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Primary Ciliary Dyskinesia

Recent Advances in Pathogenesis, Diagnosis and Treatment

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Contents

Abstract

Primary ciliary dyskinesia is a genetic disorder causing dysfunctional motility of cilia and impaired mucociliary clearance, resulting in a myriad of clinical manifestations including recurrent sinopulmonary disease, laterality defects and infertility. The heterogenous clinical presentation of primary ciliary dyskinesia and the limitations of transmission electron microscopy to assess ultrastructural defects within the cilium often delay diagnosis. Recent advances in the understanding of the basic biology and function of the cilium have led to potential diagnostic alternatives, including ciliary beat analysis and nasal nitric oxide measurements. Moreover, the identification of disease-causing mutations could lead to the development of comprehensive genetic testing that may overcome many of the current diagnostic limitations. Although the clinical manifestations of primary ciliary dyskinesia have been recognised for over a century, there are few studies examining treatments and standards of care have yet to be established. Multicentre collaborative efforts have been established in North America and Europe, which should help to develop standardised approaches to the diagnosis and treatment of primary ciliary dyskinesia.

First described more than a century ago, primary ciliary dyskinesia (PCD) is a genetic disorder affecting the functional motility of cilia resulting in a myriad of clinical manifestations including recurrent sinopulmonary disease, laterality defects and infertility. Ultrastructural defects of cilia have been linked to the clinical presentation of PCD,[1-3] and more recently our understanding of the genetic and molecular abnormalities of this disease has greatly advanced. This article describes normal ciliary structure and function, genetics and clinical characteristics of PCD, laboratory and other diagnostic studies, and treatment.

1. Ciliary Structure

The respiratory tract is exposed daily to inhaled pathogens, allergens and other noxious agents, and as such, innate host defences are critical in preventing acute pulmonary injury and infection. Complex, local defences have evolved to protect the airway, including the mucociliary escalator, which mechanically eliminates bacteria and particulates that deposit at the epithelial surface.[4,5] The regulation and function of cilia that play a role in mucociliary clearance are complex. Ciliary ultrastructure and orientation are critical for efficient clearance of the lower respiratory tract by moving fluids, mucus and inhaled foreign materials vectorially from the distal to more proximal airways. Indeed, to achieve effective mucociliary clearance, the conducting airways closely coordinate ciliary function, airway surface fluid volume, fluid composition and macromolecule (e.g. mucin) secretion.[6]

Cilia are found on the epithelial surfaces of various organs. Each of these hair-like appendages, anchored by a basal body to the apical cytoplasm and extending from the cell surface into the extracellular space,[7] consists of approximately 250 proteins organised into longitudinal microtubules, which make up the basic axonemal structure.[8,9] On the basis of the arrangement of these microtubules, cilia may be broadly classified as being motile or primary cilia.

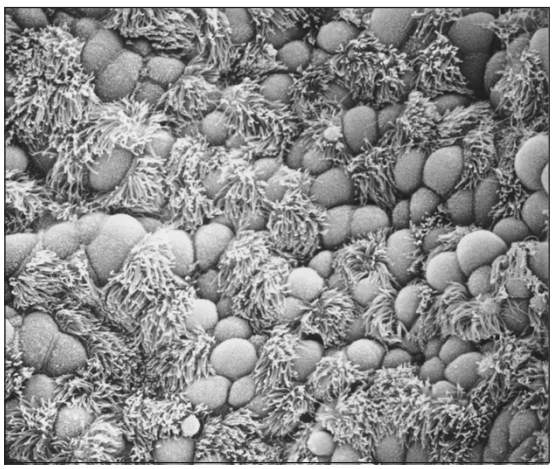

Fig. 1. Photomicrograph of airway epithelial cells grown in culture at an air-liquid interface showing ciliated and non-ciliated cells (photograph courtesy of Dr Steve Brody, Washington University, St. Louis, MO, USA).

The motile cilia are found in the apical surface of the upper and lower respiratory tract (figure 1), ependymal cells lining the ventricles of the CNS, the oviducts of the female reproductive system and as the flagellum of male spermatozoa. Each motile cilium is organised into nine microtubule pairs (or doublets) surrounding a central doublet in a characteristic '9+2' arrangement as seen by cross-sectional views by electron microscopy (figure 2).[8,9] An array of radial spoke proteins link the membrane sheath surrounding the central pair with the surrounding microtubule doublets, and nexin proteins link the peripheral doublets into a circumferential network. Powered by adenosine triphosphatase-containing inner and outer dynein arms located on the peripheral microtubules, the coordinated action of these multiple proteins and sliding of the microtubules act to generate ciliary motion.[5] The nexin links and radial spokes act to restrict the degree of sliding between microtubules and allow the cilium to bend.[10,11] The wavelike pattern of ciliary motion is thought to have important functions in fluid and cell movement, and any disturbance in the precise, orchestrated movement of the cilia can cause disease.[11-13]

In contrast, primary cilia lack a central microtubule doublet and outer dynein arms, creating a '9+0' arrangement and leaving these structures immotile. Primary cilia exist in the retina, the renal nephron and within hair cells of the inner ear. The interactions between microtubule structures provide sensory function, and defects in primary cilia have been linked to different pathologies, including polycystic kidney disease, cystic liver disease, retinitis pigmentosa, and pancreatitis in rodent models and humans.[14-16]

A third distinct classification of cilia also exists, but they are only transiently present during embryonic development. These nodal cilia share a similar '9+0' microtubule arrangement as primary cilia, but they exhibit rotational movement resulting in leftward flow of extracellular fluid. Several investiga-

tors have shown that abnormalities in nodal cilia play a role in establishing sidedness and left-right body orientation.[17-19]

2. The Genetics of Primary Ciliary Dyskinesia (PCD)

PCD is usually described as an autosomal recessive condition, although rare cases of autosomal dominant and X-linked inheritance have been reported.[20,21] The calculated frequency of PCD ranges from 1 in 15 000 to 30 000 live births, but these measures probably underestimate the disease in the general population.[22-25]

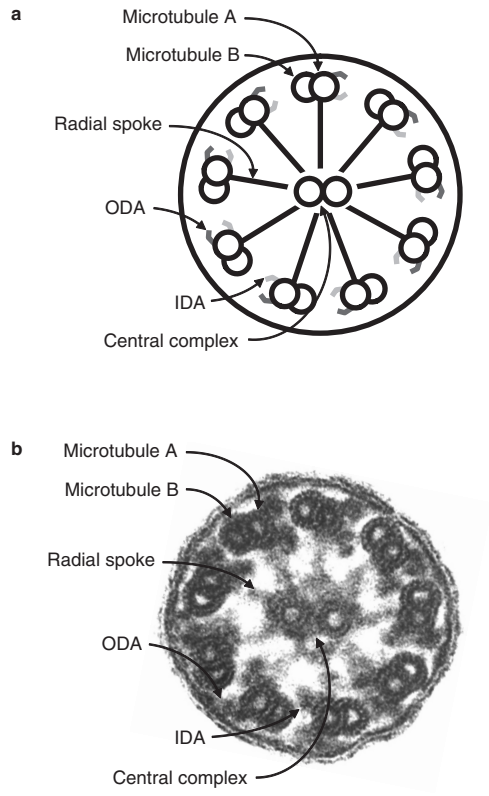

Fig. 2. Representations of healthy human cilium. (**a**) Cross-sectional diagram depicting the complex structure and arrangement of axonemes and dynein arms. Each inner dynein arm (IDA) and outer dynein arm (ODA) is composed of numerous proteins and multiple dyneins. (**b**) Electron photomicrograph showing the same elements (photographs courtesy of Dr Frances White, Washington University, St. Louis, MO, and John Carson, University of North Carolina, Chapel Hill, NC, USA).

Theoretically, mutations in any of the 250 proteins that make up the complex structure of the cilium could cause impaired mucociliary clearance, and is consistent with the wide spectrum of clinical presentations. Indeed, linkage analyses has confirmed that substantial locus heterogeneity exists, making correlations between ciliary defects and the underlying mutations difficult.[26]

The basic structure of cilia is highly conserved across species and numerous animal models exist, including those in the mouse, rat, dog and pig.[27] However, a simple green alga, *Chlamydomonas reinhardtii*, has provided extensive insights into the structure, function and genetics of the human cilium.[28-30] Axonemal structure of *Chlamydomonas* flagellum shares a high degree of homology with the mammalian cilium and spermatozoa tail, making this protozoan organism a powerful model to identify candidate proteins and genes important for ciliary function.[9,31,32] On the basis of such studies with this protozoan, defective dynein proteins lead to ultrastructural changes and dysmotile flagella, providing several candidate, PCD-causing genes for examination.[33,34]

Human investigations into the genetic basis of PCD have focused on dynein arm proteins and, to date, 11 different genes have been screened.[27] Mutations in *DNAI1*, homologous to the *C. reinhardtii* gene *IC78*, have been found in PCD patients with outer dynein arm defects and functional ciliary abnormalities,[35-37] and have been estimated to occur in 10% of PCD patients with an insertion of a T nucleotide at +3 position of intron 1 (219+3insT) being the most commonly identified mutation.[38]

Another gene, *DNAH5*, homologous to *C. reinhardtii* gene HCγ, has also been associated with PCD.[39-42] A recent study has indicated that 53% of PCD patients with known outer dynein arm defects exhibited mutations in *DNAH5* clustered in five exons,[43] making it a promising target for genetic screening. Two other dynein protein genes (*DNAH11* and *DNAH7*) have also been implicated in PCD, although their relationship to disease is not well established.[43,44] Genome-wide linkage studies of PCD families have also identified a number of

other loci, which may be important in the development of PCD and could be promising targets for further study.[45] Although the genetic basis of this disease is far from being completely understood, the identification of disease-causing mutations could lead to the development of comprehensive genetic testing that may overcome many of the current diagnostic limitations.

3. Clinical Manifestations of PCD

Ciliated cells line the nasopharynx, middle ear, paranasal sinuses and lower respiratory tract from the trachea to the bronchioles.[46] Each ciliated cell has ≈200 cilia projecting from its surface in the same orientation, which beat in a coordinated fashion. Orchestrated movement of the cilia work to sweep the periciliary fluid and the mucus overlying it, resulting in vectoral movement of mucus out of the respiratory tract. Indeed, the mucociliary escalator acts as the primary clearance mechanism of the airways[5,6] and any disruption, whether the cilia are immotile or disoriented in their movement, can manifest in chronic sinopulmonary symptoms (table I).

The majority of PCD patients present in the immediate newborn period with significant respiratory distress, suggesting cilia are critical for effective clearance of fetal lung fluid.[47-49] Case reports emphasise unexplained recurrent atelectasis or pneumonia early in life, which can manifest as tachypnoea, hypoxaemia or even respiratory failure requiring mechanical ventilation.[49-51] Despite this early clinical feature, the association of newborn respiratory distress with PCD has been underappreciated and diagnosis is often delayed. In a retrospective review, investigators have found the mean age of PCD diagnosis was slightly >4 years despite unambiguous pulmonary manifestations in infancy.[52] Chronic cough and persistent rhinitis, too, are frequently present since early infancy. Consequently, the infant may struggle with poor feeding and failure to thrive in the early years of life,[53] thus mimicking cystic fibrosis and making prompt diagnosis a challenge.[45,54,55]

Table I. Common clinical manifestations and features of primary ciliary dyskinesia

Middle ear
Chronic otitis media
Conductive hearing loss
Paranasal sinuses
Chronic nasal congestion
Mucopurulent rhinitis
Chronic pansinusitis
Nasal polyposis
Lung
Neonates: atelectasis, pneumonia, respiratory distress
Chronic cough
Recurrent pneumonia
Bronchiectasis
Digital clubbing
Left-right laterality defects
Situs inversus totalis
Heterotaxy
Congenital cardiac disease
Genitourinary tract
Male infertility
Female infertility

Impaired mucociliary clearance of the lower respiratory tract leads to recurrent episodes of pneumonia or bronchitis. Bacterial cultures of sputum or bronchial aspirates most commonly yield nontypeable *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. *Pseudomonas aeruginosa* infection has also been reported, most often found in older individuals with advanced disease.[48] Chronic lung involvement and inflammation leads to bronchiectasis in many individuals. Digital clubbing may also be apparent as a sign of long-standing pulmonary involvement. Pulmonary function testing typically shows progressive, obstructive defects.

The upper respiratory tract is frequently involved in PCD. Inadequate innate mucus clearance commonly manifests as chronic sinusitis. Some patients develop nasal polyposis. Middle-ear disease is described in virtually all cases of PCD with varying degrees of chronic otitis media often leading to conductive hearing loss and intervention with myringotomy tube placement.[48,55,56]

Left-right laterality defects are found in PCD and almost half of the patients have *situs inversus totalis* with reversal of the thoracic and abdominal organs.[57,58] Without functional nodal cilia in the embryonic period, thoracoabdominal orientation is random and not genetically preprogrammed. Other forms of left-right asymmetry have also been reported in association with PCD including left and right isomerism, which may include anatomical deformities of the heart, liver and spleen.[12,59]

Men with PCD are typically infertile as a result of impaired spermatozoa motility secondary to defective sperm flagella, although male infertility is not a universal finding in this disease.[58] In fact, 50% of men with PCD may have intact spermatozoa motility, suggesting sperm tails may preserve some function or could actually be under different genetic control than cilia.[60] Fertility issues in women have also been reported, possibly due to the ciliary dysfunction in the fallopian tubes.[61,62]

Other clinical manifestations of PCD are rare and less well understood. Several reports have associated hydrocephalus with PCD, hypothetically due to impaired cerebrospinal fluid flow secondary to dysfunctional motor cilia that line the ventricular ependymal cells.[63,64] Retinitis pigmentosa has recently been linked to some forms of PCD[20,65] and there have been rare reports of associations with oesophageal atresia.[66]

4. Diagnosis of PCD

Current diagnostic criteria for PCD is based on the presence of phenotypic features and identification of ultrastructural ciliary defects.[47,48] However, those standards can be problematic and confusing for the physician. As predicted by the complexity of the human cilium, the clinical presentation of PCD is heterogeneous. The clinical presentation of PCD is highly variable and may be subtle in patients with milder forms of this disease who have partial preservation of ciliary function. Respiratory distress in the immediate newborn period is the earliest sign of PCD in many individuals, but is often also overlooked by clinicians.[52] Even with other clues, such as chronic sinopulmonary or rhinitis symptoms in infancy or even *situs inversus totalis*, diagnosis is often delayed. Moreover, ciliary movement and normal ultrastructure does not exclude the diagnosis of PCD. Thus, physicians must have a high index of suspicion for PCD.

Transmission electron microscopy is the current 'gold standard' to assess ultrastructural defects within the cilium. Curettage or brushing from the nasal epithelium along the inferior surface of the nasal turbinate provides an adequate specimen for review. Cilia may also be recovered from bronchial brushing, but this procedure requires sedation and bronchoscopy. Identification of a discrete defect in any aspect of the ciliary structure (inner and outer dynein arms, nexin links, radial spokes or microtubule configuration) with concurrent phenotypic features is sufficient to make the diagnosis of PCD. However, careful interpretation of the ultrastructural findings is necessary, as nonspecific changes may be seen related to exposure to airborne pollutants or infection by respiratory pathogens.[67-69] Acquired or secondary ciliary dyskinesia can occur following environmental insults[10] and need to be distinguished from genetic causes.

Often, the diagnosis of PCD can be hampered by inadequate sample processing and the lack of an experienced pathologist or microscopist who can confidently distinguish between primary and acquired ciliary defects.[70] Additionally, there are numerous reports of patients with classic PCD who have normal cilia ultrastructure,[71,72] which indicates that PCD can occur in the absence of unambiguous ciliary defects. Some have advocated culturing airway epithelial cells at an air-liquid interface, allowing the injured epithelium to recover; secondary changes resolve and PCD can then be diagnosed based on identification of ciliary defects.[73] Nevertheless, ultrastructural examination of cilia using electron microscopy to diagnose PCD has significant limitations. It is an expensive and time-consuming technique that makes it impractical as a screening tool in clinical practice.

The saccharin test has been used as a screening tool to qualitatively assess mucociliary function that may be useful in older children and adults.[74] A

normal test can potentially exclude PCD, but this approach lacks well validated diagnostic criteria, does not distinguish between primary and acquired ciliary dyskinesia, and cannot be used in young children.

Ciliary beat analysis has emerged as another method for assessing ciliary function. Determination of ciliary beat frequency using conventional microscopic techniques has been used as a screen for PCD, but this method alone will miss some patients with PCD, such as those associated with ciliary transposition and central microtubular agenesis, as these conditions usually have normal beat frequency.[75] Recent advances in high-resolution, high-speed, digital imaging of ciliary motion in multiple planes allows for precise analysis of abnormal cilia beat patterns, which have been associated with specific ultrastructural defects.[76] Immunofluorescence imaging has also been used to demonstrate the mislocalisation of dynein arm proteins in some patients with PCD.[42] Both approaches are currently research tools, and such imaging techniques are not widely available. Additionally, the use of ciliary beat frequency and motility imaging as diagnostic tools are prone to misinterpretation when attempting to distinguish PCD from acquired defects.

Another emerging screening methodology exploits the highly reproducible observation that exhaled nasal nitric oxide (NO) levels are extremely low in individuals with PCD.[48,77-79] This finding appears to be relatively unique to patients with PCD and may become a useful screen for the disease. The link between epithelial NO production and abnormal cilia function is unclear, although some speculate it may play a role in ciliary beat regulation and inflammatory processes.[80,81] Since measuring nasal NO concentrations are noninvasive and relatively easy to perform, this method is a promising screen for PCD in patients >5 years of age. Unfortunately, studies evaluating this technique in younger children have not been reported. Case reports describing low nasal NO concentrations in infants exist,[82] but its usefulness in this age group has not been well established.

Genetic testing is becoming closer to reality as a clinical tool, and may overcome some of the pitfalls of currently available diagnostic tests. Genetic tests have been developed for *DNAI1* and *DNAH5*, and mutations in these two genes may account for >30% of PCD.[83] However, mutations in these two genes certainly do not account for all PCD. The genetic basis of PCD is emerging, and limitations in other diagnostic techniques argue for a concerted effort to define disease-causing mutations so that more comprehensive genetic testing can be applied to patients.

5. Clinical Management of PCD

Respiratory tract involvement is the leading cause of morbidity and mortality. Airway disease develops early in childhood with increasing airway obstruction,[84-86] infection and inflammation that eventually results in development of bronchiectasis, but the progression and extent of lung disease can be slowed with early diagnosis and therapy. Thus, routine surveillance studies recommended that the care of patients with PCD include spirometry to monitor lung function, chest radiographs, and sputum or throat cultures to assess airway flora.[87] Patients with PCD typically have slower decline in pulmonary function compared with those with cystic fibrosis; its prognosis and long-term survival is better.[48] In fact, the majority of patients with PCD have a normal or near normal life span. However, some will develop progressive bronchiectasis and may have severe lung disease in adulthood, which may even lead to respiratory failure.

At this time, no therapies have been adequately studied to definitively prove their efficacy in the treatment of PCD. Moreover, there are no treatments shown to correct ciliary dysfunction. Since its features are similar to those found in cystic fibrosis, many treatment options for PCD are those used for disease states characterised by impaired airway clearance and bronchiectasis.

Strategies to augment mucociliary clearance are central to PCD therapy. Routine airway clearance with postural drainage, percussion vests, positive expiratory pressure devices or other techniques should be instituted on a daily basis. Because ciliary

function is impaired, cough essentially becomes the sole mechanism for mucus clearance and thus should not be suppressed. Exercise may also enhance airway clearance in PCD patients and should be encouraged.[88]

Deoxyribonuclease (dornase alfa), an enzyme which hydrolyses eukaryotic DNA released from disintegrating neutrophils to effectively diminish mucus viscosity and enhance clearance, is routinely used in cystic fibrosis care.[87,89] Isolated case reports demonstrate improvements in lung function after use of dornase alfa,[90] but larger studies supporting these findings are needed before its long-term use in the regular maintenance therapy of PCD can be recommended.

When signs and symptoms of a respiratory infection are present, PCD patients require treatment with antimicrobial therapy based on airway culture results and sensitivities.[48] As previously stated, *H. influenzae*, *S. aureus* and *S. pneumoniae* are commonly isolated from the airway cultures of individuals with PCD. Isolation of nontuberculous mycobacteria in adults has been described, but is less common. Chronic colonisation of the lung with mucoid strains of *P. aeruginosa* can also occur in PCD, but this phenomenon appears to occur later in life compared with cystic fibrosis. No evidence supporting early eradication of *Pseudomonas* colonisation exists in the context of PCD. Maintenance therapy with inhaled or oral antibacterials may also be used cautiously in patients with PCD who have bronchiectasis or frequent exacerbations, though again, current literature lacks any evidence supporting such use of long-term antimicrobial therapy.

Although β-andrenoceptor agonists have been shown to enhance ciliary beat frequency in cell models,[91,92] there are little data indicating that such agents improve function of dyskinetic cilia. Moreover, such drugs do not provide a bronchodilation effect in PCD patients with obstructive pulmonary disease.[88]

Arginine has been proposed as having a potential therapeutic role in PCD, in that it augments production of airway NO and could enhance ciliary beat frequency. However, in preliminary studies, this treatment did not increase nasal NO levels to those found in healthy individuals and no improvement in pulmonary function was shown.[93]

Uridine-5′-triphosphate (UTP) is another novel approach that stimulates chloride ion secretion and mucin release in goblet cells resulting in increased airway fluid hydration and enhanced cough clearance in healthy individuals. A clinical trial of aerosolised UTP in a small group of PCD patients enhanced airway clearance during cough, but the evidence for its long-term efficacy is still lacking.[94]

Surgical resection of bronchiectatic lung has been performed on some PCD patients who have localised disease with severe haemoptysis or refractory, recurrent febrile illnesses, but this approach has not been prospectively studied and it remains unclear if any long-term benefit is achieved.[95]

Progression of bronchiectasis to end-stage lung disease has been reported in patients with PCD, and some have undergone successful heart-lung, double lung or living donor lobar lung transplantation.[96,97] The presence of *situs inversus* presents more of challenge in these procedures due to anatomic considerations at the anastamotic sites, but otherwise long-term survival is expected to be similar to other transplant recipients.

The treatment of chronic otitis media and middle-ear effusions, which are almost universally present in individuals with PCD, is controversial. Myringotomy tubes are frequently used in children with PCD,[48,55] yet they are not without complications, as they may lead to chronic mucoid discharge, permanent membrane perforation and tympanosclerosis. In one of the few studies, myringotomy tubes were not shown to improve hearing.[98] Moreover, hearing acuity in PCD tends to improve with time.[98,99] Hearing should be routinely screened for development of conductive hearing loss and hearing aids used when necessary.

Finally, chronic rhinitis and sinusitis are common findings in PCD. No treatments have been systematically studied or shown to be effective, although many patients are treated with intranasal corticosteroids, sinus lavage and antibacterials. As with any overuse of antimicrobial agents, development of

resistant organisms is a concern. When sinus symptoms are severe or refractory to medical management, endoscopic sinus surgery can be used in patients with PCD to promote drainage and local delivery of medications.[100]

6. Conclusions

PCD is a genetic disorder that results in dysfunction of motile cilia resulting in chronic infections of the upper and lower respiratory tracts, male infertility and *situs inversus totalis* in approximately half of affected individuals. Although its clinical manifestations have been recognised for over a century, the genetic and molecular basis of this disease are only now beginning to be understood. The presentation of PCD is heterogeneous, which presents challenges in both detection of subtle disease and in definitive diagnosis. At this time, the diagnosis of PCD requires a compatible clinical phenotype and detection of specific ultrastructural defects by electron microscopic inspection of cilia, which has its limitations. Emerging developments in measuring ciliary beat frequency, ciliary beat patterns and nasal NO show promise as diagnostic or screening tools. Ultimately, comprehensive genetic testing may serve as a diagnostic test to identify those with PCD in infancy so that prompt medical therapy can be initiated to limit morbidity and the progression of lung disease.

Unfortunately, current literature lacks adequate evidence from large prospective clinical studies evaluating the efficacy of various treatment modalities. Multicentre collaborative efforts have been established in North America and Europe, which should provide a platform for studies designed to optimise and standardise our approach to the diagnosis and treatment of PCD.

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