SUPPLEMENTARY INFORMATION:

Diagnosis of PCD by nasal nitric oxide, light microscopy and electron microscopy and management:

A multi-disciplinary consensus diagnosis of PCD was made. Clinical history and nasal nitric oxide (NO) results were considered in conjunction with light and electron microscopy (EM) findings. A diagnosis of PCD was made when a compatible clinical history was accompanied by a recognised defect of ciliary beat and/or ultrastructure. Amongst the patient cohort, 55% of patients were diagnosed based on clinical features suggestive of PCD in addition to a known EM ciliary ultrastructural defect, abnormal ciliary beat pattern at light microscopy and nasal NO levels; 25% on clinical, EM and cilia beat; 9% on clinical, cilia beat and nasal NO; 7% on clinical and abnormal cilia beat; 2% on clinical and EM abnormalities. 3% of patients were diagnosed based on a definitive clinical picture (All had recurrent respiratory infections, childhood bronchiectasis, respiratory distress at birth, chronic rhinosinusitis and situs inversus) but light and electron microscopy results were not available.

Nasal NO was measured using a chemiluminescent analyser sampling at a flow rate of 250ml/min (LR2000, Logan Research, Rochester Kent, UK) during a breath hold manoeuvre. Nasal NO was used at the start of the PCD diagnostic pathway. Once a diagnosis was confirmed by a test with good specificity, such as TEM, it was not our standard practice to revisit the beginning of the diagnostic pathway. Nasal NO was not used as a monitoring tool in PCD. Nasal brush biopsy was undertaken if the nasal NO concentration was less than 250ppb or there was a highly suggestive clinical history. Only 2 patients had normal nasal NO levels with abnormal electron microscopy and cilia beat pattern consistent with PCD. Patients seen prior to 1995 or too young or unable to perform the test did not have nasal

NO measurements. From our cohort, 16 patients were under the age of 4 at diagnosis and were unable to perform nasal NO measurement and 25 patients had a diagnosis prior to 1995 and hence did not have nasal NO performed. A nasal NO record was not found in an additional 13 patients where a diagnosis was made based on suggestive clinical findings and diagnostic TEM.

To biopsy the nose, strips of mucosa were scraped from the inferior turbinate with a cytology brush. The biopsy sample was placed in M199 maintenance medium and examined by light microscopy to assess ciliary beat pattern and frequency. From 1988-2006 ciliary beat frequency was measured by photometry.[1] From 2006 high speed video microscopy was used. [2] All ciliary beat frequency was measured at 37°C. 66 patients had ciliary light microscopy performed only prior to 2006 where high-speed video microscopy was not available. PCD was considered where cilia were immotile, slow, hyper-frequent or had a uncoordinated, stiff, incomplete or circling beat pattern. The epithelial strips were processed for TEM. Samples were analysed and quantified for dynein and microtubular defects by a trained microscopist as previously described.[3] PCD was diagnosed where patients had a consistent defect of the outer dynein arm, inner and outer dynein arm, inner dynein arm +/- microtubular disorganisation or a central pair and transposition defect.

Management of PCD

All bronchiectasis patients diagnosed at our centre receive a detailed explanation of their condition along with a full clinical and physiotherapy review with regular follow-up and access to a dedicated bronchiectasis nurse if necessary. Standardised management protocols for non-cystic fibrosis bronchiectasis was thereafter applied to adult PCD patients.

High resolution CT imaging:

HRCT was available in 93 patients. The extent of bronchiectasis, severity of bronchial dilatation, bronchial wall thickness, mucus plugging in large and small airways, mosaicism and emphysema were scored for each lung lobe (the lingula was considered as a different lobe, making a total of 6 lobes), according to a modified Bhalla system which has been shown to have low interobserver variation. The scoring system was as follows: 1) extent of bronchiectasis (0 = none, 1 = one or partial bronchopulmonary segment involved, 2 = two or more bronchopulmonary segments involved, 3 = generalized cystic bronchiectasis); 2) severity of bronchial dilatation (0 = normal, 1 = less than twice the diameter of the adjacent pulmonary artery, 2 = more than twice the diameter of adjacent pulmonary artery); 3) severity of bronchial wall thickening (0 = normal, 1 = <0.5 × the diameter of the adjacent pulmonary artery, 2 = 0.5 - 1.0 × the diameter of the adjacent pulmonary artery, 3 = ≥ 1.0 × the diameter of the adjacent pulmonary artery); 4) presence of mucous plugging in large airways (0 = none, 1 = minimal, 2 = extensive 5) presence of mucous plugging in small airways (0 = none, 1 = minimal, 2 = extensive); 6) extent of mosaicism (to nearest 5%) and 7) extent of emphysema (to nearest 5%). Patients with previous lobectomies had scores adjusted to represent the maximum score available. Scores for extent of bronchiectasis, severity of bronchial dilatation and thickening and mucus plugging in small and large airways are expressed as percentages of maximum in the results section.

Statistical analysis

To model the effects of HRCT, ciliary ultrastructure and ciliary beat frequency on lung function decline, HRCT scores with available longitudinal FEV1 measures (n=75) were grouped into terciles (except large airway mucus plugging where there was a bimodal

distribution) and ciliary beat frequency was split into ≤8 and >8Hz. Given the limitations of numbers within our cohort for analysis of the effects of electron microscopy (EM) on longitudinal FEV1, patients were grouped into outer ± inner arm defects, microtubular defects (including inner arm defect with microtubular disorganisation and central pair and transposition defect) and normal/inconclusive. Inner arm defects alone were excluded from analysis. The groupings were chosen to represent defects in the beat amplitude (outer dynein arms) versus defects in the beat regulation (determined by the nexin links and radial spokes) and patients with no defect found on ciliary ultrastructure. Similar groupings have also been used in a recent paper characterising a multi-centre US cohort.[4] See Table 1 and supplementary Table 1 for details regarding grouping.

While we have performed a number of analyses within the study, the study is exploratory and the tests were hypothesis generating. As such, we did not adjust for multiple testing.

Supplementary Table S1

Categorical terciles of HRCT scores		
		Frequency (%)
HRCT scores	Extent of bronchiectasis • 1 (<44) • 2 (44-52) • 3 (≥52)	 21 (28) 28 (37) 26 (35)
	Severity of bronchial wall dilatation • 1 (<33) • 2 (33-67) • 3 (≥67)	 13 (17) 46 (61) 16 (21)
	Bronchial wall thickness • 1 (<28) • 2 (28-39) • 3 (≥39)	 29 (39) 21 (28) 25 (33)
	Small airway mucus plugging • 1 (<50) • 2 (50-52) • 3 (≥52)	 20 (27) 30 (40) 25 (33)
	Large airway mucus plugging • 0 (0) • 1 (>1)	 63 (85) 11 (15)

Sensitivity analysis

Subsequent to the analysis of FEV1 measures using linear mixed models, and recognising that HRCT images may have been performed after certain FEV1 measures were taken, we re-ran our models of HRCT variables restricting the analysis to those FEV1 values taken on or after the HRCT imaging was performed.

Supplementary results

Analysing the association between FEV1 and other lung function parameters after adjusting for ciliary ultrastructure and age showed that FVC (%predicted) was positively associated with FEV1 (β=0.84% predicted increase in FEV1 per unit increase in FVC (95%CI:0.79,0.89)) as was TLC (β=5.30% (95%CI:3.58,7.01)) and TLCO (β=1.95% (95%CI:1.34,2.55)). RV was negatively associated with FEV1 (β=-10.30% (95%CI:-12.20,-8.39)). Mixed models of these lung function measures adjusted for ciliary ultrastructure showed that FVC (%predicted) declined by 0.27%/year (95% of patients' changes varied between -1.50 and 0.97) and TLCO by 0.07units/year (95% between -0.19-0.04). TLC increased by 0.005l/year (95% between -0.01-0.07) and RV increased by 0.02l/year (95% between -0.02-0.06).

Supplementary Table S2

	Outer and inner dynein arm defect	Outer dynein arm defect	Inner dynein arm defect	Inner dynein arm defect with microtubular disarrangement	Central pair and transposition defect	Normal	P value
Age at diagnosis (yrs)	30 (8-72)	11 (0.5-63)	26 (26)	32.5 (5-40)	35 (9-63)	29 (9-49)	0.06
FEV1% predicted at diagnosis	73.0±20.8	73.5±20.5	88.5±3.5	62.3±23.3	60.2±19.2	73.0±19.1	0.26
FEV1/FVC at diagnosis	65.5±13.6	72.0±12.9	68.0±1.4	59.6±15.0	62.6±15.1	70.5±7.6	0.07
Extent of bronchiectasis (%)	44.4 (0.0-66.7)	50.0 (0-100.0)	44.4 (44.4)	44.4 (11.4-50.1)	66.7 (33.3-72.2	47,2 (0.0-66.7)	0.17
Severity of bronchial wall dilatation (%)	33.3 (0.0-83.3)	58.8 (0.0-83.3)	33.3 (33.3)	41.7 (8.3-66.7)	66.7 (25.0-83.3)	45.8 (0.0-83.3)	0.56

Age at diagnosis, Extent of bronchiectasis and Severity of bronchial wall dilatation is presented as median (range). FEV1% predicted at diagnosis and FEV1/FVC at diagnosis is presented as mean±SD.

References:

1. Rutland J, Cole PJ. Non-invasive sampling of nasal cilia for measurement of beat frequency and study of ultrastructure. Lancet. 1980 Sep 13;2(8194):564-5.
2. Chilvers MA, Rutman A, O'Callaghan C. Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. The Journal of allergy and clinical immunology. 2003 Sep;112(3):518-24.
3. Shoemark A, Dixon M, Corrin B, Dewar A. Twenty-year review of quantitative transmission electron microscopy for the diagnosis of primary ciliary dyskinesia. Journal of clinical pathology. 2012 Mar;65(3):267-71.
4. Davis SD, Ferkol TW, Rosenfeld M, Lee HS, Dell SD, Sagel SD, et al. Clinical features of childhood primary ciliary dyskinesia by genotype and ultrastructural phenotype. American journal of respiratory and critical care medicine. 2015 Feb 1;191(3):316-24.