ORIGINAL ARTICLE

Clinical Features of Childhood Primary Ciliary Dyskinesia by Genotype and Ultrastructural Phenotype

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Abstract

Rationale: The relationship between clinical phenotype of childhood primary ciliary dyskinesia (PCD) and ultrastructural defects and genotype is poorly defined.

Objectives: To delineate clinical features of childhood PCD and their associations with ultrastructural defects and genotype.

Methods: A total of 118 participants younger than 19 years old with PCD were evaluated prospectively at six centers in North America using standardized procedures for diagnostic testing, spirometry, chest computed tomography, respiratory cultures, and clinical phenotyping.

Measurements and Main Results: Clinical features included neonatal respiratory distress (82%), chronic cough (99%), and chronic nasal congestion (97%). There were no differences in clinical features or respiratory pathogens in subjects with outer dynein arm (ODA) defects (ODA alone; n = 54) and ODA plus inner dynein arm (IDA) defects (ODA + IDA; n = 18) versus subjects with IDA and central apparatus defects with microtubular disorganization (IDA/CA/MTD; n = 40). Median FEV_1 was worse in the IDA/CA/MTD group (72% predicted) versus the combined ODA groups (92% predicted; $P = 0.003$). Median body mass index was lower in the IDA/CA/MTD group (46th percentile) versus the ODA groups (70th percentile; $P = 0.003$). For all 118 subjects, median number of lobes with bronchiectasis was three and alveolar consolidation was two. However, the 5- to 11-year-old IDA/CA/MTD group had more lobes of bronchiectasis (median, 5; $P = 0.0008$) and consolidation (median, 3; $P = 0.0001$) compared with the ODA groups (median, 3 and 2, respectively). Similar findings were observed when limited to participants with biallelic mutations.

Conclusions: Lung disease was heterogeneous across all ultrastructural and genotype groups, but worse in those with IDA/CA/MTD ultrastructural defects, most of whom had biallelic mutations in *CCDC39* or *CCDC40*.

Keywords: Kartagener syndrome; cilia; respiratory function tests; X-ray computed tomography scanners; ultrastructure

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At a Glance Commentary

Scientific Knowledge on the Subject: Chronic bronchitis, airflow limitation, and bronchiectasis have been demonstrated in retrospective studies of subjects with primary ciliary dyskinesia. However, the natural history of disease and association of ultrastructural defects and genotype with respiratory phenotypes are poorly elucidated in children.

What This Study Adds to the Field: This multicenter, prospective study systematically evaluates clinical features of primary ciliary dyskinesia in childhood and demonstrates that those with biallelic mutations in *CCDC39* and *CCDC40* or associated ultrastructural defects (inner dynein arm/central apparatus/microtubular defects) have worse lung disease and poorer growth compared with those with outer dynein arm defects (defined by ultrastructure and mutations in associated genes).

Primary ciliary dyskinesia (PCD) is a rare lung disease characterized by abnormal ciliary biogenesis, ultrastructure, and function. Impaired mucociliary clearance results in chronic infection and inflammation of the upper and lower respiratory tract (1–4). Manifestations include neonatal respiratory distress, chronic bronchitis leading to obstructive airways disease and bronchiectasis, persistent otitis media, and chronic rhinosinusitis. Other features include laterality defects, such as situs inversus totalis or heterotaxy (5). Retrospective studies suggest that airway disease begins early (4, 6, 7); however, characterization of clinical features of PCD in children is incomplete. Multicenter collaborations have provided a rigorous approach to diagnosis and disease characterization, permitting assessment of age-related trends in clinical features, pulmonary function, and airway abnormalities.

Recent advances in PCD genetics have identified disease-associated mutations in over 30 genes and strong correlations between genotype and ultrastructural defect (1, 2, 8). The most commonly identified ultrastructural defect, absence of outer dynein arms (ODA defect), is typically caused by mutations in genes encoding an ODA protein, whereas absence of both ODA and inner dynein arms (ODA + IDA defect) is frequently associated with mutations in genes encoding cytoplasmic proteins involved in ciliary assembly. Mutations in genes encoding radial spoke proteins are linked to central apparatus (CA) defects (missing central pair, eccentric central pair, and transposition of outer doublet to the central pair), which may be apparent in a small proportion of cilia cross-sections (9). Two recently described PCD genes, *CCDC39* and *CCDC40*, are associated with a distinct ultrastructural pattern that had not been recognized previously: absence of IDA in conjunction with CA defects and microtubular disorganization (IDA/CA/MTD) (9). Other genes (e.g., *DNAH11*, which encodes an ODA protein) are associated with normal axonemal ultrastructure (10, 11). Present estimates suggest that 70% of patients with PCD have a recognizable ultrastructural defect, and approximately 70% have biallelic mutations in a recognized disease-associated gene (1, 8). Considering the pace of gene discovery, the percentage with a defined PCD gene defect will continue to rise.

Although genetic defects have been linked with specific ultrastructural anomalies of the ciliary axoneme, the relationship between genotype and clinical phenotype has not been established. Natural history of pulmonary involvement has not been systematically evaluated or linked to mutated genes, except for a recent report showing milder lung disease in patients with biallelic mutations in *RSPH1* (12). In this prospective, cross-sectional study of children with PCD from North America, we systematically performed diagnostic tests and defined clinical features, focusing on structural and physiologic abnormalities of the lower airways, and correlated these features with ultrastructural defect and genotype. We hypothesized that specific ciliary ultrastructural defects and PCD disease-causing mutations are associated with distinct pulmonary phenotypes, characterized by differing degrees of airflow obstruction and bronchiectasis.

Methods

Study Sites, Subjects, and Procedures

Sites in the Genetic Disorders of Mucociliary Clearance Consortium included University of North Carolina at Chapel Hill (lead site), Washington University (St. Louis, MO), Hospital for Sick Children (Toronto, Canada), University of Colorado (Aurora, CO), University of Washington (Seattle, WA), and Stanford University (Palo Alto, CA). Study subjects were recruited through PCD clinics at the participating sites; referral to these clinics occurred through primary care physicians, pediatric pulmonologists, or self-referral. Subjects were younger than 19 years of age with a confirmed diagnosis of PCD based on ciliary electron microscopy or identification of biallelic mutations in a known PCD-associated gene. Institutional review board approval was obtained at each site. Informed consent and assent were acquired from parents and participants, when appropriate. An observational safety monitoring board reviewed and approved the protocols.

Standardized procedures were used for diagnostic testing and assessment of clinical features. Subjects underwent nasal epithelial scrape biopsies for ciliary ultrastructural analysis. Three masked reviewers at University of North Carolina at Chapel Hill evaluated electron micrographs for ciliary defects defined as absent or truncated ODA, absent ODA and IDA, absent IDA with MTD (9), and central pair defects with absent or off-central pair associated with transposition of peripheral doublet to the center. IDA defects were not considered diagnostic except when apparent on repeat biopsies (13). Genetic testing was performed through Sanger sequencing at the Molecular Genetics Research Core at the University of North Carolina at Chapel Hill. Genetic workup included screening for coding regions and splice junctions for up to 26 PCD-associated genes, including mutations in 14 genes identified in our participants (Table 1; *see* Table E1 in the online supplement) (*ARMC4*, *CCDC114*, *CCDC39*, *CCDC40*, *DNAAF1* [*LRRC50*], *DNAAF2* [*KTU*], *DNAH5*, *DNAI1*, *DNAI2*, *HEATR2*, *LRRC6*, *RSPH4A*, *RSPH9*, and *SPAG1*). Nasal nitric oxide measurements were performed as previously published (14). Sputum or deep pharyngeal culture was processed for bacterial pathogens at each site's local clinical laboratory. Spirometry (in children >3 yr old) and infant lung function testing under sedation (in children <3 yr old) were performed according to American Thoracic Society/European Respiratory Society criteria (15–17). All pulmonary function test data

Table 1. Ciliary Ultrastructural Defects and Mutations in 118 Pediatric Subjects with PCD

Ciliary Defect Type	Mutated Gene	<5 yr [*N* (%)] (*n* = 29)	5–18 yr [*N* (%)] (*n* = 89)	All [*N* (%)] (*n* = 118)
ODA only	*DNAH5*	1	27	28
	DNAI1	0	7	7
	DNAI2	0	5	5
	CCDC114	0	2	2
	ARMC4	1	0	1
	No gene identified	3	8	11
Total		5 (17%)	49 (55%)	54 (46%)
ODA + IDA	*LRRC6*	1	2	3
	HEATR2	2	0	2
	SPAG1	0	1	1
	DNAAF2 (KTU)	0	1	1
	DNAAF1 (LRRC50)	0	1	1
	No gene identified	3	7	10
Total		6 (21%)	12 (13%)	18 (15%)
IDA/CA/MTD	*CCDC39*	7	6	13
	CCDC40	8	9	17
	No gene identified	1	9	10
Total		16 (55%)	24 (27%)	40 (34%)
CA or IDA alone	*RSPH4*	1	1	2
	RSPH9	0	1	1
	No gene identified	1	2	3
Total		2 (6%)	4 (4.5%)	6 (5%)

Definition of abbreviations: CA = central apparatus; IDA = inner dynein arm; MTD = microtubular disorganization; ODA = outer dynein arm; PCD = primary ciliary dyskinesia.
71% of patients had identified biallelic gene mutations.

from children younger than 8 years of age were overread for quality. Spirometric measurements were expressed as percent predicted (18) and infant lung function as *z* scores (19, 20).

Chest computerized X-ray tomographic (CT) images were obtained according to a protocol limiting radiation exposure. The controlled breathing technique under sedation/anesthesia was used for the youngest subjects as previously described (21). A radiologist (M.L.C.) systematically and blindly reviewed chest CTs for the presence of bronchiectasis and parenchymal disease in six lobes, including the lingula as a lobe, using previously described criteria (22).

Statistical Analyses

Summary statistics are presented for the entire cohort and for four groups: (*1*) ODA defect only, (*2*) ODA+IDA defect, (*3*) IDA/CA/MTD defect, and (*4*) CA or IDA defect alone. Group 1 (ODA defect only) was combined with group 2 (ODA + IDA defect) and compared with group 3 (IDA/CA/MTD defect). Group 4 was not part of the comparison analyses because of low sample size (n = 6). Fisher exact test was used to compare categorical variables, and Wilcoxon rank-sum test for continuous variables. A general linear model was used to examine whether the relationship between spirometric measurements and age was different between the combined groups (1 and 2) and group 3 or the IDA/CA/MTD group. Two-sided *P* values less than 0.05 were considered statistically significant. Analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, NC).

Results

Participant Characteristics, Ciliary Ultrastructural Defects, and PCD Mutations

One hundred eighteen participants with PCD were enrolled between 2006 and 2012. Distribution of ultrastructural defects was 46% ODA alone, 15% ODA + IDA, 34% IDA/CA/MTD, and 5% CA or IDA alone (Table 1). IDA/CA/MTD defects were more prevalent in subjects younger than 5 years of age than in older children (55% vs. 27%), whereas ODA alone defects were less prevalent (17% vs. 55%). Seventy-one percent (84 of 118) had biallelic gene mutations (*see* Table E1).

Demographic and clinical features of the entire cohort and groups of ultrastructural defects are described (Table 2). Most participants had clinical manifestations of PCD, including (*1*) neonatal respiratory distress (82%) requiring a median of 9.5 days of supplemental oxygen and a median of 12 days of hospitalization; (*2*) chronic daily, "wet" cough (99%) with onset occurring at a median of 1 month of age; (*3*) chronic nasal congestion (97%) with onset occurring at a median of 1 month of age; and (*4*) chronic or recurrent otitis media (92%). Prevalence of these clinical manifestations did not differ for the IDA/CA/MTD group versus the combined ODA and ODA + IDA groups. Laterality defects (situs inversus totalis and heterotaxy) were observed in 55% of the 118 participants. Nasal nitric oxide levels were low for children who were able to cooperate with palate closure maneuvers (resistor method). The median value was 13.6 nl/min as reported previously (14), but did not differ across groups based on ultrastructural defects. The only characteristic that differed significantly was age of evaluation, because the IDA/CA/MTD group was younger ($P = 0.04$) as compared with the combined ODA and ODA + IDA groups.

Spirometry, chest CT, and growth parameters are described in Table 3. Most children had normal spirometric indices or mild obstructive impairment, but there was a wide range with median FEV_1 of 89% predicted and median forced expiratory flow, midexpiratory phase ($FEF_{25–75}$) of 68% predicted. Infant lung function tests in 13 children showed a median $FEV_{0.5}$ *z* score of 0.22 that was normal, whereas the median $FEF_{25–75}$ *z* score was diminished (−0.91). Chest CT data revealed prominent bronchiectasis (median number of lobes involved, three) and alveolar consolidation (median number of lobes involved, two). Growth parameters for most children were normal, showing median height at 42nd percentile, median weight at 52nd percentile, and median body mass index (BMI) at the 63rd percentile.

Lung disease severity and growth parameters were evaluated by groups (Table 3). The IDA/CA/MTD group had worse airflow obstruction, more radiographic disease, and poorer growth, compared with the combined ODA and ODA + IDA groups. Median FEV_1 and $FEF_{25–75}$ % predicted values were significantly reduced in the IDA/CA/MTD group compared with the combined ODA

Table 2. Demographics and Clinical Characteristics

	All (*n* = 118)	ODA Only (*n* = 54)	ODA + IDA (*n* = 18)	IDA/CA/MTD (*n* = 40)	CA or IDA Only (*n* = 6)	*P* Value*
Male sex, %	53	50	61	53	50	1.00
White race, %	81	87	61	80	100	1.00
Age, yr	8 (5–11)	8 (6–12)	5 (3–11)	7 (2.5–10)	12 (4–14)	0.04
Nasal nitric oxide (nl/min, palate closure plateau, resistor method)	13.6 (9.3–18.5) n = 84	13.6 (7.9–16.8) n = 48	12.2 (10.3–23.9) n = 12	13.9 (7.7–24.95) n = 20	17.1 (14–23.9) n = 4	0.53
Laterality defect, %	55	59	67	50	17	0.32
Neonatal respiratory distress, %	82	78	89	88	67	0.44
Supplemental oxygen at birth, d	9.5 (3–18.5)	9 (4–15)	12.5 (4–20)	11 (7–25)	0 (0–4.5)	0.23
Newborn hospitalization, d	12 (7–20)	10.5 (7–18)	14 (7–20.5)	13 (8–24)	10 (6–10)	0.25
Chronic cough, %	99	100	100	98	100	0.36
Age of onset of wet cough, mo	1 (1–3)	1 (1–6)	1 (1–1)	1 (1–5.5)	1 (1–1)	0.7
Pneumonias, %	81	80	72	83	100	0.63
Number of acute pneumonias over 5 yr (or lifetime if <5 yr old)	3 (1–6)	3 (1–6)	2 (1–5)	1.5 (1–6)	5 (3–10)	0.5
Number requiring hospitalizations	1 (0–2)	1 (0–2)	1 (1–2)	1 (0–2)	1 (0–2)	0.59
Chronic nasal congestion, %	97	100	89	98	100	1.00
Age of onset of chronic nasal congestion, mo	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	0.64
Chronic/recurrent otitis media, %	92	93	89	90	100	0.74
Age of onset of otitis media, mo	4 (2–8)	3 (2–7)	6 (2–13.5)	3.5 (3–6)	4.5 (2–6)	0.94

Definition of abbreviations: CA = central apparatus; IDA = inner dynein arm; MTD = microtubular disorganization; ODA = outer dynein arm.
% or median (first quartile–third quartile).
**P* values for the comparison between the group of IDA/CA/MTD defect and the combined groups of ODA defect only and ODA+IDA defect.

and ODA + IDA groups ($P = 0.003$ and $P = 0.002$ for FEV_1 and $FEF_{25–75}$, respectively). The IDA/CA/MTD group had lower FEV_1 and $FEF_{25–75}$ values (72 and 49% predicted, respectively) compared with the combined ODA and ODA + IDA groups (FEV_1, 92%; $FEF_{25–75}$, 73% predicted). Among the children who performed infant lung function tests, median $FEF_{25–75}$ *z* score was diminished in the IDA/CA/MTD group (*z* score, −1.02) compared with the combined ODA and ODA+IDA groups (*z* scores, 0.77 and −0.84, respectively; $P = 0.023$) (Table 3).

The median number of lobes with alveolar consolidation was greater in the IDA/CA/MTD group (median, three) compared with the combined ODA and ODA + IDA groups (median, two) ($P = 0.001$). The IDA/CA/MTD group weighed less ($P < 0.0001$), were shorter ($P = 0.036$), and had a decreased BMI ($P = 0.003$) compared with the combined ODA and ODA + IDA groups. The IDA/CA/MTD group had a median weight at the 39th percentile, median height at the 36th percentile, and median BMI value at the 46th percentile compared with the combined ODA and ODA + IDA groups (68th, 48th, and 70th percentile, respectively). When analyses were limited to children who had defined biallelic genetic mutations (n = 84), findings were similar except for height ($P = 0.129$).

In Figures 1A and 1B, FEV_1 and $FEF_{25–75}$ % predicted, respectively, are plotted against age in this cross-sectional study. Although FEV_1 and $FEF_{25–75}$ % predicted remained constant with increasing age among participants with ODA (group 1) and ODA + IDA (group 2) defects, both measures declined with advancing age in participants with IDA/CA/MTD (group 3) defects; slopes for the combined ODA groups (ODA [group 1] and ODA + IDA [group 2]) versus the IDA/CA/MTD group are significantly different ($P = 0.003$ and $P = 0.010$ for FEV_1 and $FEF_{25–75}$, respectively). The slope of FEV_1 change in the IDA/CA/MTD group was −3.88% predicted per year, whereas the slope of the combined ODA and ODA + IDA groups was nearly flat, 0.085% predicted per year. For $FEF_{25–75}$ measures, the slopes were −4.09% predicted per year and 0.30% predicted per year for the IDA/CA/MTD and combined ODA and ODA + IDA groups, respectively.

In Figures 2A and 2B, the number of lobes with bronchiectasis and alveolar consolidation, respectively, are plotted according to age groups. For all ages, the extent of bronchiectasis and alveolar consolidation varied, ranging from zero to six affected lobes. For subjects younger than 5 years, the number of lobes with alveolar consolidation or bronchiectasis in the combined ODA and ODA + IDA groups versus the IDA/CA/MTD group was not different ($P = 0.860$ and 0.466, respectively). Among children 5–11 years of age, the IDA/CA/MTD group had more lobes with bronchiectasis ($P = 0.0008$) and alveolar consolidation ($P = 0.0001$) than the combined ODA and ODA + IDA groups. For the 12–18 years age group, the IDA/CA/MTD group did not seem to have more lobes involved, although there were relatively few subjects with IDA/CA/MTD defects in this age group (n = 6).

Table 4 describes the respiratory microbiology. For all 118 subjects, predominant pathogens were *Staphylococcus aureus* (19%), *Haemophilus influenza* (22%), *Streptococcus pneumonia* (14%), and *Moraxella catarrhalis* (8%). Nonmucoid and mucoid *Pseudomonas aeruginosa* were recovered in 6 and 3% of subjects, respectively. Samples were expectorated sputum in 66% and pharyngeal swabs in 34%. There were no significant differences in respiratory bacterial isolates between children with IDA/CA/MTD defects compared with those with ODA and ODA + IDA defects.

Table 3. Markers of Disease Severity by PCD Group

	All (*n* = 118)	ODA Only (*n* = 54)	ODA+IDA (*n* = 18)	IDA/CA/MTD (*n* = 40)	CA or IDA Alone (*n* = 6)	*P* Value*
Height, percentile†	42 (19 to 70) n = 106	42 (20 to 83) n = 51	63 (15 to 77) n = 16	36 (13 to 60) n = 34	44 (24 to 62) n = 5	0.036
Weight, percentile	52 (17 to 80) n = 118	67 (30 to 91) n = 54	76 (34 to 82) n = 18	39 (13 to 52) n = 40	62 (47 to 81) n = 6	<0.0001
BMI, percentile†	63 (32 to 82) n = 106	68 (32 to 92) n = 51	74 (41 to 82) n = 16	46 (26 to 65) n = 34	80 (71 to 84) n = 5	0.003
FEV_1, % pred	89 (67 to 99) n = 86	93 (78 to 101) n = 46	91 (74 to 99) n = 12	72 (58 to 88) n = 24	86 (77 to 93) n = 4	0.003
$FEF_{25–75}$, % pred	68 (48 to 80) n = 86	73 (57 to 80) n = 46	78 (59 to 94) n = 12	49 (32 to 64) n = 24	75 (53 to 88) n = 4	0.002
Infant $FEV_{0.5}$, *z* score	0.22 (0.12 to 0.31) n = 13	1.00 (0.57 to 1.43) n = 2	0.20 (0.02 to 0.31) n = 3	0.14 (0.01 to 0.22) n = 7	0.38 n = 1	0.144
Infant $FEF_{25–75}$, *z* score	−0.91 (−1.02 to −0.81) n = 13	0.77 (0.57 to 0.97) n = 2	−0.84 (−0.91 to −0.82) n = 3	−1.02 (−1.25 to −0.91) n = 7	−0.05 n = 1	0.023
Chest CT						
Number of lobes with bronchiectasis	3 (1 to 5) n = 118	3 (1 to 4) n = 54	3 (0 to 5) n = 18	3.5 (1 to 5) n = 40	4.5 (3 to 6) n = 6	0.243
Number of lobes with alveolar consolidation	2 (1 to 3) n = 118	1.5 (1 to 3) n = 54	2 (1 to 3) n = 18	3 (2 to 4) n = 40	2 (1 to 2) n = 6	0.001

Definition of abbreviations: BMI = body mass index; CA = central apparatus; CT = computed tomography; $FEF_{25–75}$ = forced expiratory flow, midexpiratory phase; IDA = inner dynein arm; MTD = microtubular disorganization; ODA = outer dynein arm; PCD = primary ciliary dyskinesia.
Median (first quartile to third quartile).
**P* values for the comparison between the group of IDA/CA/MTD defect and the combined groups of ODA defect only and ODA+IDA defect.
†Subjects <2 years not included.

Historical review of prior respiratory culture results revealed transient infection with several organisms in most participants: *S. aureus*, *H. influenzae*, *S. pneumonia*, and *M. catarrhalis* were recovered in 35, 50, 30, and 19% of the children, whereas nonmucoid and mucoid *P. aeruginosa* were recovered in 20 and 5% of the children, respectively. There was no identifiable

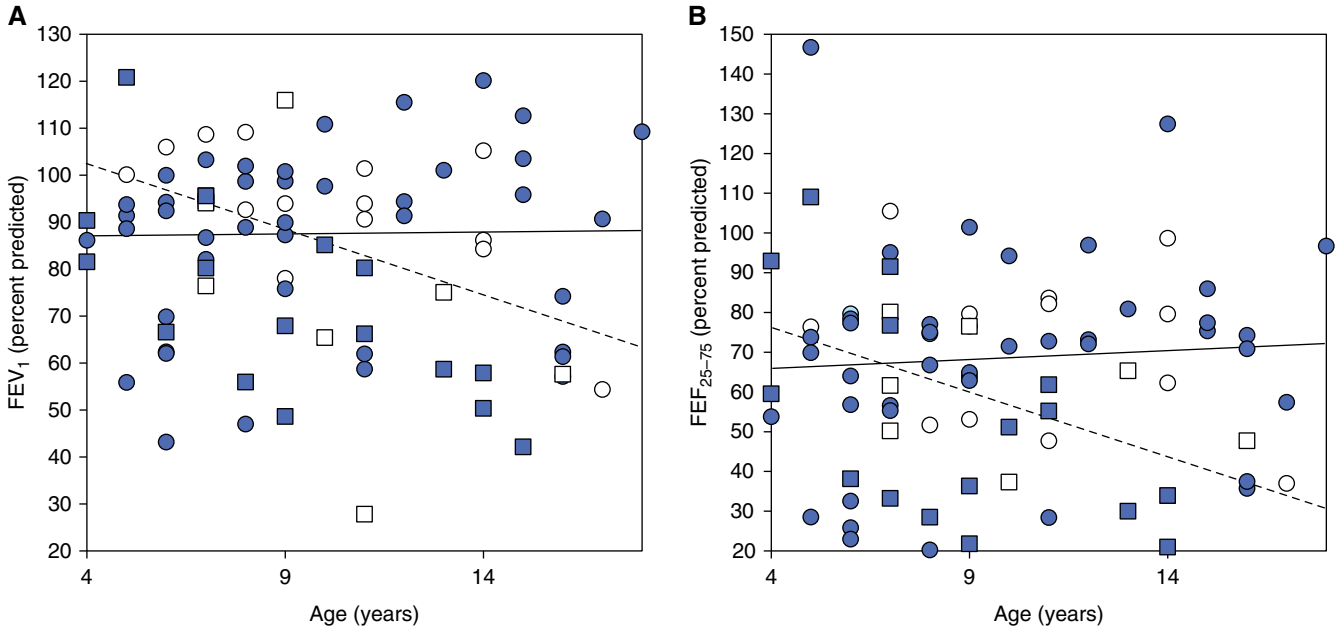

Figure 1. Pulmonary function measures (*A*) FEV_1 and (*B*) forced expiratory flow, midexpiratory phase ($FEF_{25–75}$) % predicted plotted as a function of age in pediatric subjects with primary ciliary dyskinesia (n = 82). *Circles* represent subjects with primary ciliary dyskinesia who have outer dynein arm (ODA) defects that include absent or truncated ODA and combined absence of ODA and inner dynein arm (IDA) (groups 1 and 2). *Squares* represent individuals who have microtubular disorganization (MTD) associated with IDA and central apparatus (CA) defects (IDA/CA/MTD, group 3). *Filled symbols* identify subjects who have known biallelic genetic defects, whereas *open symbols* indicate those subjects for whom the genetic basis of their disease is yet unknown. In this cross-sectional study, the association of both FEV_1 and $FEF_{25–75}$ with age declines for subjects with IDA/CA/MTD derangements (group 3) compared with the combined ODA groups, ODA (group 1) and ODA + IDA (group 2) ($P = 0.003$ and 0.010, respectively). The slope of FEV_1 change in the IDA/CA/MTD group was −3.88% predicted per year (*dashed line*), or −3.90% predicted per year for subjects with known genetic defects. The FEV_1 slope of the combined ODA and ODA + IDA groups was 0.085% predicted per year (*solid line*), or 0.64% predicted per year in known genetic defects. The slope of $FEF_{25–75}$ change was −4.09% predicted per year (*dashed line*), or −5.03% predicted per year in known genetic defects, in the IDA/CA/MTD cohort, and 0.30% predicted per year (*solid line*), or 0.70% predicted per year in known genetic defects, for the combined ODA and ODA + IDA groups.

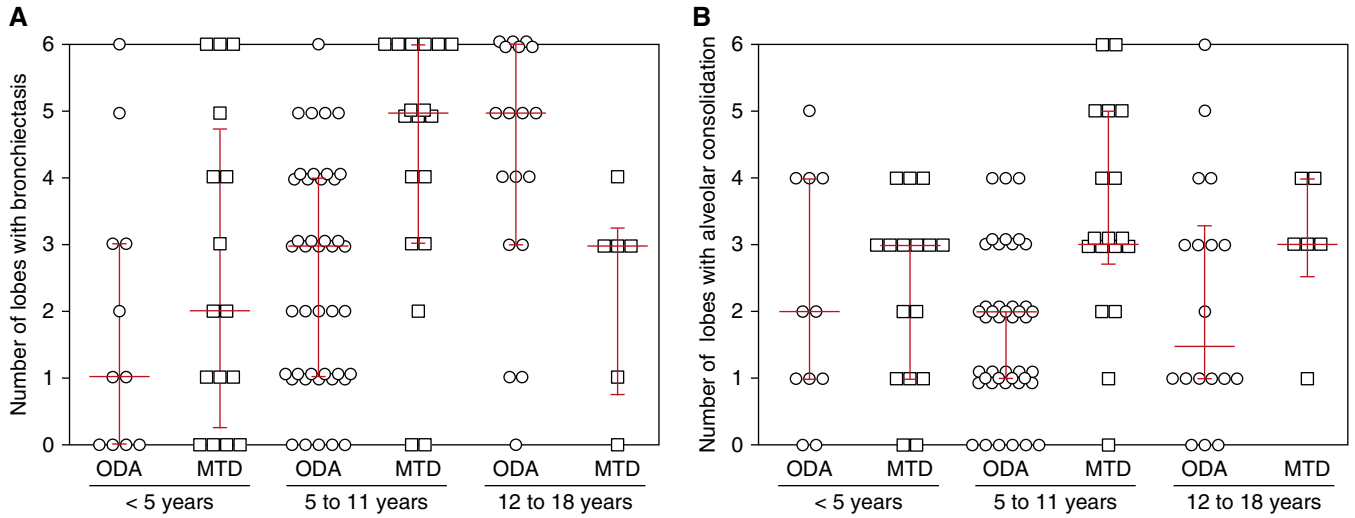

Figure 2. Radiographic findings of (*A*) bronchiectasis and (*B*) alveolar consolidation in individual lobes and lingula in pediatric subjects with primary ciliary dyskinesia (n = 112), segregated into different age groups (<5 yr, 5–11 yr, and 12–18 yr of age). *Circles* represent subjects with primary ciliary dyskinesia who have outer dynein arm (ODA) defects, which include absent or truncated ODA and complete absence of both ODA and inner dynein arm (IDA) (groups 1 and 2). *Squares* represent individuals who have microtubular disorganization (MTD) associated with IDA and central apparatus (CA) defects (IDA/CA/MTD, group 3). The *vertical lines* represent median and first and third quartile. Subjects with IDA/CA/MTD defects between the ages of 5 and 11 years have increased bronchiectasis ($P = 0.0008$) and alveolar consolidation ($P = 0.0001$) compared with the combined ODA groups, ODA (group 1) and ODA + IDA defects (group 2).

association between pathogen and indices of lung disease severity.

Discussion

We describe clinical features and spectrum of lung disease in a multicenter cohort of children with confirmed PCD and/or biallelic PCD-associated mutations. Prominent clinical features included neonatal respiratory distress, early onset persistent chronic cough and nasal congestion, and recurrent otitis media. Situs inversus totalis or situs ambiguous was identified in slightly more than half (55%) of the subjects, likely reflecting ascertainment bias associated with recognition of laterality defects. Ultrastructural and genotype analysis did not reveal significant differences in prevalence of clinical features across groups. However, structural and physiologic markers of lung disease severity were heterogeneous across groups, and worse in those with an IDA/CA/MTD ultrastructural defect, most of whom had biallelic *CCDC39* and *CCDC40* mutations.

Previous studies evaluating clinical features of PCD during childhood have been limited by retrospective data collection, small sample sizes, addition of adult data, and less rigorous diagnostic confirmation, although most were conducted before newer diagnostic tests became available (4, 7, 23–25). Individuals with recently recognized ultrastructural defects, such as IDA/CA/MTD, may not have been included in earlier studies or may have been classified as IDA defect. In some prior studies, up to a third of subjects included in the "PCD" group had an isolated IDA defect, which can be a nonspecific, transient finding unrelated to genetic defects. A recent study (13) revealed that a substantial portion of subjects with isolated IDA

Table 4. Respiratory Microbiology in PCD Participants

	All (*n* = 118)	ODA Only (*n* = 54)	ODA+IDA (*n* = 18)	IDA/CA/MTD (*n* = 40)	CA or IDA Alone (*n* = 6)	*P* Value*
Oropharyngeal flora only, %	34	35	33	33	33	0.84
Staphylococcus aureus, %	19	22	17	15	17	0.61
Haemophilus influenza, %	22	22	28	23	0	1.00
Streptococcus pneumoniae, %	14	13	22	13	0	0.78
Moraxella catarrhalis, %	8	6	6	10	17	0.45
Nonmucoid *Pseudomonas aeruginosa*, %	6	4	0	13	0	0.10
Mucoid *P. aeruginosa*, %	3	2	0	5	17	0.29
Nontuberculous mycobacteria, %	3	4	0	5	0	0.62
Other, %	15	13	28	13	17	0.78

Definition of abbreviations: CA = central apparatus; IDA = inner dynein arm; MTD = microtubular disorganization; ODA = outer dynein arm; PCD = primary ciliary dyskinesia.
**P* values for the comparison between the group of IDA/CA/MTD defect and the combined groups of ODA defect only and ODA+IDA defect.

defects had normal axonemal ultrastructure on repeat testing. Clinical features in the current study are described in more detail than in our earlier studies (4). Specifically, we define age of onset of "wet" cough, nasal congestion, and otitis media, and document that these hallmark features typically begin during the first 3 months of life. We collected more details regarding neonatal manifestations, including number of days requiring supplemental oxygen support (median, 9.5 d) and days hospitalized (median, 12 d). Given our detailed clinical phenotyping, we can now define key clinical features early in PCD.

In the past, PCD has been considered a "milder" lung disease than cystic fibrosis (CF). A previous cross-sectional study in pediatric and adult subjects with PCD showed that FEV_1 decline (−0.8% predicted per year) was slower than that reported for CF at the time (−3.6% predicted per year) (4). The first longitudinal study in PCD (24 adults and 12 children) demonstrated a wide range of lung function, but values remained stable over time after initiation of airway clearance techniques and culture-directed antibiotic therapy (24). In another multicenter study of 158 children with PCD, pulmonary function and growth parameters varied widely at time of presentation, but remained stable over a period of 2–6 years (26).

In contrast, a longitudinal study of 74 subjects with PCD (median age at diagnosis, 8 yr) found significant decline in lung function over three decades (7). This variability in lung function and observed rate of decline emphasizes the need to better characterize factors contributing to pulmonary deterioration in the PCD population. Unlike a recent study suggesting that lung disease is not influenced by ultrastructural phenotype (27), our study found that children with a specific PCD ultrastructural defect, IDA/CA/MTD, many of which are associated with biallelic mutations in *CCDC39* and *CCDC40*, have worse pulmonary function than that seen in similar aged children with ODA defects and similar to that found in patients with CF. These novel findings open new lines of research to better understand mechanisms involved and implications of these observations for clinical care.

This study is the first to systematically assess structural changes on chest CT by ultrastructural defect or genotype in a young PCD population and demonstrates that alveolar consolidation and bronchiectasis were common findings among all ultrastructural groups. The IDA/CA/MTD group had significantly more lobes of consolidation compared with the combined ODA and ODA + IDA groups. Relatively few studies have evaluated structural disease in a large PCD population and most included only older children (23, 25, 28, 29). Case reports have shown that bronchiectasis can occur in infants with PCD (6).

Growth parameters of most children in our study were normal, but subjects with the IDA/CA/MTD defect and/or biallelic mutations in *CCDC39* or *CCDC40* had impaired growth parameters, when compared with the combined ODA and ODA + IDA groups. Studies in other lung diseases including chronic obstructive pulmonary disease (30, 31), bronchopulmonary dysplasia (32), and CF (33–35) have shown that subjects with impaired growth and/or low BMI tend to have poorer lung function and pulmonary outcomes. The poor growth parameters in the IDA/CA/MTD group may reflect or contribute to severity of lung disease. Parallel to our results, other investigators (26) reported normal growth parameters in children with PCD, with relatively few subjects who had a low BMI; however, these investigators did not evaluate growth parameters based on ultrastructural ciliary defects or gene mutation. Unlike a recent publication (36) that reported that BMI was reduced in subjects with *DNAH5* and *DNAI1* (both ODA genes), our subjects with ODA defects (which include individuals with biallelic *DNAH5* and *DNAI1* mutations) had normal growth parameters.

The higher prevalence of IDA/CA/MTD defects in our preschool age group (55%) compared with those 5–18 years of age (27%) is worth highlighting. Perhaps patients with this defect are diagnosed earlier because they have more severe symptoms in infancy and early childhood, although we were unable to identify differences in onset of respiratory symptoms or management among subjects with different ultrastructural defects. Interestingly, infant lung function testing showed that expiratory flow rates were diminished in the group with IDA/CA/MTD defects compared with those with ODA and ODA + IDA defects. Although infant lung function testing was performed in only a small subset of patients based on age at enrollment, these findings provide preliminary evidence of the presence of early airflow limitation and airway disease in infancy.

Association of PCD with biallelic *CCDC39* and *CCDC40* mutations has only recently been recognized, and understanding of pathophysiology is rapidly evolving. Mutations in *CCDC39* and *CCDC40* are relatively common in PCD (9) and both mutant genes lead to similar defects in axonemal architecture with inconsistent ultrastructual abnormalities, characterized by absent IDA with displacement or absence of the central pair and mislocalized peripheral doublets in some but not all cilia. Associated motility defects range from stiff cilia with reduced mean ciliary beat frequency and amplitude to complete immotility (9, 37, 38). *CCDC39* and *CCDC40* are evolutionarily conserved genes and encode structurally related coiled-coil domain-containing proteins localized to the axoneme. The coiled-coil is a common structural domain in eukaryotic and prokaryotic proteins and can act as an adapter between molecules (39). *CCDC39* and *CCDC40* interact with components of the nexin-dynein regulatory complexes and are involved in IDA attachment (40). *CCDC39* seems to confer stability of the nexin-dynein regulatory complexes or is involved in protein transport, because it contains structural maintenance of chromosome-like domains analogous to those found in other ciliary and centrosomal proteins involved in intracellular transport (37). *CCDC40* mutant cells lack *CCDC39* in the axoneme, indicating that it is required for transport of *CCDC39* into the axoneme (38). *CCDC39* and *CCDC40* may have roles in nexin-dynein regulatory complexes assembly, microtubule attachment, or serve as a docking domain. Other mutated genes, particularly the ODA + IDA genes, can lead to marked ciliary dysmotility or immotility, thus abnormal ciliary motion cannot fully explain the severe pulmonary phenotype in PCD subjects with *CCDC39* and *CCDC40* mutations.

In summary, we report clinical features of childhood PCD based on ultrastructural axonemal defects and genotype. In this cross-sectional study, there was significant heterogeneity of lung disease beginning at an early age, but subjects who had an IDA/CA/MTD defect, usually linked

to mutations in PCD-associated genes (*CCDC39* and *CCDC40*), presented earlier and had worse lung disease by both functional and structural assessment (spirometry and CT scans) and poorer nutritional status. Given these findings, longitudinal analyses are needed to confirm this pattern in individual patients and better characterize pulmonary function decline with age. We expect that other genotype-phenotype relationships will be identified in PCD, which will more fully define the influence of specific genetic defects on lung disease. PCD has classically been difficult to diagnose because of technical challenges associated with electron microscopy and video microscopy; patients often have to be evaluated at specialty centers to confirm the diagnosis (41). Genetic testing offers the benefit of not only confirming diagnosis, but also, as demonstrated in this study may contribute to prognosis. These findings provide greater insights into development and progression of lung disease beginning in infancy, and could potentially allow for more personalized monitoring and treatment of these children. ■

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