Online data supplement

Clinical features described at last review	N	*CCDC103* p.His154Pro	N	Comparator group
Age (mean SD)	16	11.8 (4.8)	16	11.9 (8.8)
Gender (% Male)	16	67%	16	63%
History of neonatal respiratory distress	14	71%	13	77%
Wet Cough	14	93%	15	100%
Nasal Symptoms	13	85%	15	100%
Glue ear	10	70%	16	56%
Bronchiectasis on HRCT	8	50%	7	86%
Sputum microbiology*	14		15	
Pseudomonas aeruginosa		0%		7%
Haemophilus influenza		29%		7%
Streptococcus pneumonia		14%		7%
No growth/ oral flora		57%		87%

Supplementary Table 1. Comparison of clinical features between the *CCDC103* p.His154Pro mutation group of patients versus the South Asian age matched comparator group carrying other dynein arm-associated gene mutations. Data obtained from the most recent clinical review by the UK PCD management team, obtained via a retrospective review of clinical records. * Some individuals grew multiple organisms

Supplementary Methods

Diagnostic tests

Nasal Nitric Oxide

Nasal Nitric oxide testing using a breath hold maneuver was performed using one of 3 analyzers: a Logan LR2000 (Logan Research Ltd., UK) or a NIOX Mino or NIOX Flex (Aerocrine AB, Sweden). Results are reported in nl/min for standardization between analyzers (1).

Light microscopy

To biopsy the nose, strips of mucosa were scraped from the inferior turbinate with a cytology brush (2). The biopsy sample was placed in M199 maintenance medium at 37°C and examined by light microscopy to assess ciliary beat pattern and frequency. High speed video microscopy was performed using an oil immersion 100x lens on a minimum of 10 strips of epithelium. Beating cilia were recorded at 500 frames per second and played back at 60 frames per second to analyse cilia beat pattern and frequency (in Hz) of both top and side profiles (3).

Electron microscopy

Samples were fixed in 2.5% glutaraldehyde in cacodylate buffer and processed for electron microscopy. Defects were quantified using the method described by Shoemark et al 2012 (4). Briefly, cells were washed in sodium cacodylate buffer, post-fixed with 1% osmium tetroxide and centrifuged in agar or agarose to generate a pellet. Using a series of increasing concentrations of methanol followed by propylene oxide, cells were dehydrated before embedding in resin then 70-90nm sections were cut using an ultramicrotome, mounted onto copper grids. Heavy metal staining was with uranyl acetate and lead citrate. Assessment of the respiratory epithelium and ciliary ultrastructure were made on a transmission electron microscope. Quantification of cells, microtubular arrangement in the axoneme and presence of dynein arms was performed by a clinical electron microscopist blinded to the case information. Care was taken to assess cilia from a number of healthy cells from locations proximal and distal to the epithelial cell surface. Transverse sections of cilia were methodically quantified until either the entire section or 300 cilia had been counted.

References

1. Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013; 10: 574-581.
2. Rutland J, Dewar A, Cox T, Cole P. Nasal brushing for the study of ciliary ultrastructure. *J Clin Pathol* 1982; 35: 357-359.
3. Chilvers MA, Rutman A, O'Callaghan C. Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. *J Allergy Clin Immunol* 2003; 112: 518-524.
4. Shoemark A, Dixon M, Corrin B, Dewar A. Twenty-year review of quantitative transmission electron microscopy for the diagnosis of primary ciliary dyskinesia. *J Clin Pathol* 2012; 65: 267-271.