Primary ciliary dyskinesia

Key points

- Primary ciliary dyskinesia is just one of a group of conditions where a heterogeneous array of genetic mutations affect the assembly or structure of motile cilia.
- Overlapping phenotypes between motile and nonmotile ciliopathies are redefining the diagnostic and therapeutic approach to encompass all ciliopathy patients with a respiratory phenotype.
- An extended diagnostic algorithm may be required to capture the majority of cases with a respiratory ciliopathy, including patients with syndromic ciliopathies.
- The terminology around disorders of motile cilia is becoming more descriptive to better reflect the heterogeneity and underlying disease mechanisms across the spectrum of respiratory ciliopathies.

Educational aims

- To summarise the existing knowledge base around the disease mechanisms for respiratory ciliopathies, including primary ciliary dyskinesia (PCD).
- To explore and understand the reasons for changing terminology around respiratory ciliopathies.
- To emphasise key messages around the diagnosis and treatment of all ciliopathies.
- Diagnosing PCD is complex and time consuming, and there is no single stand-alone test that can confirm or exclude a diagnosis in all cases.
Ciliopathies: understanding the role of cilia in human health and disease

Cilia are organelles that project from the surface of most cells within the body, playing an important role in many regulatory processes including cell signalling, reproduction, development, organogenesis and as a primary defence mechanism in some organs [1–3]. Throughout evolution, the function and structure of cilia has been preserved, and they are found on nearly all vertebrate cells [4]. It is helpful to differentiate between the two categories of cilia, primary (nonmotile) and motile cilia. Primary cilia are single, nonmotile organelles on the surface of almost all cells in the human body that act as sensors picking up chemical and physical stimuli, moderating signal pathways in the environment around them [2].
Primary cilia determine organogenesis and normal development [5], and so loss of function mutations in nonmotile cilia genes result in a group of rare syndromic disorders [6]. In contrast, motile cilia are present on a group of specialised epithelial surfaces, usually several hundred to a cell, and act as a mucociliary escalator moving fluid and debris in a synchronised and unidirectional way at the air–liquid interface [3, 7]. Multiple motile cilia are located throughout the airway and sinuses, Eustachian tube, brain ependyma, fallopian tubes and as a single cilium in the sperm tail. The exception to the rule for motile cilia are the nodal cilia. Here, single motile cilia on the cells within the embryonic node have a different axonemal structure with a circular waveform resulting in leftward flow of liquid and calcium across the node during organogenesis. This transient function in the developing embryo determines organ laterality and is often known as the left–right organiser [8]. Loss of function mutations in motile cilia genes result in multisystem disorders potentially impacting on lung health, fertility, organ situs, congenital heart defects and the normal circulation of spinal fluid [9].

Collectively, these inherited disorders of human cilia are called ciliopathies. In most cases, these rare genetic conditions affect either primary cilia resulting in syndromic ciliopathies, or motile cilia such as in primary ciliary dyskinesia (PCD). These previously disparate disorders are now recognised as part of a ciliopathy spectrum where an overlapping clinical phenotype may be evident [4, 10]. Variation in the severity of syndromic features suggests that, in some cases, where overlapping phenotypes exist, genetic modifiers determining disease expression come into play [11]. Individually and combined, >35 inherited ciliopathy diseases are known, resulting from dysfunction of primary or motile cilia, or both. Globally, one in 2000 individuals are affected with significant comorbidity, challenging healthcare systems to improve diagnostics and management for patients with these complex multisystem conditions [10].

A broader perspective of respiratory ciliopathies

Ciliary dysfunction, in motile and nonmotile cilia, leads to a wide spectrum of clinical phenotypes that may overlap genetically and phenotypically. Cilia play an important role in organ function and development, with an increasing recognition of the complex conditions associated with ciliary defects [12]. These defects manifest in a variety of ciliopathy syndromes that have a different set of cardinal signs from patients with PCD that include polyductyly, visual and hearing defects, obesity, and renal and cardiac defects. Loss-of-function mutations in primary and motile cilia were thought to have distinct phenotypes relating to sensory or motor functions, respectively. However, it has become clear that motile cilia in the airway also have a sensory role [13, 14] and that defects in certain ciliary proteins, that are important for the function of both motile and nonmotile cilia, can lead to a range of ciliopathy phenotypes with overlapping clinical features. For example, patients with Bardet–Biedl syndrome (BBS), when investigated for a high background prevalence of respiratory PCD-like symptoms (neonatal respiratory distress, rhinitis and otitis media), had some curious diagnostic findings. These patients were found to have normal structure of the motile cilium but with some unique inclusions near the tip of the cilary axoneme; however, function was apparently preserved [15]. Similar findings have been recorded in other ciliopathy syndromes, where long, whip-like cilia and inclusion bodies near the axonemal tip, like those seen in the BBS cohort, have become a recognised feature in a subgroup of patients where an overlapping respiratory phenotype exists. Loss of function mutations in RPGR, causing progressive blindness in patients with retinitis pigmentosa (RP), are also known, although rare, mutations leading to a syndromic PCD-RP phenotype. Like in BBS, in these patients, assessment of ciliary structure using transmission electron microscopy (TEM) was normal but with disruption of ciliary orientation leading to a functional defect [16]. A further example of phenotypic overlap is seen in patients with autosomal dominant polycystic kidney disease (ADPKD). Polycystin 1 and 2, the protein abnormalities associated with ADPKD, are also found in cilia and smooth muscle cells in the airway, implicating their role in an increased prevalence of bronchiectasis in these patients [17,18]. Finally, a small group of patients with a de novo mutation in FOXJ1 presenting with a collection of clinical anomalies related to defects in motile cilia (hydrocephalus, bronchiectasis and randomisation of left–right asymmetry) appear to have a heterozygous autosomal dominant mode of inheritance [19]. Diagnostic tests, including high-speed video microscopy, immunofluorescence (IF) and TEM, indicate paucity of cilia with mislocalisation of the basal bodies. Specific staining for protein tyrosine kinase 2, a focal adhesion protein, confirms aberrant localisation in the cytoplasm of the respiratory epithelial cells in patients with this mutation [19]. This group of patients represent the first reported cases of a dominantly inherited motile ciliopathy, further highlighting the complexity of this heterogeneous group of disorders

Importantly, syndromic patients that are referred with symptoms in keeping with a respiratory ciliopathy should undergo the diagnostic pathway that is in place for PCD to confirm or exclude functional abnormalities related to concomitant defects of the motile cilium. However, when seeing patients who have a strong history for PCD but do not follow the “norm”, we should consider the presence of other ciliopathies.
PCD as a model for ciliopathies

PCD is a heterogeneous multisystem disorder of motile cilia leading to a common clinical phenotype of rhinosinusitis, middle ear disease, bronchiectasis and subfertility. Situs abnormalities occur in almost half of patients, and associated congenital heart diseases and other organ abnormalities occur in a subgroup of patients with situs ambiguous or heterotaxy [20, 21]. PCD is the most common and well phenotyped of the respiratory ciliopathies, with clearly defined functional and structural defects that can be determined from an array of tests across a comprehensive, but not fail-proof, diagnostic algorithm. Despite rapid advances bringing new diagnostic tools from bench to bedside [22, 23], gaps in our knowledge remain. A greater understanding of the underlying disease mechanisms and genetics, where next-generation sequencing has advanced gene discovery with loss-of-function mutations in more than 50 respiratory ciliopathy genes now reported [24]. Increased knowledge of cilia genes and observed functional defects in a wider array of ciliopathy conditions is challenging perceptions of the clinical phenotype, where overlapping features between motile and nonmotile ciliopathies are emerging. PCD, as a disease, offers clinical scientists a unique research model with the opportunity to examine both the structure and function of motile cilia directly from the airway epithelium, or following regeneration of ciliated epithelium in cell culture [25]. It has, therefore, been possible to interrogate the downstream impact of novel gene variants, determining pathogenicity and expanding our knowledge of a wider set of ciliopathy syndromes.

Diagnostic tests: state-of-the-art algorithms and basic rules to secure a diagnosis

The diagnosis of PCD involves a complex, time-consuming and expensive diagnostic pathway that has determined that reliable testing is only available or practical in specialist centres where expertise has grown around a clinical academic interest in this rare condition. Separate diagnostic guidelines have been published by the European PCD Consortia [26, 27] and their North American counterparts [28], leading to controversy around which tests, and how many, should be employed to optimise diagnostic accuracy. A welcome European–North American review of the guidelines has outlined why different approaches to PCD diagnostics has arisen, clarifying why a uniform diagnostic algorithm across all reference centres is a challenge from a pragmatic perspective [29]. This review will not debate that issue but aims to outline the diagnostic tests available that, in various combinations, can be used in a targeted cascade to optimise diagnostic potential in the majority of cases (figure 1). The take home message is that to diagnose respiratory ciliopathies, including PCD, rarely does a single test suffice. In particular, as we expand our expertise across a wider group of ciliopathy conditions, a robust approach is required to ensure the functional and structural findings are consistent with candidate gene variants to support diagnostic confidence.

Historically, diagnosis has relied on determining the function and structure of motile cilia by using high-speed video microscopy (HSVM) and TEM, respectively. Genotyping and novel imaging techniques, moving in from the research environment, offer improved accuracy when used in combination with HSVM and TEM, and include immunofluorescent antibody testing (IF) and super-resolution three-dimensional (3D) electron tomography imaging.

The majority of cases do not need the full panel of tests to be deployed. Rather, a staged approach is appropriate to ensure a clear diagnosis is made, or indeed excluded. Clinical assessment is key to deciding which cases need referral for testing and having some basic rules or a scoring system, such as PICADAR [30], can be helpful. If a patient exhibits some or all of the cardinal signs - situs abnormalities, unexplained neonatal respiratory distress, persistent rhinitis from early life, middle ear disease with complications such as otorrhoea post-grommet surgery, or congenital heart disease - then they warrant referral to a PCD centre where the following screening and diagnostic tests might be applied.

Nasal nitric oxide

The measurement of nasal nitric oxide (nNO) represents an accurate and immediate screening tool for patients >5 years of age [31]. It has largely replaced the more archaic techniques using saccharine or charcoal to assess ciliary function in the nose [32] and, in centres with a chemiluminescent analyser and specialist team to undertake accurate and routine NO measurements, plays an invaluable role in the diagnostic pathway. Limitations relate to patient cooperation, and other causes of nasal obstruction or conditions where lower nNO measurements are characteristic, such as in cystic fibrosis (CF) [33]. Recently, it has also been recognised that some respiratory and syndromic ciliopathies have normal nNO and, in these cases, the pursuit of a diagnosis must continue with the implementation of additional diagnostic testing [34]. For a screening tool to fulfil its potential, it would ideally be widely available to clinicians. The quest for a portable nNO device that matches the accuracy of the chemiluminescent analysers based at reference centres continues. NIOX, with the MINO [35] and, more recently, the VERO [36] device, have provided the potential for screening patients in a wider setting that should result in direct referrals to a reference centre if...
Figure 1  Flowchart to demonstrate the pathways to diagnosis of PCD as per the a) European Respiratory Society and b) American Thoracic Society consensus statements. It is important to note that not all patients will require all investigations. §: cystic fibrosis (CF) should be ruled out before performing nasal nitric oxide (nNO) measurement, as roughly one-third of CF patients can have nNO values below PCD diagnostic cut-offs. nNO measurements should only be performed with chemiluminescence analysers using standardised protocols at centres with specific expertise in nNO measurements. Some nNO analysers have not received approval from federal agencies worldwide (US Food and Drug Administration and Health Canada), which may have implications for clinical implementation. ¶: genetic panel testing for mutations in >12 disease-associated PCD genes, including deletion/duplication analysis. +: as nNO levels can be significantly decreased by viral respiratory tract infections, a repeat nNO measurement, at least 2 weeks after the initial low value (expert opinion), is recommended to ensure the initial low value is not secondary to a viral process. A normal nNO value upon repeat testing is not suggestive of PCD, as nNO values remain consistently low in PCD. §§: most forms of PCD resulting in normal nNO levels have normal or non-diagnostic electron microscopy studies. Thus, genetic testing is recommended in these cases. #: or presence of variants of unknown significance. ##: additional corroborative testing may provide information on clinical prognosis, further disease understanding, and potential future therapeutic considerations. ¶¶: known disease-associated TEM ultrastructural defects include outer dynein arm (ODA) defects, ODA plus inner dynein arm (IDA) defects, IDA defect with microtubular disorganisation, and absent central pair, identified using established criteria. Of note, the presence of IDA defects alone is rarely diagnostic for PCD. ++: up to 30% of PCD cases can have normal ciliary ultrastructure on electron microscopy. Consider referral to PCD specialty centre if there is a strong clinical phenotype but all electron microscopy and genetic testing are negative. HSVMA: high-speed video microscopy analysis. Reproduced from [29] with permission.
clinical phenotype and nNO measurements raise the possibility that a patient may have a respiratory ciliopathy.

Ciliary beat pattern and frequency

Despite not being diagnostic in its own right, ciliary function, measured by beat pattern and frequency using HSVM, remains consistently the most abnormal test in confirmed cases of PCD [37]. However, as a technique, it requires significant experience to develop accuracy in the interpretation of a complex and growing array of specific beat patterns, and so it is not always available in the clinical diagnostic algorithm at some reference centres. In expert hands, it is an invaluable tool where recognised beat pattern abnormalities are correlated with specific structural defects and genetic mutations, and in some cases, consistent beat pattern anomalies have led to the pursuit of a difficult diagnosis where TEM or genetic evidence was elusive [23] (table 1).

Ciliary structural analysis

In contrast, the determination of hallmark structural defects using TEM (table 1) is a recognised diagnostic test, albeit with an accurate pick-up in only 75–80% of cases [38]. Using TEM to determine loss of specific proteins within the ciliary axoneme was considered the gold standard to secure a diagnosis (figure 2) but it is increasingly recognised that in some cases where a clear clinical phenotype was present, TEM was normal. Like all PCD diagnostic tests, TEM should be used as part of a wider set of investigations.

Immunofluorescent antibody staining of ciliary proteins

IF staining represented the first real opportunity to develop a reliable, cost-effective test within the PCD diagnostic pathway that can potentially be used in a wider laboratory setting. IF has long been used in PCD genetic research [39] to determine localisation of protein compounds within the ciliary axoneme, proving instrumental in confirming specific mutations as pathogenic variants. IF also offers value in clinical diagnostics where an established antibody panel has been validated using antibodies for specific proteins identifiable using TEM [22]. Additional fluorescent antibodies are now in clinical use helping to bridge diagnostic gaps when using TEM, such as in cases of DNAH11.

Table 1 Beat pattern assessment using HSVM, and correlations with TEM structural phenotype and genotype

<table>
<thead>
<tr>
<th>Ciliary beat pattern defect</th>
<th>Functional defect</th>
<th>TEM defect</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static</td>
<td>ODA</td>
<td>ODA</td>
<td>DNAH5, DNAI1, DNAI2, DNAL1, NME8, CCDC114, ARNC4, CCDC151, TTC25</td>
</tr>
<tr>
<td></td>
<td>ODA docking</td>
<td>ODA</td>
<td>DNAAF1, DNAAF2, DNAAF3, DNAAF4, DNAAF5, LRRC6, ZMYND10, SPAG1, C21orf59, PIH1D3, CFAP300</td>
</tr>
<tr>
<td></td>
<td>Pre-assembly factors</td>
<td>ODA and IDA</td>
<td></td>
</tr>
<tr>
<td>Normal/stiff at tip</td>
<td>ODA</td>
<td>ODA type 1</td>
<td>DNAH9</td>
</tr>
<tr>
<td>Hyperfrequent/dyskinetic</td>
<td>ODA</td>
<td>Normal</td>
<td>DNAH11</td>
</tr>
<tr>
<td>Hyperfrequent/</td>
<td>N-DRC</td>
<td>MTD/normal</td>
<td>CCDC164, CCDC65, GAS8</td>
</tr>
<tr>
<td>dyskinetic/normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Static/normal</td>
<td>ODA</td>
<td>ODA/normal</td>
<td>CCDC103</td>
</tr>
<tr>
<td>Circling from top view</td>
<td>Radial spoke</td>
<td>Central pair</td>
<td>RSPH1, RSPH4A, RSPH9, RSPH3, DNAJ1B13</td>
</tr>
<tr>
<td>Dyskinetic/circling</td>
<td>Central pair</td>
<td>Normal</td>
<td>STK36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal/C2b</td>
<td>HYDIN</td>
</tr>
<tr>
<td>Reduced amplitude/stiff</td>
<td>Axonemal ruler</td>
<td>MTD and IDA</td>
<td>CCDC39, CCDC40</td>
</tr>
<tr>
<td>Normal</td>
<td>ODA docking</td>
<td>ODA</td>
<td>MNS1</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>Normal</td>
<td>CCDC11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown</td>
<td>ENKUR</td>
</tr>
<tr>
<td>Normal/dyskinetic</td>
<td>Unknown</td>
<td>Normal</td>
<td>GASL2, LRRC56</td>
</tr>
<tr>
<td>Ciliary aplasia</td>
<td>RGMC</td>
<td>Misllocalisation of basal bodies and reduction of cilia</td>
<td>CCNO, MCIDAS</td>
</tr>
</tbody>
</table>

ODA: outer dynein arm; IDA: inner dynein arm; N-DRC: nexin-dynein regulatory complex; MTD: microtubular disorganisation; RGMC: reduced generation of multiple motile cilia.
mutation (figure 3), adding further value to its use in the clinical setting. This test is now utilised in most reference centres and continues to play a key role in genetic research laboratories engaged in novel variant analysis of candidate genes.

3D electron tomography

This technique uses a tilt series of electron micrograph images that are then aligned and stacked to generate 3D super resolution rendered reconstructions of cilia giving detailed ultrastructural information not visible using TEM. Although largely a research tool, its application clinically has led to confirmation of difficult cases, determination of pathogenic mutations and a better understanding of protein localisation within the axoneme. HYDIN mutation is an excellent example where tomography demonstrated loss of the protein, C2b, in the central pair apparatus, confirming the downstream deleterious effect of the mutation. Defects using TEM for patients with HYDIN mutation have been difficult to detect, but easily visualised using tomography [40] (figure 4). Correlation with genetic variants and beat pattern anomalies has led to a growing confidence in the recognition of subtle TEM defects in the central pair that may previously have been dismissed. Likewise, tomography has played a key role in the determination of DNAH11, where typically patients have low nNO and a stiff hyperkinetic beat pattern but normal TEM findings. Tomography clearly identifies loss of the DNAH11 protein in the proximal part of the outer dynein arm (ODA), correlating with the proximal stiffness on beat pattern analysis using HSVM [41] (figure 5). Patients with DNAH11 mutation are now easily diagnosed using both genetics and IF staining but it is an example where experts consistently identified beat pattern anomalies using HSVM, recognising that

Figure 2 TEM images of cross-sections of cilia from nasal brushing samples. a) Normal; b) outer and inner dynein arm defects; c) inner dynein arm defects plus microtubular disorganisation; d) central pair defect; and e) transposition defect.

Figure 3 IF staining in a healthy control where a) tubulin stains green to indicate presence of cilia on the cell surface, and b) staining with DNAHS antibody for the outer dynein arm (ODA) in red is identical, indicating normal ODA structure; and in c) and d) a patient with DNAH11 mutations, where c) tubulin staining green for cilia is seen whereas in d) absence of staining for DNAH11 using red labelled antibody is evident.
this cohort were highly likely to have PCD before the techniques described here were available to confirm pathogenic variants in DNAH11.

Genotyping

The advance in genome sequencing has had significant impact on the diagnosis of PCD. To date, mutations in at least 50 genes have been identified as pathogenic in PCD [24, 42, 43]. Biallelic recessive, hemizygous X-linked and, more recently, heterozygous de novo dominant [19] pathogenic mutations in known ciliopathy genes are accepted as diagnostic, but around 20–25% of cases cannot be confirmed using genetic testing. Genes encoding ciliary structure and function in most cases correlate with the findings on TEM, IF and HSVM. However, in many cases, VUS (variants of unknown significance) require diagnostic confirmation using one or more of these tests to avoid misdiagnosis where missense nonpathogenic variants are identified, and all genetic diagnoses must be supported in this way [42].

Heterogeneity and disease mechanisms

The clinical impact of mutations affecting motile cilia results from disruption of the normal mucociliary escalator with mutation specific phenotypic variations. Genotypic–phenotypic correlations have shown some mutations are not associated with laterality defects, others more likely to affect fertility, have a higher prevalence of hydrocephalus or affect the severity of pulmonary disease [21, 24, 44–46].

Understanding the spectrum of clinical symptoms and disease correlations requires some knowledge of the underlying disease mechanisms and where specific mutations impact ciliary development. Table 2 outlines how the 50 known mutations fall into categories impacting pre-assembly, assembly, transport or docking of ciliary proteins, and where clear correlations have been identified. For instance, it is recognised that defects in genes encoding ODA components, assembly and docking as well as the proteins anchoring the dynein regulatory complex are associated with randomisation of left–right asymmetry or situs
Table 2. Known genes associated with defects in cilium structure seen on TEM or HSVM

<table>
<thead>
<tr>
<th>Defect in cilia</th>
<th>Associated genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODA</td>
<td>DNAH5, DNAH11, DNAI1, DNAI2, DNAL1, DNAH9, NME8</td>
</tr>
<tr>
<td>IDA</td>
<td>DNAH1, TTC12, CFAP57</td>
</tr>
<tr>
<td>Pre-assembly</td>
<td>DNAAF1, DNAAF2, DNAAF3, DNAAF4, DNAAF5, LRRC6, ZYMND10, SPG1, PIH1D3, C21orf59, CFAP300</td>
</tr>
<tr>
<td>factors</td>
<td>MTD/central complex HYDIN, STK36</td>
</tr>
<tr>
<td>96-nm axonemal</td>
<td>Radial spoke RSPH1, RSPH4A, RSPH9, RSPH3, DNAJB13</td>
</tr>
<tr>
<td>ruler/IDA</td>
<td>96-nm axonemal CCDC39, CCDC40</td>
</tr>
<tr>
<td>RGMC</td>
<td>Nexin link/N-DRC CCDC164, CCDC65, GAS8</td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>RGMC MCIDAS, CCNO, FOXJ1</td>
</tr>
<tr>
<td>ODA docking</td>
<td>CCDC114, ARM4C, CCDC151, TTC25, MNS1, CCDC103</td>
</tr>
<tr>
<td>PCD spectrum</td>
<td>CFAP53 (CCDC11), ENKUR, GAS2L2, LRRC56, NEK10</td>
</tr>
</tbody>
</table>

ODA: outer dynein arm; IDA: inner dynein arm; MTD: microtubular disorganisation; N-DRC: nexin–dynein regulatory complex; RGMC: reduced generation of multiple motile cilia.

defects, due to involvement of the nodal cilia during embryogenesis [47, 48]. In contrast, defects in the radial spoke [49], central pair [40] and dynein regulatory complex [50] or where ciliogenesis is impacted [46], nodal cilia are not affected and there is no association with laterality defects.

Clinically, what does this mean?

Identification of a gene may lend itself to recognition of a certain phenotype and can be useful in the clinical assessment of and treatment decisions for individual patients. For example, mutations encoding DNAAFs (dynein axonemal assembly factors) cause completely immotile cilia with inner dynein arm (IDA) and ODA defects, and males with these particular mutations are more likely to have fertility problems due to sperm dysmotility [51].

Patients with PCD but no laterality defects have been found to have different structural and motility defects but often present a diagnostic challenge as they may have normal nNO, as in those with HYDIN mutations [39], or normal ciliary motility, like those with nexin link defects [42]. They are also less likely to have congenital heart disease or other structural organ abnormalities. Likewise, mutations in MCIDAS and CCNO, where reduced generation of motile cilia (RGMC) effectively presents as ciliary aplasia, a severe progressive decline in lung function is typical. Specific TEM appearances show severe reduction and mislocalisation of the basal bodies that anchor the ciliary axoneme, and diagnosis is reliant on genetic testing [46]. Finally, patients with mutations in CCDC39 and CCDC40 with an IDA and microtubular defect tend to be diagnosed earlier, despite having no association with situs abnormalities, because of a more severe respiratory phenotype and lower body mass index when compared with patients who have DNAH5 mutations [42].

Genotype-phenotype correlations will become increasingly useful when counselling and managing patients.

Establishing a PCD diagnostic reference centre

The growing complexity of securing a diagnosis across such a heterogeneous group of conditions has led to attempts to develop comprehensive guidelines. The two international consensus guidelines, despite their differences, have led to useful debate around how best to improve diagnostics for PCD across a diverse landscape where resources and expertise can be limiting factors. This is particularly true for emerging centres but also is relevant for existing diagnostic services. The pursuit of a diagnosis has moved on from a binary approach based on the presence or absence of a trademark TEM defect. Similarly, identifying biallelic mutations in PCD genes cannot currently confirm >80% of cases and where negative, cannot exclude the diagnosis. However, despite the growing number of diagnostic tests and tools at our disposal. significant challenges remain in establishing a reference centre with the comprehensive array of clinical tests needed to optimise diagnostic accuracy (figure 1).

Where expertise and resources are limited, a pragmatic approach to developing a pathway towards a diagnosis is key. What is feasible will depend on human factors as much as specialised microscopes. Having the required electron, confocal and light microscopes is only useful if a ciliary biologist is available. The expertise to analyse and interpret ciliary defects requires high throughput of samples to acquire the skill sets to achieve accurate diagnosis across an array of subtle but consistent anomalies. Working in isolation is also difficult in a condition where cross-checking and quality control rely on a team approach. A network of experts who collaborate to provide shared expertise has arisen from successive PCD consortia where training schools, workshops and conferences have facilitated skill acquisition, collaboration and research (BEAT-PCD, BESTcilia and ERS task forces). As a result, partnerships have developed between reference centres and emerging services, resulting in improved access to diagnostics and driving research.

With all of this in mind, a diagnostic centre requires the ability to perform at least two tests to secure a firm or highly likely diagnosis. Ideally, this will include TEM and/or genetic testing. Since both tests leave around 15–30% of cases undiagnosed, additional tests are required where the phenotype is convincing. nNO and IF are ideal back-up investigations that can be established.
across a wider laboratory setting, in particular where a hub-and-spoke network model might be more practical. They are useful as screening tests in centres that can link into a reference centre for the required additional testing to confirm the diagnosis. HSVM remains the single most abnormal test in confirmed cases of PCD, making it potentially the most valuable tool in the diagnostic algorithm. However, it requires exposure to many cases and acquired expertise to develop the necessary skills in accurate interpretation. It should only be used in combination with TEM or genotyping where specific beat patterns should correlate with structural defects and specific mutations to underpin the accuracy of the diagnosis (table 2).

Clinical management of respiratory ciliopathies

Protocols for the management of PCD have largely been extrapolated from treatment programmes for CF with a respiratory centric focus. Both conditions have ciliary dysfunction-related retention of secretions, primary in PCD and acquired in CF, requiring airway clearance and treatment of chronic and recurrent infection. However, it is here that the overlap in these multisystem disorders ends with each requiring its own specialist multisystem approach for monitoring and treatment. The management of all respiratory ciliopathies will follow these strategies for patients with PCD, RGMC or syndromic ciliopathies in terms of respiratory, ear, nose and throat, and fertility medicine. However, some patients with ciliopathy syndromes will need additional comorbidity care focused on their specific needs.

PCD management approach

It should be noted that evidence-based medicine is in short supply for this condition, with clinical trials addressing treatments in non-CF bronchiectasis [52] or looking at small numbers of patients with PCD. The first randomised clinical trial studied the long-term use of azithromycin in patients with PCD and showed a significant reduction in infectious exacerbations [53]. There was no change in the other outcome measures (forced expiratory volume in 1 s and quality of life scores). The commonest pathogen found in spumt of patients with PCD is Haemophilus influenzae [52, 54]. Others include Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis and Pseudomonas aeruginosa [55]. Prompt and more prolonged treatment of respiratory tract infections guided by microbiological findings is one of the pillars of managing PCD lung disease [52].

Mucolytic therapies are the second pillar and the first line of managing airway clearance. Here, practice between CF and PCD is beginning to diverge. In CF, sticky secretions are tackled using DNase, hypertonic saline or mannitol alone or in combination based on expert physiotherapist assessment. In PCD, experience has shown that hypertonic saline is most effective, loosening secretions and inducing cough to help clear secretions. Studies of the use of mucolytics are scarce and often include PCD in cohorts of patients with non-CF bronchiectasis [56, 57]. Studies such as that by Paff et al. [57] are amongst the first to include only patients with PCD.

It is clear that despite the lack of clinical evidence, respiratory ciliopathies require specialised and directed comorbidity care. Common sense and sharing of expertise have led to a pragmatic approach but more randomised clinical trials are needed to establish truly effective therapies for this group of patients.

Factors to consider for patients with more complex and syndromic ciliopathies

For patients with syndromic ciliopathies, comorbidity care should be designed around their multiple needs. In the UK, specialist services have been designed around specific conditions, PCD, BBS, Alström
syndrome and RP all being examples where a national approach to complex care needs has transformed clinical service provision and driven research programmes, leading to improved diagnosis and targeted therapeutic programmes. Patients with complex coronary heart disease with an underlying ciliopathy may be late to have their respiratory phenotype assessed as the severity of their other morbidities dominates the clinical picture. A wider group of syndromic ciliopathies with no dedicated care pathway may represent the most vulnerable group, where clinicians unfamiliar with the rarity of their condition and their complex needs may focus on dominating signs and symptoms, with the result that their progressive respiratory disease remains untreated, leading to relentless decline. As we continue to recognise the overlap in the motile and syndromic ciliopathies, greater collaborative working will be required to ensure that these patients are appropriately managed for their complex needs.

**Conclusion**

Respiratory ciliopathies are complex multifactorial pulmonary diseases demonstrating genetic heterogeneity with a common respiratory phenotype. PCD remains the best understood ciliopathy, with an established diagnostic pathway that has become well recognised. Over the last decade, new terminology is developing in response to a better understanding of the underlying cell biology and disease mechanisms. Some mutations affect assembly and transport of cilia proteins within the cell, resulting in effective aplasia rather than dyskinesia, and are now known as RGMC disorders within the wider respiratory ciliopathy class. So, it seems that PCD is just one of a wider group of respiratory ciliopathies where a better understanding of the heterogeneous nature of this group of conditions will lead to more accurate and descriptive terminology.

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**Conflict of interest**

None declared.

**References**