Primary ciliary dyskinesia (PCD) is a genetic disorder of cilia structure and function, chronic infections of the respiratory tract, fertility problems and disorders of organ laterality. Establishing a definitive diagnosis can be challenging, requiring a compatible phenotype and detection of ciliary functional and ultra-structural defects, along with newer screening tools such as nasal nitric oxide and genetics testing. 10 known PCD-causing mutations within two genes are now available in a clinical panel, and in the future, comprehensive genetic testing may serve to identify young infants with PCD to improve the long-term outlook for patients with the disease. Therapy includes regular pulmonary function testing and monitoring of sputum flora to allow a targeted approach to treatment. Referral to an academic centre with expertise in bronchiectasis and/or PCD is prudent to ensure access to the most recent diagnostic testing and therapies. With increased understanding of the disease it is likely that we will expand the definitions of classic and non-classic PCD, as well as its relationship to less common ciliopathies.

Keywords: Bronchiectasis, cilia, dynein, mucociliary clearance, nitric oxide, primary ciliary dyskinesia

Ciliary dyskinesia refers to a syndrome of oto-sino-pulmonary disease with other accompanying phenotypic features. It is often referred to as primary ciliary dyskinesia (PCD) and sometimes referred to as immotile cilia syndrome (ICS) or Kartagener syndrome, or occasionally the motile ciliopathies [1–3]. PCD is currently the preferred term [4]. Although secondary ciliary dyskinesia may be seen in diseases associated with acute and chronic airway inflammation and infection, this chapter will focus primarily on the genetically transmitted form of the disease, that is PCD, rather than nongenetic, generally secondary forms of the syndrome [5]. Since the hallmarks of the disease are chronic lung disease with bronchiectasis, a brief discussion of the major respiratory features (bronchiectasis) is included, as well as a brief review of airway host
defence. This will allow a better understanding of the role of cilia in health and disease. This chapter will focus on disease in adults, as an excellent review of PCD in children was recently published [6].

PCD is a rare, usually autosomal recessive disease characterised by oto-sino-pulmonary disease, including bronchiectasis, organ laterality defects and male infertility. First described early in the 20th century, its disease origins as a defect in ciliary structure and function were described in Sweden in the 1970s [7, 8]. The last decade or so has seen resurgence in interest in PCD, specifically a new focus has emerged from several groups worldwide on more precisely defining the major aspects of the disease phenotype, including elucidating the molecular basis for the ciliary abnormalities. Such data will help clinicians establish a diagnosis of PCD (which can be difficult in many circumstances), which in turn, will hopefully allow more targeted therapeutic approaches. Cystic fibrosis (CF) has long been recognised as a prototype genetic disease associated with severe pulmonary disease and bronchiectasis, with intense research activity devoted to CF pathogenesis and treatment over the last several decades. PCD offers a similar disease model to CF, albeit with a different basic aetiology, offering complementary insights into significant human disease associated with dysregulation of the mucociliary clearance (MCC) apparatus in the respiratory tract.

The major clinical characteristics of PCD are chronic ear, sinus and lower airways symptoms and signs from birth because of the failure of one of the major airway defence mechanisms, that of MCC. By adulthood, bronchiectasis is invariable and is characterised by an abnormal and permanent dilation of bronchi. It is the consequence of inflammation and destruction of the structural components of the bronchial walls, usually in the walls of the medium-sized airways, often at the level of segmental and sub-segmental bronchi. Most experts accept that a “vicious cycle” of infection and inflammation is created by the basic defect in airway host defence. This generates airway damage and further impairment of airway clearance, eventually with chronic colonisation/infection with a variety of microorganisms, leading to further infection and inflammation and eventually destruction of conducting airways and even alveolar surfaces. In its most severe form, bronchiectasis may lead to respiratory failure and death. For the clinician faced with a patient with bronchiectasis, the diagnostic algorithm involves sifting through the various causes of the disease, with a predominant cause often elusive; thus, it may be labelled either as idiopathic or, with an appropriate history, as post-infectious bronchiectasis [9]. However, a careful clinical history, together with focused tests, may find an underlying cause such as CF or PCD, which is almost always helpful from genetic, prognostic, therapeutic and healthcare system standpoints.

Thus, structural and functional abnormalities of motile cilia and human flagellated cells (sperm) explain the complex PCD phenotype involving various organ systems. The motile cilia in the respiratory tract are vital components of the mucociliary apparatus used in airway clearance and the flagellated structures are important in the male and female reproductive systems. Left–right asymmetric organ defects may also be part of the phenotype, for example, situs inversus totalis, commonly known as Kartagener syndrome [10].

**Normal cilia structure and function**

In addition to humoral, cellular and innate immune systems, the respiratory tract has developed complex local physical defences to protect the airways from the myriad of inhaled pathogens, allergens and other inhaled noxious particles. One such mechanism is the mucociliary escalator, which mechanically eliminates bacteria and particulates that deposit on the epithelial surface of the respiratory tract.

Cilia are hair-like attachments found on the epithelial surfaces (~200 per cell) of various organs and are anchored on by a basal body to the apical cytoplasm and extend from the cell surface into the extracellular space. Each cilium is composed of approximately 250 proteins organised into longitudinal microtubules, which make up the basic axonemal structure [11]. Based on the
Figure 1. Diagram of a cross-section of the basic ciliary structure.

arrangement of the microtubules, cilia are classified into motile cilia, primary cilia and nodal cilia [12]. Motile cilia are the cilia found in the apical surfaces of the upper and lower respiratory tract, the ependymal cells lining the ventricles of the central nervous system, the oviducts of the female reproductive tract and the flagellum of the male sperm. Motile cilia are organised into nine microtubule pair doublets, surrounding a central pair creating a distinctive 9+2 arrangement (fig. 1) [3]. The central pair is linked to the surrounding pair doublet through an array of radial spoke proteins and the surrounding pair doublets are linked to one another via nexin linked proteins. Through ATP-containing dynein arms on the peripheral microtubules, the microtubules slide by one another to produce ciliary motion [13]. The protein links between the microtubules limit the degree of sliding and allow the cilium to bend. Dyneins can be sub-divided into axonemal and cytoplasmic dyneins. Axonemal dyneins move cilia and flagella, as described previously, while cytoplasmic dyneins are involved in the organisation of spindle poles during mitosis [14]. Axonemal dyneins form two structures, the inner and outer arms, and are attached to the microtubules of the nine outer doublets throughout the length of the axoneme, thus they are central to the process of the bending of the cilium or sperm tail. Through coordinated and synchronised bending, wave like movements occur at ~16 Hz, which function to propel mucus and adherent particles/bacteria on the surface of the airway. Integral to the normal function of cilium is normal airway periciliary fluid layer composition and function. One of the main pathogenetic mechanisms in CF is thought to be dysregulation of this fluid layer, which bathes cilia with a thin mucus layer on top [15]. It can be readily seen, therefore, that two discrete abnormalities of MCC, one involving the cilia themselves, the other involving the fluid that bathes the cilia, may result in a broadly similar airway phenotype (bronchiectasis).

Finally, nodal cilia occur during embryonic development. In contrast to the 9+2 structure of motile cilia, they have a 9+0 configuration. They have a very interesting rotational movement, resulting in leftward flow of extracellular fluid, which is important for cell signalling during the development of normal human left–right asymmetry (situs solitus) [12]. Defects in the nodal cilia may cause errors with left–right body orientation; for example, dextrocardia, situs inversus totalis and situs ambiguous [16–18]. This explains the association of organ laterality defects in PCD, as well as other rare genetic diseases such as polycystic kidney disease, Senior–Loken syndrome, Alstrom syndrome, Bardet–Biedl syndrome and retinitis pigmentosa [19].

**Clinical manifestations**

The clinical signs and symptoms of PCD are shown in table 1.

The clinical phenotype that occurs with defective ciliary structure and function is fairly predictable. Cells lining the nasopharynx, middle ear, paranasal sinuses, the lower respiratory tract and the reproductive tract contain cilia and are generally affected in PCD when the disease is fully expressed. In contrast to CF, pancreatic function is preserved, and hepatobiliary disease is usually not a feature. In general, the clinical course of the disease is milder, with absence of the systemic problems associated with CF such as nutritional issues and diabetes. Although there are few data
on life expectancy in PCD, it is believed from clinical experience, and some cross-sectional and longitudinal studies, that PCD carries a more favourable prognosis than CF [20, 21]. Nonetheless, the disease may be quite severe and some patients develop respiratory failure requiring consideration for lung transplant [21].

As with CF, a clue to the diagnosis is a family history of PCD, particularly in populations with high levels of consanguinity [22]. For example, there is a reported 1 in 2,200 prevalence of PCD in the Asian population of Britain [23]. The prevalence of PCD in the general population is unknown, although estimates based on mass radiology studies in differing countries (Scandinavia and Japan) suggest a range of 1:16,000 to 1:40,000 depending on the techniques and calculations involved, and taking into account the likelihood that its prevalence is almost certainly underestimated, even in these focused studies [6].

**Oto-sino-pulmonary disease**

At birth, newborns with PCD often present with a clinical syndrome of neonatal respiratory distress, indicating the importance of ciliary function in clearing the fetal lung [24, 25]. It is useful to consider PCD with this clinical history, whether in later childhood or adulthood (although adult recall of neonatal events may not be reliable). Early childhood atelectasis, pneumonia, hypoxaemia or respiratory failure can be seen [4, 26]. Frequently, these problems may be attributed to other aetiologies (for example wet lung, aspiration or pneumonia), and PCD maybe overlooked for some time. This is borne out by data showing the mean age at diagnosis in children with PCD was >4 years even when persistent pulmonary symptoms occurred, such as chronic cough and persistent rhinitis [27]. Children with wheezing may also be labelled as having “atypical” asthma that is unresponsive to appropriate therapy [28]. Frequently, infants and young children have recurrent upper respiratory tract symptoms, including chronic rhinosinusitis and chronic otitis media [27]. Nasal polyps and conductive hearing loss from the recurrent infections and inflammation is common [29]. Most expert paediatricians discourage placement of drainage

<table>
<thead>
<tr>
<th>Table 1. Clinical signs and symptoms of primary ciliary dyskinesia</th>
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<tbody>
<tr>
<td><strong>By system affected</strong></td>
</tr>
<tr>
<td>Middle ear</td>
</tr>
<tr>
<td>Chronic otitis media with tube placement</td>
</tr>
<tr>
<td>Conductive hearing loss</td>
</tr>
<tr>
<td>Nose and paranasal sinuses</td>
</tr>
<tr>
<td>Neonatal rhinosinusitis</td>
</tr>
<tr>
<td>Chronic nasal congestion and mucopurulent rhinitis</td>
</tr>
<tr>
<td>Chronic pansinusitis</td>
</tr>
<tr>
<td>Nasal polyposis</td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>Neonatal respiratory distress</td>
</tr>
<tr>
<td>Chronic cough (lifelong)</td>
</tr>
<tr>
<td>Recurrent pneumonia</td>
</tr>
<tr>
<td>Bronchiectasis</td>
</tr>
<tr>
<td>Genitourinary tract</td>
</tr>
<tr>
<td>Male and female fertility problem or history of in vitro fertilisation</td>
</tr>
<tr>
<td>Laterality defects</td>
</tr>
<tr>
<td><em>Situs inversus totalis</em></td>
</tr>
<tr>
<td>Heterotaxy (± congenital cardiovascular abnormalities)</td>
</tr>
<tr>
<td>Central nervous system</td>
</tr>
<tr>
<td>Hydrocephalus (rare)</td>
</tr>
<tr>
<td>Eye</td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
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<td></td>
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tubes ("grommets"), as these frequently lead to otorrhoea, worsening of the tympanic scarring and hearing loss over the long term [6, 25]. Although adult nutritional issues are generally not a feature of PCD, infants with PCD may have significant issues with severe gastro-oesophageal reflux, feeding and ability to obtain adequate nutrition and tend to be on the lower end of the growth curve [30]. In later childhood and early adulthood, the impaired MCC in the lower respiratory tract leads to recurrent episodes of bronchitis and pneumonias, which eventually leads to bronchiectasis of the middle and lower lobes [31, 32]. In all age groups, chronic cough is a predominant feature of the disease (often reported by family members), both in response to the chronic inflammation and as a compensatory mechanism for defective ciliary function and MCC [33]. Adults may develop clubbing as a marker of long standing pulmonary disease. By the time patients present to adult clinics, many adults frequently have a history of lobectomy in early life, prior to the diagnosis being established. Since this procedure cannot usually correct what is, after all, a general problem in the lung, it can rarely be recommended [21]. Typically the disease manifests itself as intermittent exacerbations of infectious symptoms, but always with a baseline level of chronic symptoms (as is usual for most patients with bronchiectasis, whatever the cause) [34]. At all stages of the disease the focus should be on minimising symptoms, improving quality of life and slowing declines in lung function (see later). Another unusual, but recently reported complication of chronic airway diseases in older patients with PCD is that of lithoptysis, that is, expectoration of stone-like masses from the airways [35]. The hypothesis is that calcite stone formation is a bio-mineralisation response to the chronic airway inflammation and retention of infected airway secretions in some patients with PCD.

Airway microbiology/imaging

It is not infrequent that adults present to bronchiectasis clinics having rarely had sputum cultures [21]. However, monitoring of the flora of the airway is important, since older adults often harbour problematic organisms that may require specific treatment [21]. Based on monitoring protocols developed for CF and small studies in PCD, it is recommend that airway cultures be performed every 3 to 6 months [20, 21]. Initially, cultures of airway secretions (sputum cultures) grow Haemophilus influenzae, Streptococcus pneumoniae and Staphylococcus aureus. Once bronchiectasis is evident on chest imaging (high-resolution computed tomography (HRCT)), smooth and mucoid Pseudomonas aeruginosa and other opportunistic pathogens such as nontuberculous mycobacteria (NTM) may be present. In cross-sectional studies series, all adults >30 years of age had evidence of bronchiectasis, with an increasing prevalence of these organisms [21, 36].

Pulmonary function testing

Most patients demonstrate progressive obstructive defects with advancing age. Although there are few longitudinal data, cross-sectional studies suggest that the disease is milder than CF in terms of the progression of loss of lung function [21, 36]. Nonetheless, it is important in order to guide treatment, to obtain baseline and serial measures of lung function and assess disease severity and progression, as some patients will develop severe or end-stage lung disease [21]. Ongoing studies, involving larger numbers of patients in multiple centres, will better define longitudinal markers and the natural history of the disease.

Radiology

With more abundant and specialised imaging, bronchiectasis is being observed more frequently in general. Thus, PCD may be considered in patients with HRCT-proven bronchiectasis. The computed tomography scan characteristics of bronchiectatic airways are well described [37]. However, HRCT features alone do not usually allow a confident distinction between cases of idiopathic versus post-infectious bronchiectasis versus known causes or associations of bronchiectasis, although there are certain patterns of disease distribution that support a diagnosis of PCD, for example, a predilection for the middle and lower lobes has been reported in patients.
with PCD, in contrast to the upper lobe distribution of cylindrical bronchiectasis in patients with CF [38]. Some authors suggest that absence of bronchiectasis on a HRCT scan may have a role in excluding the diagnosis of PCD, at least in adults [31].

**Reproductive tract abnormalities**

Infertility in both males and females is also a prominent feature. 98–99% of males with PCD have impaired spermatozoa motility secondary to defective sperm flagella [39]. Data are scattered in females, but a consistent feature is that of normal or delayed fertility in some, while other females show impaired fertility with an increased risk of ectopic pregnancy, presumably because of impaired ciliary function in the oviduct [40].

**Organ laterality and other anatomic defects**

During the embryonic period, thoraco-abdominal orientation is determined via the unidirectional, rotating beat of nodal cilia. With abnormal nodal ciliary structure and function, thoraco-abdominal organ orientation is random. This leads to situs inversus with reversal of the thoracic and abdominal organs in ~50% of patients with PCD [16, 21]. Occasionally, laterality defects are not “pure”, that is, situs ambiguous/heterotaxy may be present. This is the phenomenon of left–right asymmetry within specific organ systems, leading to either sole or randomly combined anatomical deformities of the heart, liver and spleen. A recent series found that at least 6% of patients with PCD have heterotaxy, including complex congenital heart defects [17]. Defects in the outer dynein arm (ODA) may be a more common cilia abnormality in patients with laterality defects than that of the inner dynein arm (IDA) or central apparatus [17].

**Rare associations of PCD**

PCD is occasionally seen with rare diseases linked to abnormalities in primary cilia or sensory cilia, for example in the kidney, retina and embryonic node, which may lead to a wide spectrum of clinical features. An example is PCD with retinitis pigmentosa. Mutations in the X-linked retinitis pigmentosa GTPase regulator gene (RPGR) gene have been identified in a few cases of PCD co-segregating with retinitis pigmentosa [41, 42]. Ciliary dysfunction in both respiratory epithelium and the photoreceptors of the retina seems to be the common factor [42]. Hydrocephalus may be seen in mice with PCD, but its association in humans is less clear, the problem may be secondary to the impaired cilia that line the ventricular ependymal cells of the central nervous system, which helps cerebrospinal fluid flow through the sylvian aqueduct [43–45].

**Diagnostic approaches**

**Overview**

Since the first reports of abnormal ciliary structure as the cause of PCD, the diagnosis of PCD has usually been established by obtaining nasal samples of airway cilia for examination under light and electron microscopy. With appropriate techniques, ciliary motion (absent or dyskinetic in PCD) may be defined and ciliary ultra-structure examined for abnormalities, the classic being absent or short/stubby dynein arms. However, these tests are quite dependent on technical factors and local expertise, and thus it can sometimes be a challenge to definitively diagnose PCD. Recently, however, the diagnostic work-up for PCD has evolved to encompass other methodologies, for example, measures of nasal nitric oxide (NO), more sophisticated analyses of ciliary structure and function and genetic testing (see later). Often, the resources needed to make a definitive diagnosis are only available in specialised centres. Nonetheless, a history yielding the symptomatic clues above should prompt consideration of the diagnosis and, if necessary, referral to the growing number of centres with an interest and expertise in the diagnosis and treatment of PCD and related diseases. An algorithm of currently available tests is presented to help the clinician work through the process (fig. 2). It goes without saying that prior to consideration of the diagnosis of PCD, and embarking on a detailed
work-up, other diseases can be considered and ruled out as appropriate [9]. As there are wide variations in PCD presentation and phenotype there may be overlap with CF, immunological deficiencies, allergic bronchopulmonary aspergillosis (ABPA) and other causes of bronchiectasis. Other chapters in this Monograph address these diseases in considerable detail.
Screening tests versus diagnostic tests

Tests of ciliary function can be divided into those that are indirect and may be used to screen patients (e.g. the nasal saccharin test) and those that definitively assess function and structure (ciliary beat frequency (CBF)/pattern tests and electron microscopy studies). Newer screening/diagnostic tests currently undergoing study include nasal NO, which may reflect ciliary structure function indirectly, immunofluorescent analysis of ciliary proteins, high-speed video-microscopy to quantitate ciliary motion and clinically available panels of genetic tests known to be associated with ciliary structural abnormalities.

MCC: the saccharin test

The saccharin test is cheap and can be readily performed in the clinical setting as a screening test. However, it is subject to an array of technical factors that render it less reliable than other methodologies. A 1–2 mm particle of saccharin is placed on the inferior nasal turbinate 1 cm from the anterior end (if too far anterior cilia actually beat forwards from the nose). The difficult part is that the patient must sit quietly with the head bent forward without sniffing, sneezing, coughing, eating or drinking. The time it takes for the patient to taste the saccharin is a rough measure of nasal MCC. Generally, tasting saccharin in <30 minutes is normal. Patients with rhinosinusitis commonly taste it within 60 minutes. If it is not tasted within 60 minutes, PCD can be considered. The test is not suitable for small children who will not sit still for 60 minutes, patients with a poor sense of taste and patients with a cold within the past 6 weeks [46].

NO levels

NO is present in high concentrations in the upper respiratory tract and is produced by the paranasal sinus epithelium [47]. NO is produced enzymatically from L-arginine by several isoforms of NO synthase. NO appears to contribute to local host defence, modulate ciliary motility and serve as an aerocrine mediator in helping to maintain adequate ventilation–perfusion matching in the lung [48]. Abnormal values of nasal NO have been reported in various sinus and lung diseases; for example, acute and chronic sinusitis, CF and nasal polyposis [48]. Quite fortuitously, low nasal NO levels were first reported in PCD in the early 1990s by a Scandinavian group researching exhaled NO in a variety of normal and diseased states [49]. The observation has been replicated on several occasions and, although not fully understood at a mechanistic level, it seems to be a robust index of classic PCD [21, 50]. In individuals with PCD, levels of exhaled NO are extremely low (~10% of normal values) even when compared with patients with CF and other sinus disorders, where nasal NO may be low, although not usually in the PCD range [51, 52]. Interestingly, in one study, carriers (nonsmoking parents of patients with PCD) had intermediate levels of nasal NO [21]. Confirmation of the diagnosis of PCD requires further diagnostic tests. Nevertheless, the highly reproducible nature of low nasal NO levels make it a valuable screening tool [53].

Ciliary function

Transnasal brushings or nasal scrape samples may be obtained fairly readily via direct visualisation of the inferior turbinate, without local anaesthesia or sedation [54]. Ciliary beat patterns and frequency can be seen under direct visualisation using a microscope, and classed as qualitatively normal, dyskinetic or immotile [21, 55]. For more quantitative measures, CBF can be measured and the ciliary waveform can be analysed using high-speed digital video imaging to differentiate between abnormal beating cilia and the normal beat patterns [56]. A cilium can be viewed in slow motion or frame by frame, with 40 to 50 frames per ciliary beat cycle [57]. Normal cilia beat forward and backwards within the same plane, with no sideways recovery sweep. Recent advances in computer image processing software may help standardise measures of waveform and direction of multiple cilia, as a measure of the effectiveness of ciliary transport [58, 59]. This software may
also efficiently compute ciliary activity with accuracy and reproducibility. CBF and beat pattern abnormalities have been associated with specific ultra-structural defects such as isolated outer arm defects, isolated inner arm or radial defects or transposition defects [58]. If patients have both a normal CBF and a normal beat pattern, then classic PCD can reasonably be excluded. However, if one or the other is abnormal, further studies are necessary. As with any studies of cilia structure and function, it is critical to exclude ongoing inflammation as a cause of secondary ciliary dysfunction leading to false positives [5].

**Ciliary structure**

In patients with an appropriate phenotype, suspicion for PCD for other reasons (for example, respiratory symptoms and a sibling with disease) or positive screening tests (saccharin test, nasal NO or CBF abnormalities), the axonemal structure of the respiratory cilia should be studied using transmission electron microscopy (TEM) [8]. Inflammatory influences can be avoided by sampling the patient in a stable state, post-antibiotics or, if *in vitro* testing, by culturing the epithelial cells in an inflammation-free environment. The yield may be higher in patients with sino-pulmonary symptoms rather than isolated upper or lower respiratory tract symptoms [60]. There are a number of structural phenotypes associated with PCD [61]. Most cases of PCD are due to a lack of ODA, or a combined lack of both IDA and ODA [21]. Less common defects include IDA defects alone or defects in combination with radial spoke defects, or central microtubule pair defects such as transposition or central microtubular agenesis [62–64]. In a proportion of patients with PCD, no structural defects were defined using existing TEM techniques [53, 60]. This, despite a strong phenotype, defined ciliary functional abnormalities and demonstrated genetic defects, underscoring the notion that the disease is almost certainly under-diagnosed, due to the hitherto reliance on TEM as the “gold standard” for diagnosis of the disease. As seen later, advances in molecular techniques will probably allow a broader definition of PCD (classic and non-classic PCD, akin to the situation with CF), leading to more efficient diagnosis with subsequent beneficial downstream effects for earlier diagnosis, treatment and improved long-term clinical outcomes.

**Immunofluorescent stains**

Immunofluorescent analysis using antibodies directed against the main axonemal components has recently been used to facilitate identification of structural abnormalities of cilia, and is used in diagnosis in some centres in Europe [65, 66]. PCD patients with ODA defects have absence of DNAH5 staining from the entire axoneme and accumulation of DNAH5 at the microtubule-organising centre as compared with normal individuals with normal DNAH5 staining along the ciliary axoneme [66]. Recent work has also shown that antibody-based techniques can diagnose not only ODA but also IDA abnormalities caused by KTU mutations in PCD [67]. In the future, it may be possible to develop a panel of antibodies directed towards multiple ciliary proteins that may enable the screening of respiratory epithelial samples.

**Genetic testing**

**Overview**

As the molecular underpinnings and the genetics of PCD become more defined, genetic testing may overcome some of the drawbacks of the currently available diagnostic tests. Given the complexity of ciliary structure and the genetic heterogeneity of PCD, finding gene mutations causative for PCD has been challenging. Fortunately, non-human models have helped in the process of discovery. Since the basic structure of cilia is highly conserved across species, an example being a simple green alga, *Chlamydomonas reinhardtii*, extensive information has been gleaned regarding the structure, function and genetics of human cilia, specifically identifying candidate proteins and genes from mutant *Chlamydomonas* that are critical for normal ciliary function (e.g. slow swimmers with ODA defects and mutant γ-heavy chain dynein) [68]. Initial mutations
found using the candidate gene approach include mutations in DNAI1, homologous to the Chlamydomonas genes IC78. This was discovered in PCD patients with ODA defects and functional ciliary abnormalities [69, 70]. Since then, there have been several more PCD-causing gene mutations published, using a variety of approaches (table 2). Homozygosity mapping in large families that may or may not be consanguineous, but have multiple affected and unaffected siblings can be successfully used to identify disease-causing genes. This method utilises the marker analysis to look for the shared region of the genome from affected and unaffected individuals, to identify the chromosomal locus/loci shared between the affected siblings. Genes within the shared locus/loci are candidates, which can be further aided by the candidate gene approach to prioritise the genes to be tested from the shared locus. OMRAN et al. [91] successfully used this method to localise the shared locus in affected individuals from a large consanguineous family and identified mutations in the DNAH5 gene. Genome-wide linkage analysis, another approach to find disease-causing mutations, using 31 multiplex families with PCD failed to identify disease-causing genes [92]. The main limitation of genome-wide linkage analysis is the extensive genetic and ultra-structural heterogeneity in PCD that limits the comparison of data across the families to get meaningful log of odds (LOD) genetic linkage scores that helps indicate the possible disease-causing loci. Other methodologies include the comparative computational analysis approach, which identifies candidate genes using the DNA information collected from various sequencing projects from various distinct species. It assumes that higher-level organisms independently lost certain genetic information during evolution once the information coding for the specific processes was obsolete. Using subtraction analysis it is possible to find candidate genes necessary for cilia formation and function by comparing the genome of a ciliated eukaryote with eukaryotes not dependent on cilia [93, 94]. A comprehensive discussion of the molecular basis of PCD is beyond the scope of this chapter; the reader is referred to Knowles et al. [4].

**Table 2. Primary ciliary dyskinesia-causing genes in humans showing extensive locus heterogeneity**

<table>
<thead>
<tr>
<th>Human gene</th>
<th>Chromosomal location</th>
<th>Axonemal component</th>
<th>Ultra-structure of patients with mutations</th>
<th>[Ref.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNAI1</td>
<td>9p13.3</td>
<td>ODA IC</td>
<td>ODA defects</td>
<td>[69–73]</td>
</tr>
<tr>
<td>DNAI2</td>
<td>7q25</td>
<td>ODA IC</td>
<td>ODA defects</td>
<td>[74, 75]</td>
</tr>
<tr>
<td>DNAH5</td>
<td>5p15.2</td>
<td>ODA HC</td>
<td>ODA defects</td>
<td>[76–78]</td>
</tr>
<tr>
<td>DNAH11</td>
<td>7p21</td>
<td>ODA IC/LC</td>
<td>Normal ultra-structure</td>
<td>[79–83]</td>
</tr>
<tr>
<td>TXNDC3</td>
<td>7p15.2</td>
<td>Cytoplasmic*</td>
<td>ODA defects</td>
<td>[84]</td>
</tr>
<tr>
<td>KTU/PF13</td>
<td>14q21.3</td>
<td>Cytoplasmic*</td>
<td>ODA-HDA defects</td>
<td>[85, 86]</td>
</tr>
<tr>
<td>LRRCC50</td>
<td>16q24.1</td>
<td>Ciliary Axoneme</td>
<td>Axonemal disorganisation</td>
<td>[87, 88]</td>
</tr>
<tr>
<td>CCDC39</td>
<td>3q26.33</td>
<td>Ciliary axoneme</td>
<td>Axonemal disorganisation</td>
<td>[87, 89]</td>
</tr>
<tr>
<td>CCDC40</td>
<td>17q25.3</td>
<td>RS</td>
<td>Transposition defect</td>
<td>[90]</td>
</tr>
<tr>
<td>RSPH4A</td>
<td>6q22.1</td>
<td>RS</td>
<td>CP defects and normal ultra-structure</td>
<td>[90]</td>
</tr>
<tr>
<td>RSPH9</td>
<td>6p21.1</td>
<td>RS</td>
<td></td>
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</tr>
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</table>

DNAI1 and DNAH5 are associated with ODA defects in PCD

Mutations in DNAI1 and DNAH5 [69, 70, 71–73, 76–78] that encode dynein axonemal intermediate chain 1 and heavy chain 5, respectively, have been well documented in several studies as causative for PCD. DNAI1 accounts for ~2–10% of patients with PCD, although if one “selected” the phenotype to include patients with ODA defects alone, this increases to ~4–14%.

The commonest mutation (founder mutation) in DNAI1 is IVS+2_3insT, accounting for >50% of mutations. DNAH5 is a heavy chain dynein and mutations in the gene were initially found in a large inbred family of Arab descent. Subsequent studies show mutations in DNAH5 to be present in ~15–28% of patients with PCD. Together therefore, DNAI1 and DNAH5 account for ~20–40% of patients with classic disease with ODA defects. Despite extensive allelic heterogeneity, four exons in DNAI1 and five exons in DNAH5 represent mutation clusters, which became the basis of development of the clinical genetic testing for PCD.

Miscellaneous other mutations associated with PCD

Mutations have been identified in other genes in patients with PCD, specifically, DNAH11, DNAI2, KTU, RSPH9, RSPH4A, TXNDC3 and LRRCT50, CCDC39 and CCDC40 (table 2) [26, 67, 74, 79–81, 84, 85, 88–90]. Some genotype–phenotype associations have been defined amidst the plethora of mutations found, primarily at the ultra-structural level (rather than at the clinical level). Mutations in DNAH5, DNAI1 and DNAI2 are exclusively seen in patients with ODA defects, whereas mutations in KTU and LRRCT50 are exclusively seen in patients with combined ODA+IDA defects [4]. The genetics of the DNAH11 (which encodes dynein axonemal heavy chain 11) mutation are quite Interesting as it was found in a patient with proven CF and situs inversus. It was not clear if this patient had PCD/Kartagener syndrome, or isolated situs inversus, as there is an obvious phenotypic overlap between the CF and PCD. However, the patient had abnormal ciliary beat pattern as seen in PCD, normal ciliary ultra-structure, but with a mutation in the DNAH11 gene that was assumed to be linked to the situs inversus [95, 96]. Subsequently, mutations in DNAH11 were unequivocally shown to be PCD causing in a large German kindred and more recently two patients with PCD were found to harbour mutations in DNAH11 [80, 81]. All of the patients with DNAH11 mutations presented with normal dynein arms. This phenotype highlights the difficulty in diagnosis in those patients with a strong clinical phenotype, but with normal cilia on TEM analysis. Mutations in DNAI2 that encode for a dynein axonemal intermediate chain 2 have been identified in 4% of PCD patients with ODA defects [79]. In contrast to the above proteins and genes, which encode for dynein proteins, KTU is a cytoplasmic protein, required for the assembly of the dynein complex [67]. First noted to be mutated in Mekada fish with laterality defects, and subsequently Chlamydomonas, it was then found to be mutated in PCD patients with both IDA and ODA defects (logical since it is required for normal ODA and IDA assembly and transportation). Mutations in KTU are seen in ~12% of PCD patients with combined ODA and IDA defects. RSPH9, which encodes for the radial spoke head protein 9, was identified as being a PCD-causing gene using homozygosity mapping in two Arab Bedouin families. Subsequently, an identical homozygous 3-bp inframe deletion mutation was identified in both families. Interestingly, the ultra-structure analysis of patients from one family depicted 9+2 or 9+0 microtubular configuration, and from the other family normal ciliary ultra-structure was seen [90]. Using homozygosity mapping in three inbred Pakistani families, RSPH4A was identified as a PCD-causing gene that encode another radial spoke head protein 4A. Ultra-structural analysis showed transposition defects with the absence of a central pair and 9+0 or 8+1 configuration. TXNDC3 (encoding thioreduxin domain-containing protein 3) is a component of the sperm flagella ODA, and a nonsense mutation on one allele and a splice mutation on the other allele were found in one PCD patient [84]. Large genomic deletions, as well as point mutations involving LRRCT50 (leucine-rich repeat containing 50), are responsible for a distinct PCD variant that is characterised by a combined defect involving assembly of the ODA and IDA. Functional analyses shows that LRRCT50 deficiency disrupts assembly of distally and proximally DNAH5 and DNAI2 containing ODA complexes, as well as DNAI11-containing IDA complexes, resulting in immotile cilia [85]. Multiple other candidate genes have been tested in patients and families with PCD, and were found to be negative.

Other genetic associations

X-linked retinitis pigmentosa, sensory hearing deficits and PCD have been associated via mutations in the RPGR, essential for photoreceptor maintenance and viability [41]. In addition, a
single family was reported with a novel syndrome that is caused by oral-facial-digital type 1 gene (OFD1) mutations, and characterised by X-linked recessive mental retardation, macrocephaly and PCD [45].

Future directions

Animal models for PCD have been reported to occur in nature, although they have rarely been studied in depth [97]. Similarly animals with a PCD phenotype have been constructed using molecular techniques, mainly in mice [4]. Other than the Mdnah5 deficient mouse and the Dpcd/poll knock out mouse, the causative gene in the other models are unknown. The Mdnah5 deficient mice were created via transgenic insertional mutagenesis that leads to a frame shift mutation. The mice have the classic PCD phenotype and the ultra-structural analysis reveals absent ODA [98, 99]. The Dpcd/poll knock out mice present a phenotype of sinusitis, situs inversus, hydrocephalus, male infertility and ciliary IDA defects [43]. Recently, a murine mutation of the evolutionarily conserved adenylate kinase 7 (Ak7) gene resulted in animals presenting with pathologic signs characteristic of PCD, including ultra-structural ciliary defects and decreased CBF in respiratory epithelium [100]. The mutation is associated with hydrocephalus, abnormal spermatogenesis, mucus accumulation in paranasal passages and a dramatic respiratory pathology upon allergen challenge. Ak7 appears to be a marker for cilia with 9+2 microtubular organisation. Mutations of the human equivalent may underlie a subset of genetically uncharacterised PCD, although no human mutations have been identified as yet. Finally, a novel method of developing a mouse model with a PCD phenotype was recently published [101]. A transgenic mouse lacking an ODA was developed by deleting Dnaic1, a mouse intermediate chain dynein. Importantly, the mice did not develop many of the problems that usually result in an early death for the animals, such as hydrocephalus or other severe developmental defects. Thus, the survival of the animals allowed the investigators to show that the animals did experience problems consistent with defective MCC, at least in the upper airway (severe rhinosinusitis). Objective measures of MCC were also consistent with defective ciliary function in the nasal passage, though interestingly not in the lower airway, possibly reflecting differing turnover of ciliated epithelium in various regions of the respiratory tract (upper versus lower). This animal model may allow studies that attempt to dissect out the relative importance of the various components of the MCC apparatus in different airway regions.

Summary: an algorithm for testing

As there is no easy, single diagnostic test to diagnose PCD, it is recommended that the diagnosis be based on multiple contributing pieces of data (fig. 2). A typical clinical presentation to suggest additional testing for PCD includes recurrent respiratory tract infections (either upper or lower, or both), neonatal respiratory distress, childhood ear infections, adult bronchiectasis in the absence of a diagnosis and male/female fertility problems. Additional features to provoke further tests include organ laterality defects, and complex congenital heart or other organ defects and retinitis pigmentosa. Ciliary dyskinesia, sperm immotility or identification of specific defects of axonemal structures on electron microscopy are also suggestive of the diagnosis. The reader should bear in mind that patients with PCD with atypical histories may have no demonstrable ciliary ultra-structural defects on standard TEM. Nasal NO, if available, helps exclude the disease if normal or very high and, if very low, strongly suggests the diagnosis. Recently, clinically available genetic testing, a rapidly evolving field, may assist in an increasing number of patients with PCD.

Therapeutic approaches

Overview

The goal for the management of PCD is to prevent exacerbations and complications as much as possible, and to slow the progression of disease. As the disease is generally not as severe as CF, and
the diagnosis may be delayed, adults with the disease may not fully appreciate or understand the nature and/or severity of the disease. Thus, education as to the diagnosis, prognosis and therapeutic avenues need to be discussed thoroughly with the patients once the diagnosis is secure (usually on several occasions). Although there are few literature-based studies in PCD, there are enough studies in CF and non-CF bronchiectasis to allow significant extrapolation (although not total, see later) into patients with PCD, to at least frame a plan of treatment depending on disease severity, sputum microbiology and patient circumstances. Medical therapy has been shown to slow the deterioration in lung function [20, 102]. Ellerman and Bisgaard [20] reported longitudinal lung function in 24 patients diagnosed before and after the age of 18 years. They observed worse lung function in patients diagnosed in adulthood, but did not find further deterioration in lung function in either group once the diagnosis was established and routine care initiated. This suggests that aggressive treatment could prevent further lung damage. It should be noted, however, that other larger patient cohorts followed for a longer time period suggest that PCD may be a serious threat to lung function as early as pre-school, with a high degree of variation in the loss of lung function once diagnosed [103]. There was no link to either age or level of lung function at diagnosis and early detection did not slow the rate of decline in lung function. These data support the genetic and phenotypic heterogeneity of PCD. Despite this, regular clinical surveillance is strongly recommended to establish trends of disease progression, and to detect exacerbations early to attempt to prevent irreversible lung damage. This should include at least lung function testing, sputum or throat cultures to assess airway microbiology and annual chest radiographs [104]. Pulmonary function in PCD patients appears to decline slower compared with patients with CF and the majority of patients with PCD seem to have a normal to near normal life span [21]. However, there are patients that develop progressive bronchiectasis, leading to severe lung disease and respiratory failure.

**Specific therapies**

There are no therapies to date that have been shown to correct ciliary dysfunction in PCD patients. Some pilot or single case reports suggest benefit for some of the underlying pathogenetic pathways in PCD, but none are yet available on a general basis, or proven in randomised controlled studies (although patients will often inquire as to their availability) [33, 105, 106]. Thus, therapies to enhance airway clearance, as well as to suppress or kill bacteria are the cornerstones to PCD care.

**Airway clearance**

As with CF, routine airway clearance with cardiovascular exercise, percussion vests, chest physical therapy and various valve/positive expiratory pressure devices should be performed on a daily basis. The aims of respiratory physiotherapy include mobilising and aiding expectoration of broncho-pulmonary secretions, improving efficiency of ventilation, maintaining or improving exercise tolerance, improving knowledge and understanding and reducing breathlessness and chest pain. There are no data in either CF or PCD to support any one method of airway clearance over another, and in adults a good practice is to facilitate a consultation with a chest physiotherapist for an education “class”, and to determine what modality of airway clearance and what devices the patient prefers. As with any chronic lung disease, exercise is highly recommended for cardiovascular fitness and specifically for airway clearance. Even though a chronic cough is a major complaint, it should not be suppressed as it is a compensatory mechanism for mucus clearance with dysfunctional cilia [33].

**Antibiotics**

Antibiotics are the mainstay of treatment for bacterial infections of the airways associated with PCD. The microbiological flora of the airways is broadly similar to that of CF, although with a delayed appearance of *P. aeruginosa*. Antibiotic therapy should be based on regular sampling of
airway secretions for Gram-positive, Gram-negative and acid fast pathogens to build a pattern of the main pathogens in any given patient’s airways [21, 107]. In adults, sputum is usually easy to acquire and bronchoscopy is not usually necessary to gather specimens. When PCD patients have symptoms of a respiratory tract infection, they require treatment with antibiotics based on airway cultures and sensitivities. H. influenzae, S. aureus, and S. pneumoniae are commonly isolated from the airways of PCD patients. There are no randomised placebo-controlled studies evaluating the efficacy of antibiotics in exacerbations in adults or children although numerous studies indicate that antibiotics can improve symptoms and hasten recovery. Antibiotics are recommended for exacerbations that present with an acute deterioration (usually over several days) with worsening local symptoms (cough, increased sputum volume or change of viscosity, increased sputum purulence with or without increased wheeze, breathlessness and haemoptysis) and/or a decrease in lung function based on lung function testing. Expert consensus is that 2 weeks of therapy is reasonable. The choice of antibiotics may be initially empirical, based on the likely microbial agent or guided via previous sputum cultures in an individual (hence the recommendation to gather serial samples). The recommended route of antibiotics needs further study to address the optimal regimen, but most clinicians use oral antibiotics for milder exacerbations and combined anti-pseudomonal intravenous drugs for more significant deteriorations. Previous studies suggest that the combination of intravenous and inhaled antibiotics might have greater efficacy than intravenous therapy alone [34]. In patients chronically colonised with P. aeruginosa, the addition of nebulised tobramycin to high-dose oral ciprofloxacin for 14 days led to a greater reduction in microbial load at day 14 although there was no clinical benefit [108]. Attempts at early eradication of newly acquired bacteria are recommended as in CF, although there are no data that show that such an approach prevents the progression of lung disease. Long-term antibiotics or nebulised antibiotics (tobramycin, colomycin or aztreonam) may be used in patients with chronic or frequent exacerbations. Some patient do well on “rotating” cycles of oral antibiotics, although there are no data to support such an approach and there is a general concern about inciting microbial resistance.

Modulation of airway secretions

In the CF population, nebulised hypertonic saline (7% hypertonic saline) is beneficial by modulating the liquid content of the periciliary fluid layer, thereby thinning thick secretions and triggering a cough reflex [109, 110]. However, in PCD, its utility is less clear as it stimulates cough to help clear secretions but its role in thinning secretions is not known [111]. A small study of 24 patients with non-CF bronchiectasis showed that hypertonic saline resulted in greater expectorated sputum weight and a greater reduction in sputum viscosity compared with the active cycle of breathing technique alone [112]. Thus, it may be considered in the PCD population as it can augment mucus clearance with little to no risk, other than time. Other aerosolised hypertonic agents such as dry powder mannitol are currently being investigated and may be promising in the future [113]. Deoxyribonuclease (dornase alfa), an enzyme that hydrolysates eukaryotic DNA released from decaying neutrophils to diminish mucus viscosity and enhances clearance, is beneficial in CF patients, but its use by extrapolation into PCD patients remains unproven and may even be detrimental to lung function [114, 115].

Other airway treatments

Bronchodilators are not particularly effective in PCD or CF unless a coexisting asthmatic component exists [116]. PCD patients may be initially misdiagnosed as asthmatics unresponsive to conventional therapy, including β-agonists and inhaled corticosteroids. β-Adrenoceptor agonists have been shown to augment CBF in functional cilia but there is little data in the dyskinetic cilia seen in the PCD population [117]. Anti-inflammatory strategies such as alternate-day prednisolone have not been shown to be effective in CF; there are no studies in PCD [118]. Inhaled steroids may or may not be of benefit in individual patients with PCD; a recent Cochrane
review concluded no benefit in non-CF bronchiectasis overall [119]. As with other inflammatory diseases of the lung, the macrolide antibiotics may exert long-term benefits for the modulation of airway inflammation and thus disease expression [106, 120].

**Miscellaneous lung treatments**

L-Arginine might hypothetically have a therapeutic role in PCD patients, in augmenting the production of airway NO, theoretically enhancing CBF (although the exact role of NO in this process is unknown). However, in the small studies performed, L-arginine did not normalise nasal NO levels and no improvement in lung function was observed [121]. Uridine-5-triphosphate (UTP), or its analogues, is also a potential therapy for CF and similar diseases [122]. UTP stimulates chloride ion secretion and mucin release in goblet cells, therefore increasing airway fluid hydration and enhancing cough clearance in healthy individuals. A small acute clinical trial of nebulised UTP in PCD demonstrated enhanced airway clearance during cough, but no long-term benefits in pulmonary function have been shown [33]. Localised surgery may be considered in situations that resemble that of CF or idiopathic bronchiectasis, where occasionally very localised lung disease is considered to be problematic in causing severe systemic symptoms, frequent exacerbations and/or life threatening haemoptysis [123, 124]. Patients with such localised disease, haemoptysis or refractory pulmonary infections, have undergone surgical resection of the bronchiectatic lung but the long-term effects are unknown [124]. If PCD does progress to end-stage lung disease, lung transplantation must be considered. PCD patients have undergone successful heart-lung, double lung or living donor lobar lung transplant [125]. In patients with *situs inversus*, the anatomic disorientation adds an extra challenge when considering the anastomotic sites but is not a contraindication. The long-term survival appears similar to other lung transplant recipients.

**Treatment of extrapulmonary disease in adults**

As PCD affects other aspects of the respiratory tract other than the lungs, treatment of those areas must be considered. Chronic rhinitis and sinusitis may cause significant morbidity in patients with PCD. As of now, no treatments have been shown to be unequivocally effective, although most patients are treated with intranasal corticosteroids, sinus lavage procedures and antibiotics. Antibiotics should be used sparingly for sinus symptoms as resistance occurs quickly and antibiotics should be reserved for more pressing pulmonary symptoms. If sinus symptoms persist despite aggressive medical management or are severe, endoscopic sinus surgery can be used to promote drainage and better delivery of topical medications [126]. Male infertility due to sperm immotility can be overcome by assisted fertilisation techniques such as intracytoplasmic sperm injections [127]. Females, who are infertile secondary to fallopian tube dysfunction, can have direct ovum harvesting from the ovaries and can get *in vitro* fertilisation.

**Statement of interest**

P.G. Noone is principal investigator on an industry sponsored study (multicentre) looking at the effects of inhaled mannitol in non-cystic fibrosis bronchiectasis (Pharmaxis). He is also principal investigator on an industry sponsored study (multicentre) looking at the effects of inhaled aztreonam in non-cystic fibrosis bronchiectasis (Gilead).

**References**