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Genome sequencing reveals underdiagnosis of primary ciliary dyskinesia in bronchiectasis

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Take home message: Primary Ciliary Dyskinesia is underdiagnosed as a cause of idiopathic bronchiectasis. Whole genome sequencing reveals variants in motile ciliopathy genes

Abstract (247/250)

Background: Bronchiectasis can result from infectious, genetic, immunological and allergic causes. 60-80% cases are idiopathic, but a well-recognised genetic cause is the motile ciliopathy,

primary ciliary dyskinesia (PCD). Diagnosis of PCD has management implications including addressing co-morbidities, implementing genetic and fertility counselling and future access to PCD-specific treatments. Diagnostic testing can be complex, however PCD genetic testing is rapidly moving from research into clinical diagnostics and would confirm the cause of bronchiectasis.

Methods: This observational study used genetic data from severe bronchiectasis patients recruited to the UK 100,000 Genomes Project and patients referred for gene panel testing within a tertiary respiratory hospital, Patients referred for genetic testing due to clinical suspicion of PCD were excluded from both analyses. Data was accessed from the British Thoracic Society audit, to investigate whether motile ciliopathies are underdiagnosed in people with bronchiectasis in the UK..

Results: Pathogenic or likely pathogenic variants were identified in motile ciliopathy genes in 17/142 (12%) individuals by whole genome sequencing. Similarly in a single centre with access to pathological diagnostic facilities, 5-10% patients received a PCD diagnosis by gene panel, often linked to normal/inconclusive nasal nitric oxide and cilia functional test results. In 4,898 audited patients with bronchiectasis, <2% were tested for PCD and <1% received genetic testing.

Conclusions: PCD is underdiagnosed as a cause of bronchiectasis. Increased uptake of genetic testing may help to identify bronchiectasis due to motile ciliopathies and ensure appropriate management.

Introduction

Bronchiectasis is both a clinical disease and a radiological appearance that has multiple causes and can be associated with a range of conditions[1].It can result from infectious, genetic, immunological or allergic causes but the majority of cases are of unknown cause and termed 'idiopathic' [2]. Guidelines recommend investigation of aetiology since it can alter management [3, 4]. A targeted approach to aetiology can reduce the number of idiopathic cases reported [5]Genetic causes include rare *CFTR* genotypes, channelopathies, immunodeficiencies, and primary ciliary dyskinesia (PCD) [2]. The estimated PCD prevalence among adults with bronchiectasis is 1-13% [6-11]. Testing for PCD is suggested in patients with supporting clinical features, including a history of neonatal distress, symptoms from childhood, recurrent otitis media, rhinosinusitis, or infertility [3]. Patients with adult-onset bronchiectasis arising from PCD are described as younger than their idiopathic bronchiectasis counterparts, having moderate impairment of lung function and higher rates of chronic infection with *Pseudomonas aeruginosa* [11-13]. Due to these risk factors and the multi-system nature of PCD, in the UK, diagnosed PCD patients have access to a specific and multidisciplinary team approach to management in specialist PCD referral centres. Recently the first randomised control trial for evidence-based medicine in PCD was completed and specific therapies which target genetic defects are in development [12, 13]. A diagnosis therefore is becoming increasingly important as it impacts on clinical care.

Mutations in at least fifty different genes cause PCD and its spectrum of associated motile ciliopathies [14]. Diagnosing PCD is complex due to the requirement for a multi-test approach requiring specialist expertise and equipment [15-17]. Therefore, the risk of late or missed diagnosis is high. Late diagnosis is associated with poorer prognosis [18]. In England, PCD testing is available at 3 specialists centres and includes evaluation by nasal nitric oxide (NO) measurement, high-speed video microscopy, immunofluorescence, tissue culture at air liquid interface and

transmission electron microscopy(TEM) [15, 19]. Genetic testing for PCD has now moved from a research-based test into clinical practice [14].

In this study we investigated the contribution of primary motile ciliopathies in bronchiectasis using three datasets: whole genome sequencing (WGS) of patients recruited to the UK 100,000 Genomes Project, clinical gene panel sequencing of patients within a large tertiary PCD and bronchiectasis centre, and PCD diagnostic data from the British Thoracic Society national audit. Specifically, the aim was to identify motile ciliopathies in which diagnosis was not strongly suspected. Our analysis demonstrates that despite comprehensive national PCD testing facilities, motile ciliopathies remain underdiagnosed in people with idiopathic bronchiectasis.

Methods

The 100,000 Genomes Project

The UK 100,000 Genomes Project, overseen by Genomics England Ltd (GEL; https://www.genomicsengland.co.uk), was initiated in 2013 to sequence 100,000 genomes from NHS patients and family members in the UK affected by rare diseases or cancer [20].

142 people were recruited as 'non-CF bronchiectasis' (with 107 additional family members in a total cohort of 249 individuals). All participants provided written informed consent. Inclusion criteria were severe disease (<30 FEV_1% predicted), or individual <50yr old with involvement of >1 lobe or suspicion of an inherited cause (including ciliopathies); full recruitment inclusion/exclusion criteria are shown in the online supplement. Importantly, participants with strong clinical suspicion of PCD were recruited to the 100,000 Genomes Project as a separate group and were excluded from the present study [21].

WGS was carried out using Illumina short-read sequencing. GEL developed standardised data analysis pipelines (detailed in the supplementary methods) to filter and tier variants most likely to be clinically relevant.

We carried out expert curation of all variants tiered by GEL in non-CF bronchiectasis patients and applied additional complementary variant analysis pipelines. These interrogated an expanded panel of 91 genes associated with motile ciliopathies (**Table E3**) for exonic or splice donor/acceptor single nucleotide, short insertion/deletion, copy number (CNVs) and structural variants that were predicted to be protein altering. Recently developed variant annotation tools (SpliceAI [22],

UTRannotator [23]), were also applied to screen for additional, potentially protein-altering variants. Pathogenic and likely pathogenic variants in known disease genes for motile ciliopathies were categorised using the ACMG/AMP guidelines [24]. A summary of methodology and participant numbers can be found in **Figure E1**.

Here we report individuals with genetic variants associated with motile ciliopathies only; other potentially disease-causing variants identified, for example those in *CFTR*, will be reported elsewhere.

Royal Brompton Hospital Clinical Genetics and Genomics Laboratory bronchiectasis panel audit results

In 2017 the Royal Brompton Hospital set up clinical genetic diagnostic testing for patients with respiratory disease, including targeted analysis of genes for bronchiectasis and PCD, as part of a custom "Respigene" gene panel (Agilent Technologies, Inc.). Sequencing was performed on an Illumina NextSeq550, and reads were mapped to human genome reference (GRCh38). Variants were classified for pathogenicity according to ACMG/AMP guidelines [24]. An in-house CNV caller was and all likely pathogenic and pathogenic SNVs and CNVs were confirmed by Sanger sequencing or digital droplet PCR (ddPCR), respectively. The 52-gene bronchiectasis panel consisted of *CFTR*, *SCNN1A*, *B*, *D* and *G* genes and 47 PCD genes (**Tables E2, E3**).

Results of all clinical referrals for the bronchiectasis panel between 2017-2020 were included. Patients with a strong clinical diagnosis of PCD were referred only for testing of a PCD gene sub-panel and were excluded from this study. PCD diagnostic investigations (nasal NO, high speed

video microscopy, immunofluorescence and TEM) were performed as described previously [25-27].

British Thoracic Society audit

The 2017 British Thoracic Society (BTS) bronchiectasis audit was carried out across 105 hospitals with 4,845 records. The audit focused on diagnosis and management of adult bronchiectasis. Audits applied to patients who had a follow-up or review outpatient appointment for bronchiectasis between 1 Oct – 30 Nov 2017. Data were collected from patient notes. Participants were asked to enter all eligible cases, or where this was not possible due to large numbers, to take care to avoid bias in case selection [28]. For the purpose of this analysis, cases in which the answer was yes to two or more of the following questions were considered to have severe disease: 'advanced disease / considering transplantation', 'recurrent exacerbations (>3 per year)'; 'deteriorating bronchiectasis with declining lung function', 'pseudomonas isolated 2 or more times in the past 12 months'.

Results

WGS of patients with severe and familial bronchiectasis reveals mutations in genes associated with motile ciliopathies.

17 of 142 (12%) individuals with a clinical diagnosis of bronchiectasis screened by WGS as part of the 100,000 Genomes Project had pathogenic or likely pathogenic variants in a motile ciliopathy gene as listed in **Tables E2 and E3**. Results for these individuals are shown in **Table 1**. The mean

age of those with bronchiectasis with variants suggestive of an inherited motile ciliopathy was 45, median age 46.5, range 21-75. The male:female ratio was 6:11, in keeping with a female predominance in patients with bronchiectasis [29]. These 17 patients were recruited across seven genomic medicine centres, including the three associated with specialist PCD diagnostic centres.

Two patients from consanguineous families were found to be homozygous for the relevant pathogenic variants. All other families were not knowingly consanguineous, and all patients had compound heterozygous variants except for 1 homozygous and 2 hemizygous X-linked patients. All individuals with causal variants in motile ciliopathy genes were reported to have sinusitis and recurrent respiratory infections. Where distribution of bronchiectasis was noted (10 cases), this was generally bilateral (9/10). Where age of onset had been recorded (5 cases), this was always childhood onset (5/5). Three patients had dextrocardia, 2 had hydrocephalus, 2 had hearing impairment, 1 had bilateral otitis media.

Mutations in 13 different motile ciliopathy genes were recorded amongst the affected individuals from this cohort (**Table 1**). Genetic diagnoses included 10 cases with pathogenic or likely pathogenic variants (also 3 variants of unknown significance, VUS) identified in several known PCD genes: *CCDC39* (2 cases), *DNAI1* (2 cases), *DNAI2, DNAH5* (2 cases), *DNAH11, RSPH1* and *RSPH4A*. Two cases shared the same single de novo dominant *FOXJ1* variant initially classified as a Tier 3 VUS, until *FOXJ1* was subsequently confirmed as a novel PCD gene associated with dominant inheritance through the identification of additional patients and further experimental analysis described elsewhere [30]. Two other affected individuals carried likely diagnostic X-linked PCD-causing variants in genes associated with additional clinical phenotypes [31, 32]: first, an *OFD1* variant c.3G>A identified as likely to affect the start codon and protein

translation, however this remains a Tier 3 VUS without further experimental evidence since the parental genotypes were not available to confirm familial segregation, and furthermore since PCD-linked *OFD1* mutations tend to be located towards the 3' end of the gene [33]; secondly, an *RPGR* variant c.602A>G creating a predicted missense amino acid substitution that also remains a VUS without experimental validation or parental genotypes available. Finally, autosomal recessive variants classified as pathogenic or likely pathogenic were also identified in *CEP164*, *CFAP53* and *NEK10* in three affected individuals. All three genes have previously been connected to motile ciliopathy phenotypes, with *NEK10* and *CEP164* mutations directly linked to causing bronchiectasis in humans [34-36]. *CFAP53* mutations were previously associated with situs inversus but only mild respiratory symptoms (recurrent cough, sinusitis) [37, 38].

Additional detailed analyses identified nine more cases with variants in ciliary genes, but of less certain significance (**Table E5**).

Of note, mutations in 6 of the 13 reported genes in **Table 1** are associated with non-classical PCD clinical diagnostic findings of normal TEM and / or normal NO (*FOXJ1, NEK10, OFD1, RPGR, DNAH11* and *RSPH1)*. Nasal NO and nasal brushing data were not available for the 100,000 Genome cohort and therefore we sought to replicate the findings through audit of genetic testing in bronchiectasis patients in a tertiary respiratory hospital.

Gene panel testing of bronchiectasis patients referred to a tertiary care centre reveals mutations in genes associated with motile ciliopathies

56 patients with idiopathic bronchiectasis were referred to the Royal Brompton Hospital for diagnostic genetic testing (cases referred specifically for PCD genetic testing were excluded from this study). Four (7%) received a definite PCD genetic diagnosis, with two pathogenic or likely pathogenic variants identified in known PCD genes (*CCDC103, CCDC40, DNAH11*) (**Table 2**). There were a further three potential diagnoses, two with a likely pathogenic variant plus a second variant in the same gene classified as a VUS (*DNAH11, GAS2L2*) and one apparently homozygous for an exon duplication (*DNAL1*). This increases the total number of cases in which bi-allelic mutations were identified to 12.5%, similar to the frequency of PCD gene variants seen in the 100,000 Genomes patient cohort. In a further 4 patients, a single heterozygous pathogenic/ likely pathogenic variant was identified but no second variant, precluding definitive diagnosis.

33/56 patients had cilia function tests prior to referral for genotyping. Two of the 4 definite genetic PCD diagnoses had normal nasal NO (>77nl/min). Normal functional tests associated with *CCDC103 p.(His154Pro)* variants are in keeping with previous descriptions [39]. Another patient was homozygous for a variant in *CCDC40* (c. 940-1G>C) affecting an essential splice acceptor site. *CCDC40* causal variants normally confer microtubular disorganisation and absent inner dynein arms (absence in IF of GAS8 and DNALI1) [40, 41]. However, this complex case had unusual HSVM, TEM and IF with some features not being the classical phenotype, as there was microtubular disorganisation and absent GAS8 but with the inner arms present when tested by TEM and IF [40]. Furthermore, the individual has a brother who is a heterozygous carrier of the *CCDC40* splice variant, who does not have respiratory symptoms but has dextrocardia. These results make interpretation of the variant difficult, however as demonstrated by *CCDC103* H154P compared to loss of function mutations in CCDC103 mutations in the same gene do not always present functionally in the same way.

Functional analyses were also available on 6/7 individuals with a potential PCD genetic diagnosis. One case with a single likely pathogenic *DNAH11* variant, c.3020T>G, and no second pathogenic allele had a typical HSVM pattern for *DNAH11* defects, making the diagnosis highly likely. Another case with a single frameshift deletion of *DNAAF1* (exon 2-3) and no second allele had atypical findings on HSVM for a *DNAAF1* defect. Strikingly, this was the only individual in this cohort with low nasal NO.

BTS bronchiectasis audit data suggest access to testing in the UK may limit diagnosis

4,898 adults with bronchiectasis from 89 centres were included in the BTS audit. Only 95 were tested for PCD (1.9%). 47 had nasal NO, 45 TEM and 45 HSVM measured. 23 patients had nasal NO only, which is known to be normal in several motile ciliopathies. Evaluation by all 3 tests, as would be appropriate according to the ERS guidelines [15], was performed in 22 patients (0.4%). 597 people had a severe disease phenotype. Testing was more likely in this group and conducted in 15 people (2.5%) of which 6 (1%) received full testing. Given that the 100,000 Genome Project recruitment criteria included a category for <50 years and PCD patients tend to be younger than their idiopathic counterparts, we analysed the data according to age. 56/534 (11%) of those under 50 were referred for testing and 12/534 (2%) received full testing. These findings, taken together with the results of the 100,000 Genomes Project, suggest that there is insufficient testing for PCD in patients with bronchiectasis to identify the majority of affected patients.

Discussion

This multi-centre study is one of the first to analyse WGS in bronchiectasis. Our study highlights under-diagnosis of PCD. We identified motile ciliopathy associated genes in 12% of idiopathic bronchiectasis patients recruited for WGS due to severe, familial disease or <50years of age.

The WGS Project had the option to recruit patients under a PCD phenotype category, this infers that clinicians recruiting to the non-CF bronchiectasis category had not clinically diagnosed PCD in their patients but did have suspicion of an inherited cause. Hence this level of diagnosis in a large portion of people recruited as non-CF bronchiectasis suggests either there are barriers to accessing PCD testing and/or clinicians struggle to ascertain which cases have features suggestive of PCD. We have to question whether these truly are all cases where PCD should not have been suspected given for instance that three had dextrocardia, upper airway symptoms and bronchiectaisis.

To identify if access to PCD testing was a barrier to diagnosis, we audited data from the specialist respiratory genetics service at the Royal Brompton Hospital which runs alongside a PCD diagnostic service. This identified that 7% patients referred for genetic testing with a clinical diagnosis of idiopathic bronchiectasis had pathogenic mutations in motile ciliopathy genes. Including those with a single pathogenic mutation with an abnormal functional test and/or variant of unknown significance, this rises to 12.5%. An additional 7% were found to be heterozygous for a single pathogenic variant in a known PCD gene. Typically, the gene panel-based genetic diagnosis of PCD is based upon a sequencing strategy that covers only the coding regions and canonical spice sites of the known genes, hence it is postulated that in at least some of these patients, the second pathogenic variant may be in the non-coding regions of the relevant genes. Future work to achieve a higher diagnostic rate for bronchiectasis in this cohort could likely benefit from a more detailed

interrogation of promotor and intronic regions of the relevant genes, that were not studied here, as well as further functional experiments to determine whether some of the variants identified in this study can provide a likely diagnosis.

Many of the patients with bronchiectasis in the Royal Brompton Hospital analysis were referred for PCD testing before genotyping, but the functional testing had given equivocal results. Normal nasal NO and normal TEM were present in most cases where tested. Normal NO and TEM have been described previously in some of the genes reported [34, 39, 42-44]. Importantly, our data suggest nasal NO testing alone is not sufficient to exclude a diagnosis of PCD in bronchiectasis [45]. We suggest that NO is not a screening test and should be used as part of a diagnostic testing algorithm alongside other testing modalities such as genetics and nasal brushing.

Amongst the WGS and gene panel genetic diagnoses, several affected individuals carried causal variants in PCD genes that confer classic cilia structure and function defects. However, a number also carried variants in PCD genes linked to less classic defects (*FOXJ1, OFD1, RPGR, RSPH1, CCDC103, GAS2L2*). In three such cases (two also reported elsewhere [46]), variants were revealed in *CEP164, CFAP53* and *NEK10*, genes currently linked to motile ciliopathy rather than clinically defined PCD. Previously, bronchiectasis in addition to syndromic features but no cilia functional testing was reported for *CEP164* mutation patients; *NEK10* was reported to cause bronchiectasis in patients with normal nasal NO levels, normal nasal ciliary ultrastructure and negligible ciliary beating abnormalities; and *CFAP53* mutation patients also had normal NO and negligible reduction in airway ciliary beat frequency [34, 35, 37]. Patients with these milder respiratory features could therefore escape detection during standard PCD clinical evaluation.

Both the predefined cohorts (100,000 Genomes and Royal Brompton Hospital) were biased towards selection of more severe and familial disease and the prevalence in an unselected cohort may be less. A future unbiased study of all cases of bronchiectasis will define the rate of PCD in an unselected cohort. There is possible greater genetic heterogeneity than has been considered. In bronchiectasis, our cases of incomplete diagnoses imply that particular attention to sequencing of non-classical PCD genes and ciliary gene variants located outside of exonic coding regions, with potentially less clear-cut effects on cilia motility, may be warranted. Both WGS and gene panel testing were successful at identifying undiagnosed motile ciliopathies. Using a panel of known PCD genes may be a cost effective first step for referring patients with features of a motile ciliopathy, severe or familial bronchiectasis. Our data showing a significant contribution of ciliopathies within bronchiectasis cohorts supports the need for a change in policy for genetics testing in bronchiectasis, as is now reflected in the National Test Directory guidelines for the UK NHS Genomic Medicine Service which includes the clinical indication "Respiratory ciliopathies including non-CF bronchiectasis".

The BTS audit data shows only 0.4% people with bronchiectasis in the UK have guideline-recommended testing for PCD, despite the presence of a network of 3 specialist diagnostic services [47]. Better access will not resolve all the issues: the genetic cause is not identified in up to 30% of well-defined PCD patients, therefore this may be an underestimate of the true prevalence of motile ciliopathy defects in a bronchiectasis cohort, and the true number of ciliopathy cases could account for up to 16% of the cohort [48-50].

We conclude that PCD is an underdiagnosed cause of severe adult bronchiectasis and that people with bronchiectasis who are young or have severe or familial disease should be tested for motile ciliopathies.

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Data collection: AS, CH, JSL, MC, ML, DM-R, ADeS

Data analysis: AS, HG, GW, DB, CC, JDC, DM-R, HMM

Writing of the manuscript: AS, HG, GW, CH, JSL, JDC, DM-R, HMM, ADeS

Revising critically for important intellectual content and final approval: all authors

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Table 1. Genotypes of people recruited to the UK 100,000 Genomes Project as non-CF bronchiectasis carrying variants suggestive of PCD

Family code	Gene	Genomic location (GRCh38)	Mat/Pat origin	cDNA change	Protein change	Tier	ACMG/ AMP class	GEL/literature reported	dbSNP [Reference]
1	*CCDC39 (NM_181426.2)*	3:180659582T>C 3:180616872TTAC>TA	Mat Pat	c.610-2A>G c.2357_2359delinsT	Splice variant p.(Ser786fs)	Tier 1 Tier 1	5 5	GMC exit questionnaire GMC exit questionnaire	rs756235547 [51] rs587778821 [51]
2	*CCDC39 (NM_181426.2)*	3:180651496delT 3:180616604CTG>C	Unknown phase	c.1072del c.2497_2498del	p.(Thr358fs) p.(Gln833fs)	Tier 1 Tier 1	5 5	Tiered only Tiered only	rs587778822 [51] rs1007345781 [52]
3	*DNAI1 (NM_012144.4)*	9:34514436G>A 9:34514436G>A	Mat Pat	c.1612G>A c.1612G>A	p.(Ala538Thr) p.(Ala538Thr)	Tier 2 Tier 2	4 4	GMC exit questionnaire GMC exit questionnaire	rs368248592 [53] rs368248592 [53]
4	*DNAI1 (NM_012144.4)*	9:34459054G>GT 9:34513112G>A	Unknown phase	c.48+2dup c.1490G>A	Splice variant p.(Gly497Asp)	N.T. N.T.	5 3	RIPD Form RIPD Form	rs397515363 [54] rs376252276 [54]
5	*DNAI2 (NM_023036.6)*	17:74309345G>A 17:74309345G>A	Mat Pat	c.1304G>A c.1304G>A	p.(Trp435*) p.(Trp435*)	Tier 1 Tier 1	5 5	Tiered only Tiered only	rs752924362 [54] rs752924362 [54]
6	*DNAH5 (NM_001369.2)*	5:13708286A>C 5:13870868G>A	Unknown phase	c.13175T>G c.3733C>T	p.(Phe4392Cys) p.(Arg1245Cys)	Tier 2 Tier 2	3 3	Tiered only Tiered only	rs145400611 [NR] rs149609746 [NR]
7	*DNAH5 (NM_001369.2)*	5:13753290delT 5:13780960C>A	Mat Pat	c.10815del c.8821-1G>T	p.(Pro3606fs) Splice variant	Tier 1 Tier 1	5 5	Tiered only Tiered only	rs397515540 [55] rs1060501454 [NR]
8	*DNAH11 (NM_001277115.2)*	7:21620016C>T 7:21899361C>T	Unknown phase	c.4438C>T c.13075C>T	p.(Arg1480*) p.(Arg4359*)	Tier 1 Tier 1	5 5	Tiered only Tiered only	rs72657321 [44] rs774903187 [44]
9	*FOXJ1 (NM_001454.4)*	17:76137652delG	De novo, dominant	c.967del	p.(Glu323fs)	Tier 3	5	RIPD Form	rs1598372791 [30]
10	*FOXJ1 (NM_001454.4)*	17:76137652delG	De novo, dominant	c.967del	p.(Glu323fs)	Tier 3	5	GMC exit questionnaire (VUS*)	rs1598372791 [30]
11	*OFD1 (NM_003611.3)*	X:13735074G>A	Parental unknown	c.3G>A	p.(Met1?)	Tier 3	3	Tiered only	rs778840618 [NR]
12	*RPGR (NM_000328.3)*	X:38317333T>C	Parental unknown	c.602A>G	p.(His201Arg)	Tier 2	3	Tiered only	NR
13	*RSPH1 (NM_080860.4)*	21:42486463T>G 21:42486463T>G	Mat Pat	c.275-2A>C c.275-2A>C	Splice variant Splice variant	Tier 1 Tier 1	5 5	Tiered only Tiered only	rs151107532 [56] rs151107532 [56]
14	*RSPH4A (NM_001010892.3)*	6:116630553G>GTT 6:116628100C>T	Mat Pat	c.1916+2_1916+3insTT c.1393C>T	Splice variant p.(Arg465*)	Tier 3 Tier 3	4 5	RIPD Form RIPD Form	NR rs755782051 [57]
15	*CEP164 (NM_014956.5)*	11:117387204C>T 11:117411859C>T	Mat Pat	c.1726C>T c.4228C>T	p.(Arg576*) p.(Gln1410*)	Tier 3 Tier 3	5 4	Tiered only Tiered only	rs145646425 [58] rs147398904 [NR]
16	*CFAP53 (NM_145020.5)*	18:50262051G>A 18:50242969C>A	Unknown phase	c.238C>T c.1144G>T	p.(Arg80*) p.(Glu382*)	Tier 3 Tier 3	5 5	RIPD Form RIPD Form	rs374898373 [NR] rs200321140 [NR]
17	*NEK10 (NM_152534.4)*	3:27352882T>C 3:27304746C>A	Unknown phase	c.1A>G c.1028+1G>T	p.(Met1?) Splice variant	Tier 3 Tier 3	4 4	RIPD Form RIPD Form	rs1363654282 [NR] rs1323610713 [NR]

*Initially classified as a VUS, done prior to the finding of additional patients and further studies describing *FOXJ1* as a new ciliopathy gene [30]. Mat, maternal; Pat, paternal; NR, not reported. RIPD (Researcher Identified Potential Diagnosis) is the notification submitted by researchers from within the GEL Research Environment for potential mutations that are not Tier 1 or Tier 2 and have not already been reported in a GMC (NHS Genomics Medical Centre) exit questionnaire. ACMG/AMP classification and GEL tiering criteria are outlined in methods section.

Table 2. Genotypes of people referred with non-CF bronchiectasis to the Royal Brompton Hospital carrying variants suggestive of PCD

Family code	Confirmed diagnosis	Gene	cDNA change	Protein change	ACMG/ AMP class	dbSNP [Reference]	Ciliary function studies			
							nNO (nl/min)	HSVM (CBF in Hz)	TEM	IF
RBH-1	Yes	*CCDC103 (NM_181426.2)*	c.461A>C c.461A>C	p.(His154Pro) p.(His154Pro)	5 5	rs145457535 [59] rs145457535 [59]	371	Slow and stiff (7.4)	Normal	DNAH5, GAS8, RSPH9 present
RBH-2	Yes	*CCDC103 (NM_181426.2)*	c.461A>C c.461A>C	p.(His154Pro) p.(His154Pro)	5 5	rs145457535 [59] rs145457535 [59]	NA	NA	NA	NA
RBH-3	Yes	*CCDC40 (NM_017950.4)*	c.940-1G>C c.940-1G>C	Splice variant Splice variant	4 4	NR NR	77	Normal in areas, reduced bending and amplitude in areas (10.6)	Microtubular disorganisation but normal IDAs	GAS8 inconclusive DNAH5, DNALI1, RSPH9 present
RBH-4	Yes	*DNAH11 (NM_001277115.2)*	c.2569C>T c.2569C>T	p.(Arg857*) p.(Arg857*)	5 5	rs72655998 [44] rs72655998 [44]	NA	NA	NA	NA
RBH-5	Potential diagnosis	*DNAH11 (NM_001277115.2)*	c.4669C>T c.8072A>G	p.(Arg1557*) p.(Gln2691Arg)	4 3	rs759040005 [NR] rs183682756 [NR]	NA	Static and stiff areas (9.3)	Normal	NA
RBH-6	Potential diagnosis	*DNAL1 (NM_031427.4)*	Exon 5 dup Exon 5 dup	Exon 5 dup Exon 5 dup	4 4	NR NR	NA	Mixed non-specific findings	Normal	DNAH5, GAS8, RSPH9 present
RBH-7	Potential diagnosis	*GAS2L2 (NM_139285.4)*	c.887_890del c.307G>A	p.(Val296Glyfs*13) p.(Ala103Thr)	4 3	rs587633197 [42] NR	77	Normal beat pattern (10.6)	Normal	NA
RBH-8	No – no 2nd pathogenic variant identified	*CCDC103 (NM_181426.2)*	c.461A>C NA	p.(His154Pro) NA	4 NA	rs145457535 [59] NA	118	Reduced beat amplitude and mucus impeded (9.7)	Normal	NA
RBH-9	No – no 2nd pathogenic variant identified	*DNAAF1 (NM_178452.6)*	Del exons 2-3 NA	p.? NA	4 NA	NR NA	47	Reduced beat amplitude (8.1)	Partial absence of outer dynein arms	DNAH5 partial absence DNALI1, RSPH9, GAS8 present
RBH-10	No – no 2nd pathogenic variant identified	*DNAAF1 (NM_178452.6)*	c.882G>A NA	p.(Trp294*) NA	4 NA	NR NA	150	Reduced beat amplitude, one twisting area (12.1)	Normal	DNAH5, GAS8, RSPH9, RSPH1, RSPH4A present
RBH-11	No – no 2nd pathogenic variant identified	*DNAH11 (NM_001277115.2)*	c.3020T>G NA	p.(Leu1007*) NA	4 NA	rs1480698078 [60] NA	86	Hyperfrequent (16.4)	Normal	NA

nNO, nasal nitric oxide level; HSVM, high speed video microscopy; CBF, ciliary beat frequency; TEM, transmission electron microscopy; IF, immunofluorescence; IDA, inner dynein arm. ACMG/AMP classification as outlined in methods section.

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