Primary Ciliary Dyskinesia

Recent Advances in Diagnostics, Genetics, and Characterization of Clinical Disease

Michael R. Knowles[1], Leigh Anne Daniels[1], Stephanie D. Davis[2], Maimoona A. Zariwala[3], and Margaret W. Leigh[4]

[1]Department of Medicine, [3]Department of Pathology & Laboratory Medicine, and [4]Department of Pediatrics, University of North Carolina School of Medicine, Chapel Hill, North Carolina; and [2]Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, Indiana

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous recessive disorder of motile cilia that leads to oto-sino-pulmonary diseases and organ laterality defects in approximately 50% of cases. The estimated incidence of PCD is approximately 1 per 15,000 births, but the prevalence of PCD is difficult to determine, primarily because of limitations in diagnostic methods that focus on testing ciliary ultrastructure and function. Diagnostic capabilities have recently benefitted from (*1*) documentation of low nasal nitric oxide production in PCD and (*2*) discovery of biallelic mutations in multiple PCD-causing genes. The use of these complementary diagnostic approaches shows that at least 30% of patients with PCD have normal ciliary ultrastructure. More accurate identification of patients with PCD has also allowed definition of a strong clinical phenotype, which includes neonatal respiratory distress in >80% of cases, daily nasal congestion and wet cough starting soon after birth, and early development of recurrent/chronic middle-ear and sinus disease. Recent studies, using advanced imaging and pulmonary physiologic assessments, clearly demonstrate early onset of lung disease in PCD, with abnormal air flow mechanics by age 6–8 years that is similar to cystic fibrosis, and age-dependent onset of bronchiectasis. The treatment of PCD is not standardized, and there are no validated PCD-specific therapies. Most patients with PCD receive suboptimal management, which should include airway clearance, regular surveillance of pulmonary function and respiratory microbiology, and use of antibiotics targeted to pathogens. The PCD Foundation is developing a network of clinical centers, which should improve diagnosis and management of PCD.

Keywords: primary ciliary dyskinesia; Kartagener syndrome; nasal nitric oxide; genetics; newborn respiratory distress syndrome

Primary ciliary dyskinesia (PCD; online Mendelian inheritance in man no. 244400 [http://www.ncbi.nlm.nih.gov/Omim/]) is a genetically heterogeneous recessive disorder of motile cilia that results in neonatal respiratory distress, chronic oto-sino-pulmonary disease, male infertility, and organ laterality defects in approximately 50% of cases (1–4). Initial recognition of this syndrome in 1933 was based on the triad of chronic sinusitis, bronchiectasis, and situs inversus, known as Kartagener syndrome (5). In 1976, Afzelius (6) reported that these patients had "immotile" cilia and defective ciliary ultrastructure; however, subsequent studies demonstrated that most patients had cilia with stiff, uncoordinated, and/or ineffective ciliary beat. Therefore, "primary" was adopted for the term PCD for this heterogeneous disorder, to distinguish it from secondary or acquired ciliary defects associated with infection and inflammation.

PCD has an estimated incidence of 1 per 10,000–20,000 births, based on population surveys of situs inversus and bronchiectasis in Norway and Japan (7, 8). The prevalence of PCD in the United States is difficult to determine, largely due to inadequacies of diagnostic methods (9). There are fewer than 1,000 patients in the United States with a well established diagnosis of PCD, because many providers do not appreciate the cardinal signs and symptoms, especially in infants and children. Furthermore, diagnostic tests of cilia ultrastructure and/or function are not standardized or readily available. Therapy is not specific for PCD, but rather extrapolated from other diseases, particularly cystic fibrosis (CF) and idiopathic bronchiectasis.

Even though the quality of tests of ciliary ultrastructure (9–11) and/or function (9, 12, 13) has improved in a few specialized centers, we now recognize that some patients cannot be diagnosed by these tests, because as many as 30% of patients with PCD have normal ciliary ultrastructure and/or subtle, nondiagnostic changes in ciliary waveform (9–13). Diagnostic capabilities have recently benefited from the development of a new screening test for PCD, involving measurement of nasal nitric oxide (nNO) (14, 15). Genetic testing is also becoming a reality, with recent discovery of PCD-causing genetic mutations (2–4, 16, 17). Furthermore, better definition of genotype/phenotype has led to better recognition of the early onset and severity of clinical disease in children with PCD (4, 18–21). This progress has benefited from an National Institutes of Health–sponsored Rare Disease Network (http://rarediseases.info.nih.gov/) and our rare disease consortium focused on studying genetic disorders of mucociliary clearance (http://rarediseasesnetwork.epi.usf.edu/gdmcc/), as well as expansion of U.S. PCD centers that recently occurred on the 10th anniversary of the founding of the PCD Foundation (http://www.pcdfoundation.org/).

This review provides an overview of the rapidly evolving state of the art for clinical disease in PCD, including limitations in the diagnosis and treatment of PCD and description of progress that promises to revolutionize the identification and treatment of patients with PCD (1–4). Some results have been previously reported in abstract form (19).

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Correspondence and requests for reprints should be addressed to Michael R. Knowles, M.D., Cystic Fibrosis/Pulmonary Research and Treatment Center, 7011 Thurston-Bowles Building, CB# 7248, University of North Carolina, Chapel Hill, NC 27599-7248. E-mail: knowles@med.unc.edu

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STRUCTURE AND FUNCTION OF MOTILE AND NONMOTILE CILIA

Overview of Different Types of Cilia

Motile cilia in the respiratory tract have a complex axonemal structure for generation of functional ciliary motility (22, 23). Specialized motile cilia in the embryonic node have a rotary motion to direct laterality of organs during embryogenesis (24). There are also nonmotile (sensory or primary) cilia, which do not contain components necessary for motility (25, 26). Mutations in genes encoding proteins involved in biogenesis, structure, and/or function of motile and nonmotile cilia cause ciliopathies, which have a broad range of phenotypes (25, 26) (*see* subsequent text).

Normal Motile (9 + 2) Cilia

Cilia are evolutionarily conserved organelles, as evidenced by homologies with *Chlamydomonas* (2, 16). Motile cilia have microtubules of α- and β-monomers of tubulin, and associated accessory elements (23) (Figure 1). Outer dynein arms (ODAs) and inner dynein arms (IDAs) are present along the length of the peripheral microtubules (doublets), and contain enzymes for ATP hydrolysis (22, 23). Nexin links (nexin–dynein regulatory complex) connect the doublets, and radial spokes extend from the doublets to the central pair to provide structural support necessary for cilia bending (27). Mutations in genes encoding the axonemal structure or functional components of motile cilia, or necessary for the biogenesis of cilia, including cytoplasmic proteins, can result in PCD.

Embryonic Nodal Motile (9 + 0) Cilia

During early embryonic development (gastrulation), cells in the ventral node contain a single motile cilium per cell. This specialized motile cilium has nine peripheral doublets and dynein arms, but lacks the central pair of microtubules, and has a rotary motion, which facilitates development of organ laterality during embryogenesis (24). In the absence of normal nodal ciliary function, organ placement is random. Mutations in genes that encode components of the nine outer doublets result in a laterality defect (situs inversus or situs ambiguus) in approximately 50% of patients with PCD, but mutations in genes that encode for components of the central apparatus (central pair, radial spokes, etc.) do not cause laterality defects (2, 16).

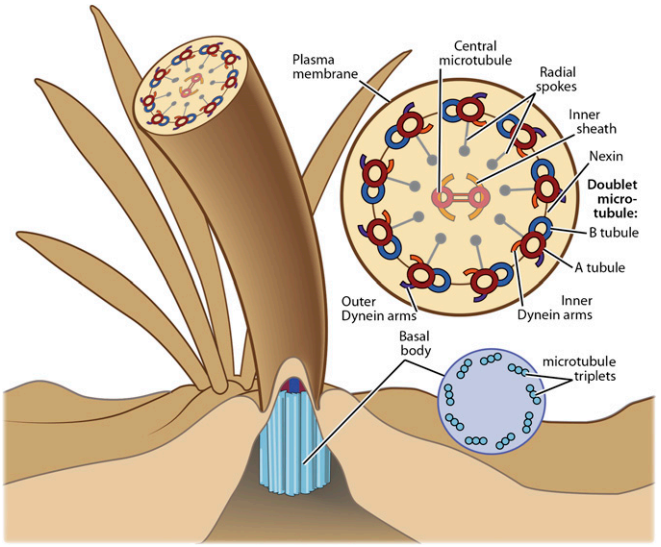

Figure 1. Normal motile cilia (9 + 2) configuration. Motile cilia are composed of highly complex microtubules of α- and β-monomers of tubulin, and associated accessory elements.

Nonmotile, Primary, or Sensory (9 + 0) Cilia

Most cells of the body have a single nonmotile cilium, which contains specialized proteins/receptors to sense the local environment. These cilia are involved in signaling pathways, and play a key role in planar cell polarity (25, 26). Mutations in genes encoding proteins necessary for biogenesis or function of nonmotile cilia cause disorders involving multiple organs (e.g., Bardet-Biedl syndrome, nephronophthisis, retinitis pigmentosa, Joubert syndrome, and autosomal dominant polycystic kidney disease, which sometimes has associated bronchiectasis) (25, 26, 28).

Function of Normal Motile Cilia

Mucociliary clearance is the most important innate defense mechanism in the lung, and cilia provide the coordinated motive force for clearing bacteria and toxic substances from the conducting airways (29). ATP hydrolysis in dynein arms generate force to induce sliding of adjacent axonemal structures, and the complex ciliary waveform in human airways (12, 13, 22, 23, 30). Approximately 200 cilia per cell beat in a coordinated fashion across each cell and across multiple cells, and this coordinated vectorial synchrony results from both planar orientation and training by cilia-driven fluid flow (31, 32). The cilia beat in-plane, and the forward (power) stroke is more rapid and extends a bit higher into the mucus layer than the recovery stroke (30). The wave-like movement across cells results from tightly packed cilia beating at approximately 6–12 Hz. Regulation of ciliary beating involves signaling molecules, including cAMP, cGMP, Ca^{2+}, and NO (22). The ability for 200 cilia on each cell to beat so rapidly reflects the very low friction among cilia, which results from negatively charged glycoproteins that coat the ciliary shaft (33).

CLINICAL PHENOTYPE EARLY IN LIFE

Overview

Mucociliary clearance is a primary innate defense mechanism, and the clinical features of PCD reflect defective function of motile cilia in the conducting airways, paranasal sinuses, middle ear (eustachian tube), and the reproductive tract, as well as specialized motile cilia in the ventral node during embryogenesis (29) (Table 1). Manifestations of PCD occur at birth and within the first several months of life (18, 19). Knowledgeable clinicians should be able to establish the likelihood of PCD, based on the clinical phenotype (9, 18), which has recently been rigorously defined through systematic analysis in our rare disease consortium (*see* subsequent section, Clinical Phenotype) (19).

Respiratory Distress in Term Neonates

Early manifestations of PCD occur in the neonatal period, as over 80% of full-term neonates with PCD have respiratory distress. These infants have tachypnea, increased work of breathing, and frequently require supplemental oxygen for a few hours to weeks (18–20). These infants are often diagnosed with transient tachypnea of the newborn or neonatal pneumonia. This neonatal presentation implies that normal ciliary function plays a critical role in the clearance of fetal lung fluid, although the mechanism is unknown. The presence of unexplained respiratory distress and radiographic abnormalities (e.g., atelectasis and/or increased perihilar/interstitial markings), along with supplemental oxygen requirements in a full-term infant, should raise suspicion for PCD, particularly in neonates with situs inversus (20, 21).

TABLE 1. AGE-RELATED PREVALENCE OF PRIMARY CILIARY DYSKINESIA CLINICAL FEATURES BY SITE OF DYSFUNCTIONAL CILIA

Location of Dysfunctional Cilia (or Sperm)	Clinical Features	Youngest Age when Present in >50%	Youngest Age when Present in >80%
Embryonic node	Laterality defect (situs inversus totalis or heterotaxy)	Neonatal to school age*	—
Eustachian tube	Chronic/recurrent otitis media	Infancy	Infancy
	Transient hearing loss/speech delays	Infancy to preschool	—
Nose/paranasal sinuses	Year-round, daily nasal congestion	Infancy	Infancy
	Chronic pansinusitis†	Preschool	School age
Trachea/bronchi	Unexplained neonatal respiratory distress	Neonatal	Perinatal
	Recurrent lower respiratory infections	Infancy	Preschool
	Bronchiectasis	School age	Adult
Sperm	Infertility	Adult	—

*For all children with primary ciliary dyskinesia (PCD), prevalence of laterality defect is greater than 50%; however, for all individuals (children and adults) with PCD, prevalence of laterality is likely close to, but not greater than, 50% (37). This discrepancy likely reflects earlier pursuit of PCD diagnostic testing in children with situs inversus totalis.

† Pansinusitis is seen in almost all patients with PCD who have sinus imaging studies, but these studies are not done very often in pre-school age patients.

Oto-Sino-Pulmonary Manifestations

Common findings in infants and children with PCD include daily nasal congestion (rhinitis) and year-round wet cough occurring soon after birth. Chronic otitis media that results in temporary or sometimes permanent hearing loss and recurrent sinus infections are classic features. Often, sinusitis is not diagnosed in young children secondary to lack of radiographic imaging. Bronchiectasis occurs in some infants and preschoolers (21). Chest computed tomography (CT) scans show a variety of abnormalities, including atelectasis, air trapping, thickened airway walls, and mucous plugging (21, 34–36). Because respiratory manifestations overlap with other common early childhood diseases (e.g., recurrent respiratory viral infections, gastroesophageal reflux), the diagnosis of PCD is frequently missed, despite the presence of cardinal clinical features, even when coupled to situs inversus. This lack of knowledge highlights the importance of education for many specialties, including neonatology, pulmonology, cardiology, otolaryngology, and for primary care providers.

PROGRESSION OF LUNG DISEASE: INFANCY TO ADULTHOOD

Overview

Nearly 100% of infants and children with PCD have a persistent daily wet cough. Cough clearance may partially compensate for defective mucociliary clearance, but recurrent/chronic bacterial infections in the lower airways inevitably occur, and there is age-dependent development of bronchiectasis, which is universal in adults with PCD (18, 34, 37).

Respiratory Microbiology

Pulmonary bacteriology of children is dominated by *Haemophilus influenza*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*; however, a surprising number of children, including infants/preschoolers, also intermittently culture *Pseudomonas aeruginosa*, which emerges as chronic airway infection in teenagers and young adults (18, 37). Some patients culture more than one type of bacteria in the same sample. The respiratory microbiology in PCD parallels that in CF, although chronic *P. aeruginosa* infection occurs at an older age in PCD and *S. pneumoniae* is a frequent pathogen in PCD, but not CF (37). The prevalence of nontuberculous mycobacteria (NTM) also parallels that seen in CF, with approximately 15% of adults with PCD having positive NTM cultures, but a lower prevalence in children with PCD (37)

Lung Function

Lung disease in children with PCD is not mild, in contrast to general perceptions. Abnormal lung function can develop early in life, as many infants and young children demonstrate abnormal airflow mechanics (21, 38–41). The range of FEV_1 is heterogeneous in PCD at age 6–8 years; indeed, some young patients have severe obstructive impairment that is worse than patients with CF at a comparable age. Cross-sectional and longitudinal data show that spirometry worsens with increasing age (39). However, airflow obstruction does not progress as rapidly in late childhood and early adulthood compared with CF, which is possibly related to preservation of cough clearance. In one longitudinal study, lung function remained stable in PCD children who received aggressive treatment (40). Patients with PCD have spirometric evidence of decreased $FEF_{25–75}$ values and ventilation inhomogeneity, as measured through multiple breath washout (42).

Lung Imaging

Imaging studies show that lung disease in PCD begins in infancy or early childhood, as revealed on high-resolution chest CT. These findings manifest as subsegmental atelectasis, peribronchial thickening, mucus plugging, and/or evidence of air trapping and ground-glass opacity. Bronchiectasis can occur during infancy, and inevitably develops as disease progresses (21). Approximately 50–75% of older pediatric patients and nearly all adults with PCD have bronchiectasis with predilection for middle lobe, lingula, and basal segments (34–36). Similar to CF, chest CT scanning is more accurate to assess early PCD lung disease compared with spirometric indices (38). Chest high field 3T magnetic resonance imaging appears to be as effective as high-resolution chest CT in assessing the extent and severity of lung abnormalities in PCD, making this radiation-free imaging technique an intriguing option for longitudinal assessment and research studies (43).

NONPULMONARY MANIFESTATIONS

Laterality Defects: Situs Inversus Totalis or Situs Ambiguus

Situs abnormalities are present in approximately 60% of pediatric patients, as compared with approximately 50% of adults,

which suggests that abnormal situs serves as a marker to aid earlier recognition. Situs abnormalities reflect defective function of the 9 + 0 motile cilium at the node during embryogenesis (24). A subset of patients with situs ambiguus have heterotaxia syndromes, including polysplenia (left isomerism) and asplenia (right isomerism). At least 6% of patients with PCD have situs ambiguous, and these patients have a 200-fold increased likelihood of having structural congenital heart disease compared with the general population with heterotaxy (44, 45). This observation links congenital heart disease with cilia defects. There are increased postoperative respiratory complications in patients with heterotaxy and congenital heart disease, which suggests that some of these patients may have PCD (46). Indeed, studies in mice with mutations (*Dnai1*) in motile cilia suggest that cilia are required for normal heart development (looping) (47). Moreover, patients with heterotaxy and congenital heart disease have a high prevalence of respiratory ciliary dysfunction, which highlights the critical role of cilia function in normal cardiac development (48, 49).

Fertility Status

Infertility is almost universal in men with PCD, secondary to dysmotility of spermatozoa. However, some men with PCD are azoospermic, and a few have normal sperm motility and have fathered children (50). Females have impaired ciliary function in the fallopian tubes, and may have reduced fecundity or a history of ectopic pregnancies related to delayed ovum transit through the fallopian tube (51).

Pectus Excavatum and Scoliosis

Pectus excavatum occurs in approximately 10% of patients with PCD, as compared with 0.3% in the general population (34, 44). We have also noted a high prevalence of scoliosis (5–10%) in PCD, as noted by others (52). Thus, PCD should be considered in patients with pectus excavatum and/or scoliosis with concomitant sinopulmonary disease.

DIAGNOSTIC APPROACHES

Overview

The diagnosis is delayed in European children with PCD (median age of diagnosis, ~5.5 yr) (53). We note similar findings in our North American rare disease consortium (median age of diagnosis, ~5 yr for children and 22 yr for adults); therefore, physicians should increase their index of suspicion for PCD in patients with pertinent phenotypes. As most institutions do not have adequate resources for a rigorous diagnostic evaluation, referral to specialized centers is strongly recommended. Patients appropriate for referral include those with situs inversus with any respiratory disease, unexplained neonatal respiratory distress, early onset and persistent nasal congestion and daily cough, bronchiectasis without a defined etiology, and/or a family history of PCD in a sibling. For adults, all males with dyskinetic (or immotile) spermatozoa should be considered for PCD if they have respiratory symptoms. Females who are infertile or subfertile without an obvious cause, particularly if they have features of PCD, should be evaluated. Several medical disorders and phenotypes coexist with PCD, including complex congenital heart disease, laterality defects, retinitis pigmentosa, hydrocephalus, pectus excavatum, and scoliosis (1–4, 44, 52, 54). We are trying to define the prevalence of overlap of PCD with primary/sensory ciliopathies, such as Bardet-Biedl or Alstrom syndromes, but a family history of an associated "ciliopathy" should raise the index of suspicion for PCD. It is noteworthy that there is an increased incidence (~1 in 2,000) of PCD in a highly consanguineous Asian population (55).

Clinical Phenotype

A key factor in evaluation of patients with chronic respiratory disease is identification of phenotypic features that characterize PCD as compared with other diseases (9). This is particularly true for infants or children who have not developed bronchiectasis (34–36) (Table 1). A history of neonatal respiratory distress, despite term birth, is a prevalent feature (over 80%), and a useful marker of PCD (18–20). Situs abnormalities are a relatively specific marker for distinguishing PCD from other children with chronic respiratory symptoms. The early onset and persistence of respiratory symptoms may help distinguish PCD from other disorders with more episodic respiratory symptoms (18, 19). Typically, the chronic nasal congestion/drainage and chronic wet cough in PCD occur on a daily basis throughout the year, and usually start in infancy. Most children with PCD can expectorate sputum with surprisingly little effort. Chronic otitis media often appears in the first months of life, and is characterized by the persistence of middle ear fluid, despite antibiotics or placement of tympanostomy tubes (18, 19). Chest CT scans are rarely performed in young children with recurrent respiratory symptoms; however, if bronchiectasis is defined in a young child, PCD is highly likely, particularly if CF has been ruled out (21, 34–36). Symptoms that comprise a strong clinical phenotype in PCD in young children include (*1*) neonatal respiratory distress, and/or (*2*) chronic, persistent lower respiratory symptoms (early onset and persistent wet cough), and/or (*3*) chronic, persistent upper respiratory symptoms (nasal congestion and otitis media), and/or (*4*) a laterality defect (situs inversus or ambiguus). Indeed, the presence of any two of these four hallmark clinical features provides a strong clinical phenotype for PCD, assuming that CF has been excluded (19).

Ciliary Ultrastructure in PCD

Identification of ultrastructural defects on electron microscopy (EM) has been the traditional test used to confirm a diagnosis of PCD, but this approach can no longer be the sole "gold standard" for diagnosis. This approach cannot be used to diagnose the growing number of patients with PCD (at least 30%) with normal ultrastructure. For those patients with PCD with ultrastructural defects, the majority of defects (~55%) involve the absence or shortening of ODAs (40%), or an ODA defect in conjunction with an IDA defect (15%) (Figure 2). Isolated IDA defects comprise only a small fraction (<5%) of confirmed PCD, and false-positive diagnoses commonly occur with these IDA defects (9, 56). Before an isolated IDA defect can be used to validate a diagnosis of PCD, a ciliary biopsy for EM must be repeated when the nasal epithelium is healthy. The vast majority of verified IDA defects have associated central apparatus abnormalities that may occur in only a small subset (5–20%) of cilia (Figure 2). These central apparatus defects include microtubular disorganization and transposition of outer doublets into the central area of the cilia cross-sections, and frequently reflect mutations in *CCDC39* or *CCDC40* (57, 58). Early reports suggested that ciliary "disorientation" (misalignment of the central pair) was a cause of PCD (59, 60), but more recent studies have demonstrated that misalignment of the central pair is a secondary change, and should not be used as a diagnostic ultrastructural criterion (61). A few patients are reported to have absence of cilia on multiple biopsies, suggesting ciliary aplasia, but this finding is difficult to prove, as no genetic etiology has been identified for these patients.

Transmission EM testing of cilia has additional limitations, due to challenges in obtaining adequate specimens, technical factors in processing specimens, and interpretation of images (9, 62). This perspective was highlighted by the experience in our

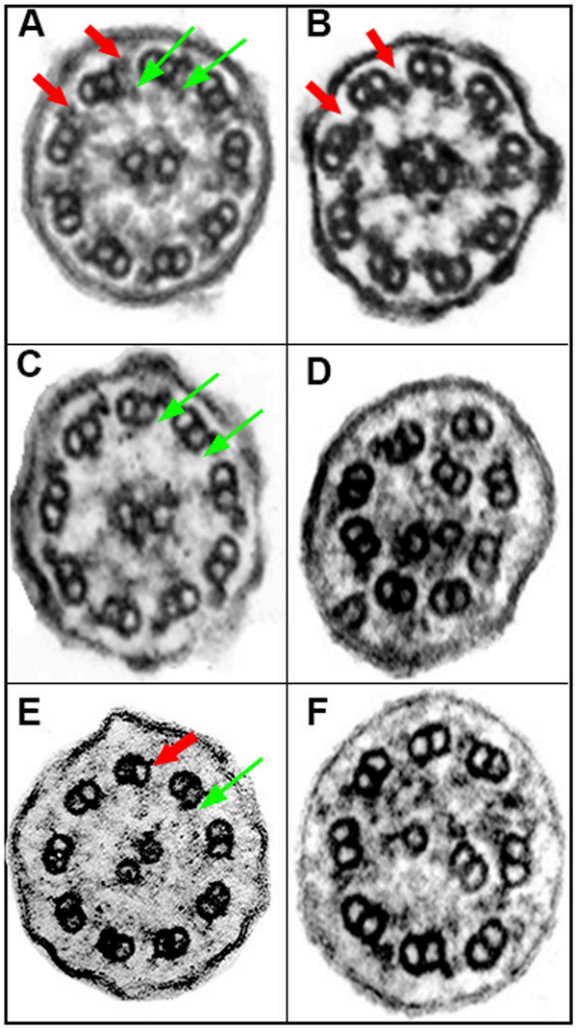

Figure 2. Ciliary ultrastructure by electron micrographs (EMs) from (*A*) normal subject and (*B–F*) patients with primary ciliary dyskinesia (PCD). (*A*) The 9 + 2 axonemal structure is shown, with *thick red arrows* showing the outer dynein arm (ODA) and *thin green arrow* showing the inner dynein arm (IDA). (*B*) ODA defect denoted by *thick red arrows* (*CCDC114* mutations). (*C* and *D*) Patient with PCD with *CCDC40* mutations: (*C*) IDA defect denoted by *thin green arrows,* and (*D*) microtubular disorganization with one outer doublet translocating (disruption of 9 + 2 axonemal structure) into the central region in approximately 10% of cilia. (*E*) ODA + IDA defects noted by *thick red arrow* and *thin green arrow.* (*F*) Central apparatus defect; translocation of outer doublet into central region and loss of one of the central pair (seen in ~5% of cilia with radial spokehead gene mutations).

rare disease consortium, where as many as 15–20% of the patients referred with a "confirmed" diagnosis of PCD (based on ciliary ultrastructure) had a false-positive diagnosis of PCD. For successful ciliary EM testing, the specimen can be from either the nose or bronchi, but cilia should be obtained when there is no significant inflammation, because the normal 9 + 2 microtubular pattern can be altered during respiratory infections, or if cells are poorly fixed. Processing of ciliary biopsy specimens is highly specialized, but not well standardized. Many EM cross-sections (at least 30 sections containing >60 high-quality cilia images is desirable) should be examined by experienced readers, and a quantitative approach to interpretation is critical, because ciliary EMs from normal subjects may have only seven or eight visible ODAs, and only three visible IDAs, per cilium (9, 63). EM analysis is more feasible in biopsies from adults versus children, likely due to the technical challenges of obtaining adequate "scrape" biopsies from narrow airways (10, 62). Acquired (secondary) ciliary defects result from airway damage from recurrent infections, and can sometimes be difficult to differentiate from PCD.

It is critical to recognize that at least 30% of patients with a strong PCD clinical phenotype and low nNO have normal ciliary EMs. Many of these have been confirmed by genetic testing; for example, a recent study demonstrated that 22% of 61 such patients have PCD-causing mutations in *DNAH11* (64). Thus, normal ciliary ultrastructure cannot rule out PCD.

Ciliary Beat Frequency and Motility

Assessment of ciliary motility from fresh biopsies of airway (usually nasal) epithelium has been a traditional approach to confirm a diagnosis of PCD; however, due to substantial limitations, this cannot be the sole diagnostic approach. Although a useful technique in patients with immotility or gross dysmotility of all cilia, accurate recognition of more subtle motility defects requires experienced investigators (9, 27, 65). It is recognized that standard light microscopic analysis is not sufficient (66). Even with high-speed video microscopy, there are recognized limitations due to: (*1*) effects of infection and inflammation on cilia; (*2*) disruption of epithelium during sample collection; (*3*) lack of quantitative data to allow rigorous interpretation of waveform analyses; and (*4*) overlap of ciliary beat frequency between PCD and disease-control and normal subjects (67, 68). Approaches to improve accuracy include ciliated air–liquid cultures to reduce the effects of infection/inflammation, and cooling the specimen to slow cilia beat frequency for waveform analysis; however, these modifications do not eliminate all of the limitations (69–71). Another problem that has been recognized through genetic testing is that many forms of PCD have no, or only subtle, abnormalities of ciliary ultrastructure and/or beat frequency and waveform. Therefore, these biological assays of ciliary structure/function are not sensitive to detecting the growing range of ciliary phenotypes in PCD.

nNO

nNO levels in patients with PCD are low (10–20% of normal values, which range from 125 to 867 nl/min; mean, 287 nl/min), suggesting that nNO may be a useful test for PCD (3, 9, 14–16, 37). nNO can be measured by aspirating nasal air through a catheter placed at the opening of one nostril and directed to an NO analyzer (72). Because exhaled air from the lower airways has a much lower concentration of NO than nasal gases, maneuvers must be instituted to close the soft palate and thereby limit contamination of nasal air by air from the lower airways (72). These techniques have been used reproducibly in adults and children over 5 years of age, but often are not possible in younger children. As an alternative, some centers have measured nNO during tidal breathing in infants and young children (73). In healthy subjects, nNO values during tidal breathing are approximately 40% lower than values obtained at plateau during palate closure. Low nNO levels have also been reported in some patients with CF (9, 37); therefore, sweat testing or *CFTR* genetic studies should be done to rule out CF, if nNO is low. Low nNO values have also been reported during acute viral infections, acute sinusitis, and panbronchiolitis; therefore, measurements should be taken when there are no acute changes in respiratory status, and repeated on a separate day for confirmation (3, 9). To date, nNO has been used in a limited number of specialized centers as a reliable test for PCD, and is currently being extended to the growing network of PCD

centers, recognizing that there must be meticulous attention to details of the standard operating procedures. However, with standardization, and definition of appropriate cut-off values, nNO measurement could be used more widely as a diagnostic test for PCD.

Other Methods of Diagnosis

Fluorescence-labeled antibodies have been successfully used to demonstrate absence of cilia axonemal and cytoplasmic preassembly of proteins, but only a few laboratories have tested this approach (2, 74–77). Measurement of mucociliary clearance in the lung has been used by some investigators, but has been reported to be inadequate, because it cannot be performed in young children and is confounded by involuntary cough and bronchiectasis from other etiologies (78). The nasal saccharine clearance test has no role as a current diagnostic method, due to multiple limitations in performance and interpretation of the test.

GENETIC TESTING

Disease-Causing Genetic Mutations in PCD

PCD is a Mendelian recessive and genetically heterogeneous disorder. Between 1999 and 2010, PCD-causing mutations were described in 11 genes based on homozygosity mapping and candidate gene testing (*see* review articles, References 2, 16, 17, and Table 2). Since early 2011, there has been an explosion of discovery, facilitated by exome sequencing, whereby PCD-causing mutations in 10 additional genes have been published (57, 58, 75–77, 79–85) (*see* Table 2). The vast majority (85%) of the mutations are loss-of-function variants (nonsense, frameshift, or defective splice mutants), and approximately 15% are conservative missense mutations. There are founder (or recurring) mutations in some genes that are common to many patients, but most of the mutations occur in only one family/patient. There are multiple other recently discovered novel genes with mutations in patients with PCD (henceforth, referred to as "PCD genes") that are in different stages of validation and publication. Based on identification of genetic mutations in over 200 patients with PCD in our rare disease consortium, it is estimated that approximately 65% of patients with PCD can be identified as having biallelic mutations in one of these 21 published PCD genes. The upcoming publications of recently discovered novel genes and additional discoveries from exome sequencing make it likely that over 80% of patients with PCD can be identified by genetic testing within the next few years using high-throughput genetic technologies. If true, this will revolutionize the diagnostic approach in PCD, and lead to early identification and initiation of clinical monitoring and treatment.

The roles of PCD genes in normal ciliary biogenesis and function, and the effect of mutations on cilia structure and function, are detailed in many publications (2, 16, 17, 57, 58, 75–77, 79–83), but several key observations are emerging. First, there is very strong correlation between mutations in specific genes and effect on ciliary ultrastructure. Most of the genes code for proteins in the ciliary axonemes in the ODA, or IDA, or radial spoke, but six of these genes are expressed in the cytoplasm, and play a role in preassembly of the cilia. Mutations in these six cytoplasmic genes (*see* Table 2) lead to loss of both ODA and IDA, and are associated with ciliary immotility or severe dysmotility. Second, biallelic loss-of-function mutations lead to low nNO (<77 nl/min), regardless of the PCD-causing gene. Third, genetic studies confirm the relationship between ultrastructural dynein arm defects and situs abnormalities (i.e., mutations in genes that code for dynein arm components are associated with laterality defects, but mutations in genes that code for central apparatus components are not). Fourth, genetic discovery provides opportunity to examine ciliary EMs from multiple patients with mutations in the same PCD-causing gene, and allows recognition of subtle changes, such as IDA defects associated with microtubular disorganization involving only 10% of cilia (e.g., mutations in *CCDC39* and *CCDC40*). Finally, genetic mutations in patients with PCD with normal ciliary ultrastructure offer irrefutable confirmation that PCD can occur in the absence of ciliary EM defects. Furthermore, the ciliary beat frequency in patients with mutations in these genes (e.g., *DNAH11*, *RSPH4A*, *RSPH9*) with normal EMs can be normal (or higher than normal), and the waveforms can appear normal, or have only subtle defects. We anticipate that additional novel PCD-causing genes will emerge from ongoing research, and that some of these patients may have a different (milder) phenotype than seen in classic PCD with ultrastructural defects.

MANAGEMENT OF LUNG DISEASE

Overview

Pulmonary disease in PCD reflects the loss of the key innate lung defense mechanism, mucociliary clearance, and symptoms begin early in life. There are no validated PCD-specific therapies; thus, therapies for PCD are extrapolated from another disease with defective mucociliary clearance, CF, and from patients with non-CF bronchiectasis, particularly as relates to antibiotic therapy and macrolides as antiinflammatory agents (86–90). We concur with recommendations of the European Respiratory Society for management of lung disease in PCD, including routine airway clearance, the use of antibiotics to control infection, and the elimination of exposure to inflammatory triggers, including passive smoke (3).

Monitoring Pulmonary Status

Clinic visits at least twice per year are suggested for spirometric monitoring and respiratory culture surveillance, including NTM. Patients with PCD can readily expectorate sputum for this purpose. Periodic imaging studies are also indicated to monitor the extent of disease. Preventive measures include infection control training and routine immunizations, as well as pneumococcal and influenza vaccine.

Antibiotic Therapy for Lung Disease

Studies in CF and non-CF bronchiectasis (including some patients with PCD) demonstrate that systemic antibiotics are effective at treating "exacerbations" (worsening respiratory symptoms) of lung disease (86, 87). Antibiotics should be chosen based on respiratory culture data. Surprisingly, early (small) studies of chronic inhaled antibiotics in non-CF bronchiectasis did not show benefit in spirometric parameters, although some studies showed a reduction of neutrophil elastase activity in sputum (91). A recent 12-month-long, controlled study of inhaled gentamicin demonstrated striking benefit to reduce frequency of exacerbations, and reduce the burden of bacteria and markers of pulmonary and systemic inflammation (92). For patients with PCD who develop airway infection with *P. aeruginosa*, it is noteworthy that one study in patients with non-CF bronchiectasis demonstrated high rates of eradication of *P. aeruginosa* (90). A goal for the PCD community is to develop the requisite elements (patients, sites, outcome measures) to perform prospective studies of pertinent therapies in PCD.

Osmotic Agents and Dornase α

Stimulation of Cl^- (and liquid) secretion by an inhaled agent (P2Y2 receptor agonist) improved cough clearance of radiolabeled

TABLE 2. MUTATIONS IN THE GENES THAT CAUSE HUMAN PRIMARY CILIARY DYSKINESIA

Human Gene	Human Chromosomal Location	*Chlamydomonas* Ortholog	Ciliary Ultrastructure in Subjects with Biallelic Mutations	Presence of Laterality Defects	% of Individual with Biallelic Mutations	MIM No.	References
DNAH5	5p15.2	*DHC γ*	ODA defect	Yes	15–21% of all PCD, 27–38% of PCD with ODA defects	608644	2, 16
DNAI1	9p21-p13	*IC78*	ODA defect	Yes	2–9% of all PCD, 4–13% of PCD with ODA defects	244400	2, 16
DNAI2	17q25	*IC69*	ODA defect	Yes	2% of all PCD, 4% of PCD with ODA defects	612444	16
DNAL1	14q24.3	*LC1*	ODA defect	Yes	na	614017	16, 81
CCDC114	19q13.32	*DC2*	ODA defect	Yes	6% of PCD with ODA defects	615038	83, 84
TXNDC3 (NME8)	7p14-p13	*LC5*	Partial ODA defect (66% cilia defective)	Yes	na	610852	16
DNAAF1 (LRRC50)	16q24.1	*ODA7*	ODA + IDA defect	Yes	17% of PCD with ODA + IDA defects	613193	16
DNAAF2 (KTU)	14q21.3	*PF13*	ODA + IDA defect	Yes	12% of PCD with ODA + IDA defects	612517, 612518	16
DNAAF3 (C19ORF51)	19q13.42	*PF22*	ODA + IDA defect	Yes	na	606763	75
CCDC103	17q21.31	*PR46b*	ODA + IDA defect	Yes	na	614679	77
HEATR2	7p22.3	Chlre4 gene model 525994 Phytozyme v8.0 gene ID Cre09.g39500.t1	ODA + IDA defect	Yes	na	614864	79
LRRC6	8q24	*MOT47*	ODA + IDA defect	Yes	11% of PCD with ODA + IDA defects	614930	80
CCDC39	3q26.33	*FAP59*	IDA defect + axonemal disorganization	Yes	36–65% of PCD with IDA defects + Axonemal disorganization	613798	16, 82
CCDC40	17q25.3	*FAP172*	IDA defect + axonemal disorganization	Yes	24–54% of PCD with IDA defects + Axonemal disorganization	613808	16, 82
RSPH4A	6q22.1	*RSP4, RSP6*	Mostly normal, CA defects in small proportion of cilia	No	na	612649	16
RSPH9	6p21.1	*RSP9*	Mostly normal, CA defects in small proportion of cilia	No	na	612648	16
HYDIN	16q22.2	*hydin*	Normal, very occasionally CA defects	No	na	610812	76
DNAH11	7p21	*DHC β*	Normal	Yes	6% of all PCD, 22% of PCD with normal ultrastructure	603339	16
RPGR	Xp21.1	na	Mixed	No	PCD cosegregates with X-linked Retinitis Pigmentosa	300170	16
OFD1	Xq22	*OFD1*	nd	No	PCD cosegregates with X-linked mental retardation	312610	16
CCDC164 (C2ORF39)	2p23.3	*DRC1*	Nexin (N-DRC) link missing; axonemal disorganization in small proportion of cilia	No	na	312610	85

Definition of abbreviations: CA = central apparatus; IDA = inner dynein arm; MIM = Mendelian inheritance in man; na = not available; N-DRC = nexin–dynein regulatory complex; ODA = outer dynein arm; PCD = primary ciliary dyskinesia.

MIM number is the online MIM (www.ncbi.nlm.nih.gov/omim), which is a continuously updated catalog of human genes, genetic disorders, and traits, with particular focus on the molecular relationship between genetic variation and phenotype expression.

particles in a small study of adults with PCD (93). Recently, nebulized 7% hypertonic saline over 3 months improved lung function and quality of life, and reduced antibiotic use in non-CF bronchiectasis (94). The presumption is that stimulation of cough and increased hydration of airway secretions with hypertonic saline or other osmotic agents can benefit tracheobronchial clearance in non-CF bronchiectasis. By analogy, hydrating airway secretions may facilitate cough clearance in PCD, even though mucociliary clearance remains defective. In contrast to beneficial effects in CF, dornase alfa has not been shown to improve pulmonary status in non-CF bronchiectasis; indeed, adults with non-CF bronchiectasis taking dornase alfa for 24 weeks experienced more pulmonary exacerbations and a more significant decline in FEV_1 compared with the placebo group (95).

Antiinflammatory Therapy

Oral macrolides are clearly effective in reducing exacerbations in CF through antiinflammatory mechanisms (86). Similar data are emerging in relatively small studies in non-CF bronchiectasis, as relates to reduction in exacerbations (88, 89). In contrast to CF, most of the patients with non-CF bronchiectasis have bacterial infections other than *P. aeruginosa*, so the mechanism of macrolide effects in non-CF bronchiectasis could relate to

antibiotic effect, as well as an antiinflammatory effect. The development of microbial resistance with chronic macrolides is of concern. Current practice guidelines suggest that patients should not receive chronic oral macrolides unless they have been tested and shown to not harbor NTM (86, 88, 89). The widespread use of inhaled or systemic corticosteroids in CF and non-CF bronchiectasis has been debated due to the risk–benefit ratio.

Lung Resection and Lung Transplantation

PCD affects all regions of the lung. Resection of lung in PCD is occasionally useful, but should be undertaken only after careful consideration and consultation with PCD experts. Patient selection should focus on those with severe localized bronchiectasis with recurrent febrile relapses or severe hemoptysis, despite aggressive medical management (96). Patients with PCD with end-stage lung disease are candidates for lung transplantation, and a small number of transplants have been successfully performed.

MANAGEMENT OF OTOLARYNGOLOGIC MANIFESTATIONS

Middle Ear Disease

Management of otitis media with effusion is controversial, even though it affects almost all children with PCD, and has implications for conductive hearing loss, delayed speech and language development, and cholesteatoma formation (18, 97). Advocates of tympanostomy (ventilation) tubes suggest that hearing may be improved (long term) in some patients, and otorrhea can be controlled (97). In contrast, the European Respiratory Society Consensus Statement recommends against placement of tubes for chronic otitis media in patients with PCD, because resultant otorrhea is a problem, and spontaneous resolution of chronic otitis media may occur in the teenage years (3, 98). Episodes of otitis media should be treated by standard approaches, but data are not available to address the question of surgical intervention. Although middle-ear symptoms typically decrease over time, it should be recognized that chronic otitis media may persist into adulthood, and conductive hearing loss (glue ear) occurs in some patients. Therefore, audiology assessments, hearing aids, and communication assistance should be encouraged.

Chronic Sinusitis

Sinus disease is a major problem for many patients with PCD. Initial management includes nasal steroids, nasal lavage, and intermittent courses of systemic antibiotics. Polyps may require surgery. Functional endoscopic sinus surgery is helpful in many patients that are refractory to medical therapy, particularly if there is aggressive postsurgical treatment to maintain adequate drainage (99, 100).

FUTURE DIRECTIONS

PCD Diagnostic and Treatment Centers

Treatment of patients with PCD is not standardized, and many patients with PCD receive suboptimal management, including many who do not have regular surveillance of respiratory microbiology, and many have not received regular treatment with antibiotics targeted to pathogens. A multidisciplinary approach to management of chronic disease is well recognized to benefit long-term outcomes, particularly for rare diseases. Because CF shares many features of PCD lung disease, and has established a successful clinical network, the PCD Foundation is developing a network of PCD clinical centers, which frequently involve CF center clinicians. In addition to the eight sites already active in our rare disease consortium, the PCD Foundation has recently selected additional sites to be recognized for the care of patients with PCD, which should alleviate some of the inconsistency in care. This approach will also facilitate the identification of patients with PCD for participation in prospective clinical trials, and create the opportunity to partner with European PCD centers for trials.

Note added in proofs: Since the preparation and acceptance of this manuscript, the following have been identified as PCD disease-causing mutations and published in the literature (MIM# given in parentheses): *ARMC4* (615408), *DYX1C1* (608706), *ZMYND10* (607070), *CCDC65* (611088), *RSPH1* (609314), and *SPAG1* (603395).

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