Primary ciliary dyskinesia (PCD): A genetic disorder of motile cilia

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Abstract. Primary ciliary dyskinesia (PCD) is a genetic disorder of motile cilia. Clinical features include chronic oto-sino-pulmonary disease, laterality defects, and male fertility reflecting impaired function of respiratory cilia in the upper and lower respiratory tracts, nodal cilia in the embryonic node and sperm tails, respectively. Recent studies have identified over 40 PCD-associated genes that encode proteins involved in ciliary biogenesis, assembly, structure, or function. Mutations in these genes account for approximately 70% of PCD cases; therefore, further gene discovery is expected. The diagnosis of PCD is challenging because no single test has the required diagnostic accuracy. Recent efforts have focused on standardizing and validating a panel of tests (including assessment for key clinical features, nasal nitric oxide measurement, ciliary ultrastructure analysis, and PCD genetic testing) to be used at PCD Centers to accurately diagnose PCD. Multi-center research programs focused on PCD in North America and Europe have been crucial for PCD gene discovery, advancing our understanding of the natural history of PCD and launching multi-center clinical trials.

Keywords: Cilia, dynein, mucociliary clearance, *situs inversus totalis*, *situs ambiguus*, heterotaxy, bronchiectasis, nitric oxide

1. Introduction

Primary ciliary dyskinesia is a heterogeneous, usually recessive, genetic disorder of motile cilia, and is characterized by a diverse array of clinical features, including chronic/recurrent upper and lower respiratory tract infections, laterality defects (including *situs inversus totalis* and heterotaxy), and male infertility [78]. Early names for this disorder included "Kartagener Syndrome" (triad of bronchiectasis, chronic sinusitis and *situs inversus totalis*) [73] and "Immotile Cilia Syndrome" (after identification of immotile respiratory cilia and immotile sperm in individuals with Kartagener Syndrome) [1, 41].

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Electron microscopy studies demonstrated absence of dynein arms on ciliary cross-sections from patients with "Immotile Cilia Syndrome" [1, 41]. Similar dynein arm defects occurred in patients with bronchiectasis and sinusitis, but without *situs inversus totalis* [41]. Subsequent studies showed that respiratory cilia from some patients with dynein arm defects were not immotile, but were dyskinetic; hence, the names "dyskinetic cilia syndrome" [129] and "ciliary dyskinesia" [130] were introduced and then modified in 1981 to "primary ciliary dyskinesia" to reflect the congenital nature of this disorder of motile cilia [141]. Advances over the past 25 years have greatly expanded our understanding of the genetics, pathophysiology, clinical features and clinical progression of primary ciliary dyskinesia.

Primary ciliary dyskinesia (PCD) is a separate entity from the "non-motile ciliopathies" or "primary ciliopathies" that are inclusive terms used over the last 10–15 years for an array of genetic disorders of the non-motile or sensory primary cilium [33, 147]. The term non-motile ciliopathies is preferred to avoid confusion between primary ciliopathies and primary ciliary dyskinesia. While the general structure of motile and non-motile cilia are similar, each type has distinct structures and functions.

2. Cilia: Structure and function

2.1. Types of cilia

Cilia are highly conserved organelles that can be divided into two major types: motile cilia and non-motile (sensory) cilia. Specialized motile cilia are apparent during early embryonic development within the ventral node and hence known as "nodal cilia".

2.1.1. Motile cilia

Motile cilia are distinct structures at the apical surface of cells that beat rhythmically and are essential for muco-ciliary clearance and transport of fluid, and play a role in sexual reproduction. Motile cilia are abundant in the upper respiratory tract (including nasal passage, paranasal sinuses and Eustachian tube), lower respiratory tract (tracheobronchial tree), as well as the ependyma of the brain and the fallopian tubes [127]. Sperm tails are structurally similar to motile cilia [68].

Motile cilia typically occur as multiple protrusions oriented in an organized array. The axonemal structure of motile cilia is very similar to flagella in single-cell organisms such as the green algae, *Chlamydomonas reinhardtii*, that has two flagella that are used for locomotion and reproduction [69]. These similarities have provided insights into the structure, function and genetics of human motile cilia [57].

Motile cilia are complex structures composed of hundreds of proteins. A cylindrical scaffold of nine microtubule doublets encircling a central pair of microtubules creates the characteristic $9 + 2$ arrangement typically seen on transmission electron microscopy [144] (Fig. 1). The basal body is a specialized structure at the base of the cilium that anchors the cilium in the apical cell membrane thereby directing the direction of ciliary beat and playing a pivotal role in assembly and transport of proteins into the cilium [122]. Emanating from each microtubule doublet are the outer and inner dynein arms that occur at distinct intervals. These dynein arms are complex structures that contain ATPase and function as "motors" for the cilia. Dynein arms emerge from the A microtubule of the outer doublets and slide onto the B-microtubule of the adjacent doublet, generating a force that translates to a bending motion of the cilium. The outer dynein arm is a large protein complex of heavy, intermediate and light dynein chains grouped into three stalks, with a tilt axis perpendicular to the microtubule. Such an arrangement allows the outer dynein arms to provide the power stroke needed for bending the cilium [69]. Inner dynein arms are composed of two stalks, and are important for controlling the rhythmic motion of cilia as part of a complex often referred to as the Nexin-dynein regulatory complex (N-DRC) [17, 57]. Cilia also have nexin links that connect adjacent outer microtubular doublets and limit the
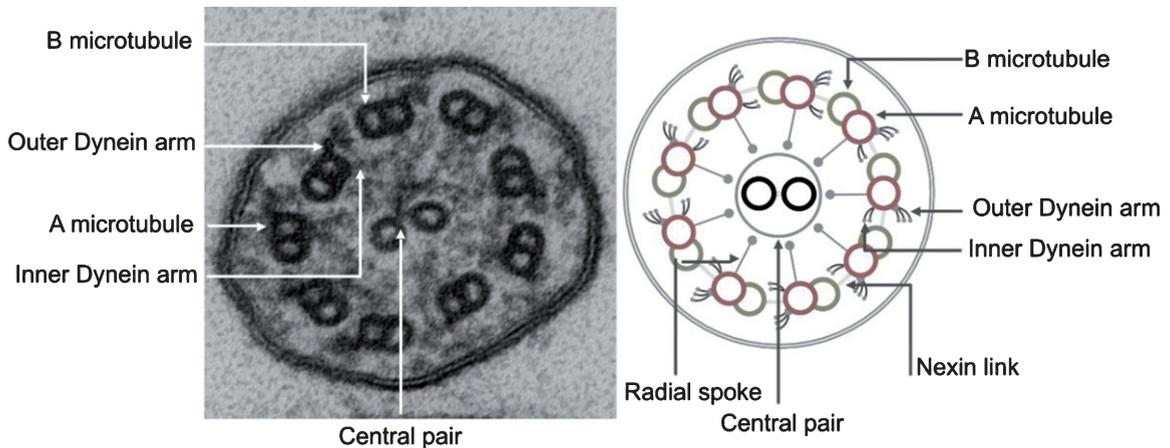
Fig. 1. Transmission electron photomicrograph (left) and schematic diagram (right) of a normal motor cilium in cross-section showing the ultrastructural features of the ciliary axoneme.

degree of sliding between microtubules, as well as radial spokes that control dynein arm activity and relay signals from the central apparatus to the dynein arms [47]. The central structure of a cilium is separated from the cell cytoplasm by a functional barrier, which regulates flux of proteins into the cilium [5, 122]. Movement of proteins along the length of the cilium is done through a specialized mechanism of intra-flagellar transport (IFT), that was first described in lower order organisms [27, 126].

An airway epithelial cell has approximately 200 motile cilia that are oriented in the same direction and move in unison at a constant frequency that ranges between 8–20 Hz. Motile cilia beating is influenced by changes in the ciliary microenvironment, such that the cilia beat frequency can change in response to triphosphate nucleotides, the depth and viscosity of the apical surface fluid, changes in redox conditions, and exposure to pathogens and airborne pollutants [72, 140].

2.1.2. Nodal cilia

A subtype of motile cilia, known as nodal cilia, transiently appears during early embryonic development. Nodal cilia are motile solitary cilia, with a "9 + 0" arrangement that emerges in the ventral node of the gastrula. Because these cilia lack a central pair, they have a rotatory motion that generates leftward movement of fluid across the surface of the embryonic node. This motion is sensed by immotile sensory cilia around the rim of the node, and is thought to be responsible for determining body laterality [43, 106, 151]. In PCD associated with immotile nodal cilia, left-right orientation becomes random, resulting in laterality defects such as *situs inversus totalis*, *situs ambiguus*, and heterotaxy syndromes [8, 58, 76, 86].

2.1.3. Non-motile (sensory) cilia

The non-motile cilium typically occurs as a solitary cilium per cell and serves as an "antenna" to sense the surrounding environment and transmit signals [147]. Unlike motile cilia, non-motile cilia lack dynein arms and thus are immotile. They also lack a central pair, thus forming a "9 + 0" configuration. Non-motile cilia protrude from the surface of most mammalian cells during interphase and are essential for normal development and tissue differentiation, harboring many signaling receptors such as sonic hedgehog (SHH), epidermal growth factor receptor (EGFR), and platelet-derived growth factor receptor (PDGFR) [23, 26, 51]. Non-motile cilia have specialized functions in sensory organs, forming the retinal photoreceptors and cochlear stereocilia [44, 51]. Not surprisingly, mutations affecting the function of non-motile cilia, result in multiorgan involvement, leading to syndromes and conditions that are

known as non-motile ciliopathies [6, 47, 63]. These include conditions such as the autosomal recessive polycystic kidney disease and Bardet-Biedl Syndrome.

2.1.4. Overlap functions

Although motile and primary sensory cilia are often organized as having separate functions, it is important to recognize that sensory receptors have also been identified on motile cilia, including bitter taste receptors [132], and the Polycystin-1 (PKD1) and polycystin-2 (PKD2) flow sensors which are associated with increased risk of bronchiectasis [70].

3. Genetics of primary ciliary dyskinesia

Motile cilia are composed of hundreds of proteins. Genes encoding proteins involved in ciliary biogenesis, assembly, structure, or function are potential candidate genes for PCD. Gene discovery studies have identified 40 PCD-associated genes (Table 1). Mutations in known PCD genes account for approximately 70% of all PCD cases [81]. It is expected that more PCD genes will be identified, as more patients with clinical phenotypes suggestive of PCD are evaluated.

Mutations in PCD genes usually cause one of the "hallmark" ultrastructural axonemal defects, including absence of the outer dynein arm, absence of inner and outer dynein arms, absence of inner dynein arms associated with microtubular disorganization and/or central pair organization defects [78]. Approximately, 30% of genetically proven PCD cases have normal or normal-appearing ciliary axonemal structure which can reflect limitations of electron microscopy to detect subtle changes in structure [61, 79].

Early gene discovery relied on homozygosity mapping with sequencing of candidate genes, comparative genomics, transcriptomics, and proteomics [47, 90, 100, 115]. Recent advances in genetic approaches, such as next generation sequencing technologies including the use of whole exome sequencing, has allowed the discovery of increasing numbers of new PCD genes in subjects with symptoms suggestive of PCD [62, 80, 116] and enabled identification of PCD genes that encode non-structural proteins in cilia. As shown in Table 1, PCD-associated genes can be classified by location of protein (e.g., outer dynein arm, radial spoke, cytoplasmic) or by associated ultrastructural defect (e.g., absent outer dynein arm, absent outer and inner dynein arms, absent inner dynein arm with microtubular disorganization). For most structural protein genes, alteration in the encoded structural protein results in a distinctive ultrastructural defect (such as absent outer dynein arms with mutated *DNAH5* that encodes an outer dynein arm protein). However, mutations in some genes (such as *DNAH11* that encodes a different heavy chain isoform of the outer dynein arm), does not result in a detectable ultrastructural defect on transmission electron microscopy [125], yet, absence of DNAH11 is detectable by three dimensional tomography [138].

3.1. Genes encoding outer dynein arm components

This group includes some of the most prevalent genes implicated in PCD. Mutations in two of these genes, *DNAI1* (MIM 604366) [52, 123] and *DNAH5* (MIM 603335) [110], are associated with absence of the outer dynein arms, dyskinetic and slow ciliary beat pattern and account for more than 30% of all PCD cases [90]. *DNAI1* was the first identified PCD gene through a candidate gene approach based on screening mutants that cause abnormal flagellar motility in the green alga *C. reinhardtii* [123]. *DNAI1* mutations account for about 9% of all PCD subjects [156]. *DNAH5* was first cloned using homozygosity mapping in affected endogamous families with PCD [114], and later identified by sequencing subjects

Table 1
Genes associated with primary ciliary dyskinesia

Gene name	Alias	Locus	Protein location	Ultrastructure Phenotype
DNAH5		Chromosome 5	Outer dynein arm	ODA absent or truncated
DNAI1		Chromosome 9	Outer dynein arm	ODA absent or truncated
DNAI2		Chromosome 17	Outer dynein arm	ODA absent or truncated
DNAL1		Chromosome 14	Outer dynein arm	ODA absent or truncated
NME8	*TXNDC3*	Chromosome 7	Outer dynein arm	ODA absent or truncated
DNAH8		Chromosome 6	Outer dynein arm	Not defined
DNAH11		Chromosome 7	Outer dynein arm	Normal structure
TTC25		Chromosome 17	Outer dynein arm docking	ODA absent or truncated
CCDC114		Chromosome 19	Outer dynein arm docking	ODA absent or truncated
ARMC4		Chromosome 10	Outer dynein arm docking	ODA absent or truncated
CCDC151		Chromosome 19	Outer dynein arm docking	ODA absent or truncated
CCDC103		Chromosome 17	Dynein arm attachment	ODA absent
DNAH1		Chromosome 3	Inner dynein arm	Not defined
LRRC6		Chromosome 8	Cytoplasmic	ODA + IDA absent or truncated
DNAAF1	*LRRC50; ODA7*	Chromosome 16	Cytoplasmic	ODA + IDA absent or truncated
DNAAF2	*C14orf104; KTU*	Chromosome 14	Cytoplasmic	ODA + IDA absent or truncated
DNAAF3	*C19orf51; PF22*	Chromosome 19	Cytoplasmic	ODA + IDA absent or truncated
DNAAF4	*DYX1C1*	Chromosome 15	Cytoplasmic	ODA + IDA absent or truncated
DNAAF5	*HEATR2*	Chromosome 7	Cytoplasmic	ODA + IDA absent or truncated
SPAG1		Chromosome 8	Cytoplasmic	ODA + IDA absent or truncated
ZMYND10		Chromosome 3	Cytoplasmic	ODA + IDA absent or truncated
CFAP298	*C21orf59; kurly*	Chromosome 21	Cytoplasmic	ODA + IDA absent or truncated
PIH1D3		Chromosome X	Cytoplasmic	ODA + IDA absent or truncated
CFAP300	*C11orf70*	Chromosome 11	Cytoplasmic	ODA + IDA absent or truncated
HYDIN		Chromosome 16	Central pair	Normal structure
STK36		Chromosome 2	Central pair	Normal/Central pair defect
RSPH4A		Chromosome 6	Radial spoke	Normal/Central pair defect
RSPH9		Chromosome 6	Radial spoke	Normal/Central pair defect
RSPH1		Chromosome 21	Radial spoke	Normal/Central pair defect
RSPH3		Chromosome 6	Radial spoke	Normal/Central pair defect
DNAJB13		Chromosome 11	Radial spoke	Normal/Central pair defect
CCDC65		Chromosome 12	Nexin link	Normal structure
DRC1	*CCDC164*	Chromosome 2	Nexin link	Normal structure
GAS8		Chromosome 16	Nexin link	Normal structure
CCDC39		Chromosome 3	N-DRC	IDA absent and microtubular disorganization
CCDC40		Chromosome 17	N-DRC	IDA absent and microtubular disorganization
CCNO		Chromosome 5	Apical cytoplasm for centriole replication	Reduced cilia
MCIDAS		Chromosome 5	Nucleus as transcription co-regulator	Reduced cilia number
RPGR		Chromosome X	Transition zone	Normal structure
OFD1		Chromosome X	Centriole	Normal structure

ODA: outer-dynein arm; IDA: inner-dynein arm; N-DRC: nexin-dynein regulatory complex.

with PCD, accounting for approximately 25% of PCD patients [110]. Other outer dynein arm genes, including *DNAL1* (MIM 610062) [66], *DNAI2* (MIM 605483) [91], and *NME8* (MIM 607421) [39] are associated with outer dynein arm defect, but are much less prevalent (<3% of PCD). Mutations in *DNAH11* (MIM 603339), which also encodes an outer dynein arm protein, produces an intriguing phenotype [79, 125] with normal ciliary ultrastructure, but very rapid beat frequency with subtle limitation of the bend of the cilia. Even though the ciliary beat frequency appears normal or supra-normal, mutations in *DNAH11* cause a subtle abnormality in the ciliary waveform with inadequate mucociliary clearance and hence disease.

3.2. Genes encoding nexin-dynein regulatory complex (N-DRC) components

Coordinated beating of motile cilia relies on the function of the N-DRC that functions as a biochemical complex maintaining the alignment between microtubule doublets during cilia beating, interacting with the radial spokes and IDA, and includes the nexin link that connects outer microtubules [16, 57]. Mutations in coiled-coil domain containing proteins, *CCDC39* (MIM 613798) [98] and *CCDC40* (MIM 613799) [9, 11] cause defects in both the assembly of the N-DRC and inner dynein arms, and disruption of this periodicity. The latter results in inconsistent ultrastructual abnormalities, characterized by disordered microtubules in some, but only a minority, of all cilia. Axonemal disorganization and inner dynein arm defects represent about 12% of all PCD cases, and mutations in *CCDC39* and *CCDC40* account for about 70% inner dynein arm defects with microtubular disorganization [2]. Interestingly, individuals with mutations in *CCDC39* and *CCDC40,* and the axonemal disorganization and inner dynein arm defect group, have worse pulmonary function and more rapid decline compared to other PCD patients [34, 35].

3.3. Genes encoding radial spoke components

This group includes *RSPH9* (MIM 612648) [24], *RSPH4A* (MIM 612647) [24], *RSPH3* (MIM 61586) [71], and *RSPH1* (MIM 609314) [111]. Mutations in *RSPH9* and *RSPH4A*, both associated with absence of some of the central pairs and motility defects [24], were identified using homozygosity mapping in consanguineous families. These proteins encode radial spoke head proteins, and screening in the alga *C. reinhardtii* identified mutations in orthologous genes that showed similar ultrastructural defects [154]. In PCD subjects with mutant radial spokes proteins, cilia motility is circular. Because nodal cilia do not normally have central pairs, this group of patients does not present with *situs* abnormalities.

3.4. Genes encoding cytoplasmic proteins involved in assembly

Cilia are complex organelles, and the current paradigm is that at least part of the dynein arms are preassembled in the cytoplasm prior to transport into the ciliary axoneme [49, 126]. Recently, PCD-causing mutations in genes encoding cytoplasmic proteins that result in absence of ODA and IDA were identified, indicating that they play a role in the preassembly of dynein motor complexes. *DNAAF2* (*ktu;* MIM 612517) was the first cytoplasmic gene to be identified in patients with PCD [115]. DNAAF2 interacts with intermediate dynein chain DNAI2 and the chaperone heat shock protein HSP70. Other PCD genes encoding cytoplasmic proteins that are associated with absence of ODA and IDA when mutated include: *DNAAF1* (*LRRC50, ODA7*, MIM 613190) [90], *LRRC6* (MIM 614930) [64, 83], *DNAAF5* (*HEATR2*; MIM 614864) [62], *DNAAF3* (*PF22*; MIM 614566) [100], *DNAAF4 (DYX1C1; MIM 608706)* [145], *SPAG1* (MIM 603395) [81], *CCDC103* (MIM 614677) [121], *ZMYND10* (MIM 607070) [102, 155], *CFAP298* (*C21orf59*; *Kurly*; MIM 615494)) [4], *CFAP300* (*C11orf70*; MIM

618058)) [59] and *PIH1D3;* MIM 300933) [112, 119] (Table 1). The exact function of these proteins is not known, but evidence points to close interactions among several proteins, as well as protein complexes and heat-shock proteins suggesting that they may function as chaperones during dynein arm protein assembly [65, 92], e.g., LRRC6 for instance interacts with ZMYND10 [155]; DNAAF5 interacts with SPAG1 and DNAAF2 during the earliest stages of cilia preassembly [65]; DNAAF2 interacts with DYX1C1 and PIH1D3 at later stages of cilia preassembly, possibly acting as linker proteins [65, 119, 145]. These proteins also interact with protein chaperones HSP70 and HSP90, facilitating the organization of dynein arm assembly [25, 115, 120, 145].

3.5. Genes encoding proteins involved in centriole replication

The multi-ciliated airway cell undergoes a process by which the cell centrosome multiplies to form over 200 centrioles. These centrioles form the basal bodies from which motile cilia nucleate. This process is poorly understood, but appears to be regulated by two factors, *MCIDAS* (MIM 614086) and *CCNO* (MIM 607752). While mutations in most PCD-associated genes result in structural changes in the ciliary axoneme, mutations in *MCIDAS* and *CCNO* are associated with reduced numbers of normally appearing motile cilia [15, 148]. MCIDAS is a co-regulator that regulates CCNO and FOXJ1, a master cilia regulatory protein; however, CCNO regulates centrosome replication, which is essential for the formation of multiple cilia. Patients present with a clinical phenotype compatible with PCD. TEM evaluation shows sparse cilia, thus the term "reduced generation of multiple motile cilia" disease (RGMC). RGMC should be considered part of the spectrum of PCD, and not a separate entity. The findings in these patients is consistent with the syndrome previously known as ciliary aplasia [37, 96, 128], and mutations in these factors should be considered in patients with symptoms suggestive of PCD with repeated ciliary biopsies that report inadequate numbers of motile cilia.

3.6. Genes encoding centrosomal proteins and the transition zone

The cilia transition zone is an area at the base of the cilium that is active in intra-ciliary transport of signaling molecules and other components into the ciliary axoneme [105]. Many of the gene mutations associated with the transition zone have been associated with non-motile ciliopathies. Although most non-motile ciliopathy genes do not influence motile cilia function [63], one gene stands in contrast. *RPGR* (MIM 312610) encodes a protein in the outer segment of the retinal rod photoreceptor, which is a modified cilium. In airway epithelia, RPGR localizes to the transition zone [60] where it provides cilia stability and ciliary protein trafficking. Mutations in *RPGR* have also been reported in patients with symptoms combining X-linked Retinitis Pigmentosa and PCD [101]. These patients had typical PCD respiratory symptoms, associated with normal ultrastructure and, in some cases, variable cilia axonemal structural defects, including truncated or absent dynein arms or central pair defects [101].

OFD1 (MIM 300170) encodes a centrosomal protein that is also important in WNT signaling and body axis patterning. Mutations in *OFD1* are associated with non-motile cilia dysfunction and X-linked oral-facial-digital type I (OFD1) syndrome [48]. Mutations in *OFD1* were also reported in a few patients with OFD1 with respiratory symptoms and severely disorganized motile cilia beating [20].

4. Clinical features of PCD

The clinical features of PCD reflect defective motile cilia function in the respiratory tract, sinuses, middle ear, and reproductive tract, as well as in the ventral node during embryogenesis (Table 2) [78, 137].

Table 2
Age-Related Prevalence of Clinical Features in Primary Ciliary Dyskinesia[1]

PCD clinical feature	Youngest age when feature present in >50% of PCD	Youngest age when feature present in >80% of PCD
Neonatal respiratory distress	12 hr of life	24 hr of life
Organ laterality defects (SIT or SA)	Neonatal to school age	—
Recurrent otitis media with effusion	Infancy	Infancy
Year-round, daily cough	Infancy	Infancy
Year-round, daily nasal congestion	Infancy	Infancy
Chronic pansinusitis	Preschool	School age
Recurrent lower respiratory infections	Infancy	Preschool
Bronchiectasis	School age	Adult
Male infertility	—	Adult

[1]Table reproduced from Shapiro, *Pediatric Pulmonology*, 2015 [137].
SIT, *situs inversus totalis*; SA, *situs ambiguus*.

4.1. Lower respiratory tract

4.1.1. Neonatal respiratory distress

Respiratory manifestations of PCD are present from birth. The majority (75–91%) of patients diagnosed with PCD have a history of unexplained neonatal respiratory distress [46, 67, 87, 107]. Despite term gestation, PCD neonates present with tachypnea and increased work of breathing, and usually require supplemental oxygen, continuous positive airway pressure ventilation (CPAP) or, in rare cases, intubation with mechanical ventilation. Frequently, these neonates will be diagnosed with transient tachypnea of the newborn (TTN) or neonatal pneumonia; however, the PCD clinical presentation differs from that of other etiologies of neonatal respiratory distress. In a case-control study comparing PCD term neonates versus term neonates with neonatal respiratory distress (disease controls), PCD presented with later onset of neonatal respiratory distress (median 12 hours of life versus 1 hour of life), required a longer duration of oxygen therapy (mean 15.2 days versus 0.8 days), and had a higher frequency of atelectasis and/or lobar collapse on chest radiographs (70% versus 0%) [103]. Mechanisms for lobar collapse may include preferential gas trapping and hyperinflation of lower lobes leading to atelectasis/compression in the upper lobes, or intraluminal airway mucous obstruction in the upper lobes [103]. Lobar collapse in the setting of unexplained neonatal respiratory distress should prompt consideration for PCD. The combination of neonatal respiratory distress and *situs inversus totalis* warrants PCD evaluation. Similarly, *situs ambiguus* with neonatal respiratory distress out of proportion to the associated cardiac lesion should prompt PCD evaluation.

4.1.2. Chronic cough

Patients with PCD have persistent, year-round wet cough that typically starts before 6 months of age [87]. They have intermittent exacerbations and recurrent lower respiratory tract infections, including pneumonia and bronchitis. Nearly 80% of pre-school children with PCD have recurrent lower respiratory tract infections [78]. While cough can improve with antibiotics and aggressive airway clearance, it does not completely resolve. The most prevalent pathogens recovered in sputum from PCD children are *Haemophilus influenza, Staphylococcus aureus, Streptococcus pneumonia* and *Moraxella catarrhalis* [35]. In children with PCD, the age-specific prevalence of *Pseudomonas aeruginosa* is lower than in CF, with reported prevalence of 5–7% for mucoid phenotypes in children and adolescents [107]. In adult patients with PCD, the prevalence is approximately 27% for mucoid *P. aeruginosa*,

with increased prevalence with age, especially after 30 years [107]. In contrast to CF, cough clearance is preserved in PCD, which may decrease the risk of chronic colonization with *P. aeruginosa* during childhood [35]. However, after years of impaired mucociliary clearance and progressive bronchiectasis, the diseased airways of many patients with PCD develop chronic *P. aeruginosa* infection [152]. While rare in childhood, non-tuberculous mycobacteria has been reported in more than 10% of adults with PCD [107].

4.1.3. Progressive obstructive lung disease

Obstructive lung disease can develop in early life, yet the severity and progression of lung disease in PCD is highly heterogeneous. Early cross-sectional and longitudinal studies show that airflow obstruction worsens with increasing age [42, 56, 95]. One cross-sectional study in pediatric and adult subjects with PCD showed that the mean FEV_1 decline (–0.8% predicted per year) was slower than that reported for CF at that time (–3.6% predicted per year) [107]. In a recent longitudinal study of children with PCD, mean annual change in percent predicted FEV_1 was –0.57% per year, with children with inner dynein arm/central pair/microtubular disorganization mutations having the greatest rate of decline [35]. Similarly, in a retrospective, international cohort study of 991 children and adults with PCD, lung function was reduced in all age groups when compared to reference values, particularly for those PCD patients with microtubular defects [54].

4.1.4. Bronchiectasis

Patients with PCD develop progressive bronchiectasis that is essentially universal by adulthood [18, 107]. Chest radiographic studies have demonstrated evidence of lung disease during infancy and early childhood [78]. Chest CT findings may include subsegmental atelectasis, mucous plugging, air-trapping, ground-glass opacities, peribronchial thickening, and bronchiectasis. Bronchiectasis is the permanent dilation of bronchi resulting from a vicious cycle of recurrent infection, impaired mucociliary clearance, and destruction of the bronchial wall and elastic connective tissue. Progressive airway obstruction and bronchiectasis can lead to end stage lung disease [107]. In a recent retrospective study of 151 adults with PCD with a median age of 35 years, 7-year incidence of all-cause mortality was nearly 5%, and respiratory mortality was 3.3% [131].

4.1.5. Other

Chest wall abnormalities with unexplained sinopulmonary disease should prompt consideration of PCD. Pectus excavatum occurs in as many as 10% of patients with PCD, compared to 0.3% in the general population [75]. Additionally, there is a higher prevalence of scoliosis (5–10%) in PCD [78].

4.2. Upper respiratory tract

4.2.1. Nasal congestion and chronic sinusitis

Chronic, year-round daily nasal congestion occurs in 76–100% of children with PCD, and is frequently associated with hypoplastic frontal and sphenoid sinuses [124]. Onset typically begins within the first 6 months of life. Recurrent sinusitis is also common, and most PCD patients will have pansinusitis on computed tomography (CT) [99]. Nasal polyps can occur in up to 15% of PCD patients [22].

4.2.2. Chronic otitis media

Recurrent otitis media (ROM, "glue ear") occurs in up to 80% of PCD children [137]. In one study, 38% of PCD children required more than 30 lifetime antibiotic courses [143]. ROM complications can include conductive hearing loss, speech and language delay, and cholesteatoma formation [40].
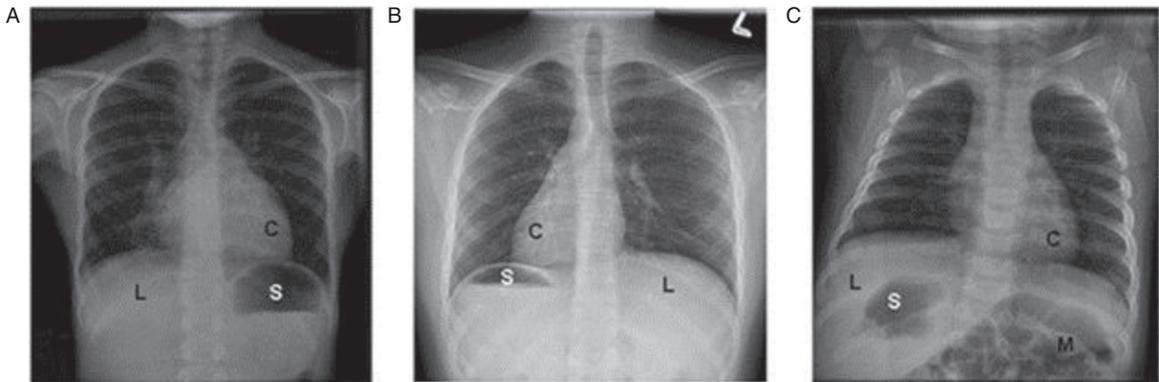
Fig. 2. Examples of laterality defects on radiology imaging in various situs groups [133]. Examples of various laterality defects on radiology imaging in PCD. Different situs arrangements found in PCD, including (A) a participant with situs solitus, or normal organ arrangement, with left cardiac apex, left-sided stomach bubble, and right-sided liver; (B) a patient with situs inversus totalis (SIT), or mirror-image organ arrangement, with right cardiac apex, right-sided stomach bubble, and left-sided liver; (C) a patient with situs ambiguus (SA), with left cardiac apex, right-sided stomach bubble, right-sided liver, and intestinal malrotation; This patient also had right-sided polysplenia visualized on a CT scan. C, cardiac apex; S, stomach; L, liver; M, intestinal malrotation. Image and legend reproduced from Shapiro et al, *Chest*, 2014, with permission from Elsevier [133].

PCD children have nearly universal conductive hearing loss due to persistent otitis media with effusion [137]. Hearing abnormalities can improve during adolescence, but may persist into adulthood [137].

4.3. Laterality defects

Cilia dysfunction in embryonic nodal cells can result in laterality defects and congenital heart disease [78, 133]. Nodal cilia contain a $9+0$ axonemal composition and lack the components of the central apparatus and radial spokes. Patients with mutations in genes that encode the central complex and radial spokes do not have *situs* abnormalities, since they have normal rotatory function essential for left-right asymmetry [111]. Organ laterality defects in PCD include *situs inversus totalis* (SIT—mirror-image arrangement), and *situs ambiguus* (SA—arrangement falls between mirror image and normal, Fig. 2) [133]. SIT occurs in slightly <50% of PCD patients, whereas SA occurs in at least 12% of PCD patients and is associated with a 200-fold increased probability of having structural congenital heart disease compared to the general population [19, 55, 133]. Therefore, echocardiograms are indicated to evaluate for CHD in patients with PCD and SA, while in general they are not necessary for asymptomatic patients with PCD and SIT or *situs solitus*. Patients with SA may have heterotaxy syndromes (i.e. intestinal malrotation, interrupted inferior vena cava, or polysplenia) and imaging studies (i.e. abdominal ultrasound and echocardiogram) should be considered.

4.4. Infertility

Males with PCD are typically infertile, but some males report normal fertility. Females may have hypofertility and increased risk of ectopic pregnancy.

4.4.1. Male infertility

Sperm motility is driven by a flagellum containing motile axonemes consisting of $9+2$ microtubules, dynein arms, and regulatory structures, yet the motor protein composition of the axoneme of the sperm tail differs from that of motile respiratory cilia [68]. Abnormal axonemal structure results in reduction or loss of motility, leading to infertility. The most frequent ultrastructural defects of the

sperm flagella are outer dynein arm defects, microtubular translocation, and absent radial spokes [68]. *CCDC114* mutations may have no significant effect on fertility, since there is low transcript expression of *CCDC114* in testis compared to respiratory ciliated cells [116]. Genetic counseling is recommended and reproductive strategies for immotile/dysmotile sperm include *in vitro* fertilization and intracytoplasmic sperm injection [68].

4.4.2. Female hypofertility

Females with PCD with dynein arm defects can be fertile [68]. The motor protein composition and ciliary beating of human fallopian tube cilia is identical to respiratory cilia; however, the transport of the ovum towards the uterus is not totally cilia-dependent [127]. Females with PCD may also experience increased risk of ectopic pregnancy [127].

4.5. Clinical features overlapping with other disorders

PCD can co-exist with other rare diseases involving ciliary genes, including retinitis pigmentosa (an inherited cause of blindness from retinal ciliary dysfunction), and Orofaciodigital Syndrome (mental retardation, craniofacial abnormalities, macrocephaly, digital anomalies, and cystic kidneys) [150]. Overlapping ciliopathies have been reported, particularly with genetic disorders of non-motile cilia that result in cystic kidneys, cystic or cholestatic liver, skeletal malformations, developmental delay, hydrocephalus, blindness, or deafness [150]. PCD can also occur with other disease due to proximity of disease causing mutations at the same chromosomal locus, such as Cri du Chat syndrome, which results in a large deletion on chromosome 5p that includes the region of *DNAH5* [136].

4.6. Genotype-phenotype correlation

Genotype-phenotype relationships are emerging in PCD, particularly regarding variability in severity and progression of lung disease. Patients with a distinct ultrastructural defect (inner dynein arm defect with microtubular disorganization), that is usually linked to mutations in *CCDC39* and *CCDC40,* typically present earlier in life, have worse pulmonary function by both functional and structural assessment (spirometry and CT scans), and poorer nutritional status compared to similar-aged patients with outer dynein arm defects [34, 35]. In contrast, patients with *RSPH1* mutations typically have milder disease, higher levels of nasal nitric oxide, and better lung function [133].

5. Approach to diagnosis of PCD

PCD is a rare disease and is significantly underdiagnosed. Currently in North America, there are approximately 1,000 individuals with PCD followed by PCD clinical centers; however, prevalence estimates suggest that there should be at least 16,000 individuals with PCD in this region of the world [85, 87]. Substantial advances in the definition of PCD phenotype, ciliary ultrastructure analysis, PCD genetic testing, and nasal nitric oxide (nNO) measurement have provided more accurate tools for PCD diagnosis. Interpretation of these tests requires specialized expertise. Most PCD Centers employ a panel of tests because no single test has the diagnostic accuracy needed at this time.

5.1. PCD clinical phenotype

The first step in making a diagnosis of PCD is recognizing the specific clinical symptoms consistent with the disease (Table 2). Most children with PCD are diagnosed between 3 and 6 years of age, yet clinical symptoms for these children are present in the first year of life [85]. Children with PCD who

do not have an organ laterality defect are diagnosed later than children who present with respiratory symptoms and *situs inversus totalis* [85]. These findings suggest both a need for improved understanding of the clinical phenotype of PCD and the possibility of insufficient availability and/or utilization of PCD diagnostic testing.

A detailed clinical history is the first key step to recognize individuals at high likelihood of having PCD [87]. Any patient with the Kartagener triad (bronchiectasis, chronic sinusitis and *situs inversus totalis*) is highly likely to have PCD. However, early diagnosis relies on early recognition of chronic sino-pulmonary disease before progression to chronic sinusitis and bronchiectasis. A challenge for pediatricians is distinguishing the chronic respiratory symptoms of PCD from the recurrent episodes of viral respiratory infections. A recent multi-center study identified four key clinical features that enhance the likelihood of PCD in children: year-round, wet-sounding cough that starts in the first 6 months; year-round nasal congestion that starts in the first 6 months of life; a history of neonatal respiratory distress requiring supplemental oxygen for more than 24 hours despite term gestation; and organ laterality defect that is typically *situs inversus totalis*, but may be heterotaxy or *situs ambiguus* [87, 137]. Other clinical features, such as chronic otitis media, are part of the PCD phenotype but have not proven to have the same discriminatory diagnostic functionality as these specific 4 features [87, 137]. Presence of any one of these key clinical features should raise suspicion of PCD (sensitivity 0.96, specificity 0.41); presence of two key clinical features warrants diagnostic testing (sensitivity 0.80, specificity 0.72); and presence of all 4 key clinical features is highly likely PCD (sensitivity 0.21, specificity 0.99) [87, 137].

5.2. Ciliary biopsy

Several approaches have been used for ciliary biopsy, including scraping the respiratory epithelium from the nasal cavity (inferior surface of inferior nasal turbinate), brush biopsy from the nasal turbinate or brush biopsy of the bronchial mucosa during a bronchoscopy [113, 137, 139]. An important aspect of this procedure is obtaining an adequate sample of cilia for evaluation, accounting for changes in the epithelial surface from inflammation, including loss of cilia and other secondary changes to the cilia [113, 137].

5.2.1. Ultrastructural defects in cilia

In the past, ciliary biopsy with analysis of cilia structure by electronic microscopy (EM) was the gold standard diagnostic test for PCD; however, genetic testing has shown that biallelic mutations in some of the PCD genes can be associated with normal or near-normal ciliary ultrastructure. While this finding limits the sensitivity of EM analysis, this test still has diagnostic utility [139, 146]. It is important to have enough cilia in the sample to determine if a structural abnormality is present consistently [139]. Assessment of 200 or more individual cilia in cross-section is recommended for an adequate EM evaluation [36, 78, 113]. Hallmark defects of ciliary ultrastructure that are considered diagnostic for PCD include absence (or truncation) of outer dynein arms, absence (or truncation) of both outer and inner dynein arms and absence of inner dynein arms associated with microtubular disorganization [137, 149]. Each of these hallmark defects are associated with a specific group of genes that encode either proteins that are components of the defective structures or proteins that are involved in assembly of these structures (Table 1). Central apparatus defects (missing central pair, central pair off-center, transposition of outer doublet into central position) may occur in a subset of cilia and should be considered suggestive of PCD. These central apparatus defects can occur in small subset of cilia from healthy individuals or individuals recovering from viral illnesses. Therefore, central apparatus defects are not considered diagnostic unless observed on repeated biopsies or confirmed by genetic testing that identifies biallellic mutations in a radial spoke gene (Table 1) [108]. Similarly, absence of

inner dynein arms is frequently a secondary change in cilia and should not be considered diagnostic unless this defect occurs on repeated biopsies [108]. Of note, isolated inner dynein arm defects have not been associated with any of the presently defined PCD genes [108]. It is also important to remember that normal ciliary ultrastructure does not rule-out PCD, as it is estimated that up to 30% of individuals with PCD will have normal ciliary structure on EM [78].

5.2.2. Altered ciliary beat pattern

Diagnosis of PCD by ciliary movement analysis requires significant expertise and careful considerations. Although once a commonly utilized diagnostic test, evaluation of ciliary movement by light microscopy should not be used as a stand-alone test to diagnose PCD [134, 137]. High-speed video microscopy (HSVM) is an evolving alternative approach to ciliary movement analyses [134, 137]. HSVM involves recording of ciliary movement at high speed and then assessing the recording at low speed for altered ciliary beat pattern. At present, limitations of HSVM include subjectivity and low inter-rater reliability for HSVM ciliary evaluations; therefore, HSVM is not recommended as a PCD confirmatory test [134]. However, when performed in specialized centers, in conjunction with EM evaluation of cilia, it can have diagnostic utility.

5.2.3. Immunofluorescence using antibodies to ciliary proteins

Immunofluorescence technologies have been used in genetic discovery studies to confirm that mutations in a ciliary gene alter the encoded protein within ciliated cells. In some cases, immunofluorescence studies have identified protein alterations that are not easily detectable by EM. For example, cilia with radial spoke head defects often have near-normal appearance on EM; but, immunofluorescence studies with specific antibodies to radial spoke proteins can detect altered expression of respective proteins [50, 117]. Similarly, individuals with mutations in *DNAH11* have normal ciliary ultrastructure on EM [78]; however, immunofluorescence studies demonstrate that individuals with biallelic mutations in *DNAH11* have partial reduction of the outer dynein arms in only the proximal region of the cilia [38]. With further standardization and optimization of antibody panels, immunofluorescence technology has potential as a diagnostic test in the future.

5.3. Nasal nitric oxide testing

Nitric oxide (NO) is a small diffusible gas molecule that is involved in multiple functions throughout the body. Multiple studies have demonstrated that nitric oxide within the nasal cavity (particularly within the sinuses) is high in healthy individuals and much lower (<10% of normal values) in PCD, suggesting that nasal NO (nNO) measurement may be a useful test for PCD [29, 84, 88, 135]. Nasal NO is measured by a non-invasive approach using a small nasal probe to aspirate nasal gas at a rate of approximately 5 ml/second for measurement of nitric oxide content [10, 14]. Recent guidelines recommend measuring nasal NO with a chemiluminescence analyzer [137]. Alternative nitric oxide analyzers, including hand-held electrochemical devices are under study [94, 134]. Velum closure (VC) maneuvers are used to limit "contamination" of nasal air by air exhaled by the lower airways [14]. These techniques yield reproducible "plateau" measurements in children over 5 years of age, but often are not possible in younger children, who cannot cooperate with VC maneuvers. Some centers have measured nasal NO during tidal breathing in young children who are unable to cooperate with palate closure maneuvers [10, 14]. Currently, nasal NO testing is performed on a research basis at most PCD centers secondary to lack of regulatory body approval of the device for disease specific testing.

Multi-center studies and several meta-analyses have demonstrated diagnostic utility of nasal NO testing with a cut-off value of less than 77 nl/min considered consistent with a diagnosis of PCD [29, 84, 88, 135]. Most individuals with PCD have levels much lower than this cut-off. Despite tremendous

utility, nNO testing does have several important limitations. Because PCD is so rare, it is important to employ this testing in individuals with a high-suspicion of PCD based on clinical symptoms as more indiscriminate testing of individuals can result in false positives [28]. Further, while a nNO measurement less than the cut-off of 77 nl/min is consistent with a diagnosis of PCD, it is important to note that one testing measurement by itself should not be considered diagnostic of PCD [134]. Current guidelines recommend that in suspected PCD cases, the nNO measurement should be repeated at least one additional time and this repeat test should be at least one month after the initial testing [137]. Repeat testing accounts for clinical situations in which false positives can occur such as recent viral infection, acute sinusitis or occult sinus bleeding not readily assessed on clinical exam [3, 84, 88]. In addition, low nasal NO levels have been reported in cystic fibrosis [88, 107]; therefore, this disorder should be excluded if nasal NO is used as the primary diagnostic test. In an individual with a clinical phenotype consistent with PCD and with two NO measurements below the cut-off, but a normal sweat chloride test to rule out cystic fibrosis, the diagnostic accuracy of nasal NO testing is comparable to that of ciliary biopsy and genetic testing [135].

5.4. Genetic testing for PCD

Genetic discovery over the past 15 years has identified over 40 PCD genes [77, 157], and several commercial panels test for the majority (>34) of these known genes [12, 134]. At present, genetic confirmation is possible for approximately 70% of individuals with PCD with present commercial panels [30, 93, 137]. Hence, an inconclusive or negative genetic test does not rule-out PCD. Of note, many of the PCD genes are very large with greater than 10 exons; therefore, it is not uncommon for PCD genetic testing to identify a single pathogenic or likely pathogenic variant in two or more different PCD genes. It is important to understand that this finding is not diagnostic of PCD. In order to improve the sensitivity of genetic testing, further research is needed to identify additional genes associated with PCD as well as to better characterize the variants of unknown significance that are frequently identified. Expanding access and cost-effectiveness of whole-exome and whole genome sequencing creates the potential for almost all individuals with PCD to have a genetic diagnosis for PCD. Furthermore, new gene discovery informs understanding of ciliary structure and function. This collective advancement not only improves PCD diagnostic capabilities, but also sets the stage for the development of targeted PCD therapies that improve specific ciliary dysfunction abnormalities.

5.5. PCD diagnostic summary

At present, no test has the sensitivity and specificity to serve as a single diagnostic test for PCD. Most PCD Centers use a panel of tests. Recent Guidelines and Consensus Statements emphasize the importance of clinical phenotyping to better identify those individuals more likely to have PCD, followed by nasal NO testing and PCD genetic testing to make a confirmatory diagnosis of PCD [134, 137]. However, current diagnostic testing has limitations and must be used with caution to prevent inaccurate diagnoses.

6. Approach to therapy in PCD

6.1. Management of lung disease

No PCD-specific therapies are available and the evidence base with which to guide management of PCD is extremely slim. The general therapeutic approach to PCD is based on studies in cystic fibrosis and non-CF bronchiectasis. A recent state-of-the-art publication provides consensus recommendations

from the PCD Foundation for monitoring and management in PCD [137]. The aims of PCD treatment are to maintain or recover lung function by early diagnosis and to minimize progression of bronchiectasis through aggressive airway clearance and antibiotic treatment of respiratory tract infections. Ideally, long-term follow-up should be at a PCD Foundation-accredited clinical center or CF Foundation-accredited CF center with established practices for monitoring lung disease with surveillance cultures, spirometry and imaging [137].

Enhanced mucus clearance is promoted with positive pressure expiratory devices, high-frequency chest compression using vest therapy, manual chest physiotherapy, autogenic drainage, postural drainage, active cycle breathing, and exercise [32]. There have been limited studies of inhaled medications in PCD. Theoretically, nebulized hypertonic saline stimulates cough and increases airway hydration, improving cough clearance. In a 3-month study, nebulized 7% hypertonic saline demonstrated improved lung function, quality of life, and reduced antibiotic use in non-CF bronchiectasis [74]. One small randomized clinical trial evaluating hypertonic saline in PCD patients demonstrated no improvement in quality of life [118]. Dornase alpha, a deoxyribonuclease that reduces mucus viscosity, is beneficial in CF. However, its use in adults with non-CF bronchiectasis was associated with increased pulmonary exacerbations and greater lung function decline in one small study [109]. Inhaled corticosteroids are not recommended routinely for patients with PCD, except for those patients with concomitant asthma or airway reactivity.

Multiple studies demonstrate that systemic antibiotics are effective for treating worsening respiratory exacerbations in CF and non-CF bronchiectasis, including PCD. Prompt and aggressive treatment of respiratory tract infections should be guided by microbiological studies using sputum culture, and/or bronchoalveolar lavage (BAL). While preliminary studies of inhaled antibiotics demonstrated no benefit with respect to lung function, one study using inhaled gentamicin demonstrated reduced pulmonary exacerbations, bacterial load, and inflammatory markers [104]. Chronic macrolide use is well-supported in the cystic fibrosis literature given its antibacterial, anti-inflammatory, and anti-quorum-sensing properties, and its association with improvement of lung function and reduction of exacerbations [31]. Similarly, macrolide use in non-CF bronchiectasis patients is associated with reduction of pulmonary exacerbations [153]. A European clinical trial is underway to assess the efficacy and safety of a 6-month course of azithromycin in PCD patients [82].

Lobectomy is not routinely recommended for patients with PCD. Careful consideration and consultation with PCD experts should be pursued, with consideration of resection reserved for those patients with severe localized bronchiectasis and recurrent febrile relapses or severe hemoptysis, despite aggressive medical management [142]. Similarly, lung transplantation is reserved for patients with PCD who have advanced lung disease.

Additional preventative measures include avoidance of smoke exposure, infection control practices, and pediatric immunizations, including pneumococcal vaccine and annual influenza vaccine [137].

6.2. Management of otolaryngologic manifestations

Pediatric PCD patients should visit an otolaryngologist at least once to twice annually, whereas adult PCD patients should follow-up with an otolaryngologist as needed [137]. Otolaryngology care includes audiology assessment, consideration for pressure equalization tubes (PET) placement, and management of chronic rhinosinusitis. PCD patients have nearly universal conductive hearing loss due to persistent otitis media with effusion. Since chronic otitis media can persist into adulthood, audiology assessments, hearing aids, and communication assistance should be made available for those PCD patients with hearing loss. There is controversy over the clinical indication and benefit of pressure-equalizing tube placement. In studies assessing hearing in children with PCD, PET

placement was associated with greater improvement in hearing compared to medical treatment, with normalization of hearing in 80–100% of children [53]. In contrast, the European Respiratory Society Consensus Statement recommends against placement of tubes for chronic otitis media in PCD, given the complications associated with otorrhea and likely resolution of chronic otitis media in the teenage years [7].

Initial management of sinus disease includes nasal steroids, nasal lavage, and intermittent courses of systemic antibiotics. Daily nasal saline rinses are encouraged. Nasopharyngoscopy may be utilized to assess for nasal polyps. Endoscopy sinus surgery (ESS) is often performed in PCD patients and may improve lower respiratory tract disease in some patients [97].

7. PCD research and future directions

The past 15 years have brought remarkable progress to our understanding of PCD and PCD gene discovery. A major factor contributing to this progress has been the establishment of multi-center research programs in North America (Genetic Disorders of Mucociliary Clearance Consortium that is part of the Rare Diseases Clinical Research Network supported by National Institutes of Health) and in Europe (BESTCILIA program supported by European Commission) as well as multiple international collaborations involved in gene discovery. Recently, the PCD Foundation has certified and organized PCD Clinical Centers across North America and has launched a PCD Registry. These efforts laid the foundation for multi-center clinical trials that have been completed recently: a clinical trial in Europe evaluating use of chronic azithromycin in PCD and a trans-Atlantic, multi-center clinical trial evaluating agents to enhance hydration of airway secretions and, thereby, enhance cough clearance in PCD. These well-established collaborative networks are critical for advancing to the next stages of research focused on this rare disorder. Priorities for the future include further PCD gene discovery, further definition of genotype-phenotype correlations, definition of factors in early PCD that predict long-term outcomes, and definition of best outcome measures for clinical trials. This research will direct future studies to identify and test therapies to control and, possibly, correct the underlying genetic defect(s).

Addendum

Since submission of this manuscript, three additional PCD-associated genes (*DNAH9, GAS2L2, LRRC56*) have been reported [13, 21, 45, 89].

Acknowledgments

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