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Primary ciliary dyskinesia in the genomics age

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Published in:
The Lancet Respiratory Medicine

DOI:
[10.1016/S2213-2600\(19\)30374-1](https://doi.org/10.1016/S2213-2600(19)30374-1)

Publication date:
2020

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):
Lucas, J. S., Davis, S. D., Omran, H., & Shoemark, A. (2020). Primary ciliary dyskinesia in the genomics age. *The Lancet Respiratory Medicine*, 8(2), 202-216. [https://doi.org/10.1016/S2213-2600\(19\)30374-1](https://doi.org/10.1016/S2213-2600(19)30374-1)

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Title: Primary ciliary dyskinesia in the genomics age

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Key Words: primary ciliary dyskinesia, cilia, diagnosis, genetic testing, treatment

Word count: 5617

Author's contributions: JSL planned the manuscript. All authors contributed to the literature search, writing, editing, and final preparation of the manuscript.

Acknowledgements: The National PCD Centres in Southampton (JSL) and Royal Brompton Hospital (AS) are commissioned and funded by NHS England. JSL is supported by NIHR Southampton Biomedical Research Centre, NIHR Clinical Research Facility, National Institute for Health Research (RfPB PB-PG-1215-20014) and The AAIR Charity (Reg. No. 1129698). SDD is funded by the Genetic Disorders of Mucociliary Clearance Consortium (GDMCC) (U54HL096458), which is a part of the NCATS Rare Diseases Clinical Research Network (RDCRN). RDCRN is an initiative of the Office of Rare Diseases Research (ORDR), NCATS, and the GDMCC is funded through a collaboration between NCATS and NHLBI. HO is supported by grants from the German Research Foundation (Om6/4, 2 Om6/7, Om6/8, OM6/10, OM6-11/DFG KFO 326), the Interdisciplinary Center for Research (IZKF) Muenster (Om2/009/12 and Om2/015/16), BESTCILIA (FP7 grant agreement number (GA) 305404), REGISTRY WAREHOUSE (Horizon2020 GA 777295), LYSOCIL (HORIZON2020 GA 811087), NEOCYST (Federal Ministry of Education and Research (BMBF)), Schroeder Stiftung, Kindness for Kids (H. Omran), Eva Luise Köhler Foundation, Care-for Rare Foundation, and Central Innovation Programme for Small and Medium-Sized Enterprises (ZIM). JSL, AS and HO are supported by COST Action BEAT-PCD (BM 1407). HO and JSL are members of the European Reference Network on Rare Lung Diseases ERN-LUNG. The funding sources had no role in the writing of this Review.

Declaration of Interests: JSL reports grants, personal fees and non-financial support from CIRCASSIA, grants and personal fees from VERTEX, outside the submitted work; In addition, JL has a Copyright pending (QOLPCD). SDD reports grants from NIH that are pertinent to the work presented in this review; she also has had grants from the Cystic Fibrosis Foundation, Parion Science, Vertex, and Circassia Pharmaceuticals, all outside the submitted work; HO reports financial activity from ETHRIS, personal fees and other from VERTEX, outside the submitted work; AS: declares no conflicts of interest

Abstract: 191/150-200

Primary ciliary dyskinesia (PCD) is a genetically and clinically heterogeneous syndrome. Impaired function of motile cilia causes failure of mucociliary clearance. Patients typically present with neonatal respiratory distress of unknown cause, and then continue to have a daily wet cough, recurrent chest infections, perennial rhinosinusitis, otitis media with effusion and bronchiectasis. Approximately 50% of patients have *situs inversus*, and male and female infertility is common. Whilst understanding of the underlying genetics and disease mechanisms have substantially advanced in recent years, there remains a paucity of evidence for treatment. Next generation sequencing has increased the trajectory of gene discovery, and mutations in >40 genes have been reported to cause PCD, with many others likely to be discovered. Increased knowledge of cilia genes is challenging our perceptions of the clinical phenotype, as some recently reported genes are associated with more mild respiratory disease. Developments in genomics and molecular medicine are rapidly improving the diagnosis of patients, and a genetic cause can be identified in approximately 70% of patients known to have PCD. A number of groups are now investigating novel and personalized treatments, although gene therapies are unlikely to be available in the near future.

Key messages

- Primary ciliary dyskinesia (PCD) is a syndrome caused by mutations in genes responsible for structure and function of motile cilia; although mutations have been identified in more than 40 responsible genes, the genetic cause of PCD cannot yet be found in ≈25% of patients. .
- A PCD diagnosis can be confirmed by identification of a hallmark ultrastructural defect by transmission electron microscopy or bi-allelic pathogenic mutations in a known PCD gene.
- Genetic advances are identifying patients with atypical presentations of PCD and have confirmed some overlaps with non-motile ciliopathies
- No diagnostic test is perfect for PCD; therefore, diagnosis relies on a combination of tests and no test can be used in isolation.
- A number of genotype-phenotype associations have been described in small studies, and international collaboration and prospective registries are now needed to properly understand these.
- In the absence of disease-specific evidence, respiratory management of PCD is based on evidence from cystic fibrosis. PCD guidelines recommend regular airway clearance and treatment of pulmonary exacerbations with antibiotics for all patients. Early steps for personalised medicine are being taken in light of genetic understanding.

Introduction:

Primary ciliary dyskinesia (PCD) is a rare syndrome, characterized by extensive genetic heterogeneity and clinical variability. Mutations in over forty genes have been reported to cause PCD, and genes continue to be discovered. Abnormal ciliary function leads to unexpected neonatal respiratory distress in term infants, persistent wet cough from early infancy, bronchiectasis, chronic rhinosinusitis, and conductive hearing impairment; 50% of patients have situs inversus, and infertility is common¹. Disease progression is highly variable, with some patients maintaining reasonably good lung function and quality of life into later adulthood, whilst others have worse outcomes²⁻⁵. There is increasing evidence that mutations in different genes lead to variable phenotypes, for example some genes are never associated with situs anomalies, and variants in others are more likely to cause infertility^{6,7}. There is limited but increasing evidence that some genes are associated with severity of pulmonary disease⁸⁻¹¹.

PCD is estimated to affect 1:10-15,000 Europeans, and is more common in populations with closed genetic pools¹²⁻¹⁴; interestingly genetic heterogeneity is seen in socially isolated consanguineous populations^{15,16}. Estimates of prevalence are limited outside Europe, but it is expected that PCD is more common in certain populations, such as those from Arabic countries^{17,18}. A survey of European PCD specialists reported that only a small percentage of the expected patients have been diagnosed, and patients reported through an international survey that 37% had visited a doctor with PCD-related symptoms more than 40 times before being referred for testing^{12,19}. The reasons for under-diagnosis are multi-factorial including difficulty accessing diagnostic services and the lack of awareness of the syndrome amongst general physicians¹⁹. Symptoms are non-specific, and patients with situs inversus, which is rare in the general population, are diagnosed earlier than those with normal organ positioning¹². Clinical tools have been developed to help physicians identify patients for testing^{20,21}, but these are based on 'typical' symptoms. We are increasingly aware of genotypes which are associated with atypical presentations.

As with other rare diseases, the evidence base for treating patients is lacking. In addition to a handful of small PCD studies, consensus guidelines are based on evidence from more common disorders such as cystic fibrosis (CF) and chronic rhinosinusitis^{22,23}.

In this review, we discuss the current state-of-the-art regarding the underlying mechanisms of PCD and how this might inform our understanding of clinical presentation and natural disease progression. We review current diagnostic and management strategies for patients with PCD. We will discuss how the improving knowledge of PCD genetics is impacting our understanding of the

phenotype, our conduct of diagnostic testing and our management of patients. The authors reflect on the expanding phenotype, and how this challenges our definition of PCD.

Search strategy and selection criteria

This manuscript is not a systematic review but consists of the authors' expert knowledge of the disease area informed by the literature; we searched PubMed, for evidence relating to PCD in the English language since 2000: using the search term "primary ciliary dyskinesia" in combination with the following: (diagnosis; clinical symptoms/clinical presentation/phenotype; treatment/clinical trial). After identifying eligible studies, we checked for additional citations in their reference lists.

Clinical features

Patients with PCD usually present with a classic clinical phenotype^{1,20,21,24}. Defining and understanding these manifestations allows for earlier recognition and identification of these patients. In PCD, over eighty percent of the population will present with respiratory distress at birth despite being term. This distress typically occurs 12 to 24 hours after birth and many infants require prolonged oxygen due to hypoxemia²⁵. Furthermore, chest radiographs in infants with PCD often reveal lobar collapse.

Impaired mucociliary clearance in this population leads to chronic wet cough associated with rhinorrhea. These manifestations occur daily and typically begin during the first months of life. The majority of patients with PCD have recurrent acute otitis media, otitis media with effusion, and chronic otitis media^{1,26,27}. Repeat ear infections may lead to transient or permanent hearing loss, the latter causing significant morbidity later in life. Sinusitis is a common manifestation in this population, but may not be detected in younger patients due to lack of imaging^{8,23}.

Recurrent pulmonary infections due to impaired mucociliary clearance ultimately leads to bronchiectasis. Respiratory cultures (sputum, bronchoalveolar lavage) in the younger population often reveal *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus* and *Hemophilus influenzae*. *Pseudomonas aeruginosa* may be detected in children with PCD, but becomes more predominant in adults^{8,28-30}. Chest computed tomography imaging during infancy have revealed early airway manifestations, including bronchiectasis³¹. Infertility is often present in males. In females, fertility may be difficult due to impaired cilia motion in the fallopian tubes^{7,24}.

Situs inversus occurs in fifty percent of PCD patients; and situs ambiguus, including heterotaxy, has been reported in up to 12% of patients with PCD^{6,32,33}. These patients have an increased incidence of

congenital heart disease. Children and adults with situs inversus and/or heterotaxy associated with respiratory manifestations should be evaluated for PCD^{23,34}.

Diagnosing PCD has historically been difficult due to the need for specialized testing coupled with lack of general awareness leading to the failure of clinicians to recognize the classic phenotype. Recently, four clinical features were defined as predictive of PCD, including laterality defect; unexplained neonatal respiratory distress; early-onset, year-round nasal congestion and wet cough. The sensitivity and specificity of having PCD if all four features were present was reported at 0.21 and 0.99, respectively²¹. In another study, a scoring tool, PICADAR (Primary Ciliary Dyskinesia Rule) was developed to predict the diagnosis of PCD in a symptomatic population²⁰. The seven features in addition to persistent wet cough that were predictive of PCD included full-term gestation, neonatal chest symptoms, admission to the neonatal intensive care unit, chronic rhinitis, ear symptoms, situs inversus and congenital cardiac defect. PICADAR represents a diagnostic algorithm that may prove useful when delineating whether a work-up is indicated for PCD (score of 4: sensitivity 0.97, specificity 0.48; score of 5: sensitivity 0.90, specificity 0.75²⁰). However, these predictive diagnostic tools may be under-utilized if general awareness remains low. Efforts to raise knowledge, led by specialists in the field, is already improving referrals. In the UK, the age of diagnosis in the pediatric population has dropped from 4.8 years (2009)¹² to 2.6 years (2015- unpublished data). In North America, there are now 41 accredited PCD Foundation centers; this initiative is increasing the identification and recognition of this rare disease. Further research is warranted in high risk populations to investigate whether predictive tools with targeted nasal nitric oxide testing could further promote early referral. Adult bronchiectasis clinics are a likely reservoir of undiagnosed patients, and work is needed to raise awareness amongst adult pulmonologists. Modified predictive tools will be needed for this population since many adults are unlikely to recall early life events which are captured by the current questionnaires^{20,21}. For example a modified PICADAR score and low nasal nitric oxide was accurate in identifying PCD amongst adults with bronchiectasis³⁵.

Significant heterogeneity exists within the PCD population in age-matched lung function values^{2,3,8}. In some cases, genotype accounts for the variability between patients, for example mutations in *CCDC39* and *CCDC40* are associated with worse lung function, whilst *DNAH9* mutations are associated with milder disease⁸⁻¹⁰. In a recent publication, the rate of FEV₁ decline was highly variable in 137 patients with PCD followed at seven centers for five years⁹. Furthermore, ventilation inhomogeneity as measured through the multiple breath washout technique may be abnormal even with normal spirometric values^{36,37}.

Diagnosis

Multiple investigations are usually required to make a diagnosis of PCD. There are two evidence based diagnostic guidelines published by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) respectively^{34,38}. Both guidelines recognise that there is no perfect diagnostic test for PCD. There is a lack of sensitivity or specificity for most tests used, specialist equipment and expertise is required and consequently access to testing can be problematic. Guidelines recommend a combinations of the following tests:

Nasal Nitric Oxide (NO)

The level of NO gas in the nasal cavity can be measured with a gas analyser. This test is ordinarily reserved for children who have developed paranasal sinuses and are able to comply with velum closure manoeuvres (usually >4yrs old). Most individuals with PCD have NO values which are tenfold lower than healthy controls³⁹. Sensitivity and specificity are good (98% and >99.9%, respectively) at a cut off at 77nl/min when measured in patients with symptoms of PCD, with a chemiluminescent analyser, using ATS/ERS NO measurement guidelines⁴⁰. However there can be several other causes of reduced nasal NO such as smoking or nasal obstruction. Patients with cystic fibrosis are also reported to have reduced NO values^{41,42}. Conversely, although a normal nasal NO level remains helpful to exclude a diagnosis, there are exceptions where nasal NO is above the typical cut-off used for diagnosing PCD. These include individuals with mutations in *GAS8*, *STK36*, *CCDC103*, *RSPH1* and *GAS2L2*^{11,43-46}. Increasing knowledge of genetics has highlighted that nNO cannot be used in isolation as a screening test, as certain patients with PCD will be missed.

New portable analysers make NO testing increasingly accessible in the clinic, although both sensitivity and specificity when using these analysers is reduced⁴⁷.

High speed video microscopy analysis (HSVMA)

Live samples, usually obtained by nasal brushing, can be assessed for ciliary beat frequency and pattern. This functional assessment is achieved ex-vivo using a light microscope fitted with a high speed video camera. Ordinarily cilia beat in a metachronal wave with an effective forward stroke followed by a recovery stroke. Specific beat patterns are observed in patients with PCD: completely immotile, immotile with occasional residual movement, reduced bend and reduced beat amplitude, hyperfrequent with reduced beat amplitude or with a circular motion when viewed from above. In some cases a mixed phenotype can occur. Correlations between cilia beat pattern and ultrastructural defect or genotype in PCD have been demonstrated^{48,49}. Ciliary beat frequency is less accurate when used in isolation and is dependent on several factors, such as patient age, formulation of the maintenance media and sample temperature⁵⁰. Recent evidence shows excellent

accuracy of HSVMA for diagnosis, however the limitation is the specialist equipment required to record the videos and the expertise required to interpret the beat pattern. Also pathogenic variants in some genes can be associated with fairly normal ciliary beating (e.g. *RSPH1*, *CCDC103*, *DNAH9*)

Immunofluorescence:

Antibodies directed against ciliary proteins are becoming increasingly available and are used regularly in a research setting to confirm absence of ciliary protein and the pathogenicity of a genetic mutation⁵¹. Recent evidence for the use of antibody testing with a panel of six antibodies shows similar sensitivity and specificity as TEM in clinical practice⁵².

Transmission electron microscopy:

Normal ciliary ultrastructure consists of a “9+2” structure: 9 peripheral microtubule doublets, surround the central pair of single microtubules (Figure 1). Each doublet is connected with the central pair by radial spokes, and neighbouring doublets are joined by the nexin dynein regulatory complex (N-DRC). The outer microtubular doublets contain regularly repeating structures known as outer and inner dynein arms (ODA and IDA), which are responsible for the generation of ciliary motion through ATPase activity.

Since the first description of the condition by Afzelius, PCD diagnosis has relied on ultrastructural analysis by TEM⁵³. TEM remains a robust test to confirm a diagnosis although increasingly cases with normal ultrastructure have been identified^{50,54,55}. Despite poor sensitivity, the specificity of TEM for diagnosis of PCD is excellent³⁴. As noted above, normal ciliary ultrastructure may occur in those with PCD. Hallmark diagnostic defects for PCD include: absence of the ODA, absence of the ODA and IDA, and absence of the IDA with microtubular disorganisation. In addition ultrastructural defects which can support a diagnosis when used in combination with other tests have been identified. These include central complex defects, mislocalisation of basal bodies, isolated microtubular disorganisation and partial defects of the outer +/- inner dynein arms affecting <50% of cross sections. Figure 2 shows examples of the various ciliary ultrastructural changes according to PCD genotype.

Genotyping

Both ERS and ATS guidelines agree that a bi-allelic pathogenic mutation or hemizygous X-linked mutation in a known PCD gene confirm a diagnosis^{34,38}. Around 65-70% of PCD cases diagnosed by other methods can be genetically solved. However many individuals have variants of unknown

significance or mono-allelic heterozygous variants which require confirmation by functional and structural tests (HSVMA, IF and TEM). It is not unusual to identify one or several rare missense variants that are not linked to the disease, due to the large size and number of genes sequenced in PCD patients; therefore there is significant risk of false positive results unless the genetic diagnoses are supported by corresponding HSVMA or TEM results (Table 1). Since genetic testing fails to identify ≈30% of patients, it cannot exclude a diagnosis. nNO with hallmark TEM or repeatedly abnormal HSVMA can probably identify most patients with PCD associated with normal genetics to ensure they receive appropriate clinical care.

Summary

We emphasise the risks of relying on any one test to diagnose PCD, and the benefit of using a combination of tests⁵⁰. No test is perfect, and it is only by comparing results from various tests that the clinician can be confident. Importantly, all diagnostic tests should be conducted and interpreted by PCD experts who understand the relative risks of false positive and negative results for the individual tests. Due to imperfect testing by all modalities it is likely that some patients with PCD are not recognised despite adherence to ATS or ERS guidelines. Therefore research and development in PCD gene discovery and improved diagnostic techniques are crucial. Novel approaches such as radiolabelled mucociliary clearance provide additional data which may help exclude a diagnosis of PCD when this functional test is normal in difficult diagnostic cases^{56,57}. In addition new advances in electron microscopy such as 3D electron tomography provide insight into PCD cases previously thought to have normal ultrastructure⁵⁸.

Genetics and disease mechanisms

Mutations of more than 40 different genes have been reported that cause PCD (Figure 1). Most PCD variants are transmitted in an autosomal recessive fashion. However, X chromosomal recessive inheritance has also been reported for a few genes^{59,60}. Motility of respiratory cilia is generated and regulated by complex mechanisms involving several large multimeric protein complexes. Mutations affecting many of these proteins can result in PCD. In approximately 50% of cases, mutations in some genes are associated with laterality defects, whilst mutations in other genes are always associated with situs solitus. Dyskinesia of the cilia severely impairs mucociliary clearance, leading to recurrent and chronic airway infection and inflammation. IL-8, neutrophils, and neutrophil elastase activity are significantly elevated in the PCD airway, contributing to the inflammatory environment^{61,62}. Furthermore, peripheral blood monocytes from PCD patients produce higher levels of inflammatory cytokines when stimulated compared to those from healthy individuals, perhaps

contributing to the chronic airway inflammation and resulting tissue damage from infections⁶³. This recurrence and persistence of airway infections and inflammation contribute to progressive lung disease and eventually bronchiectasis.

PCD associated with laterality defects

PCD individuals with abnormal outer dynein arm (ODA) composition, abnormal ODA docking machinery, abnormal cytoplasmic dynein preassembly or tubular disorganization share the possibility during embryogenesis of randomization of left-right asymmetry. Thus, half of all affected individuals exhibit situs inversus or heterotaxy, because those disease mechanisms also affect nodal cilia function important for determination of left-right asymmetry during early embryogenesis. The association of situs inversus with PCD is also referred to as Kartagener syndrome.

Defects of outer dynein arm composition: The ODA, attached to the outer doublets, generate the main mechanical force to produce cilia bending. Genetic defects in genes that encode ODA components such as the heavy chains DNAH5, DNAH11^{64,65}, the intermediate chains DNAI1 and DNAI2^{66,67} as well as light chains such as DNAL1 and NME8^{68,69} all result in dysfunction of ODAs.

Mutations in the gene *DNAH5* result in the most frequent defect reported in PCD individuals. High-speed video microscopy analyses (HSVMA) of cilia beating reveals immotile cilia; transmission electron microscopy (TEM) and immunofluorescence microscopy analysis (IF) confirm deficient/absent ODAs.

Mutations in genes encoding intermediate chains of the ODAs such as DNAI1 and DNAI2, or encoding the light chain DNAL1 cause similar defects to *DNAH5* mutations that can be recognized by TEM, IF and HSVMA. Interestingly, mutations in the *NME8* gene have been reported to result in a variable phenotype of the ultrastructure⁶⁹. Approximately half of the cross sections showed ODA defects, the other half showed normal composition. In the original reports it had been suggested that some cilia might lack ODAs and that other cilia might assemble ODAs. However, recently it has been discovered that two distinct ODA populations are present in respiratory cilia along the ciliary lengths. ODA complexes type 1 containing the beta-heavy chain DNAH11 are located in the proximal compartment whereas ODA complexes type 2 containing the beta-heavy chain DNAH9 are located in the distal compartment^{10,51,70}. Thus, it is more likely that in *NME8* mutant cilia the distal ciliary axonemes lack ODAs type 2.

DNAH11 mutations cause a distinct PCD phenotype characterized by a hyperkinetic beating pattern with reduced beating amplitude. The bending of the proximal ciliary compartment is deficient in *DNAH11* mutant cilia. *DNAH11* mutations do not cause obvious ultrastructural defects readily

identifiable by TEM⁵⁵. Therefore, *DNAH11* mutations are often overlooked in centres that rely only on TEM to establish PCD diagnosis. Immunofluorescence microscopy with anti DNAH11 antibodies can detect some PCD individuals with mutations in *DNAH11*⁷¹. Recently mutations in *DNAH9* have been reported in PCD individuals with mild or no respiratory disease⁷⁰. *DNAH9* mutant cilia lack ODAs of the distal ciliary axonemes and exhibit only subtle beating abnormalities^{10,70}.

Immunofluorescence microscopy analysis utilizing antibodies directed against ODA components DNAH9, DNAH11, DNAH5, DNAI1 or DNAI2 can easily depict the distinct defects of ODA composition in the different genetic PCD variants.

Defects in outer dynein arm docking and targeting: ODAs are attached to the outer doublets by complicated mechanisms involving a multimeric docking machinery which are referred to as ODA docking complexes (ODA-DCs). Mutations in genes encoding different ODA-DC components such as *CCDC114*^{14,72}, *ARMC4*⁷³, *CCDC151*^{74,75} and *TTC25*⁷⁶ can result in abnormal ODA docking. Thus, mutations of these genes result in absence of ODAs from the ciliary axonemes, usually easy to detect by TEM and IF. HSVMA readily identifies predominantly static cilia. In addition, with the use of antibodies targeting the ODA docking machinery it is possible to distinguish molecular defects caused by abnormal ODA composition or by abnormal ODA docking. Interestingly, the ODA-DC protein *TTC25* appears to be the major component of the ODA docking machinery because in those mutant cells, ODA-DCs such as *CCDC114*, *CCDC151* and *ARMC4* cannot be assembled. In contrast, *ARMC4* mutations cause abnormal ODA docking that is more prominent in the distal ciliary axoneme than proximally resulting in a heterogeneous picture along the axoneme. *CCDC103*^{43,77} is not a classical ODA-DC protein but is involved in ODA targeting. *LRR56* mutations disrupt intra-flagella transport-dependent delivery of ODA components and result in subtle defects of distal ODAs⁷⁸.

Defects in cytoplasmic preassembly of dynein arms: ODAs as well as inner dynein arms (IDAs) which are important for the regulation of the ciliary beating are preassembled in the cytoplasm prior to delivery to their final docking sites at the outer doublets along the ciliary axoneme. Therefore, mutations in genes that alter cytoplasmic preassembly processes result in defects of ODAs as well as IDAs. Those preassembly factors are referred to as dynein axonemal assembly factors (DNAAFs). Mutations in genes that encode these cytoplasmic preassembly factors such as *DNAAF1*^{79,80}, *DNAAF2*⁸¹, *DNAAF3*⁸², *DNAAF4*(*DYX1C1*)⁸³, *DNAAF5*(*HEATR2*)⁸⁴, *LRR6*⁸⁵, *ZMYND10*^{86,87}, *SPAG1*⁸⁸, *C21ORF59*⁸⁹, *DNAAF6*(*PIH1D3*)^{59,60} and *CFAP300*(*C11ORF70*)⁹⁰ cause PCD with combined ODA and IDA defects. Because the cilia lack outer as well as IDAs, most mutations result in complete cilia immotility, easily identified by HSVMA. For *DNAAF2* as well as for hypomorphic mutations in

DNAAF4, some residual cilia motility can be retained due to partial assembly of outer and IDAs. The defects are usually more pronounced in the distal ciliary axoneme than proximally.

In male individuals carrying mutations in genes encoding cytoplasmic preassembly factors, sperm dysmotility indicates that the molecular disease mechanisms of cytoplasmic preassembly also plays a role in sperm flagella.

Defects of the ninety six nanometer ruler machinery: The ninety six nanometer ruler proteins *CCDC39*⁹¹ and *CCDC40*⁹² are responsible for the correct establishment of the 96 nm repeats along the ciliary axoneme. In each 96 nm axonemal repetitive unit four ODAs and various distinct IDAs are attached to the outer doublets. Those ruler proteins are also important for anchoring the dynein regulatory complex (nexin links) connecting the outer doublets as well as IDA proteins such as *DNALI1* to the axoneme. The cilia beat with a reduced bend and amplitude. The ultrastructural defects seen by TEM comprises a severe tubular disorganization as well as deficiency of IDA components; the ODA composition is not affected. In all reported cases, recessive mutations in the genes *CCDC39* or *CCDC40* have been identified. With the use of IF it has been confirmed that mutant cilia lack nexin link proteins such as *CCDC164*⁹³, *CCDC65*⁸⁹, *GAS8*^{45,94} or *LRRC48*. In addition, patients lack IDA proteins like *DNALI1* in the axonemes. Thus, HSVMA, TEM and IF can easily identify patients with defects in the genes *CCDC39* or *CCDC40*.

PCD without laterality defects

Several disease mechanisms can alter cilia motility of multiciliated respiratory cells whilst not affecting function of nodal monocilia during early embryogenesis. Those genetic defects are not easily diagnosed by TEM and they can benefit from analysis by IF.

Defects in radial spoke components: Mutations in the genes encoding radial spoke proteins *RSPH1*⁹⁵, *RSPH4A*⁹⁶, *RSPH9*⁹⁶, *RSPH3*⁹⁷ and *DNAJB13*⁹⁸ cause abnormal radial spoke composition. However, this abnormal radial spoke composition causes only subtle ciliary beating abnormalities, usually comprising of rotational or stiff beating patterns. IF shows abnormal composition by demonstrating absence of radial spoke head proteins⁹⁹.

Defects in central pair associated proteins : Genetic defects encoding central pair associated proteins such as *HYDIN*¹⁰⁰ and *STK36*⁴⁶ have been reported. Those genetic defects are not associated with obvious ultrastructural abnormalities. In some cross sections occasionally central pair abnormalities can be noted by TEM. However, those abnormalities can also occur in normal individuals and they are not pathognomonic. Genetic testing for *HYDIN* mutations is complicated. Due to a recent evolutionary event, *HYDIN* became duplicated and therefore most of the coding

exons of *HYDIN* are also present in the pseudogene *HYDIN2*. Due to this duplication DNA sequence analysis of *HYDIN* is not easily established. Therefore, some commercial genetic kits do not screen for *HYDIN* mutations or report a reduced sensitivity for this genetic testing. Diagnosis of central pair defects is also hampered by the fact that some individuals show normal nasal NO production rate and the ciliary abnormalities detected by HSVMA are subtle. Elucidating these PCD genotypes highlights potential cases which risk being missed by standard functional tests.

Isolated nexin link defects: Genetic defects encoding the so-called nexin link dynein regulator complex proteins (N-DRCs) result in abnormalities of the nexin link structure connecting the outer doublets. So far, mutations in the genes *CCDC164*⁹³, *CCDC65*⁸⁹, and *GAS8*⁴⁵ have been reported. TEM shows no discernible abnormalities. Occasionally, outer doublets are not well aligned in the periphery of the cross section. However, those abnormalities can be also observed in normal individuals. The ciliary beat pattern of patients with isolated nexin link defects is occasionally stiff, but can also appear normal. IF identifying N-DRC defects using antibodies targeting N-DRC proteins such as *GAS8* are widely used.

Reduced generation of multiple motile cilia: These individuals show a reduced capacity to generate multiple motile cilia. In patients with mutations in *MCIDAS*¹⁰¹ respiratory cells usually lack any motile cilia or show only very few motile cilia. *MCIDAS* appears to be a major regulator of ciliogenesis in multiciliated cells. Downstream of *MCIDAS*, *CCNO*¹⁰² is responsible for the function in the deuterosome dependent amplification of basal bodies and basal body docking. Therefore, mutations in *CCNO* result in a defect with a very low number of cilia covering the airway cells. Residual cilia can still show normal cilia motility. TEM can also demonstrate mislocalised basal bodies in the cytoplasm. Because the genetic defects solely alter ciliogenesis in multiciliated cells, the function of nodal monocilia is not altered.

Motile ciliopathies with subtle or no respiratory disease: Recently, genetic defects have been recognized in individuals with motile ciliopathies that result in randomization of left/right body asymmetry and/or male infertility but to date seem to not suffer from the severe chronic destructive airway disease usually reported in PCD. *MNS1*⁹⁰, *DNAH9*^{10,70}, *CCDC11*¹⁰³ and *ENCUR*¹⁰⁴⁹⁸ belong to this group.

Genotype-phenotype relationships

Delineation of genotype-phenotype relationships have recently been described in the PCD population. Patients with absent IDA with microtubular disorganisation who have a *CCDC39* or

CCDC40 mutation, have lower body mass indices and worse lung function that declines more rapidly compared to the PCD population with ODA defects and those whom have a *DNAH5* mutation, respectively⁹. Interestingly, patients with microtubular disorganization with IDA defects, many with the *CCDC39* or *CCDC40* mutation, are diagnosed earlier with PCD and even as infants have more airflow limitation compared to those with ODA defects⁸. Patients with the *CCNO* and *MCIDAS* mutations have also been reported to have a worse phenotype with significant bronchiectasis, as well as increased incidence of hydrocephalus¹⁰⁵. It seems reasonable to recommend increased surveillance and more aggressive treatment plans for these groups. Conversely those with *RSPH1* mutations appear to have less lung function impairment, a later onset of wet cough, a lower prevalence of neonatal respiratory distress and nasal nitric oxide values often outside the diagnostic range¹¹. Furthermore, recent data has suggested that mutations in *DNAH9* are associated with milder respiratory phenotypes^{6,10}. With the increase in discovery of PCD-causing genes, more genotype-phenotype relationships will be elucidated. However, caution is needed when interpreting genotype-phenotype data based on small numbers of patients with variations in any one gene. Moreover, we are aware from diseases such as CF that the phenotype can vary depending on the type of mutation within a gene, and how it effects the protein product. Similar variability is likely within PCD genes.

By definition, patients with PCD have dyskinetic cilia, and our clinical understanding has expected airway cilia to be impacted, as confirmed by HSVMA. Recently described mutations in *ENKUR* and *CCDC11* impact left-right patterning but do not appear to cause pulmonary disease^{103,104}. These findings are supported by normal ciliary motility seen by HSVMA and normal TEM in patients with *ENKUR* mutations. Given this, some have questioned whether these patients should be included within the syndrome of PCD due to the significantly different phenotype that does not benefit from the usual PCD management. Conversely, patients with mutations in *CCNO* and *MCIDAS* have reduced generation of motile cilia, rather than dyskinesia^{101,102}. These patients have a severe respiratory phenotype and a higher risk of hydrocephalus. However, despite not having dyskinetic cilia, they are best managed in specialist PCD clinics.

Syndromes associated with PCD

Advances in imaging and genotyping have revealed PCD like features in some patients with non-motile ciliopathies. Non-motile primary cilia have a role in cell signalling and sensing of the extracellular environment. Symptoms among ciliopathies include polycystic kidneys, skeletal abnormalities, developmental delay and retinal degeneration. Increasingly abnormalities in motile cilia are described in several non-motile ciliopathies including Jeune syndrome¹⁰⁶, nephronophthisis type 2 (*NPHP2*)¹⁰⁷, Leber Congenital Amaurosis (LCA)¹⁰⁸ and Bardet Biedl syndrome¹⁰⁹.

Some males with X-linked mutations in *RPGR*, which can cause retinitis pigmentosa are also reported to have PCD symptoms¹¹⁰ associated with dyskinetic ciliary beating¹¹¹. *OFD1* is an X-linked gene associated with several overlapping ciliopathies including oral-facial-digital syndrome and Joubert syndrome. When there is also a motile cilia defect the condition is termed Simpson–Golabi–Behmel syndrome. These individuals suffer from PCD symptoms, overgrowth and can also have abnormally large kidneys, liver and spleen¹¹².

Management

Since there is no cure for PCD, the aim of treatment is to delay the decline in pulmonary airways disease, whilst maintaining patients' health, social and psychological wellbeing. The variability in clinical manifestations and the scarcity of data from clinical trials make it difficult to formulate a standardised management plan that is suitable for all patients. Management is often extrapolated from other diseases such as CF and chronic rhinosinusitis. Although both CF and PCD inevitably lead to bronchiectasis, their underlying patho-mechanisms and clinical course are different^{113, 2, 114, 115}. Response to treatment is likely to vary in PCD and international collaborations are urgently needed to ensure a sufficient number of eligible patients for well-designed treatment trials.

General physicians usually have a poor knowledge of managing rare diseases. Guidelines therefore recommend that patients should be seen several times a year in PCD centres by a specialist multidisciplinary team^{22, 23, 116}.

General Health

There is growing evidence for the importance of growth and nutrition in children with PCD. The International PCD Cohort (iPCD) have reported impaired height from an early age, and a longitudinal study has shown reduced growth throughout childhood resulting in loss of body height by adulthood^{117, 118}. Several studies have observed a relationship between lower nutritional status and worse lung function^{2, 117, 119}, although other studies have found no association^{115, 120}. Furthermore PCD patients have low levels of vitamin D, a finding associated with disease severity in bronchiectasis^{119, 121}. Knowledge of genotype might indicate the patients at increased risk; e.g. patients with mutations in *CCDC39* and *CCDC40* have been observed to have diminished growth parameters in comparison to *DNAH5*⁹.

Patients with PCD often have poor aerobic fitness and regular exercise should be encouraged to improve general wellbeing and perhaps assist in mucus clearance^{122, 123}.

Pulmonary Management

There is recent evidence that patients with PCD have more impaired lung function than those with cystic fibrosis (CF) from early childhood². With the paucity of evidence, treatment is based on more common diseases, in particular CF¹¹⁶. Guidelines recommend monitoring to include culture of sputum or cough swabs every 3 to 6 months^{22,23,116}. Spirometry is routinely used, but is not as sensitive as lung clearance index (LCI) measured by multiple-breath washout for detecting disease^{36,124-127}. LCI is easier to measure than spirometry in young children, but information regarding the relationship between FEV1 and LCI in PCD is conflicting¹²⁴⁻¹²⁶. Multiple-breath washout equipment is not always available and testing can be time consuming. LCI is not able to distinguish between individuals with reversible and irreversible structural damage³⁶; therefore lung imaging is needed to characterise structural changes. Chest X-rays are probably the most commonly used imaging in clinical practice for assessing structural lung changes in PCD^{22,23}. Most infants with PCD have unexpected neonatal respiratory distress despite term birth. The majority of these infants have evidence of lobar collapse, usually involving the upper lobes²⁵. However, chest x-rays are not sensitive as a monitoring tool to detect early pulmonary changes. Due to concern over the radiation exposure HRCT is not recommended for routine annual monitoring, but can be used intermittently or when clinically indicated. Typical changes seen in PCD include bronchiectasis, peribronchial thickening, mucus plugging, atelectasis and consolidation or collapse. Lung disease, in particular bronchiectasis, predominantly occurs in the middle and lower lobes, with relative sparing of the upper lobes^{115,128-129}. As expected, structural changes increase with age and are associated with lung function^{128,129}. There are no scoring systems specific for PCD. Studies have therefore used the scoring systems derived for CF lung disease, (i.e. Brody and Bhalla)^{130,131}. However, the structural lung disease in CF and PCD differ, and PCD-specific scoring systems are needed to fully capture changes

132 114 .

To avoid the radiation doses associated with HRCT imaging, there is a growing interest in magnetic resonance imaging (MRI) to assess structural and functional changes^{37,133,134}. Further developments of lung MRI may allow longitudinal assessments over time in PCD as well as provide the ability to quantify structural changes on a more regular basis and serve as endpoints in short term intervention therapy trials. Whilst there is virtually no evidence, experts agree that a cornerstone of treatment is airways clearance with the aim of preventing infections, atelectasis, bronchiectasis and respiratory failure^{22,23,116}. Techniques include a combination of breathing manoeuvres, positioning, manual percussion, with adjuncts including 'vest therapy' (high frequency chest wall oscillation), PEP devices and oscillatory-PEP. Current practice individualises the method based on disease severity, patient age and preference¹³⁵, with clearance usually recommended at least twice daily^{22,23,116}. If patients have difficulty clearing secretions, nebulised treatments might augment mucus clearance,

although evidence is poor in PCD. The only clinical trial using hypertonic saline failed to show a significant effect in comparison to isotonic saline; the study was probably underpowered and the outcome measure might not have been ideal, demonstrating the need for multinational trials using sensitive outcome measures¹³⁶. Whilst a small number of case studies have suggested a benefit when using recombinant DNase in patients with PCD¹³⁷⁻¹³⁹, a large trial of patients with non-CF bronchiectasis reported more frequent pulmonary exacerbations and an increased decline in FEV₁^{140,141}. Therefore use of rhDNase in PCD should only be considered on a case-by-case basis.

Prevention and treatment of pulmonary exacerbations is the second facet of pulmonary care, and again evidence is extrapolated from CF¹⁴². Prevention includes immunization (influenza, pneumococcus etc.), segregation and surveillance programmes. Common isolates include *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*^{8,29,30,120,143}. Although treatment of symptomatic infection is intuitive, the treatment of asymptomatic infection is more contentious. In the absence of disease-specific evidence, it is generally accepted that treatment should follow CF guidelines, with modification from the high doses used in CF. Given the toxic effects of some of these antibiotics, adverse events should be monitored in PCD patients. In the absence of strong evidence, a sensible approach is to treat first isolates of pathogens with at least two weeks of antibiotics in an attempt to eradicate. For *P. aeruginosa* inhaled antibiotics are usually prescribed; some clinicians will also prescribe an oral antibiotic. Patients who do not respond to treatment may require intravenous therapy. Whilst prophylactic use of azithromycin can be beneficial in CF and non-CF bronchiectasis, the evidence for use in PCD is awaiting results from a recent trial¹⁴⁴.

The role of thoracic surgery in PCD is unclear, but in the authors experience is rarely indicated. Lung transplantation is an option in patients with end stage disease, and has been performed in a number of patients in the US²⁸. Recent studies indicate that transplantation is undertaken exceptionally rarely in Europe^{26,145}. Transplantation requires careful planning particularly in patients with situs abnormalities.

Upper airways and ears

Since the majority of patients with PCD have chronic rhino-sinusitis, otitis media with effusion, and chronic otitis media^{1,26,27}, an otolaryngologist should be part of the PCD multidisciplinary team.

Perennial nasal discharge often starts from early infancy, and can be debilitating, affecting quality of life^{4,5,146}. Anecdotally, sino-nasal rinsing with saline can relieve symptoms. Topical nasal steroids are often used although there is no evidence of efficacy¹⁴⁷.

Management of the ears aims to improve hearing and prevent irreversible damage of the tympanic membranes. Ear disease is very common, particularly in young children. Symptoms often improve with age, although some adults continue to require hearing aids^{27,148,149}. The conductive hearing loss associated with middle ear effusions can delay speech acquisition, impair learning, and create social isolation. Hearing should therefore be monitored regularly using age-appropriate methods. Oral antibiotic treatment of acute otitis media may prevent complications, and topical antibiotics can be prescribed for ear discharge. In PCD, the use of ventilation tubes is controversial because troublesome mucopurulent discharge is reportedly more common than in the general population (30% versus 5%), however, randomised studies have not been undertaken^{149,150}. The ERS guidelines recommend hearing aids rather than ventilation tubes, until such time as the hearing loss resolves²². Patients with mutations associated with central complex defects (Table 1), have been reported in one study to have more ear-related problems. This group might benefit from more regular monitoring¹⁴⁹.

Cardiac disease

Echocardiogram and abdominal ultrasounds should follow diagnosis in patients with genes known to effect laterality (Table 1).

Fertility

Infertility is common in PCD and patients need access to specialist care for assisted fertility⁷. There is increasing data to guide which patients require counselling regarding fertility, for example, patients with mutations in *CCDC114* appear to have normal fertility¹⁴, whilst limited data suggests male infertility is highly likely in patients with mutations in *CCDC39*, *CCDC40*, *DNAAF1* and *LRRC6*⁷. A number of men have successfully fathered children following intracytoplasmic sperm injection (ICSI). Information regarding pregnancy is limited, but anecdotally, a number of women with PCD have successfully delivered healthy babies. There is dogma that women are at high risk of ectopic pregnancy, but experience of the authors as well as lack of epidemiological evidence cast doubt on this⁷.

Potential novel therapies

There is much interest in genetic correction of mutated PCD genes as a potential cure. Several studies have reported partial recovery of ciliary beating investigated using lentiviral transfection or TALENs (transcription activator-like effector endonucleases) in ciliated cell culture models and transgenic mouse models¹⁵¹⁻¹⁵³. Other gene editing techniques exist, for example read through therapies¹⁵⁴ and CRISPR-Cas9. With recent successes in cystic fibrosis, and the increased

understanding of the genetic basis of PCD, there are a number of researchers investigating targeted small molecule therapies.

Summary

In summary, PCD is a syndrome caused by genetic mutations effecting motile cilia, causing disease of the upper and lower airways. Next generation sequencing has led to advances in the discovery of new PCD genes over the past decade, and over 40 genes are now reported to cause this rare disease. This remarkable progress is impacting diagnostic capabilities, genetic counselling and has the potential to lead to new therapeutic options and personalised care.

There is increasing understanding of the influence of genotype on clinical presentation, although research is limited by the number of patients with mutations in any one gene. There is no gold standard diagnostic test, and a combination of nNO screening, analysis of cilia ultrastructure by EM; analysis of ciliary function by HSVM, Immuno-labelling of ciliary proteins (IF) and genetic testing can be used. Because there are a large number of implicated genes and mutations, and because many genes have yet to be found, genetic testing is not yet a definitive diagnostic test for PCD. There is no cure for PCD and management focuses on careful monitoring and physiotherapy as well as treatment of infections. The lack of a definitive diagnostic test and absence of specific treatments highlight the importance of collaborative international research for this rare disease.

Discussions between patient representatives and clinicians are needed to clarify the definition of PCD in this genomic age. Whilst previously we presumed that all patients would have pulmonary involvement, there have been recent discoveries of genes (e.g. ENKUR) affecting motile cilia that cause situs inversus in the absence of significant chest disease. Should these patients be included in the syndrome? Further study is also indicated in patients with mutations in genes where there is ciliary dyskinesia, but some mucociliary clearance is maintained (e.g. DNAH9). Conversely patients with mutations in CCNO and MCIDAS, do not have dyskinetic cilia. Rather, their relatively severe respiratory phenotype, caused by reduced numbers of motile cilia, is typical of PCD. Should this group of patients have a different label for their disease, or is it clinically sensible for them to be identified as having PCD? There is large heterogeneity and we are at the tip of the iceberg in understanding the disease.

Table1. Genetic defects in PCD, and the associated phenotypes where these differ from 'classical' PCD

Functional defect	Gene	OMIM	Predominant ultrastructural defect by TEM	Predominant HSVMA	Phenotype notes	Ref.
Randomization of L/R asymmetry						
ODA	<i>DNAH5</i>	603335	ODA	Static		64
	<i>DNAH11*</i>	603339	Normal [§]	Hyperfrequent/ stiff at base		65
	<i>DNAI1</i>	604366	ODA	Static		66
	<i>DNAI2</i>	605483	ODA	Static		67
	<i>DNAL1</i>	610062	ODA	Static		68
	<i>NME8</i>	607421	ODA	Static		69
	<i>DNAH9</i>	603330	ODA-type 1	Normal/ stiff at top	Normal NO/ milder resp. phenotype	10,70
ODA Docking	<i>CCDC114</i>	615038	ODA	Static	Preserved fertility	14,72
	<i>ARMC4</i>	615408	ODA	Static		73
	<i>CCDC151</i>	615956	ODA	Static		75
	<i>CCDC103</i>	614677	ODA/ normal ^{§§}	Static/ normal ²	Normal NO/ milder resp. phenotype ^{§§}	77
	<i>TTC25</i>	617095	ODA	Static		76
	<i>MNS1</i>	610766	ODA	Normal	Milder resp. phenotype	90
Pre-assembly Factor	<i>DNAAF1</i>	613190	ODA + IDA	Static		79,80
	<i>DNAAF2</i>	612517	ODA + IDA	Static		81
	<i>DNAAF3</i>	614566	ODA + IDA	Static		82
	<i>DNAAF4</i>	608706	ODA + IDA	Static		83
	<i>DNAAF5</i>	614864	ODA + IDA	Static		84
	<i>LRRC6</i>	614930	ODA + IDA	Static		85
	<i>ZMYND10</i>	607070	ODA + IDA	Static		86,87
	<i>SPAG1</i>	603395	ODA + IDA	Static		88
	<i>C21ORF59</i>	615494	ODA + IDA	Static		89
	<i>PIH1D3</i>	300933	ODA + IDA	Static		59,60
	<i>CFAP300</i>	618058	ODA + IDA	Static		155
Axonemal ruler	<i>CCDC39</i>	613798	MTD + IDA	Reduced bend and amplitude	More severe resp. pheno and poorer nutrition ^{8,9}	91
<i>CCDC40</i>	613798	MTD + IDA	Reduced bend and amplitude	More severe resp. pheno and poorer nutrition ^{8,9}	92,156	
N-DRC	<i>CCDC164</i>	615288	MTD /normal	Hyperfrequent/ dyskinetic/ normal	Situs solitus	93
	<i>CCDC65</i>	611088	MTD /normal	Hyperfrequent/ dyskinetic/ normal	Situs solitus	89,157
	<i>GAS8</i>	605178	MTD /normal	Hyperfrequent/ dyskinetic/ normal	Situs solitus	40,8845,94
Radial spoke Subunit	<i>RSPH1</i>	609314	CC/ normal	Circling ^{\$\$\$}	Normal NO + milder resp. phenotype. Situs solitus	95
<i>RSPH4A</i>	612647	CC/ normal	Circling ^{\$\$\$}	Situs solitus, increased chance of OM ¹³²¹⁴⁹	158	
<i>RSPH9</i>	612648	CC/ normal	Circling ^{\$\$\$}	Situs solitus; increased chance of OM ¹⁴⁹	96	
<i>RSPH3</i>	615876	CC/ normal	Circling ^{\$\$\$}	Situs solitus; increased chance of OM ¹⁴⁹	97	
<i>DNAJB13</i>	610263	CC/ normal	Circling ^{\$\$\$}		98	

Central pair	<i>HYDIN</i>	610812	C2b projection/ normal	Dyskinetic ^{\$\$\$}	Situs solitus	100
Subunit	<i>STK36</i>	607652	normal	Dyskinetic ^{\$\$\$}		46
Other	<i>CCNO</i>	607752	Mislocalisation of basal body + reduction of cilia	RGMC	More severe + Hydrocephalus	102
	<i>MCIDAS*</i>	614086	Mislocalisation of basal body + reduction of cilia ODA+IDA	RGMC	More severe + Hydrocephalus	101
	<i>CCDC11</i>	614759	normal	normal	Normal/mild?	103
	<i>ENKUR</i>	611025	N.A.	normal	Normal/mild?	104
	<i>GAS2L2</i>	611398	normal	Normal/ disorientation		44
	<i>LRRC56*</i>	618227	normal	Dyskinetic		78

TEM: transmission electron microscopy; HSVMA: high-speed video microscopy analysis; RGMC: reduced generation of multiple motile cilia; ODA: outer dynein arm; IDA: Inner dynein arm; N-DRC: nexin-dynein regulatory complex NO: nasal nitric oxide; MTD: Microtubular disorganization; N.A.: not applicable; OM: otitis media

* Not always detectable by immunofluorescence using targeted antibodies

§ Ultrastructural defects can be identified by electron tomography

\$\$Refers only to the His154Pro missense mutation

\$\$\$When viewed from above. Sometimes can appear dyskinetic or more normal

Figure legends

Figure 1: Over 40 genes have been reported to cause PCD. This figure summarises the genes which can be associated with PCD by effecting ciliary proteins, transport of those proteins, or docking of structures. ODA= outer dynein arm; CP= central pair; N-DCR=nexin-dynein regulatory complex.

Figure 2: Transmission electron microscopy images of: A) Outer dynein arm defects B) Inner and outer dynein arm defects C) Microtubular disorganisation and inner dynein arm defect D) Normal ultrastructure E) Central complex defect showing separation of the central pair and translocation of an outer microtubular doublet.

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