PRIMARY CILIARY DYSKINESIA: CURRENT STATE OF THE ART

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Abstract Primary ciliary dyskinesia (PCD) is usually inherited as an autosomal recessive, and in classical form presents with upper and lower respiratory tract infection, and mirror image arrangement in around 50% cases. It is becoming appreciated that dysfunction of cilia is implicated in a wider spectrum of disease, including polycystic liver and kidney disease, central nervous system problems including retinopathy and hydrocephalus, and biliary atresia. Cilia are complex structures, containing more than 250 proteins, but recent studies have begun to locate PCD genes scattered throughout the genome. Screening tests for PCD include nasal nitric oxide and *in vivo* tests of ciliary motility such as the saccharin test. Specific diagnosis requires examination of cilia by light and electron microscopy, with epithelial culture in doubtful cases. This is only available in supra-regional centres, which have recently been centrally funded by NCG in order to provide a comprehensive, free diagnostic service in England. Treatment is unfortunately not evidence based, since there are no randomised controlled clinical trials in this condition, and recommendations are largely extrapolated from cystic fibrosis and other suppurative lung diseases. This review covers the recent advances in the field, especially focussing on the genetics and biophysics of cilia, together with recent diagnostic and therapeutic advances.

Primary ciliary dyskinesia (PCD) is an inherited (usually autosomal recessive) disease typically characterised by chronic upper and lower respiratory tract infection, and in nearly 50% cases, mirror image arrangement. The triad of mirror image arrangement, bronchiectasis and sinusitis is known as Kartagener's syndrome. It is caused by a congenital reduction or absence of the function of the normal mucociliary escalator, an important primary airway defence mechanism, due to impaired or absence of ciliary beating. PCD must be distinguished from the numerous infective and other conditions which can cause ciliary dysfunction. Clinically, PCD is manifest by chronic bronchial sepsis and bronchiectasis, sinusitis and chronic secretory otitis media [1]. It is often diagnosed late [2], after a period of chronic ill health. In this manuscript, we review the latest knowledge about the basic science and clinical management of the condition, including the view from a parent of two children with PCD. We believe that this review is particularly timely as it coincides with the development of a diagnostic screening service for PCD in England. The National Commissioning Group (NCG) is funding centres in London, Leicester and Southampton to utilize state of the art diagnostic methods to increase the numbers of patients diagnosed. Since the test is centrally funded, the referring centre incurs no costs. Early diagnosis of PCD is likely to have significant effect on both short term and long term morbidity [5, 6], but this cannot be proven by any practical study. The development of proper diagnostic services will also lead to the establishment of a national registry, which should facilitate the performance of randomised controlled trials.

If the 1990s was the decade of nitric oxide (NO), then the next ten years may well be the decade of the cilium. Initially they were regarded as merely a part of the initial airway defences, as an essential part of the mucociliary escalator, but it is now clear that they are important in many more biological processes. There have been many advances in the knowledge of the basic science, genetics and clinical manifestations of ciliary disease. New diagnostic techniques discussed below include genetic analysis, and florescence microscopy. Cilia are implicated in polycystic hepatic and renal disease, hydrocephalus, biliary atresia, retinal degeneration, and rare syndromes such as Bardet-Biedl, Alstrom and Meckel-Gruber and oro-facial-digital syndromes in addition to nephronophthisis [5, 6]. This review focuses on PCD, emphasising recent advances, and in particular, the expanding concepts of ciliopathology. As well as the motile cilia responsible for airway defences, sensory cilia are important in many aspects of embryonic development. Motile nodal cilia have long been known to be responsible for normal right-left orientation, but there may be important roles also in the development of the kidney, neural tube, the cochlear apparatus, the central nervous system and the limb bud [5, 6]. PCD, or more accurately, ciliopathy, as with cystic fibrosis (CF), has become a truly multisystem disease.

1.   Biophysics of Cilia

Over 250 proteins make up the classical ciliary axoneme that forms a "9+2" arrangement where 9 peripheral doublets surround the central microtubular pair (7) (Figure 1). The outer and inner dynein arms are attached to each A subunit of the peripheral microtubular doublet with nexin links connecting these adjacent doublets. Peripheral doublets are connected to the central microtubular pair by the radial spokes (8, 9). The central microtubular pair does not have dynein arms.

ATP and the ATPase activity of the dynein arms generate the force required for ciliary beating and bending. Hydrolysis of ATP causes conformational changes in the dynein arm attaching sub fibres A and B, which causes sliding of the adjacent microtubules. The outer dynein arms control the beat frequency and the inner dynein arm the wave form. The 'switch point' hypothesis has been put forward to explain the bending of the cilium (10). During the power stroke, the dynein on the microtubules from one half of the cilium are active, while during the recovery stroke, there is activation of dynein arms from the other half of the cilium. This hypothesis fits with recent high speed video analysis of respiratory ciliary motion showing that cilia simply beat forwards and backwards in the same plane without a side wards recovery sweep (11). Similar analysis has also defined the beat patterns associated with different ultrastructural ciliary defects responsible for PCD as described in a later section.

Situs inversus is seen in up to 50% of patients with PCD and may be explained by abnormal movement of the nodal cilia during embryogenesis. Nodal cilia lack central microtubules resulting in a rotating beat pattern. This beat pattern provides a nodal flow in a leftwards direction across the node and it is the direction of this flow that dictates situs (12). If this flow is not present due to ciliary defects causing PCD, abdominal and thoracic situs inversus will be seen in 50% of cases.

2.   Genetics

PCD has an estimated prevalence of 1:15-30,000 live births, although this is likely be an underestimate, as clinical underdiagnosis is common [1]. PCD is usually an autosomal recessive trait, however occasional instances of X-linked transmission have also been reported [13, 14]. The prevalence of PCD is increased in certain inbred or isolated populations and families; affected parents and children may be found in such populations [15].

A major goal in the study of PCD is to determine the disease-causing genes. The molecular complexity of the axonemes of cilia and sperm mean that there are a large number of potential candidate genes, and two main approaches been employed in the search for the genes that cause PCD: genetic linkage analysis, often making use of inbred family groups, or mutation screening of candidate genes [16-18]. It is now established that PCD is genetically heterogeneous, caused by mutations in a number of different genes [17-19]. This had been predicted long before the advent of molecular genetics, from the variable clinical course and range of ultrastructural defects. So far two genes encoding dyneins, structural components of the ciliary axoneme, have been confirmed to cause PCD. The *DNAH5* gene located on chromosome 5p15.2 encodes a heavy chain dynein and the *DNAI1* gene on 9p13.3 encodes a dynein intermediate chain. DNAH5 and DNAI1 are both subunits of the outer dynein arm (ODA), which is a highly evolutionarily conserved structure [20]. Defects in *DNAH5* and *DNAI1* in PCD patients are exclusively associated with ODA defects (Figure 1). The genes underlying PCD and motile ciliary dysfunction are summarised in Table 1.

In order to determine their contribution to the disease, large-scale gene sequencing of *DNAH5* and *DNAI1* has been undertaken. From a total of 134 unrelated families screened, 28% of families (38/134) had mutations in *DNAH5* [21, 22]. In the 65 of the 134 families confirmed to have ODA defects, *DNAH5* mutations were identified in 49%. The *DNAI1* gene contributes to fewer cases of PCD. A total of 226 unrelated PCD families have separately been screened and 10% (22/226) had *DNAI1* mutations [23-26]. Of families with a confirmed ODA defect, 14% (19/134) had *DNAI1* mutations. Overall, this data suggests that together *DNAH5* and *DNAI1* account for a large proportion of PCD cases, up to 38%, and that a potential 63% of families with ODA defects carry mutations in either gene. However, it should be noted that these prevalence estimates for particular genes depend on recruiting a representative sample of the PCD population; for example, if a particular gene caused mild disease, which was often undiagnosed, then the contribution of that gene to causing the disease would be underestimated.

Two additional dyneins have been associated with PCD. Homozygous mutations in the *DNAH11* gene on chromosome 7, which encodes an axonemal heavy chain, were determined in an individual with situs inversus but no apparent ciliary ultrastructural defects [27]. This patient had uniparental disomy of chromosome 7, and had cystic fibrosis due to homozygous mutations in the *CFTR* gene. Therefore a causative link between *DNAH11* defects and Kartagener syndrome is suggested, however the overlap between symptoms of these two diseases, and the lack of an ultrastructural defect in the patient, means that the contribution of *DNAH11* to PCD is not yet clear. Proteomic studies have also implicated DNAH7, a dynein heavy chain associated with the inner dynein arm complex, in PCD. In ciliated epithelia cells from an individual with PCD and an ultrastructural defect of absent inner arms, the DNAH7 protein was found to be expressed but not assembled into the cilia, remaining cytosolic. No mutations in the *DNAH7* gene were detected, so the possibility that this may be an indirect effect arising from mutations in another gene could not be excluded [17].

Genetic linkage studies have found significant evidence for several other PCD loci, notably those on chromosomes 15q13–15, 15q24–q25, 16p, 19q, and support for an additional chromosome 1 locus [18, 28, 29]. Although these loci have been mapped, the causative gene remains to be determined. A number of candidate genes for PCD with suggestive roles in ciliogenesis have been selected for mutation screening in PCD patients, but excluded from playing a role in the disease [17].

Certain features of PCD such as situs inversus may be observed in other disorders that are more closely associated with dysfunction of the non-motile or sensory cilia. Sensory cilia are more ubiquitous than motile cilia, being found in most tissues of the body including in the kidney, retina and embryonic node, and their dysfunction results in a broad range of phenotypes including renal and retinal problems [6]. The exact overlap between the functions of PCD-causing motile cilia and the non-motile sensory cilia is not yet known, but it is possible that these structures could share common components, or that motile cilia are more widespread in the body than previously thought. This putative connection is currently clearest for retinal function, since several cases are known of individuals who have features consistent with PCD in addition to retinitis pigmentosa or to Usher syndrome [14, 30]. Mutations in the X-linked retinitis pigmentosa GTPase regulator gene (RPGR) gene have recently been identified in two brothers with PCD and retinitis pigmentosa [31], and there are a number of other RPGR mutations [14].

It was recently reported that mutations in the *OFD1* gene cause an x-linked recessive mental retardation syndrome associated with macrocephaly as well as features of PCD comprising immotile nasal epithelial cilia and respiratory tract infections. It was suggested that the mental retardation could arise from defect neuronal primary cilia in the brain [32]. Mutations in *OFD1* had previously only been found to cause oral-facial-digital type 1 syndrome, a condition of craniofacial and digital abnormalities and polycystic kidneys that is transmitted as an X-linked dominant condition with lethality in males. The reasons for striking differences arising from different *OFD1* mutations are as yet unclear.

Benefits arising from genetic analysis of PCD

Discovery of the significant contribution of the *DNAH5* and *DNAI1* genes to PCD with outer dynein arm defects has significantly contributed to improved understanding of the molecular basis of PCD. This is encouraging for diagnosis since the majority of PCD patients have defects involving the outer dynein arm either as a combined inner and outer dynein arm defect or an isolated outer dynein arm defect [4, 33].

However, currently the molecular defect in the majority of PCD cases remains unknown. In the post-genomic era, the search for genes causing PCD should be aided by an emerging catalogue of information relating to the diverse structure and function of cilia and flagella. The components of the human ciliary outer dynein arm have been almost completely defined [20], and proteomes have been characterised for the entire cilium or flagella in humans and model organisms [34, 35]. Computer-based bioinformatic analysis has also been successful in defining cilia components [36].

Determination of the genetic basis of PCD provides benefits of immediate clinical relevance, and to an increased understanding of the underlying biology of cilia dysfunction that should aid future medical developments. Of primary importance, characterisation of a PCD patient's underlying gene mutation allows a definitive molecular diagnosis and confirmation of their disease status. Once the causative gene is defined, DNA-based testing can be used to provide a rapid, simple and economic screening method applicable to large numbers of PCD cases using high-throughput techniques. This allows an estimate to be made of the overall contribution of that gene to the disease, to assess its relative medical importance. Within the family of affected individuals, a DNA-based test

also allows definitive carrier screening to be done, which is not possible with any of the current diagnostic techniques, since carriers do not display an overt measurable PCD phenotype.

Genetic studies enable a more accurate estimation of the extent of heterogeneity underlying PCD, and of the relative individual impact of the known genes such as *DNAH5* and *DNAI1* to the disease. When the causative gene is as large as for dyneins, mutation screening by DNA sequencing can be prohibitively expensive for diagnostic work, but polymorphic genetic markers near the gene may be used to provide a cost-effective preliminary screening method to determine whether mutation screening is advisable. Such large-scale 'pre-screening' linkage methods have been devised for both *DNAH5* and *DNAI1* [22, 26]. Moreover, for *DNAH5* and *DNAH1* it is clear that certain mutations are more prevalent due to genetic founder effects or mutation hotspots, and this information can be used to focus patient screening and cut costs. In *DNAH5*, mutations are clustered in 5 of the 79 exons of the gene, so these can be screened first. In 53% of patients with *DNAH5* mutations, at least one mutation within these 5 exons was detected. One mutation, 10815delT, is especially prevalent in the North American Caucasian population [22]. Similarly, diagnostic screening of the *DNAI1* gene can be focussed since 57% of *DNAI1* mutations detected in patients are identical, being a common splice site mutation, IVS1+2_3insT. In addition, three *DNAI1* exons are also mutation hotspots [26].

Defining the genes that cause PCD facilitates many functional studies that reveal information about the disease. Characterisation of a mouse model with a defect in the *DNAH5* gene homologue has been informative for the origins of the hydrocephalus that is sometimes seen in association with the human disease [37].

Additional techniques of diagnostic importance may be facilitated by molecular genetic studies, and these can also impact on the understanding of the biological basis of the disease. A novel diagnostic technique that has arisen from molecular studies of PCD is high-resolution immunofluorescent imaging of respiratory cells from patients obtained by nasal brushing biopsy [22, 38]. Fluorescent antibodies that specifically detect DNAH5 and other ciliary axoneme protein components have been used to determine the nature of the defects in PCD patients since this method allows visualisation of the entire axoneme and its components. From this work it has emerged that human cilia and sperm contain at least two different types of outer dynein arm, which are distributed differently along the length of the axoneme. In patients with DNAH5 defects, either the protein is expressed in cells but is not correctly localised in the cilia, or it is mis-localised within the axoneme. Furthermore, it is apparent

that these tools may be more widely applied, to detect axonemal defects in patients who have mutations in genes other than *DNAH5* [22, 38].

This work has provided important functional information about the domains within DNAH5 that are important for its normal function, since specific gene mutations are associated with different axonemal defects. A genotype-phenotype correlation of this nature has also been shown at the ultrastructural level, since a certain *DNAH5* mutation is associated with a less severe loss of the outer dynein arm [39].

As more PCD genes and mutations are defined in future, such studies are likely to reveal further important functional information on PCD genes, and on the links between the clinical symptoms and the underlying genotype. There is also potential for medical impact, including novel pharmaco-gene therapy tools. Aminoglycoside antibiotics have been used to over-ride the effects of certain cystic fibrosis gene nonsense mutations, and since a large proportion of *DNAH5* patients have nonsense mutations as well, these defects might also be amenable to drug-mediated rescue [22].

3.   **When to suspect the diagnosis of PCD**

PCD may be suspected because of respiratory disease or mirror image arrangement (conventional diagnostic clues), or because a non-respiratory condition such as biliary atresia has been diagnosed, and consideration needs to be given as to whether this is part of an extended spectrum of ciliopathology that includes PCD (associated diagnosis). It is helpful to divide the presentations of PCD by patient age ranges [1].

The diagnosis of PCD is frequently made late [2], in part because the common presentations (rhinitis, secretory otitis media, cough) are common in the general paediatric population [40]. Above all, if PCD is to be diagnosed, general paediatricians must be alert to the condition, and take a careful, focussed history. Although there is no proven evidence that early diagnosis is beneficial, in one series, bronchiectasis at diagnosis was only seen in those diagnosed over age 4 years [2], and in a second series, lung function at diagnosis was significantly worse in those diagnosed in adult life [5]. This is at least supportive evidence that early diagnosis is beneficial.

There are no data to weight the significance of particular symptoms or construct Receiver Operating Curves, but the common diagnostic features of PCD are listed in Table 2 as a guide. A combination of upper and lower airway symptoms is usual.

3.1 Conventional diagnostic clues

3.1.a. *Antenatal presentation* The finding of heterotaxy (usually mirror image organ arrangement) on antenatal ultrasound scanning should lead to consideration of the diagnosis, although most babies with mirror image arrangement do not have PCD. In the future, antenatal genetic diagnosis (above) on chorionic villus sampling may be offered.

3.1.b. *Presentation in the newborn period*

➢ Continuous rhinorrhea from the first day of life; the significance this is completely different from intermittent rhinitis with viral colds, starting at a few weeks of age

➢ Respiratory distress or neonatal pneumonia with no obvious predisposing cause

➢ Mirror image arrangement (but see 3.1.a above)

➢ Diagnosis by screening because of a positive family history

3.1.c. *Presentation in childhood*

➢ Chronic productive or 'wet' cough. Clearly, other causes such as cystic fibrosis will likely need to be ruled out first by appropriate testing, before referral for ciliary function, unless the child also has mirror image arrangement or other features suggestive of PCD. This history correlates well with the presence of endobronchial secretions seen at bronchoscopy in a wide range of conditions [41]; chronic wet cough for more than eight weeks in a child should always be investigated; chronic cough must be distinguished from recurrent acute cough, usually with a viral cold, which is of less significance. Isolated dry cough is not likely to be due to PCD.

➢ Atypical 'asthma', non-responsive to treatment, in particular when a wet-sounding cough is present

➢ 'Idiopathic' bronchiectasis

➢   Diagnosis by screening because of a positive family history, or recent diagnosis in a relative. This accounted for 10% cases in one series [2], underscoring that there are mild phenotypes, and that the diagnosis is often missed

➢   Rhinosinusitis – Daily rhinitis is typical, without remission; and sometimes in older children severe sinusitis despite multiple surgical procedures; although nasal polyps are commonly reported in some series, in our experience, nasal polyps are rare. It should be noted that CF is a commoner cause of nasal polyposis.

➢   Chronic otitis media with effusion - Typically, if tympanostomy (ventilation tubes) are inserted, there is usually a prolonged smelly ear discharge for weeks not responding to treatment and with no improvement in hearing.

3.1.d. *Presentation in adolescence and adult life*

➢   As above, for childhood

➢   Ectopic pregnancy and subfertility in women [4, 42]

➢   Male infertility (immotile sperm – but 50% of PCD males are fertile [43], see below)

3.1.e *PCD as an associated diagnosis*

In addition to the above, PCD should be at least considered when the following diagnoses are made, in particular if there is a family history of more than one of these conditions, or if the patient has associated ENT or lower respiratory symptoms. Further information can be found in recent reviews [5, 6].

➢   Complex congenital heart disease, especially with disorders of laterality [44]

➢   Polycystic kidney and liver disease

➢   Hydrocephalus

➢   Biliary atresia

➢   Severe oesophageal disease (oesophageal atresia, severe reflux) [44]

➢   Retinal degeneration, including retinitis pigmentosa

4.   **Making the diagnosis**

Eliminating other conditions: Although a child with absolutely typical features of PCD will appropriately be referred straight for ciliary function studies, in most cases testing for commoner conditions which enter the

differential diagnosis will precede referral. The nature of the tests carried out will be determined by the individual clinical scenario.

Screening for PCD: The most popular screening tests for PCD are the saccharin test and the measurement of nasal nitric oxide (NO). The saccharin test allows a gross assessment of mucociliary function and has been used to select patients for full diagnostic testing for PCD. It involves placing a microtablet of saccharin on the inferior turbinate and recording the time taken for the subject to taste it. It is difficult to perform and can be unreliable in children [45]. An abnormal result must be confirmed by further tests (below). In PCD nasal and exhaled nitric oxide is low [46,47] for reasons that are still unclear and measurement of nasal NO, which is more discriminatory than exhaled NO, as a screening test is very attractive [48]. Nasal NO has been shown to be useful screening test in children over the age of 5 years. However, as there is occasional overlap with other respiratory conditions such as CF, confirmation of the diagnosis of PCD will always require further diagnostic testing [1, 4, 47, 49]. Interestingly, obligate PCD carriers also have low nasal NO [4], but the overlap with normal is too great to allow this to be useful for carrier screening.

Diagnostic testing: A combined approach to the diagnostic testing of patients suspected of PCD has been adopted to reduce the number of cases that are incorrectly diagnosed or missed. Diagnostic testing may include:

- ciliary beat frequency measurement
- ciliary beat pattern analysis
- electron microscopy of ciliary ultrastructure
- measurement of ciliary disorientation
- cases of suspected ciliary aplasia, ciliary disorientation, an unusual ciliary phenotype and in cases where secondary tissue damage renders diagnosis impossible, cell culture with re-growth of the ciliated epithelium is undertaken.

Strips of ciliated nasal or bronchial epithelium obtained by brushing the nose or airways are used to assess ciliary structure and function. The nasal brushing is an out-patient procedure, which takes a few seconds only. The brushing causes minor discomfort, and very occasionally, minor epistaxis. The sample may be taken by an appropriately trained paediatrician in a general hospital, and couriered to the NCG centre, by prior arrangement.

Assessment of beat pattern by slow motion analysis using high-speed video photography in conjunction with beat frequency measurements has been shown to identify defects that may have been missed by analysis of beat frequency alone [50]. For example it has been shown that certain ultrastructural defects responsible for PCD such as ciliary transposition, central microtubular agenesis and in some cases of inner dynein arm defects, ciliary beat frequency maybe within the normal range. However, all of the cilia from such patients show a typical dyskinetic beat pattern on slow motion video analysis [33]. An extensive range of normal values for children and adults using high-speed video analysis have now been published [50].

Some groups have diagnosed PCD in patients who had clinical symptoms consistent with PCD and a slow ciliary beat frequency despite a normal ciliary ultrastructure [51]. It is still to be determined whether such patients have an inherited defect affecting function that can not be seen on electron microscopy. Whilst such patients receive clinical treatment that is similar to those with PCD they can not be confidently diagnosed as having PCD.

Examination of ciliary ultrastructure remains the definitive test for PCD. There are a number of ultrastructural phenotypes that can cause PCD and these have been reviewed elsewhere [52]. The majority of cases of PCD are due to a lack of outer dynein arms or a combined lack of both inner and outer dynein arms. In such cases the cilia are static or just flicker. Other defects include isolated lack of inner dynein arms or lack of inner dynein arms combined with a radial spoke defect. These defects cause cilia to beat with a stiff beat pattern and usually a reduced beat frequency. Rarer defects include those of the central microtubular pair, such as transposition or central microtubular agenesis [53] both of which have a circular beat pattern. The currently known range of ciliary ultrastructural alterations is shown in Table 3.

Cilia on the same cell line up in the same direction with cilia in adjacent cells beating in the same direction to allow movement of mucus. A condition described as ciliary disorientation has been proposed to be a cause of PCD where cilia on the same cell tend to point in different directions resulting in ineffective movement of mucus [54]. It is likely, however, that the vast majority of cases are secondary in nature. In most cases ciliary disorientation is not seen when biopsies are repeated following a period of good health.

On occasions the diagnosis of PCD may be difficult due to damage to the respiratory epithelium secondary to infection or inflammation. To minimise secondary damage we do not take samples from patients who have had a

cold with in the last month. If secondary damage is still present making the diagnosis difficult or if the patient is suspected of having an unusual phenotype of PCD cells obtained at biopsy may be cultured. Cells grown following biopsy loose their ciliated phenotype. By exposure of these cells to an air interface or by continual movement of spheroids formed by these cells a fully differentiated ciliated epithelium can be obtained. Culture systems have been advocated by Jorrisen and colleagues who argue that secondary damage is virtually absent after ciliogenesis in a suspension culture [55]. This technique is only necessary in cases of doubt, and is not mandatory for the diagnosis of clearcut cases of PCD.

Overall, around 10% of initial tests are equivocal. A second brushing reduces the figure to 5% (unpublished data). In the absence of another gold-standard test, it is impossible to calculate false positive and false negative rates. The most important cause of false positive tests are post-viral changes, and if there is any doubt, the tests should be repeated before confirming the diagnosis.

5.   **Respiratory Management**

There is no cure for PCD, and the aim of management is to prevent progression of disease. The optimal balance of care between regional centre and district hospital has not been determined, and is likely in practice to be driven by geography, and the facilities available locally. If the family live reasonably near a centre with special expertise in PCD, a shared care arrangement, analogous to CF clinics, might be appropriate. They should be looked after only in a setting where multidisciplinary review is available. Appropriate medical therapy has been shown to prevent deterioration in lung function [2, 3]. Management is not evidence based, and perforce relies on the experience of large clinical units dealing with PCD patients, or data derived from studies involving patients with CF or other causes of bronchiectasis. Since the pathophysiology may be different in these conditions, this may lead to mistakes. Respiratory management consists of:

➢   Regular respiratory monitoring

➢   Airway clearance by combinations of physiotherapy and physical exercise

➢   Aggressive treatment of upper and lower airways infections to prevent further progression of the disease.

Respiratory monitoring Recommendations are based on CF clinic practices. In some centres, specialist PCD clinics, including respiratory, ENT and general care at the one visit, have been set up. In addition to general paediatric care, monitoring should include pulse oximetry and age appropriate lung function tests. Regular sputum or cough swab cultures should be performed [1]. Chest radiographs are probably relatively insensitive. High resolution computed tomography of the lungs is used to define the extent of bronchiectasis, and can be used to monitor the progression of the disease. However, there is even less evidence base that regular CT scans affect outcome in PCD than there is even for CF, and the potential high lifetime cumulative radiation exposure should be considered.

Medical interventions PCD lung disease is progressive if not adequately treated, and early therapeutic interventions result in better symptom control [56]. Randomised controlled trials would greatly aid in determining the best respiratory management of PCD.

The common infecting organisms in children are *Haemophilus influenza* and *Staphylococcus aureus* but *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and non-tuberculous *Mycobacteria* have also been reported, although these are usually a feature of disease in adults [4]. There is no evidence to recommend or otherwise the use of prophylactic antibiotics. If repeated courses of oral antibiotics are required, prophylaxis should be considered. High-dose oral antibiotics should be given at the first sign of worsening respiratory symptoms or deterioration in lung function. Where possible, antibiotics should be chosen on the basis of sputum or cough swab culture and sensitivity. Occasionally broncho-alveolar lavage (BAL) may be necessary to obtain adequate specimens in non-sputum producing children.

If *Pseudomonas aeruginosa* is isolated, most clinicians would prescribe an eradication regime similar to those used in CF, for example inhaled colistin and oral ciprofloxacin. For established chronic infection with this organism, long term nebulised anti-pseudomonal antibiotics are considered. If symptoms do not respond to oral antibiotics, then intravenous therapy is given. A regular programme of three monthly intravenous therapy should be considered in patients who are not doing well.

There is even less evidence for other therapies. Regular bronchodilators do not lead to worsening airway reactivity, but they are not particularly effective. No trials have been done which would support any anti-inflammatory therapy, a different situation from CF. Anecdotally, many PCD patients have previously been mis-diagnosed as asthmatic, and given asthma therapy, which is ineffective. It is very important to be pro-active in discontinuing medications which have been started in error, and which are not effective. The role of nebulised

recombinant human deoxyribonuclease (Dnase ™) in PCD patients remains unproven. However, anecdotally some patients show an improvement in respiratory symptoms. Use of nebulised normal saline may also be effective in augmenting mucus clearance. A trial of aerosolized uridine-5'-triphosphate has been shown to enhance mucus clearance in a small number of PCD patients. However more data is needed in order to recommend its use in PCD patients on routine basis [57]. There are theoretical attractions to the inhaled use of the NO synthase substrate arginine in PCD, in particular following a study showing improvement in lung function in CF [58], but at the moment this can only be recommended in the context of a randomised clinical trial. PCD patients should receive all childhood immunisations, and also influenza A, and *pneumococcal* immunisation regularly. Complications of bronchiectasis and chronic lung disease become more prominent with age [4]. The role of lobectomy in advanced bronchiectasis is similar to that in other aetiologies, and can rarely be recommended. A few patients require lung transplantation. Overall, the medical therapy of PCD is more successful than CF; once treatment is instituted, even if there is markedly impaired lung function at diagnosis, the condition should be stabilised [3,4].

6.   **Airway inflammation**

There has been surprisingly little work on airway inflammation in PCD. Examination of spontaneously expectorated sputum reveals predominantly neutrophilic cytology, similar to cystic fibrosis (CF) [59]. Sputum neutrophils correlate with cough frequency, measured objectively. There is evidence of increased oxidative stress within the airways, as shown by increased 8-isoprostane in exhaled breath condensate [60]. BAL studies show neutrophilia, and elevated hyalurin, fibronectin and albumin, in both the airway and alveolar fluid fractions [61]. Eosinophilic inflammation is not a feature, as might be expected from the low exhaled NO levels.

Given that CF is characterised by relentless deterioration, and PCD by stability, one might predict that the inflammatory response would be greater in CF. This seems not to be the case; mucus biophysical and transport properties are the same, and sputum interleukin (IL)-8 actually three times higher in PCD [62]. The paradox of a better prognosis, despite the same or worse levels of airway inflammation and similar infecting organisms, has not been resolved.

Care should be taken therefore in extrapolating the role of anti-inflammatory treatments from CF to PCD. Anti-inflammatory strategies such as prednisolone and ibuprofen have been shown to be effective in CF, but need to be the subject of clinical trials in PCD before they could be recommended.

7.   **Physiotherapy**

Research into the effectiveness of different chest physiotherapy regimens in PCD is scarce and findings variable due to inconsistencies in methodologies and outcome measures. To date, there is no evidence to support or refute the efficacy of any one particular airway clearance technique in the physiotherapy treatment of PCD. The physiotherapy options available to the patient, parent and therapist therefore should be tailored to the individual. This will be guided by the clinical picture and expert clinical best practice. The parents of babies and infants should be shown gravity assisted positioning (GAP) and intermittent chest percussion. The optimum duration and frequency in the healthy baby is not known; the regime is intensified during infective exacerbations. GAP should include positions for lower, middle and lingula areas and in infants the apical segments of the upper lobes. Jumping and blowing games should be encouraged as early as possible and replaced by the active cycle of breathing technique (ACBT) as the child becomes older [63]. GAP can used with chest percussion and shaking administered by the parent or carer during the ACBT in younger children, and independence encouraged in older children and adults. Alternative airway clearance techniques include the PEP (positive expiratory pressure) mask or mouthpiece which can also be used with GAP [64] and oscillating PEP devices such as the Flutter, best used in sitting [65], or Acapella which also allows GAP [66]. Importantly, as with all airway clearance techniques, treatment should be based on a thorough assessment. Continued evaluation of the effectiveness of physiotherapy is essential. Factors considered important determinants of adherence to treatment must also be considered [56]. The effect of physical exercise on airway clearance in PCD has not been fully investigated but may help sputum clearance. Exercise has been shown to be a better bronchodilator than the use of a β-2 agonist in PCD [67]. It is expert opinion that exercise must be encouraged from an early age in PCD to promote general health and wellbeing. A review of inspiratory muscle training in bronchiectasis found some benefit in improving exercise endurance capacity but whether this is true in PCD is not known [68]. Some PCD patients require nebulised therapy, in which case the physiotherapist, or sometimes the respiratory Nurse or technician, will check techniques, including cleaning and maintenance of the equipment.

8.   ENT management

Abnormal muco-ciliary clearance within the upper airway in PCD leads to otitis media with effusion (OME) and chronic mucus pooling within the nasal cavity and paranasal sinuses.

OME is universal amongst infants with PCD. The condition is common in all children but in PCD is often severe. The natural history is of spontaneous improvement at around the age of 13 [69]. The condition presents with either hearing loss and speech delay or as recurrent acute otitis media (RAOM). Ventilation tube insertion is associated with a high level of post-operative mucoid otorrhoea and tympanic membrane perforation [70]. This operation should be avoided if at all possible, and certainly should not be performed before consultation with a surgeon familiar with the management of PCD. The best way to treat otorrhoea is cleaning the ear, avoiding water in the ear and topical non-ototoxic antibiotic eardrops (which should have anti-*pseudomonal* activity, for example ciprofloxacin). All PCD children should have regular hearing tests at least until adolescence. If necessary, hearing aids are recommended until spontaneous resolution of hearing loss. For milder hearing loss, educational support may be all that is needed. Speech delay should be promptly managed with speech therapy. RAOM usually improves either spontaneously in infancy or as result of prolonged systemic antibiotic treatment.

Chronic mucoid rhinorrhoea is present in all patients. Reasonable periods free of nasal discharge may be achieved by using saline nasal douches. Long term topical nasal steroids are of uncertain benefit to control rhinorrhoea but will treat any degree of rhino-sinusitis which has developed as a result of mucous pooling within the upper airway. Recurrent acute or chronic sinusitis (presenting with increased nasal obstruction, discharge and facial pain) is rare. It may be managed with prolonged systemic antibiotics but occasionally requires endoscopic sinus surgery to relieve retained, chronically infected secretions. Nasal polyps are virtually never encountered in PCD in our experience. Adenoidal hypertrophy and obstructive sleep apnoea secondary to adeno-tonsillar hypertrophy is also not a typical association with PCD but if present should be surgically managed as indicated, though with special consideration for the potential risks of pneumonia in the post-operative period. Chest physiotherapy and antibiotic prophylaxis should be considered whenever a PCD child needs a general anaesthetic.

9. Other systems

Another major feature of PCD is subfertility. Around half of men with PCD are infertile, however many men have fathered children without any medical intervention [43]. Infertility in men with PCD is entirely attributed to the poor motility of the sperm and usually there is no evidence of abnormal spermatogenesis (Table 1). There are several reports of birth of healthy children after intracytoplasmic sperm injection in case of male infertility. Females are known to have reduced fertility with higher risk for ectopic pregnancy when compared with the general population [42].

Rarer association of PCD are described above. The management of these associated conditions is unaffected by coincident PCD, except in so far as respiratory issues must be remembered if a general anaesthetic is required.

10. Nursing perspective

In recent years the role of the nurse specialist in chronic disease management has broadened and they are now recognised as valuable members of the multidisciplinary team (MTD), contributing to the quality of patient care [71]. Very little has been published on the role of the paediatric nurse specialist in the care of PCD children. The respiratory nurse may be part of the initial diagnostic process. The nurse should be involved in pre-test counselling. After diagnosis, the psychological and practical support provided by the respiratory nurse is similar in PCD to that of other chronic diseases. This includes clinical management, education, social care and counselling [72]. The Nurse specialist is ideally placed to become the key worker, providing a link between hospital, home, the school, and primary care [73]. Children with PCD must be encouraged to lead a normal life and should be encouraged to participate in all usual activities at home and school including sport.

The nurse specialist should work in partnership with the family to determine and achieve treatment goals. The nurse should be able to provide information and answer questions. At the same time possible misinformation from books and unregulated websites can be put into perspective and corrected. The nurse should have access to patient friendly information and can help determine how best to meet individual information needs – neither flooding the family with unwanted literature nor withholding information sought [73]. The nurse can put interested families in touch with the PCD support group, which provides up to date information about the disease and its management as well as providing an excellent support network (www.pcdsupport.org.uk). The nurse will also

make referrals to other members of the team; co-ordinate clinic visits; liaise with shared care hospitals; and arrange admissions as necessary. Within the clinic, the respiratory nurse will check techniques with drug delivery devices, provide education about PCD and give advice about smoking. In sensitised patients, advice about allergen avoidance in the home environment should also be part of routine. A school visit may be helpful if there are educational difficulties. The nurse should be able to assist with benefits claims, including Disability Living Allowance.

11. **Summary and Conclusions**

Ciliopathy has now become recognised as a multisystem disease, of which PCD is an important subgroup. The current challenge for paediatricians is to increase diagnostic awareness, and make early and appropriate referrals to the new diagnostic services, before the child has sustained lung damage. Next, we need to find ways through multicentre collaborations of increasing the evidence base for treatment. In the future, basic science may open the way for curative treatment, as opposed to mere management of symptoms, for example inhaled arginine or pharmacological over-riding of premature stop codons. However, none of this will happen unless as a routine, a properly focused respiratory history is taken, and the diagnosis of PCD considered outside the context of specialist clinics.

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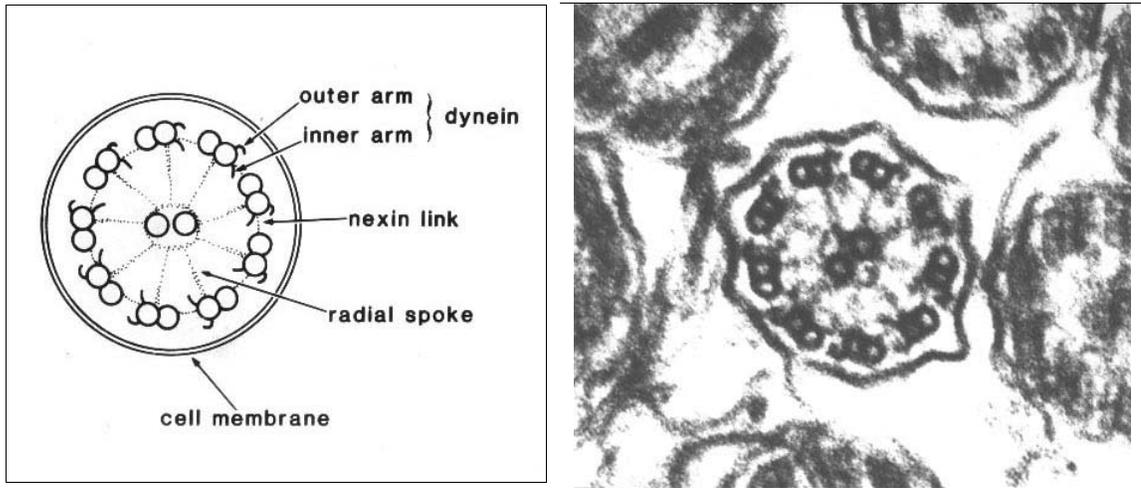**Figure 1.** Schematic diagram of the basic structure of cilia

Table 1: Genes associated with PCD and motile cilia dysfunction

Disorder	Situs inversus	OMIM #	Gene and location	Protein product	Cellular location	Function	Nature of Defect	Contribution to PCD spectrum
PCD	Yes	608644	*DNAH5*, chromosome 5p15	Dynein heavy chain 5	9+2 axoneme (motile cilia, sperm)	Outer dynein arm component	Cilia dysmotility with absent outer dynein arm, oligozoospermia and immotile sperm [76]	28% of PCD cases (49% of PCD with known outer dynein arm defects) [22]
PCD	Yes	242650	*DNAI1*, chromosome 9p21-p13	Dynein intermediate chain 1	9+2 axoneme	Outer dynein arm component	Cilia dysmotility with absent outer dynein arm [23]	10% of PCD, (14% of PCD with known outer dynein arm defects) [26]
Situs inversus and likely PCD	Yes	270100	*DNAH11*, chromosome 17p21	Dynein heavy chain 11	9+2 axoneme	Outer dynein arm component	Cilia dysmotility with normal cilia ultrastructure [27]	One case
PCD and retinitis pigmentosa	No	300455	*RPGR*, chromosome Xp21	Retinitis pigmentosa GTPase regulator	Connecting cilia of retinal photoreceptors & transitional zone of 9+2 axoneme [74]	Unknown. Proposed role in photoreceptor protein transport	Retinal degeneration, cilia dysmotility with numerous ultrastructural defects [31]	One case proven PCD, three more cases likely as discussed in [14]
Mental retardation and ciliary dysmotility	No	300170	*OFD1*, chromosome Xp22	Oral–facial–digital type 1 syndrome protein	Centrosome, cilia basal body [75]	Unknown. Required for	Developmental delay, macrocephaly, cilia dysmotility [32]	One case

Table 2 Features leading to the diagnosis of PCD, modified from [2]. Most children had multiple symptoms, typically a combination of upper and lower airway disease.

Presenting feature	Number (percent) of cases (n=55 total)
Significant neonatal respiratory distress	37 (67)
Abnormal situs	38 (69)
Cough most days	46 (84)
Sputum production	24 (44)
Rhinorrhea from the newborn period	42 (76)
'Wheeze'	16 (29)
Sinusitis	6 (11)
Serous otitis media	28 (51)
Hearing loss	14 (25)
Diagnosis made in a sibling	6 (11)

Table 3: Ultrastructural defects associated with primary ciliary dyskinesia include

- No outer dynein arms
- No outer and inner dynein arms
- No inner dynein arms
- Short outer dynein arms
- No inner dynein arms with a radial spoke defect
- No central microtubules
- No basal bodies and no cilia – ciliary aplasia
- Ciliary transposition defect
- Ciliary disorientation

Text Box: Useful contact details for PCD Diagnostic Services

<div style="border:1px solid black; padding:10px;">

Useful Contact Details

PCD DIAGNOSTIC SERVICE AT LEICESTER:
PROFESSOR CHRIS O'CALLAGHAN
PROFESSOR OF PAEDIATRICS
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