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Combining clinical, radiological and genetic approaches to pneumothorax management

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ABSTRACT

Familial spontaneous pneumothorax (FSP) accounts for 10% of primary spontaneous pneumothoraces. Appropriate investigation of FSP enables early diagnosis of serious monogenic diseases and the practice of precision medicine. Here, we show that a pneumothorax genetics multidisciplinary team (MDT) can efficiently diagnose a range of syndromic causes of FSP. A sizeable group (73.6%) of clinically unclassifiable FSPs remains. Using whole genome sequencing we demonstrate that most of these cases are not known monogenic disorders. Therefore, clinico-radiological assessment by an MDT has high sensitivity for currently known clinically important monogenic causes of FSP, which has relevance for the design of efficient pneumothorax services.

INTRODUCTION

Primary spontaneous pneumothorax (PSP) affects 1 in 10 000 people with 10% having affected relatives, so-called familial spontaneous pneumothorax (FSP).[1 2] FSPs comprise a diverse group of disorders with extrapulmonary manifestations ranging from renal cancer to arterial dissection.[1 2] We previously proposed FSPs be classified into those conditions with aberrant extracellular matrix or defective tumour-suppressor pathways.[1 2] A third group of unclassifiable FSPs currently defies diagnosis, but it is unclear if these represent *formes frustes* of known syndromes or are as yet undescribed disorders.[3] Our pneumothorax clinic provided a resource of well-phenotyped individuals who have been assessed by a respiratory-genetics multidisciplinary team (MDT) over a 12-year period. We set out to analyse this cohort, combined with additional whole genome sequencing (WGS) data from the 100 000 Genomes Project, to assess the efficiency of existing clinico-radiological pathways for the diagnosis of FSP.

METHODS

Between 2008 and 2020, 492 patients were recruited consecutively from our pneumothorax clinic. Familial cases were reviewed by a pneumothorax genetics MDT comprising respiratory physicians, clinical geneticists, and thoracic radiologists. Detailed methods are provided in online supplemental file 1.

RESULTS

Local demographics biased our cohort towards younger individuals with few cases of secondary spontaneous pneumothorax (SSP) (online supplemental table S1; figure 1A). Overall, 5-year recurrence was 38.6%±2.5%, but this rose to 44.5%±3.4% by 10 years (figure 1B). Five-year recurrence for those 50 years or younger was 41.9%±2.8%, but only 20.7%±4.8% for those >50 years, perhaps owing to higher mortality (39.3%±5.6% at 5 years for >50 years vs 1.0%±0.6% for ≤50 years; figure 1C,D). The comparatively high overall recurrence rate seen in our series may, therefore, reflect the predominance of younger patients. The sex ratio was 3:1 male:female, with no mortality difference between sexes (online supplemental table S1; figure 1E and online supplemental figure S1A). However, 10-year recurrence was significantly higher for females (63.1%±9.4% vs 41.3%±3.5%, p=0.041; online supplemental figure S1B). This persisted when patients >50 years were excluded (p=0.017; figure 1F). Lymphangioleiomyomatosis (LAM) was diagnosed in two women (1.8% of 113 female cases), while thoracic endometriosis (catamenial pneumothorax) was diagnosed in 9 (7.9% of female cases). Both patients with LAM suffered recurrences, as did 7 (77.8%) of the women with catamenial pneumothorax. These conditions may, therefore, contribute to the increased risk of recurrence we observed in women.

A positive family history of pneumothorax was elicited in 14.6% of patients (14.0% of males, 16.8% of females) and was associated with a significantly increased chance of recurrence (figure 2A,B; online supplemental table S1). Overall, 5-year FSP recurrence was 49.6%±6.6% vs 36.7%±2.7% for sporadic pneumothoraces (p=0.034; figure 2A and online supplemental figure S1C). For patients 50 years or younger, 5-year FSP recurrence was 54.7%±7.1% vs 36.2%±2.9% of sporadic pneumothoraces (p=0.025; figure 2B).

Targeted genetic testing guided by a pneumothorax MDT yielded a genetic diagnosis in 26.4% (19/72) of FSP cases (figure 2C). Birt-Hogg-Dubé syndrome was the most common, accounting for 47% of genetic diagnoses (2% of all 492 patients), followed by Marfan (21%), vascular Ehlers-Danlos (11%) and Loeys-Dietz syndromes (11%). Alpha1-antitrypsin deficiency and tuberous sclerosis were more uncommon (5%). We did not observe significant changes in rates of diagnosis over time.

A majority of individuals with FSP (73.6%) failed to fulfil the diagnostic criteria for known genetic disorders. To examine this further, 33 individuals with unclassifiable FSP (27 probands, 6 affected relatives; 64% male) were recruited to the 100 000 Genomes Project[4 5] (online supplemental table S2).

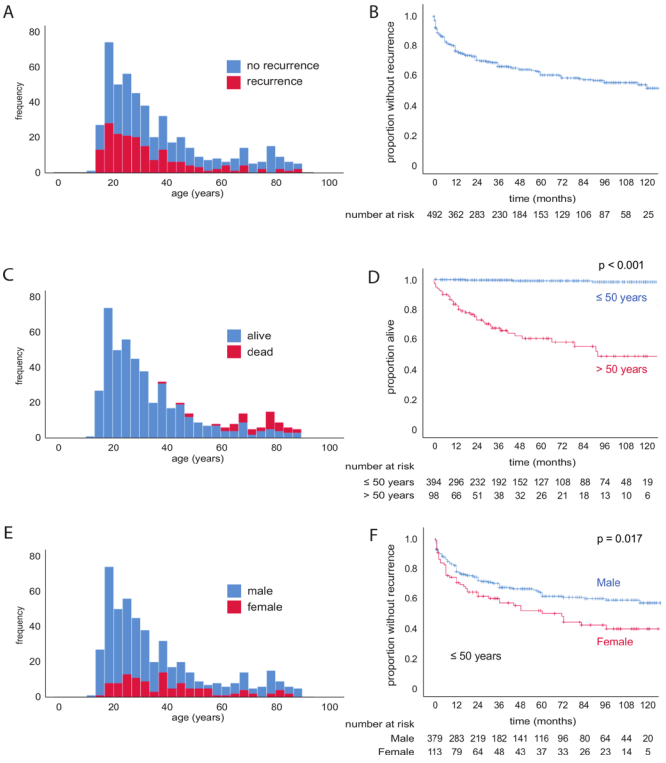

Figure 1 Pneumothorax clinic patent characteristics, survival and pneumothorax recurrence. (A) Histogram of patients; no recurrence (blue), recurrence (red). (B) Kaplan-Meier curve and number at risk table of pneumothorax recurrence. (C) Histogram of patients, alive (blue), died (red). (D) Kaplan-Meier curve and number at risk table of survival; patients 50 years of age or younger (blue), over 50 years old (red). P value calculated by log-rank test. (E) Histogram of patients; male (blue), female (red). (F) Kaplan-Meier curve and number at risk table of pneumothorax recurrence in patients 50 years of age or younger; male (blue), female (red). P value calculated by log-rank test.

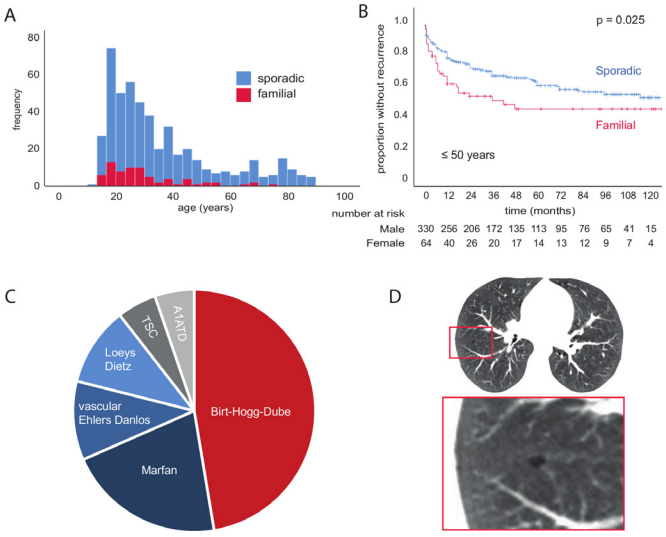

Figure 2 Familial spontaneous pneumothorax patient characteristics. (A) Histogram of patients; no family history of pneumothorax (blue), familial pneumothorax (red). (B) Kaplan-Meier curve of pneumothorax recurrence in patients 50 years of age or younger; no family history of pneumothorax 'sporadic' (blue), familial pneumothorax (red). P value calculated by log-rank test. (C) Relative proportion of monogenic disorder diagnoses made. (D) Thoracic CT scan of patient identified to have Birt-Hogg-Dubé by whole genome sequencing. noted small cyst, one of two that were present. TSC, tuberous sclerosis complex.

The crowdsourcing tool PanelApp was used to generate a pneumothorax gene panel. Candidate genes were scored by members of the Genomics England online community and ten genes received sufficient endorsement to be declared 'green genes' and included in the pneumothorax panel (table 1). WGS data from the 33 unclassifiable FSP patients were annotated for pathogenicity based on variant segregation within families, frequency in control populations, effect on protein coding, and mode of inheritance. 'Tier 1' variants were defined as high-impact variants (eg, likely loss of function) and de novo moderate-impact variants (eg, missense) in this panel. A single tier 1 variant was identified in the genome of one patient: a previously reported pathological variant of *FLCN* (c.1062+2T>G) indicating a diagnosis of Birt-Hogg-Dubé syndrome. The proband had no cutaneous features of Birt-Hogg-Dubé syndrome and no family history of renal malignancy. The thoracic CT was rereviewed and, in hindsight, noted to show two small cystic lesions (figure 2D). No other tier 1 pathogenic variants were identified indicating that clinico-radiological assessment had been highly successful in excluding known genetic causes of FSP (sensitivity 96%, 26/27 probands).

DISCUSSION

In our cohort of 492 patients with pneumothorax, 14.6% had FSP. Of these, 26.4% were diagnosed clinically with a monogenic disorder, most commonly Birt-Hogg-Dubé syndrome. Previous studies in Chinese and European populations suggested that up to 10% of pneumothoraces are due to *FLCN* mutations.[6 7] In our cohort, the overall proportion was much less at 9 of 492 (2%). To our knowledge, this is the first report of an association between FSP and increased chance of recurrence compared with sporadic pneumothorax. Female sex was also associated with an increased risk of recurrence.

WGS of 33 individuals with unclassifiable FSP revealed a single tier 1 variant indicating that clinico-radiological assessment has high sensitivity for known FSP syndromes, consequently the

Table 1 PanelApp familial pneumothorax gene panel

Gene	Mode of inheritance	Details	OMIM
COL3A1	Monoalleilic, autosomal	Ehlers-Danlos syndrome, vascular type	130050
FBN1	Monoalleilic, autosomal	Marfan syndrome	154700
FLCN	Monoalleilic, autosomal	Primary Spontaneous Pneumothorax	173600
		Birt-Hogg-Dubé Syndrome	135150
SERPINA1	Biallelic, autosomal	Alpha-1-antrypsin deficiency	613490
TGFBR1	Monoalleilic, autosomal	Loeys-Dietz syndrome type 1	609192
TGFBR2	Monoalleilic, autosomal	Loeys-Dietz syndrome type 2	610168
TGFB2	Monoalleilic, autosomal	Loeys-Dietz syndrome type 4	614816
TGFB3	Monoalleilic, autosomal	Loeys-Dietz syndrome type 5	615582
TSC1	Monoalleilic, autosomal	Tuberous sclerosis 1	191100
TSC2	Monoalleilic, autosomal	Tuberous sclerosis 2	613254

Familial primary spontaneous pneumothorax 'green gene' list generated using NHS Genomic England PanelApp crowdsourcing tool: panel V.2.20 https://panelapp.genomicsengland.co.uk/panels/105/. https://omim.org/.
COL3A1, collagen, type III, alpha-1; FBN1, fibrillin 1; FLCN, folliculin; NHS, National Health Service; OMIM, Online Mendelian Inheritance in Man; SERPINA1, alpha-1-antitrypsin; TGFBR1, transforming growth factor-beta receptor, type I; TSC1, tuberous sclerosis complex subunit 1.

majority of genetic risk in FSP remains unexplained. A previous genome-wide association study (GWAS) reported an association between PSP and the single nucleotide polymorphism (SNP) rs4733649 in the *SLC6A1* gene.[8] None of the 33 individuals nor 8854 sequenced non-pneumothorax controls had this SNP so we cannot confirm the association (not shown). COPD is a cause of SSP. Deficiency of α_1-antitrypsin is its most common monogenic cause but accounts for under 2% of prevalent cases.[9] GWASs have implicated other genes in COPD (*HHIP*, *CHRNA5*, *HTR4*, *FAM13A*, *RIN3*, *TGFB2*, *GSTCD-NPNT*, *CYP2A6* and *IL27-CCDC101*),[10] but no pathogenic variants were identified in these genes in our cases.

An important limitation of our study is its geographical restriction to one centre. National analysis of FSP will be necessary to determine its population prevalence.

In summary, assessment by a pneumothorax MDT has high sensitivity for diagnosis of monogenic causes of FSP by enabling targeted genetic testing. We find FSP increases the risk of recurrence compared with sporadic pneumothoraces. A substantial proportion of the genetic risk for FSP remains unaccounted for, but ongoing analysis of the 100 000 Genomes dataset will address this.

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Contributors SJM is the guarantor of the content of the manuscript. HLG, JB, ERM and SJM were involved in the conception and design, data analysis and interpretation. HLG performed the data collection. All authors were involved in drafting the submitted article.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the University of Cambridge Human Biology Research Ethics Committee HBREC.2020.28.

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Supplementary text

Patient ascertainment - The Cambridge Pneumothorax Clinic is a specialist service established in 2008 for the assessment of pneumothorax in a large teaching hospital in the south east of England. The practice in our hospital is that all patients presenting with pneumothorax, whether admitted or treated conservatively as outpatients, are referred to the Pneumothorax Clinic to be seen at 2-weeks (or 6-weeks if referred for surgery during their admission). Patients with traumatic pneumothorax are also referred to the Pneumothorax Clinic, but were excluded from this study. Each patient underwent standardised clinical assessment to identify syndromic causes of pneumothorax including detailed family history and physical examination such as dermatological and skeletal assessment, as we have described elsewhere (1-3). In most cases a positive family history related to the presence of pneumothorax in first- or second-degree family members (49 first-degree relatives: 68.1% of 72, 7 children, 27 parents, 15 siblings; 19 second-degree relatives: 26.3% of 72, 14 uncles/aunts, 5 grandparents), but a well-documented case in more distant relatives would also be considered significant (4 third-degree relatives: 5.6%, 2 cousins, 2 great-uncles). Low dose high-resolution thoracic computed tomographic (CT) imaging and transthoracic echocardiography were performed in certain circumstances: following pneumothorax recurrence; or all female patients; or patients whose history or clinical examination suggested a specific syndromic cause. All cases where a syndromic cause was considered possible were reviewed at a pneumothorax genetics MDT comprising respiratory physicians, clinical geneticists, and thoracic radiologists.

Data analysis - Data are presented as absolute values, percentages, mean ± standard error or median (interquartile rage). Survival data were used to generate Kaplan-Meier curves using SPSS 26 (SPSS, Chicago, IL, USA) to compare between groups including, but not restricted to: sex, age, family history, and treatment received, using the log-rank test. Survival tables provide cumulative survival and standard error; life tables provide number exposed to risk at each timepoint. P values <0.05 is considered statistically significant.

100,000 Genomes Project – Whole genome sequencing (WGS) was performed on eligible participants within the 100,000 Genomes Project (4, 5). Briefly, inclusion required a proband to have suffered a spontaneous pneumothorax and have at least one affected relative. Clinical assessment was performed and prospective participants excluded if found to suffer from a known syndromic cause. WGS data were screened for likely pathological variants; those variants with >0.005 frequency in the Genome Aggregation Consortium database (gnomAD (6)) were disregarded. Next, variants predicted to be benign by either SIFT or PolyPhen bioinformatics were excluded. Of the remaining variants, only those in protein coding-sequences were analysed further. Those found to occur in three or more individuals were identified and assessed for appropriate inheritance. Comparison was made with public databases, including gnomAD (6), and with a cohort of 8854 “control” individuals recruited to the 100,000 Genomes Project for non-syndromic cases of cancer. Phenotypic information from recruitment questionnaires, past medical history, from NHS Hospital Episode Statistics, were scrutinised.

Clinical assessment and standard operating procedures:

All new patients are assessed using a standard clinic *pro forma*:

History

Presentation

Past medical history
Pneumothorax
Hernias
Dislocation
Orthodontics
Eyes
Vascular

Family history
Pneumothorax
Hernias
Dislocation
Orthodontics
Eyes
Vascular
Renal malignancy

Social history
Tobacco/Cannabis

Examination
Height, weight, BMI
Arm span (arm / height ratio)
Uvula (e.g., bifid)
Dentition (e.g., crowding, micrognathia)
Palate (e.g., high arched)
Face (e.g., hypertelorism.)
Skin (e.g., striae, lesions)
Murdoch Walker (wrist) sign
Steinberg's (thumb) sign
Beighton score
Hind foot abnormalities
Heart sounds

Investigations

Low dose thoracic CT scan and transthoracic echocardiogram requested for:

- All patients with recurrent pneumothoraces or slow-to-resolve cases
- All patients with a family history of pneumothorax
- All female patients (may need to be delayed if pregnant)
- When clinical features are identified raising suspicion of a syndromic cause, e.g., skin lesions of Birt-Hogg-Dubé syndrome or Tuberous Sclerosis, arachnodactyly

Pneumothorax Genetics MDT format

To be quorate the following must be present:

- Respiratory consultant physician
- Clinical genetics consultant
- Thoracic radiology consultant

MDT outcomes include

- Syndromic cause unlikely – no further action
- Syndromic cause likely – targeted genetic testing arranged
- Syndromic cause possible – further investigation and/or review in Clinical Genetics clinic arranged

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Supplementary Legend:

Supplementary Figure S1. (A) Kaplan Meier curve of survival among all male (blue) and female (red) patients. (B) Kaplan Meier curve of pneumothorax recurrence in all male (blue) and female (red) patients. P value calculated by log-rank test. (C) Kaplan Meier curve of pneumothorax recurrence in all patients; no family history of pneumothorax “sporadic” (blue), familial pneumothorax (red). P value calculated by log-rank test.

Table S1. Clinic patient characteristics

Patients		N = 492	5-year recurrence (SEM)
Male sex – N (%)		379 (77.0% of 492)	
Median age (range) -- year		29.2 (12-89)	38.6% (2.5%)
	Males	27.6 (12-89)	36.6% (2.9%)
	Female	38.7 (16-84)	45.1% (5.3%)
Family history – N (%)		72 (14.6% of 492)	49.6% (6.6%)
	Males	53 (14.0% of 379 males)	43.7% (7.5%)
	Female	19 (16.8% of 113 females)	63.5% (12.4%)
Height – mean (SD) – cm			
	Males	180 (8) N = 205	
	Females	169 (7) N = 54	
Weight – mean (SD) – kg			
	Males	67.2 (11.8) N = 125	
	Females	58.7 (16.2) N = 31	
BMI – mean (SD)			
	Males	20.9 (3.5) N = 125	
	Females	21.5 (3.9) N = 31	
Arm span – mean (SD) - cm			
	Males	184 (8) N = 191	
	Females	173 (9) N = 50	
Arm span: height ratio			
	Males	1.03 (0.03) N = 188	
	Females	1.02 (0.03) N = 50	
Smoking – N (%)			
	Never	213 (47.0%)	43.8% (3.8%)
	Tobacco	Ever 151 (30.7%); current 79 (16.0%)	34.2% (4.3%)
	Cannabis	Ever 104 (21.1); current 85 (17.2%)	35.5% (5.5%)
	Missing data	6 (1.2%)	
Recurrences		N = 170	
Male – N (%)		130 (76.5% of 170)	
Ipsilateral – N (%)		120 (70.5% of 170)	

Table S2. Demographics of familial pneumothorax patients recruited to 100,000 Genomes Project

Patients		N = 33
Male sex – N (%)		21 (63.6% of 33)
Median age (range) -- year		29.0 (4-76)
	Males	24.5 (4-76)
	Female	48 (23-57)
Median age at first pneumothorax (range)		21.0 (4-76)
	Males	24.5 (4-76)
	Female	48.0 (23-57)
Ethnicity – N (%)		
	White	28 (85%)
	Black	2 (6%)
	Not stated	3 (9%)

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Supplementary Figure S1