Pediatric Pulmonology

WILEY

ORIGINAL ARTICLE OPEN ACCESS

The Association of Neonatal Respiratory Distress With Ciliary Ultrastructure and Genotype in Primary Ciliary Dyskinesia

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Received: 29 September 2024 | **Revised:** 20 February 2025 | **Accepted:** 1 April 2025

Funding: Supported by the National Institute of Health (NIH) U54HL096458 funded by the Office of Rare Disease Research (National Center for Advancing Translational Science) and administered by NHLBI and the Carolina For The Kids Grant Program, US NIH/NHLBI grant R01HL071798 to MRK and MAZ, NIH/NCATS Colorado CTSA Grant Number UM1 TR004399. This study was performed at the University of North Carolina at Chapel Hill under IRB numbers 05-2979 and 14-1225.

Keywords: genotype/phenotype correlation | neonatal respiratory distress | primary ciliary dyskinesia

ABSTRACT

Objective: To evaluate the relationship between ciliary ultrastructure/genotype and prevalence of neonatal respiratory distress (NRD) in primary ciliary dyskinesia (PCD).

Study Design: This was a retrospective analysis from a multicenter, prospective study of children and adults with PCD. Participants were classified by ultrastructural defect associated with their diagnostic genetic variants: 1) outer dynein arm defect alone (ODA), 2) outer plus inner dynein arm defect (ODA/IDA), 3) inner dynein arm defect with microtubular disorganization (IDA/MTD), 4) *DNAH11* (encodes ODA protein but has normal ultrastructure), and 5) normal/near-normal/other. The likelihood of NRD between ultrastructure groups or genotypes was evaluated by multivariate analysis using logistic regression, controlled for age, gender, race, and variant type. Similar analysis was performed within individual genotypes to assess association of NRD with the presence of 2 loss-of-function variants.

Results: Of the 455 participants analyzed, 305 (67.0%) reported NRD. The odds ratio for NRD in the *DNAH11* group was significantly lower (OR: 0.35, 95% CI: 0.16–0.76) compared to NRD in the ODA group. Within the *DNAH5* group, those with two loss-of-function variants were more likely to have NRD compared to those with possible residual function variants (OR: 3.06, 95% CI: 1.33–7).

Conclusion: NRD is less common in those with *DNAH11* variants, thus a high index of suspicion should remain for PCD in the absence of NRD. Variant type (loss-of-function vs. residual function) may explain phenotypic variability within individual PCD genes.

1 | Introduction

Primary ciliary dyskinesia (PCD) is a rare genetic disorder of motile cilia resulting in chronic oto-sino-pulmonary disease, organ laterality defects, and infertility [1]. Neonatal respiratory distress (NRD) is a common early manifestation, occurring in approximately 80% of people with PCD [1–3]. Affected neonates present with tachypnea and hypoxemia in the first days of life and often require supplemental oxygen for several days to weeks [3, 4]. Chest imaging frequently reveals upper lobar collapse [4]. The underlying cause of NRD remains unclear but may be related to retained lung fluid secondary to impaired ciliary function [5]. This presentation can be erroneously attributed to transient tachypnea of the newborn (TTN) or neonatal pneumonia, thus delaying the diagnosis [6].

To date, PCD disease-causing variants have been identified in 54 genes, and in approximately 70%, characteristic ciliary ultrastructural defects are visualized on transmission electron microscopy (TEM) [7, 8]. Multicenter studies have identified associations between genotype and clinical phenotype, while the type of genetic variants (i.e. loss-of-function vs possible residual function) may also influence phenotype [3, 9–13]. Furthermore, Wee and colleagues recently found an association between length of neonatal hospital stay and lung function measured years later in PCD [14]. However, the association between genotype and/or ciliary ultrastructure and NRD has not been systematically examined.

The aim of this project was to investigate the association between ciliary ultrastructure with NRD in PCD, and more specifically the association between genotype with NRD. We also set out to determine the effect of loss-of-function (LOF) variants on the risk for NRD. Lastly, we aimed to determine whether ciliary ultrastructure was associated with length of hospital stay after birth. We hypothesized that infants with biallelic mutations in *CCDC39* and *CCDC40*, characterized by inner dynein arm defects with microtubular disorganization (IDA/MTD) and associated with worse lung disease [3, 9], are at greater risk for NRD. Moreover, we postulated that the presence of two LOF variants in any PCD gene is also associated with a greater risk of NRD compared to those with one or more possible residual function variant.

2 | Methods

2.1 | Study Sites, Participants, and Procedures

The participants studied were enrolled in prospective, multicenter projects conducted by the Genetic Disorders of Mucociliary Clearance Consortium (GDMCC). Individuals of any age with clinical features consistent with PCD were recruited by one of nine sites in the GDMCC. Institutional review board approval was obtained at all sites. Informed consent and assent were obtained from parents and participants, when appropriate. Clinical history (including presence or absence of NRD), genetic testing [2, 3], and TEM results [1, 3, 15] were collected from participants in a standardized fashion. Individuals were included in the study if they had a diagnosis of PCD based on: 1) two pathogenic or likely pathogenic (P/LP) variants in a known PCD gene identified on molecular genetic testing, and/or 2) characteristic ciliary ultrastructural defects on TEM. Exclusion criteria included complex congenital heart disease [16], premature birth, defined as less than 37-week gestation, or if the neonatal course was uncharacterized (unknown whether full term and/or whether NRD present). NRD was defined as the need for supplemental oxygen or positive pressure ventilatory support for greater than 24 hours following birth.

Participants were grouped based on the hallmark ultrastructural defect associated with their genotype using international consensus guidelines [17], which included 1) outer dynein arm (ODA) defect, 2) outer dynein arm/inner dynein arm (ODA/IDA) defect, 3) inner dynein arm defect with microtubular disorganization (IDA/MTD), 4) normal ultrastructure associated with the *DNAH11* variants, and 5) normal or near-normal ultrastructure (includes radial spoke and central apparatus defects but excludes *DNAH11* variants). When genetic diagnosis was not available, participants were characterized by directly observed ultrastructural defect on TEM.

American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG/AMP) recommendations were used for variant classifications. Truncating or canonical splice site P/LP variants that are predicted to be null were considered as LOF, including frameshift, nonsense, start codon, canonical splice site, large copy number variants, and full gene deletion. Non-truncating P/LP variants presumably retaining some protein function were considered as possibly having residual function, including missense, inframe indels, extended splice site (exonic or intronic), and intronic variants. Gene groups were stratified as either "two-LOF" when both P/LP variants were classified as LOF or "possible residual function" when either one or both P/LP variants were classified as not-LOF [18].

2.2 | Statistical Analysis

Descriptive statistics were used to report the participants' demographic characteristics. The association between NRD and ciliary ultrastructure or genotype was evaluated by multivariable analysis using logistic regression with the ODA group as the reference group for ciliary ultrastructure and *DNAH5* as the reference group for genotype. Analysis was adjusted for age, gender, race (white vs. non-white), and presence of two LOF variants. Logistic regression was used to compare likelihood of NRD between those with two LOF variants and those with one

or more possible residual function variant in individual genes. Poisson regression was used to compare the hospital length of admission among ciliary ultrastructure groups adjusting for the same set of covariates. All analysis was performed first for the entire cohort and then separately only for those under age 18 at time of enrollment to account for potential recall bias due to age. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were reported. Significance was defined as $p < 0.05$. All analyses were performed using SAS software (version 9.4; SAS Institute, Cary NC).

3 | Results

A total of 569 participants enrolled in Consortium projects were diagnosed with PCD (Figure 1). Of those, 15 were excluded due to complex congenital cardiac disease, 50 for a history of prematurity, and 49 due to incomplete neonatal data. The remaining 455 participants were included in the analysis. 260 (57.1%) individuals were female with a median age at enrollment of 10.9 years (range: 0.01–71.9); 182 (40%) had ODA defects, 80 (17.6%) had ODA/IDA defects, 80 (17.6%) had IDA/MTD defects, 36 (7.9%) had normal ultrastructure associated with *DNAH11* variants, and 77 (16.9%) had normal/near-normal/other ultrastructure not associated with *DNAH11* variants (Table 1). NRD was reported in 305 (67.0%) individuals. By ultrastructure group, NRD was present in 63.7% of those with ODA defects, 77.5% of those with ODA/IDA defects, 75.0% of those with IDA/MTD defects, 38.9% of those with normal ultrastructure associated with *DNAH11* variants, and 68.8% of those with normal/near-normal/other ultrastructure (Table 1). Thirty-five different PCD genes were represented in the cohort (Supplemental Table 1). Aside from *DNAH11*, the other three most common genes identified were *DNAH5*, *CCDC40*, and *CCDC39*, with rates of NRD of 66.7%, 79.5%, and 72.7%, respectively.

Individuals with *DNAH11* variants were less likely to have NRD than those with ODA defects (OR: 0.35, 95% CI: 0.16–0.76) (Table 2). There was no difference in NRD in those with ODA/IDA defects, IDA/MTD defects, or normal/near-normal/other ultrastructure compared to the ODA defect group (OR: 1.68, 95% CI: 0.88–3.22; OR: 1.56, 95% CI: 0.79–3.06; and OR: 1.14, 95% CI: 0.62–2.11, respectively). When analysis was performed only in individuals less than 18 years of age, NRD was still less likely in those with *DNAH11* variants compared to the ODA defect group (OR: 0.29, 95% CI: 0.11–0.74) (Supplemental Table 2). There was still no difference in NRD in those with ODA/IDA defects, IDA/MTD defects, or normal/near-normal/other ultrastructure compared to the ODA defect group in participants less than 18 years of age (OR: 1.38, 95% CI: 0.61–3.12; OR: 1.63, 95% CI: 0.69–3.84; and OR: 1.10, 95% CI: 0.51–2.38, respectively).

When comparing specific genes to one another, those with *DNAH11* variants had a lower likelihood of NRD compared to those with *DNAH5* variants (OR: 0.36, 95% CI: 0.16–0.82) (Table 3). No difference was seen between those with variants in *CCDC39/CCDC40* and those with *DNAH5* variants (OR: 1.76, 95% CI: 0.86–3.60). In those less than 18 years of age, participants with variants in *DNAH11* still had a lower likelihood of NRD compared to those with variants in *DNAH5* (OR: 0.33, 95% CI: 0.12–0.89), and those with *CCDC39/CCDC40* variants were still not different (OR: 1.87, 95% CI: 0.75–4.63) from the *DNAH5* group.

Within the *DNAH5* group, individuals with two LOF variants had a higher likelihood of NRD compared to those with one or more possible residual function variant (OR 3.06, 95% CI 1.33-7.0) (Supplemental Table 1). No difference was found between those with two LOF variants and those with one or more possible residual function variant in either the *DNAH11* or the *CCDC39/CCDC40* groups (OR: 0.79, 95% CI: 0.20–3.15 and OR: 0.36, 95% CI: 0.04–3.25, respectively). When individuals of every genotype were pooled ($n = 424$) there was no difference in likelihood of NRD in those with two LOF variants compared to those with one or more possible residual function variant (OR: 1.44, 95% CI: 0.93–2.22).

The mean number of days hospitalized based on ultrastructure group is shown in Table 4. Those with IDA/MTD defects who had NRD and those with normal/near-normal/other ultrastructure who had NRD had a greater risk for a longer hospital admission compared to those with ODA defects who had NRD [Rate ratio (RR) 1.52, 95% CI: 1.19-1.93 and RR: 1.72, 95% CI: 1.34–2.21, respectively] (Figure 2). Length of hospital admission was not significantly different in those with variants in

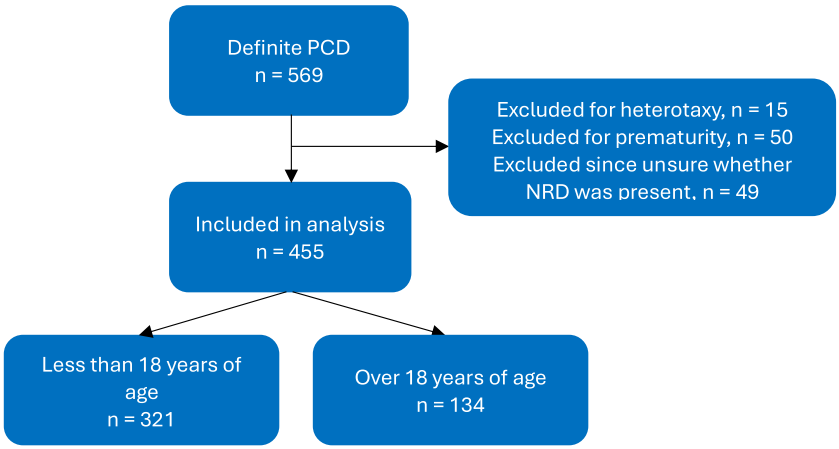

FIGURE 1 | Enrollment of participants. NRD, neonatal respiratory distress; PCD, primary ciliary dyskinesia. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 | Demographics.

	Neonatal respiratory distress	No neonatal respiratory distress	*P*-value
Total, n (%)	305 (67.0%)	150 (33.0%)	
Gender, n (%)			0.14[a]
Female	167 (54.8%)	93 (62.0%)	
Male	138 (45.2%)	57 (38.0%)	
Race, n (%)			0.008[a]
White	259 (84.9%)	112 (74.7%)	
Asian	22 (7.2%)	20 (13.3%)	
Black	5 (1.6%)	5 (3.3%)	
Mixed/other	14 (4.6%)	13 (8.7%)	
Not reported	5 (1.6%)	0	
Ethnicity, n (%)			0.17[a]
Hispanic	40 (13.1%)	13 (8.7%)	
Non-Hispanic	265 (86.9%)	137 (91.3%)	
Age at enrollment, y			< 0.0001[b]
Median (range)	9.4 (0.1–66.3)	14.7 (0.1–71.9)	
Ciliary ultrastructure			
ODA ($n = 182$)	116 (63.7%)	66 (36.3%)	
ODA/IDA ($n = 80$)	62 (77.5%)	18 (22.5%)	
IDA/MTD ($n = 80$)	60 (75.0%)	20 (25.0%)	
Normal associated with *DNAH11* ($n = 36$)	14 (38.9%)	22 (61.1%)	
Normal/near-normal/other ($n = 77$)	53 (68.8%)	24 (31.2%)	

Abbreviations: IDA, inner dynein arm; MTD, microtubular disorganization; ODA, outer dynein arm.
[a]Chi-Square test comparing white vs nonwhite individuals.
[b]Kruskal-Wallis test.

TABLE 2 | Likelihood of neonatal respiratory distress by ultrastructure group compared to ODA group (adjusted for age, gender, race, and presence of two LOF variants).

Ultrastructure group	Odds ratio relative to ODA group (95% CI)
ODA/IDA ($n = 80$)	1.68 (0.88–3.22)
IDA/MTD ($n = 80$)	1.56 (0.79–3.06)
DNAH11 ($n = 36$)	0.35 (0.16–0.76)
Normal/near-normal/other ($n = 77$)	1.14 (0.62–2.11)

Abbreviations: IDA, inner dynein arm; MTD, microtubular disorganization; ODA, outer dynein arm.

TABLE 3 | Likelihood of neonatal respiratory distress by genotype compared to *DNAH5* group (adjusted for age, gender, race, and presence of two LOF variants).

Genotype	Odds ratio relative to *DNAH5* group (95% CI)
CCDC39/CCDC40 ($n = 72$)	1.76 (0.86–3.60)
DNAH11 ($n = 36$)	0.36 (0.16–0.82)

DNAH11 with NRD compared to those with ODA defects and NRD (RR: 0.91, 95% CI: 0.61–1.36).

4 | Discussion

In this large, multicenter study examining the earliest clinical manifestations of PCD, we found that the likelihood of NRD is lower in participants with variants in *DNAH11* as compared to those with variants in *DNAH5*, the most common gene associated with PCD. Previous reports have shown PCD genotype-phenotype relationships, which focused on ciliary beat frequency (CBF), severity of lung disease, and likelihood of organ laterality defects [3, 9–12, 19]. This study expands upon these genotype-phenotype relationships by demonstrating that certain ciliary ultrastructure defects and genotypes are associated with a greater risk for NRD and the presence of two LOF variants is also associated with greater risk for NRD in certain genes.

Using topological data analysis, Shoemark and colleagues examined distinct clinical phenotypes by genotype in a large cohort with PCD and found that those with variants in *DNAH11* reported lower rates of NRD [11]. Our study examined a large

TABLE 4 | Length of hospital admission after birth in those with neonatal respiratory distress.

	Days in hospital	Standard deviation	Range
ODA ($n = 112$)	13.8	7.4	1–35
ODA/IDA ($n = 57$)	14.5	12.7	2–90
IDA/MTD ($n = 56$)	22.5	27.3	2–150
Normal associated with *DNAH11* ($n = 14$)	12.6	7.0	4–28
Normal/near-normal/other ($n = 50$)	26.0	46.7	2–285

Abbreviations: IDA, inner dynein arm; MTD, microtubular disorganization; ODA, outer dynein arm.

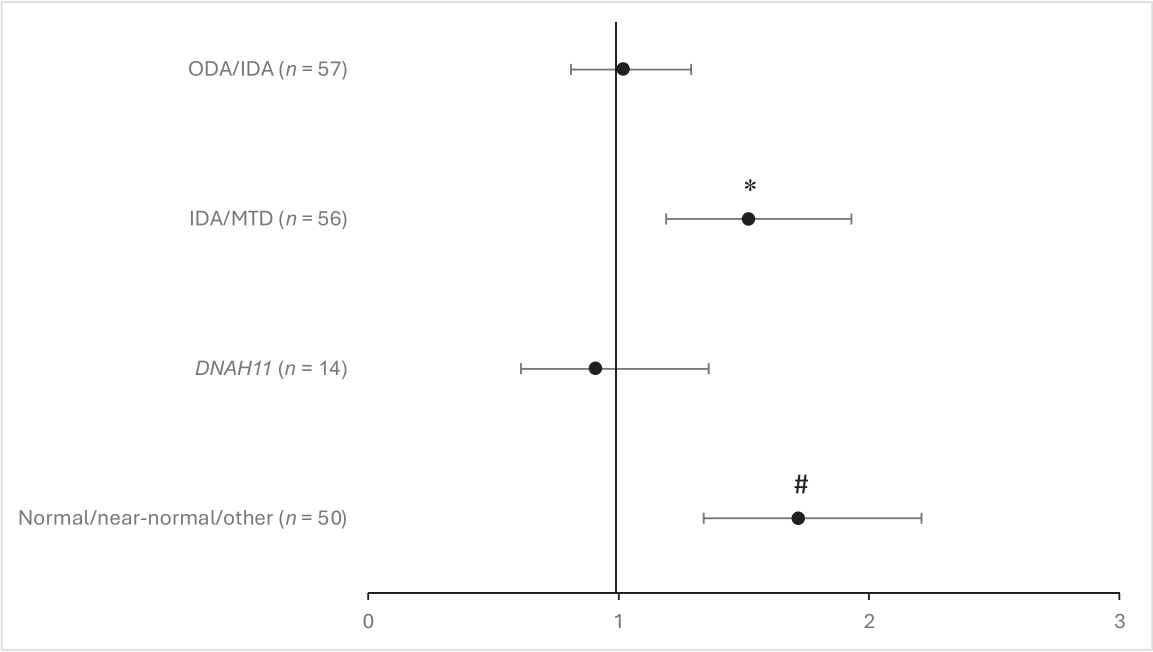

FIGURE 2 | All participants. Rate ratio for length of hospital admission for those with neonatal respiratory distress by ciliary ultrastructure group compared to ODA defect group. Asterisk, $p = 0.0008$; hashtag, $p = < 0.0001$. IDA, inner dynein arm; MTD, microtubular disorganization; ODA, outer dynein arm.

North American cohort, and using different methods, we report similar findings, further strengthening this observation.

DNAH11 was the third most common PCD gene in our cohort, accounting for almost 8% of participants. With a lower likelihood of NRD, *DNAH11* appears to have a distinct clinical phenotype from other genes that encode ODA proteins, which has implications for diagnostic genetic testing for PCD. Indeed, coupled with the absence of ultrastructural defects on TEM, the lower frequency of NRD in people with pathogenic *DNAH11* variants could contribute to the delayed or missed diagnosis of PCD compared to other genetic variants that are linked to characteristic ultrastructural defects on TEM.

The underlying cause of NRD in children with PCD is not fully understood. Furthermore, it remains unclear why some individuals present with NRD whereas others do not. Some postulate that impaired ciliary beating may lead to the retention of fetal lung fluid, resulting in atelectasis. Differences in CBF and pattern have been shown in animal models to differentially affect mucociliary transport and thus it is plausible that different genotypes and possibly even alleles within the same gene could result in variable clearance of fetal lung fluid [20]. Previous studies have shown PCD ciliary ultrastructure groups, as well as individual genes, have distinct CBF and pattern [19, 21–25]. Individuals with ODA defects have severely reduced CBF with a disorganized pattern, those with ODA/IDA defects have completely immotile cilia, and those with IDA/MTD defects exhibit a stiff beating pattern with a markedly reduced amplitude. Alternatively, individuals with PCD caused by variants in *DNAH11* have hyperkinetic, albeit stiff, cilia [26–28]. Perhaps the increased CBF seen in individuals with variants in *DNAH11* leads to improved clearance of fetal lung fluid and could explain the lower likelihood of NRD.

Despite encoding an ODA protein, *DNAH11* clearly differs from other ODA genes. The underlying reason for this difference likely lies in its location within the ciliary axoneme. Studies using immunofluorescence microscopy have shown that the dynein heavy chain DNAH5 is located along the entire axoneme, whereas dynein heavy chain DNAH11 is restricted to the proximal segment of the axoneme and dynein heavy chain DNAH9 is restricted to the distal segment of the axoneme [23, 29, 30]. Whereas pathogenic variants in *DNAH5* result in loss of

both the γ-heavy chain DNAH5 and β-heavy chain DNAH9, pathogenic variants in *DNAH11* still allow for the normal assembly of both DNAH5 and DNAH9, resulting in visibly normal cilia under TEM. The loss of DNAH11 therefore causes reduced ciliary bending in the proximal region of the cilia but not the distal region, which may have implications in functionality. Further evidence of a difference in functionality between *DNAH11* and other ODA genes can be seen in a study by Raidt and colleagues, in which individuals with variants in *DNAH11* had higher median FEV_1 z-scores compared to the rest of their cohort [31].

We also report an association between the presence of two LOF variants and NRD within the *DNAH5* group. It is well-known that in addition to phenotypic differences between PCD genes there is also phenotypic diversity within individual PCD genes. Shoemark and colleagues noted that individuals in the *DNAH5* group were phenotypically diverse and had the widest spectrum of gene variants [11]; differing ciliary function within the *DNAH5* group may contribute to the heterogeneity of disease manifestations. Whereas two variants resulting in complete absence of protein may cause a more severe phenotype, it seems plausible that a missense variant resulting in residual protein production may retain some ciliary function. This could partially explain the phenotypic diversity seen within individual PCD genes. Horani and colleagues recently identified a gene dosage effect in mice with variants in *Dnaaf5*, wherein those with homozygous missense variants had partially preserved ciliary function and improved survival compared to those with homozygous null *Dnaaf5* alleles or heterozygous missense/null alleles [13].

No difference was observed between those with two LOF variants and those with possible residual function variants in either the *DNAH11* or *CCDC39/CCDC40* groups, which may be related to inadequate statistical power due to lower sample sizes in these two groups. However, when individuals from the entire cohort with known genetics were pooled there was no difference in likelihood of NRD between those with two LOF variants and those with one or more possible residual function variant. Given that the difference was only seen within the *DNAH5* group, it may indicate differences in cilia variant functionality between different PCD genes. Recently, different disease-causing *CCDC103* variants were shown to have unique ultrastructural defects and ciliary beat patterns between variants [32]. Future investigation of ciliary beat pattern differences by variant type will likely help us better understand the phenotypic diversity in PCD, particularly within individual genes.

Wee and colleagues reported an association between the length of neonatal hospital admission and lung function measured years later in PCD [14]. Our analysis showed that those with IDA/MTD defects had a greater likelihood of a longer neonatal hospital admission, which is congruent with these findings, given that individuals with IDA/MTD defects are known to have more severe lung disease and lower spirometric indices [3]. Interestingly, we also found an association between those with normal/near-normal/other ultrastructure and a longer neonatal hospital admission. It should be noted that this analysis did not control for other factors that could affect hospital length of stay, including hospital complications (e.g., infection) or PCD genotype, which limits our interpretation. The normal/near-normal/other ultrastructure group is heterogeneous and includes various genes, including those that cause central apparatus defects, radial spoke defects, and reduced generation of multiple motile cilia. It is possible that this finding of a longer neonatal hospital admission in the normal/near-normal/other ultrastructure group may be an indicator of more severe lung disease, perhaps in a subset of genes. Indeed, a large study by Raidt and colleagues found that individuals with PCD-causing variants in *CCNO* (categorized in our study as normal/near-normal/other ultrastructure) had lower FEV_1 *z*-scores than the rest of their cohort [31]. Further studies of individuals with radial spoke and central apparatus defects are necessary to elucidate this finding.

The impact of ciliary function in genotype/phenotype relationships in PCD is complex. At present, there are various ways to measure specific characteristics of ciliary function, such as CBF, pattern, amplitude, and percent ciliary beating. However, analysis of individual ciliary traits in isolation may not accurately predict effects on mucociliary clearance and downstream complications. Novel methods are being developed to comprehensively characterize effects of individual genes and alleles on mucociliary clearance [20]. Larger studies using these new modalities are necessary to further understand differences in clinical outcomes both between PCD genes as well as between variant types within the same PCD gene.

The strengths of our study are the large number of PCD participants and the standardized approach to collection of data. Additionally, a genetic diagnosis was available in most participants, allowing us to analyze associations between the gene, variant type, and risk of NRD (although some individuals had no genetic abnormality identified and were diagnosed solely by ciliary ultrastructure defect). A weakness is that the study remains underpowered for analysis for many of the genes, which is not surprising considering the growing number of PCD-associated genes. There is also the potential for recall bias when reporting NRD, particularly among older participants, though we controlled for age at enrollment in our analyses. We also performed sub-analyses in participants under 18 years of age to limit recall bias and showed similar results to our primary analysis. The prevalence of NRD in those less than 18 years of age was 71.7%, which was not significantly different from the prevalence of NRD in the entire cohort (67.0%) ($p = 0.17$).

The overall prevalence of NRD in our cohort is lower than has been reported in previous studies. Bias could have occurred if we excluded more participants due to prematurity than is expected to occur in the general population. However, the prevalence of prematurity in our cohort was similar to that reported by the Centers for Disease Control (CDC), thus unlikely to be a cause of bias in our study. The overall percentage of individuals excluded from the ODA group was greater than the other ultrastructure groups due to higher prevalence of heterotaxy, and thus could result in underestimating NRD for those with ODA defects. This may also introduce bias in our evaluation of two LOF variants on prevalence of NRD within the *DNAH5* group. Lastly, using an absolute definition for NRD requiring supplemental oxygen use for at least one day may be a limitation. Some individuals with PCD may have tachypnea or increased work of breathing but never require supplemental oxygen and would thus be

characterized as having no NRD by our definition. This binomial classification is also a limitation in that it groups all of those with NRD together, regardless of whether they needed supplemental oxygen for one day or much longer.

Our large, multicenter cohort study found a lower likelihood of NRD in *DNAH11* compared to *DNAH5*, suggesting ciliary functional differences between these genes that manifest immediately after birth. We also found differences in likelihood of NRD between those with two LOF variants and those with possible residual function variants within the largest gene group, *DNAH5*, indicating that variant functionality may also modulate ciliary function in certain genes. Further understanding of phenotypic differences between PCD genes and variant functionality will help improve the timely and accurate diagnosis of this rare and often underrecognized disease.

Author Contributions

Andrew T. Barber: conceptualization, methodology, writing – original draft. **Stephanie D. Davis:** investigation, conceptualization, methodology, writing – review and editing, supervision. **Thomas W. Ferkol:** investigation, writing – review and editing, supervision. **Adam J. Shapiro:** investigation, writing – review and editing. **Jeff Atkinson:** investigation, writing – review and editing. **Scott D. Sagel:** investigation, writing – review and editing. **Sharon D. Dell:** investigation, writing – review and editing. **Kenneth Olivier:** investigation, writing – review and editing. **Carlos Milla:** investigation, writing – review and editing. **Margaret Rosenfeld:** investigation, writing – review and editing. **Lang Li:** methodology, writing – review and editing, formal analysis. **Feng-Chang Lin:** methodology, formal analysis, writing – review and editing. **Kelli M. Sullivan:** investigation, writing – review and editing, project administration. **Nicole A. Capps:** investigation, writing – review and editing, project administration. **Maimoona A. Zariwala:** investigation, data curation, writing – review and editing. **Michael R. Knowles:** investigation, writing – review and editing. **Margaret W. Leigh:** conceptualization, investigation, methodology, writing – review and editing.

Acknowledgments

The authors thank all participants in this study and their family members, Michele Manion and the US PCD Foundation. We thank the investigators and coordinators of the Genetic Disorders of Mucociliary Clearance Consortium (GDMCC) that is part of the Rare Disease Clinical Research Network; Dr. J. Stonebraker and E. Schecterman for technical support; Dr. Hong Dang for Bioinformatic support; and the Yale Center for Mendelian Genomics (UM1 HG006504) for providing whole exome sequencing and bioinformatics support. Supported by the National Institute of Health (NIH) U54HL096458 funded by the Office of Rare Disease Research (National Center for Advancing Translational Science) and administered by NHLBI and the Carolina For The Kids Grant Program, US NIH/NHLBI grant R01HL071798 to MRK and MAZ, NIH/NCATS Colorado CTSA Grant Number UM1 TR004399. This study was performed at the University of North Carolina at Chapel Hill under IRB numbers 05-2979 and 14-1225.

Conflicts of Interest

Stephanie D. Davis has grant support from the NIH and ReCode Therapeutics as well as support from the Primary Ciliary Dyskinesia Foundation. She also serves as a member of the of the Primary Ciliary Dyskinesia Medical and Scientific Advisory Council. Thomas W. Ferkol has grant support from the NIH (HL096458, TR003860, TR3860, AI146999, HL125241, U01HL172658, HG009650) as well as support from ReCode Therapeutics and Parion Sciences and also serves as a consultant for TransBio and Arrowhead Pharmaceuticals. He serves on the Clinical Study Advisory Board for ReCode Therapeutics. Adam J. Shapiro has received support from the Chest Foundation, the Primary Ciliary Dyskinesia Foundation, and serves as a consultant for Parion Sciences and Ethris GMBH. He serves as the medical director of the Primary Ciliary Dyskinesia Foundation and also has participated on a board for ReCode Therapeutics. Kenneth Olivier has received research grants from ReCode Therapeutics and serves on the Medical and Scientific Advisory Council of the Primary Ciliary Dyskinesia Foundation. Margaret Rosenfeld receives grant support from the NIH (U54 HL096458). Scott D. Sagel has grant support from the NIH (U54 HL096458, UL1 TR002535, R21 TR004057) and serves as a consultant for Pharming Healthcare/Precision Medicine Group. Sharon D. Dell has grant support from the BCCHRI Establishment Award and the NIH (U54 HL096458). She serves as a consultant for Sanofi and Regeneron Pharmaceuticals. She has received honoraria for speaking to AstraZeneca Canada, Sanofi Aventis Canada, and Sanofi Genzyme Corporation. She serves on the scientific advisory board of Sanofi Aventis Canada and as Deputy Editor of Annals ATS. She receives support for clinical trials from Boehringer Ingelheim Canada, Sanofi Aventis Canada, Vertex Pharmaceuticals, Merck Research Laboratories, and GlaxoSmithKline UK. Maimoona A. Zariwala receives grant support from the NIH (U54 HL096458, R01 HL071798). She serves on the Primary Ciliary Dyskinesia Medical and Scientific Advisory Council. Margaret W. Leigh has received grant support from the Primary Ciliary Dyskinesia Foundation and serves on the board of directors for the Primary Ciliary Dyskinesia Foundation.

Data Availability Statement

The authors have nothing to report.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.