social dimensions292. Persistent symptoms, daily need for treatment, poor understanding of the disease by health professionals and uncertainty for the future (for example, with regard to fertility and prognosis) contribute to the effect of PCD on QOL246,248,293. There has been little research in this area, and the quality of studies has generally been low 294. Whilst several qualitative studies have provided insights, the numbers of patients contributing to these studies were small, and almost all came from North America and the United Kingdom246,248,293. Thus, caution is needed when interpreting the data. Patients report that QOL improves following diagnosis of PCD30,295, and those with an early diagnosis have better psychosocial outcomes than patients who were diagnosed late30,295,296; these findings highlight the importance of early medical intervention21,30.

Physical symptoms mainly relate to upper and lower airways disease. Adults and children often report limitations in keeping up with peers because of coughing, breathlessness, fatigue and low energy246,248,293. Sino-nasal symptoms and hearing impairment also contribute to the physical effect of PCD246,248.

The psychological effect of PCD in children and teenagers includes frustration relating to treatment burden and the unfairness of having chronic symptoms246,248. Children describe anxiety when thinking about their health and the future, with concerns that the improvements in health following diagnosis might not be sustained293. A study from Italy reported that children with PCD had more behavioural and psychosocial issues than healthy controls297.In addition to anxiety about their future health, adults also have concerns about their ability to conceive and then being well enough to care for their family248.

Social effects include embarrassment, sometimes leading to concealment. Symptoms such as coughing up sputum and ear drainage cause embarrassment, and patients report repressing or concealing coughing and blowing their nose248,293,298,299. Some children report reluctance to reveal their diagnosis with teachers and friends or even to talk about their condition at home293. Parents of children with PCD report that treatment burden means reduced freedom in life and also expressed how other commitments, such as caring for siblings and employment, could limit their ability to complete daily treatments246,293 .

QOL-PCD is a patient-reported outcome measure that has been developed and validated, with age-specific versions available for children, teenagers and adults246–248. The instruments have been translated into a number of languages and offer a promising tool for evaluating new therapies and for measuring symptoms, functioning and QOL during routine care.

[H1] Outlook

**[H2] Nomenclature**

The phenotypic spectrum of motile ciliopathies makes it increasingly difficult to apply current nomenclature. For example, mutations in genes encoding MIPs result in clinical phenotypes characterized by male subfertility, laterality defects and mild respiratory symptoms87–89, raising the question whether we should still use the term PCD for these patients, even though PCD is usually characterized by a chronic destructive airway disease. By contrast, some patients have a severe destructive airway disease due to RGMC (for example, caused by *CCNO* mutations), but the individual motile cilia might still show a normal beating pattern. Thus, in this case the term PCD does not fit either, because muco-ciliary clearance is hampered by the lack of sufficient cilia numbers and not by the presence of dyskinetic cilia. In addition, symptoms that were rarely associated with motile ciliopathies, like hydrocephalus, have now been identified as major clinical symptoms in some individuals (for example, those with *FOXJ1* mutations)8. Furthermore, we expect to find more hereditary disorders that share features of non-motile ciliopathies and motile ciliopathies, such as those caused by *OFD1*, *RPGR* or *DYNC2H1* mutations. Thus, we also need to categorize those overlap-syndromes in more detail. On the basis of these considerations, there is an increasing need to reform the current nomenclature for motile ciliopathies.

Another urgent problem is that motile ciliopathies including PCD lack a specific code in the WHO International Classification of Diseases (ICD)300, and, therefore, clinicians use different ICD-codes for PCD in routine statistics. At present, only the Orphanet nomenclature of rare diseases offers a unique and stable identifier (ORPHA number 244 for PCD)300. As a consequence, even properly diagnosed patients with PCD are not visible in official health statistics, such as mortality or hospital admission statistics. Thus, incidence, prevalence, severity and survival of patients with PCD and other motile ciliopathies can only be studied in dedicated research projects and registries29,301.

**[H2] Diagnosis**

As PCD-associated variants are genetically heterogeneous and result in several different underlying disease mechanisms, PCD cannot be diagnosed by any standalone test (Table 2). Current diagnostic guidelines in North America (ATS) and Europe (ERS) differ13 14. Thus, it is important to perform high-quality studies comparing different diagnostic tests. Genetic panel analysis will have an increasingly important role because it is easy to perform and does not dependent on a highly specialized diagnostic centre for PCD. However, not all genetic defects are known yet. The recent discovery that PCD can be caused by dominant *de novo* mutations might facilitate gene discovery8, because dominant disease causes were not considered in most gene discovery studies.

A study of 189 individuals with PCD has demonstrated that immunofluorescence microscopy using anti-SPEF2 antibodies could detect CP-associated defects due to *HYDIN* mutations considerably more effectively than TEM82. Interestingly, in one immunofluorescence study, in the group of false-positive IF tests, a PCD individual with a novel gene defect (recessive *SPEF2* mutations) was identified82.Lack of SPEF2 as detected by immunofluorescence suggests the presence of mutations in genes encoding CP proteins; however, when a known or new causative mutation cannot be identified in those genes, further genetic analyses could lead to the discovery of new CP-associated defects60. hese studies indicate that immunofluorescence has a high potential to become a more widely used diagnostic tool. HSVM to evaluate the ciliary beat pattern has been widely used in Europe. However, this method relies on the expertise of the investigator, as it is mostly a descriptive method. Recently a method to analyze the transport of small immunofluorescence particles on the air-liquid interface surface in cultures of respiratory epithelial cells has been established8, which offers an objective assessment of ciliary transport capacity. After complete differentiation (>30 days) fluorescent particles are added to the apical cell compartment. In healthy control cells, particles are transported in a linear direction. This method enables calculation of particle transport velocity and directivity. However, further analyses are needed to determine its value as diagnostic method.

**[H2] International cooperation**

As motile ciliopathies are rare, it is crucial to join forces on an international level. Initiatives like BEST-CILIA and BEAT-PCD funded by the European Union helped to coordinate research from basic science to clinical care, with the ultimate goal to improve diagnosis and develop treatments that lead to improved long-term outcome of patients with PCD. A similar initiative (Genetic Disorders of Mucociliary Clearance Consortium) as part of the NCATS Rare Diseases Clinical Research Network (RDCRN, <https://rarediseases.info.nih.gov/diseases/4484/primary-ciliary-dyskinesia>) has been established in North America and is funded through a collaboration between NCATS and NHLBI (https://www.nhlbi.nih.gov/health-topics/primary-ciliary-dyskinesia). A prospective registry for individuals with PCD has been established to assemble sufficient numbers of individuals to assess and monitor patient data in a standardized and longitudinal way and to recruit candidates for clinical research studies; this registry is now part of the ERN-Lung PCD core301 (https://ern-lung.eu/), established by the European Reference Networks (ERNs) (<http://ec.europa.eu/health/ern/policy_en>) aiming to provide a virtual network of healthcare providers across Europe with the goal to provide highly specialised treatment, and gather knowledge and resources.

**[H2] Management**

The currently used treatment strategies in PCD are mostly based on the notions and experiences of clinicians with regards to treatments that are effective in various other medical conditions, such as cystic fibrosis. However, although PCD and cystic fibrosis have similar biophysical or transport properties of sputum, they have completely diverse underlying molecular defects302, and effectiveness of medications might differ among the diseases. All currently available treatments aim to relieve symptoms and to try to slow down disease progression. In this context, pulmonary exacerbations are indeed interesting as they probably, similarly to CF, involve detrimental and potentially irreversible effects on lung function and are a risk of poor prognosis in PCD.303 The first well-powered pharmacotherapeutic investigator initiated clinical trial studied azithromycin maintenance therapy and indicates that prophylactic antibiotic treatment can halve the number or respiratory exacerbations16. A multi-centre trial304 investigating the effects of inhibition of the epithelial sodium channel in individuals with PCD has ended, and final results are awaited. In addition, there is a growing interest in finding relevant and improved outcome parameters for both clinical use and randomized clinical research trials. In the azithromycin trial 16 , the value of N2 MBW and its most used derived parameter LCI as well as the newly developed and validated PCD-specific HR-QOL questionnaire (QOL-PCD)248, were investigated but did not provide additional information on treatment efficacy. Issues such as applicability of biomarkers for clinical or research purposes, for example, in exhaled breath condensate analysis305,306, and emerging technologies for the assessment of lung structure remain poorly addressed in PCD307,308. Overall, there is an urgent need for controlled trials to move into the age of evidence-based medicine in PCD. The characterization of the underlying molecular defects in PCD pave the way for the transition from symptom-oriented therapies to personalized (precision) medicine with the aim to correct the underlying molecular disease mechanisms. First gene correction studies in cell culture systems have been reported309, and studies using RNA-based transcript therapy are also underway. Because precision medicine approaches can only be applied in individuals with PCD with a genetically defined defect, it is important include such information in the PCD registry to identify patients potentially suitable for clinical trials and to clarify the natural history of the individual genetic defects. Future functional and clinical trials will show whether these precision medicine interventions can lead to a marked clinical improvement similar to that observed in cystic fibrosis when CFTR modulator therapy was introduced.

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**Table 1 |** Genes associated with motile ciliopathies

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Diagnosticsa** | **Clinical manifestations** | **Refs** |
| nNO | HSVM | TEM | IF abnormality82,225 | Respiratory Symptoms | Laterality defects | Subfertility137,144 | Hydrocephalus |
| ***Central pair component*** |
| *HYDIN* | reduced | subtle abnormalities | no abnormalities detected | SPEF2 | yes | n.r. | yes | n.r. | 81,82 |
| *SPEF2* | n.a. | subtle abnormalities | no abnormalities detected | SPEF2 | yes | n.r | yes | n.r. | 82 |
| *STK36* | no abnormalities detected | subtle abnormalities | no abnormalities detected | none | yes | n.r | n.r. | n.r. | 83 |
| *CFAP221 (also known as PCDP1)* | no abnormalities detected | Subtle abnormalities | no abnormalities detected | n.a. | yes | n.r | n.r. | n.r. | 84 |
| ***Radial spoke component*** |
| *DNAJB13* | no abnormalities detected | subtle abnormalities | no abnormalities detected | n.a. | yes | n.r | yesb | n.r. | 78 |
| *RSPH1* | no abnormalities detected | subtle abnormalities | no abnormalities detected | RSPH9 | yes | n.r | yesb | n.r. | 61,74,80,228 |
| *RSPH3* | Reduced | subtle abnormalities | no abnormalities detected | n.a. | yes | n.r | yesb | n.r. | 75 |
| *RSPH9* | reduced | subtle abnormalities | no abnormalities detected | RSPH9 | yes | n.r | n.r. | n.r. | 77,228 |
| *RSPH4A* | reduced | subtle abnormalities | no abnormalities detected | RSPH9 | yes | n.r | yesb | n.r. | 61,77,228 |
| **Nexin dynein regulatory complex component** |
| *DRC1 (also known as CCDC164)* | reduced | subtle abnormalities | no abnormalities detected | GAS8 | yes | n.r | n.r. | n.r. | 61,68 |
| *GAS8 (also known as DRC4)* | no abnormalities detected | subtle abnormalities | no abnormalities detected | GAS8 | yes | n.r | yes | n.r. | 70 |
| *CCDC65 (also known as DRC2)* | reduced | severely reduced amplitude/ rigid axonemes | Tubular disorganisation | GAS8 | yes | n.r | n.r. | n.r. | 69 |
| ***Ruler protein*** |
| *CCDC39* | reduced | severely reduced amplitude/ rigid axonemes | Tubular disorganisation | GAS8+ DNALI1 | yes | yes | yes | n.r. | 71,73 |
| *CCDC40* | reduced | severely reduced amplitude/ rigid axonemes | Tubular disorganisation | GAS8+ DNALI1 | yes | yes | yes | n.r. | 72 |
| **Microtubule inner protein** |
| *MNS1* | no abnormalities detected | subtle abnormalities | no abnormalities detected | none | n.r. | yes | yes | n.r. | 89 |
| *CFAP52* | no abnormalities detected | n.a. | n.a. | n.a. | mild | yes | n.r. | n.r. | 88 |
| *CFAP53 (also known as CCDC11)* | no abnormalities detected | subtle abnormalities | no abnormalities detected | none | n.r. | yes | n.r. | n.r. | 310 |
| *ENKUR* | no abnormalities detected | subtle abnormalities | no abnormalities detected | none | n.r. | yes | n.r. | n.r. | 86 |
| *NME7* | n.a. | n.a. | n.a. | n.a. | yes | yes | n.r. | n.r. | 90 |
| **Outer dynein arm protein** |
| *DNAH1* | n.a. | n.a. | n.a. | n.a. | n.r. | Yes | yesb | n.r. | 189 |
| *DNAH5* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | Yes | yesb | n.r. | 61,93,232 |
| *DNAH11* | reduced | hyperkinetic/reduced proximal bending | no abnormalities detected | none | yes | Yes | yesb | n.r. | 52,224,229 |
| *DNAH9* | no abnormalities detected | subtle abnormalities | no abnormalities detected | DNAH5 | yes | Yes | yes | n.r. | 53,94 |
| *DNAH8* | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | yes | n.a . | 101 |
| *DNAH17* | n.a. | n.a. | no abnormalities detected | none | no | no | yes | n.r. | 54 |
| **Intermediate chain protein** |
| *DNAI1* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | yesb | n.r. | 98,99 |
| *DNAI2* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | yes | n.r. | 96 |
| *NME8 (also known as TXNDC3)* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | n.r. | n.r. | 97 |
| **Dynein light chain** |
| *DNAL1* | reduced | Immotile/residual flickering | n.a. | n.a. | yes | yes | n.r. | n.r. | 100 |
| **Outer dynein arm docking complex component** |
| *CCDC103* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | yes | n.r. | 4 |
| *CCDC114* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | n.r. | n.r. | 102 |
| *ARMC4* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | n.r. | n.r. | 104 |
| *CCDC151* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | n.r. | n.r. | 103 |
| *TTC25* | reduced | Immotile/residual flickering | ODA | DNAH5 | yes | yes | n.r. | n.r. | 105 |
| ***Cytoplasmic dynein axonemal assembly factor*** |
| *DNAAF1 (also known as LRRC50)* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yesb | n.r. | 109 |
| *DNAAF2 (also known as KTU)* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yes | n.r. | 107 |
| *DNAAF3 (also known as C19ORF51)* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yesb | n.r. | 110 |
| *DNAAF4 (also known as DYX1C1)* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yes | n.r. | 108 |
| *DNAAF5 (also known as HEATR2)* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yes | n.r. | 115 |
| *CFAP298 (also known as C21orf59)* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | n.a.. | n.r. | 111 |
| *SPAG1* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yesb | n.r. | 112 |
| *LRRC6* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yes | n.r. | 114 |
| *ZMYND10* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yes | n.r.  | 113 |
| *DNAAF6 (also known as PIH1D3)c* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yes | n.r. | 12 |
| *CFAP300 (also known as C11orf70)* | reduced | Immotile | ODA | DNAH5+DNALI1 | yes | yes | yes | n.r. | 9 |
| *TTC12* | reduced | no abnormalities detected | no abnormalities detected | n.a. | yes | n.r. | yesb | n.r. | 116 |
| **Proteins involved in multiciliogenesis**  |
| *CCNO* | reduced | reduced number of cilia  | Cilia/BB reduced, Rootletin | none | yes | n.r. | yesb | yes | 120 |
| *MCIDAS* | reduced | reduced number of cilia | Cilia/BB reduced | DNAH5 | yes | n.r. | yesb | yes | 121 |
| *FOXJ1d* | no abnormalities detected | reduced number of cilia | BB mislocalized | none | yes | yes | yes | yes | 8 |
| *NEK10* | no abnormalities detected | Reduced number of cilia | Cilia reduced | none | yes | n.r. | n.a. | n.r. | 126 |
| **Ciliary base structure** |
| *RPGRc* | no abnormalities detected | n.a. | n.a. | none | n.r. | n.r. | n.a. | n.r. | 10,92 |
| *OFD1c* | n.a. | reduced number of cilia | BB mislocalized | none | n.r. | yes | n.a.. | yes | 11,311 |
| *GAS2L2*  | no abnormalities detected | hyperkinetic | n.a. | none | yes | yes | no | n.r. | 91 |
| *AK7* | no abnormalities detected | no abnormalities detected | no abnormalities detected | n.a. | - | n.r. | yes | n.r.. | 143 |
| **IFT-associated protein**  |
| *LRRC56* | reduced | no abnormalities detected | normal | none | yes | yes | n.a. | n.r. | 117 |
| *INVS (also known as NPHP2*) | n.a. | n.a. | n.a. | n.a. | yes | yes | n.a. | n.r. | 158 |
| *CEP290 (also known as NPHP6)* | n.a. | n.a. | n.a. | n.a. | n.r. | yes | n.a. | n.r. | 148,194 |
| *DYNC2H1* | no abnormalities detected | abnormal | n.a. | none | n.r. | yes | n.r. | n.r. | 162 |
| **WD-repeat domain proteins** |
| *CFAP43* | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | yes | yes | 139 |
| *CFAP44* | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | yes | n.r. | 139 |
| **Armadillo-like helikal repeats** |
| *CFAP69* | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | yes | n.r. | 138 |
| **Fibrous sheath integration protein** |
| *FSIP2* | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | yes | n.r. | 142 |
| **axonemal component, possible radial spoke function** |
| *CFAP251 (also known as WDR66)* | n.a. | uncoordinated | n.a. | n.a. | n.a. | n.r. | yes | n.r. | 190 |

aWith the exception of nasal NO measurements, analyses were performed in human respiratory epithelial cells

bsmall number of individuals reported;

cX-linked recessive inheritance

dautosomal dominant inheritance

Abbreviations:

n.a. not available

n.r. not reported

HSVM: Highspeed video microscopy

TEM: Transmission Electron Microscopy

IF: Immunofluorescence Analysis (anti-DNAH5 antibody, anti-GAS8 antibody, anti-RSPH9 antibody, anti-DNALI1 antibody, anti-SPEF2 antibody)

IFT: Intraflagellar transport

WD

BB: Basal body

**Table 2 | PCD diagnostic guidelines**

|  |  |
| --- | --- |
| **ERS guidelines** | **ATS guidelines** |
| **Evaluation** |
| Clinical history or a predictive tool (for example, PICADAR) | Clinical history or predictive tool (assessing 4 specific clinical features in PCD  |
| Nasal NO measurement and HSVM; repeat nasal NO if abnormal; repeat HSVM from a different biopsy or following cell culture  | Nasal NO measurement; repeat if abnormal |
| Order of tests depends on clinical circumstances and local resources and expertise; an algorithm for the order is suggested but can be varied | Recommended order of tests is nasal NO, genetic analysis and TEM |
| **Additional evaluation to exclude other causes** |
| Sweat chloride tests and genetic analysis for cystic fibrosis | Sweat chloride tests and genetic analysis for cystic fibrosis |
| Consider immune testing to exclude immunological causes  | Consider immune testing |
| **Poor likelihood of PCD**  |
| Weak clinical history and normal nasal NO and HSVM | ≤1 specific clinical features and normal nasal NO |
| **High likelihood of PCD** |
| Suggestive history and low nasal NO (repeated) or suggestive history and abnormal HSVM (repeated or following culture) | ≥2 specific clinical features with low nasal NO (repeated) |
| **Confirmation of PCD** |
| PCD defect on TEM and/or pathogenetic defect in PCD associated gene | PCD defect on TEM and/or pathogenetic defect in PCD associated gene |

Abbreviations:

HSVM: Highspeed video microscopy

TEM: Transmission electron microscopy

IF: Immunofluorescence analysis

PCD: Primary ciliary dyskinesia

ERS: European respiratory society

ATS: America thoracic society

**Figure 1 | Clinical manifestations of motile ciliopathies.** Motile ciliopathies can result in hydrocephalus and affect the respiratory tract, determination of the left–right body asymmetry and male and female fertility.Brain MRI exhibits massively dilated ventricles. Pelvic MRI exhibits distended fallopian tubes (marked with x) owing to fluid accumulation. Head CT scan shows pansinusitis, mucosal thickening and polyposis. Chest CT scan shows atelectasis and bronchiectasis. Chest X-ray shows situs inversus totalis.

**Figure 2 | Cilium structure in respiratory epithelial cells**. Cilia are highly complex organized structures comprising >650 proteins, contain tubulin-based microtubules as core structures and have an average length of 6 µm and a diameter of 0.2-0.3 µm. The central pair is connected with the outer microtubule doublets by T-shaped macromolecular complexes called radial spokes, which repeat in triple every 96 nm. The outer doublets are connected to each other by the nexin-dynein regulatory complex (N–DRC). The microtubule inner proteins (MIPs) stabilize the microtubule structure and project to the ODA-docking complex (ODA-DC) and other structures outside of the tubules. Whereas the outer dynein arms (ODAs) are attached to the A tubule of the doublet by the ODA-DC in 24 nm repeats, the inner dynein arms (IDAs) are repeated every 96 nm. The ODAs and IDAs are composed of dynein heavy chains (400-500 kDa), intermediate chains (45-140 kDa) and light chains (8-28 kDa). The two-headed ODAs generate the main beat force. In respiratory cilia, two different types of ODAs (type 1, proximal to the A tubule and type 2, distal) are present. Proteins are labelled using the name of the genes encoding them.

**Figure 3 | Genetic analysis.** Venn diagram summarizing the clinical manifestations and diagnostic outcome of high-speed video microscopy (HSVM) and TEM analyses of the known genes associated with motile ciliopathies affecting respiratory cilia. aGenetic defects that can be detected by immunofluorescence microscopy analyses. bHSVM results.

**Figure 4 | Cilium assembly.**

**a |** Axonemal structural components such as the outer dynein arms (ODAs) are first pre-assembled within the cytoplasm by cytoplasmic dynein axonemal assembly factors such as DNAAF1 (Ref87), DNAAF2 (also known as protein kintoun85), (DNAAF3 (Ref88), DNAAF4 (Ref86), DNAAF5 (Ref47), DNAAF6 (Ref10), cilia and flagella associated protein 298 (CFAP298)89, sperm associated antigen 1 (SPAG1)90, zinc finger MYND domain-containing protein 10 (ZMYND10)91, leucine-rich repeat-containing protein 6 (LRRC6, also known as protein tilB homologue)92 and CFAP300 (Ref7). ODAs are then transported to the intraflagellar transport (IFT) machinery that delivers the cargo to the ciliary or flagellar axoneme.

**b |** Ciliogenesis in multiple motile cilia is a complex process. Centrioles are docked at the apical plasma membrane, and motile cilia proteins are expressed. Autosomal recessive mutations in *CCNO* and *MCIDA*S as well as heterozygous *de novo* variants in *FOXJ1* cause a defect referred to as reduced generation of multiple motile cilia (RGMC). Whereas *CCNO* and *MCIDAS* defects are only known to affect the Notch 1 dependent pathway in respiratory epithelial cells, *FOXJ1* mutations also affect the NOTO dependent pathway during embryogenesis, causing lateralization defects.

**Figure 5 | TEM and immunofluorescence analysis of healthy cilia**

TEM photographs of a respiratory epithelial cell (top left) and a cilia cross-section (top right) from a healthy individual. The cross-section image exhibits the 9+2 structure of the axoneme: nine outer doublets (each comprising the A-Tubule (black arrow) and B-Tubule (red arrow)) surrounding a central pair complex (dotted black circle). The outer dynein arms (ODAs) (white arrowhead) and inner dynein arms (IDAs) (yellow arrowhead) are attached to the A-Tubule. The ODAs are present in 24 nm repeats, whereas the IDAs repeat less frequently (every 96 nm). Immunofluorescence analysis of a respiratory epithelial cell from a healthy control individual; the cell was stained with antibodies against DNAH5 (bottom row, first two panels) and GAS8 (bottom row, third panel); a Light microscopy image of the same cell (bottom row, fourth panel) is also included.

**Figure 6 | TEM and immunofluorescence analysis of cilia defects**

Immunofluorescence staining with anti-DNAH5, anti-GAS8, anti-SPEF2, anti-RSPH9 or anti-acetylated tubulin of respiratory epithelial cells obtained by brush biopsy from a healthy individual (control) and a patient with motile ciliopathy (mutant). TEM ciliary cross sections from a patient are also shown. In the TEM image of the ODA defect, the localisation of the missing ODAs is marked (red arrows). Unless otherwise stated, white scale bar, 10 µm and black scale bar, 1 µm.

**Supplementary videos**

**Video 1.** High speed video microscopy analysis of respiratory epithelial cells obtained by nasal brush biopsy of a healthy control. (40x)

**Video 2.** High speed video microscopy analysis of respiratory epithelial cells obtained by nasal brush biopsy of a patient with an ODA defect. (60x)

**Video 3.** High speed video microscopy analysis of respiratory epithelial cells obtained by nasal brush biopsy of a patient with a defect in the ruler complex. (40x)

**Video 4.** High speed video microscopy analysis of respiratory epithelial cells obtained by nasal brush biopsy of a patient with a defect in the central pair complex. (40x)

**Video 5.** High speed video microscopy analysis of respiratory epithelial cells obtained by nasal brush biopsy of a patient with a multiciliogenesis defect. (40x)

Toc blurb

Motile ciliopathies are rare genetic diseases that result in defective beating of motile cilia on epithelial cells. The pathogenetic mechanisms and clinical manifestations depend on the specific mutated gene and the affected tissues. When mucociliary clearance in respiratory epithelia is impaired, the disease is called primary ciliary dyskinesia.