Clinical and genetic study of a large Chinese family presented with familial spontaneous pneumothorax

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Background: Familial spontaneous pneumothorax (FSP) is an inherited disease, and Birt-Hogg-Dubé (BHD) syndrome is its leading cause. BHD syndrome is an autosomal dominant disorder characterized by pulmonary cysts, spontaneous pneumothorax, renal cancer, and skin fibrofolliculomas. It is caused by germline mutations in the *FLCN* gene. Thus far a variety of mutations have been reported; however, the unique characteristics of BHD syndrome-related FSP are still unclear.

Method: We reviewed the family history of a large Chinese family that presented with FSP. Genetic testing of the *FLCN* gene was performed and the special clinical characteristics of BHD syndrome-related FSP were discussed.

Results: This family comprised 5 generations and 76 members. Six of these had experienced pneumothorax episodes and 35 members had undergone genetic analysis of the *FLCN* gene, except for one member who had pneumothorax. Among the 35 members, 17 had the mutation in the *FLCN* gene. All five members with pneumothorax had the mutation. Frequency of pneumothorax in the mutation members was 29.4% (5/17). Clinical characteristics of the BHD syndrome-related pneumothorax differed from those of primary spontaneous pneumothorax, which typically affects tall, thin young men, and the recurrence rate of BHD syndrome-related pneumothorax after observation, needle aspiration or tube drainage was higher than that of primary spontaneous pneumothorax, and higher than that observed after VATS bullectomy and mechanical pleurodesis.

Conclusions: We reported the largest single family that presented with FSP from China. The clinical and genetic characteristics of the BHD syndrome-related pneumothorax differ from those of primary spontaneous pneumothorax.

Keywords: Birt-Hogg-Dubé syndrome (BHD syndrome); familial spontaneous pneumothorax (FSP); FLCN gene

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Introduction

Primary spontaneous pneumothorax (PSP; OMIM 173600) is a condition in which air enters the pleural space, without obvious underlying lung disease. Approximately 11.5% of patients presenting with spontaneous pneumothorax report a positive family history of the condition (1). Familial

spontaneous pneumothorax (FSP) was initially reported in 1921 by Faber (2). Several monogenetic disorders are associated with FSP, including Marfan syndrome, homocystinuria, Ehlers-Danlos syndrome, α 1-antitrypsin deficiency and Birt-Hogg-Dubé (BHD) syndrome (3). A significant fraction of the reported FSP cases are associated with BHD syndrome (4-7). 1968



Figure 1 Simplified family pedigree (spouses are omitted due to space limitation). The index number of generations is listed on the left using Roman numerals. The index number of certain individuals in each generation is listed on the lower right using Arabic numerals. The proband is indicated by an arrow with a "P" on the lower left. Patients with pneumothorax are highlighted in black.

BHD syndrome (OMIM 135150) is a rare autosomal dominantly hereditary disease, which was firstly described in 1977 by Birt et al. (8). This disease is characterized by skin fibrofolliculomas, pulmonary cyst, spontaneous pneumothorax, and kidney neoplasms (9). The phenotype of BHD syndrome is highly heterogeneous and varies among patients (10). Pulmonary cyst is one of the most common manifestations. About 80% of patients have multiple pulmonary cysts, as visible on chest computed tomography (CT), and about 30% of patients develop spontaneous pneumothorax (11). In 2001, the gene responsible for BHD syndrome was mapped to the short arm of chromosome 17 (17p11.2) and was named folliculin gene (FLCN, OMIN 607273, GenBank accession number NM 144997). This gene encodes folliculin (FLCN) protein, which is expressed in a variety of tissues, including the skin and its appendages, the distal nephron of the kidney, stromal cells, and type I pneumocytes of the lung (12,13). Thus far, over 100 germline mutations have been identified in all 14 exons of the FLCN gene (14). The correlation between the mutation genotype and phenotype is unclear (10).

Here, we reported a large Chinese family in which six members from five generations developed spontaneous pneumothorax. A genetic study revealed that 17 of the 35 members in this family harbored the same *FLCN* germline mutation. The clinical characteristics of BHD syndrome-related FSP were discussed. To the best of our knowledge, this is the largest single-family report on BHD syndrome-related FSP from China.

Methods

A large family from eastern China was recruited for this study. The family history of the members was obtained through the proband and several family members. The detailed clinical information of the six patients who developed spontaneous pneumothorax, including their medical history, smoking status, body weight and height, chest CT imaging result, and the choice of treatment choice were retrospectively reviewed. During health checkup, chest CT images of two other normal members were obtained, and all CT images were evaluated by thoracic radiologists from our hospital.

In total, 55 individuals underwent genetic analysis, namely 5 patients with pneumothorax, 30 normal family members (members related through marriage were excluded) and 20 unrelated healthy controls. Peripheral blood samples were obtained and genomic DNA was extracted from blood leukocytes according to standard procedures. The coding regions of the *FLCN* gene consisting of exon 4 to 14 and flanking introns were amplified using polymerase chain reaction (PCR) with oligonucleotide primers and were sequenced using the Sanger method (details are available upon request). Any detected sequences variants were compared with the reference sequences using Sequencher software (Gene Codes, Ann Arbor, MI, USA).

Patient characteristics were analyzed by basic descriptive data analysis, using SPSS 20.0 (IBM Corporation, Chicago, USA). Data were expressed as median with interquartile range (IQR), and count data were expressed as percentage.

Results

Figure 1 shows the simplified pedigree of this family (spouses are omitted). There were 5 generations and 76 members in this family. Six members, namely II-1, II-3, II-6, II-7, III-19, and IV-8 had experienced at least one episode of pneumothorax. The clinical information and characteristics of the six members with pneumothorax are listed in *Tables 1* and 2. There was no known inherited disease or connective tissue disease among the members of the extended family.

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Family	Sov	Height E (cm) wei	Body	Smoking history	Pulmonary cysts on CT scan	Age of first episode	No. of pneumothoraxes	Ages at pneumothorax (treatment)	
index No	D.		weight (kg)					Right lung	Left lung
II-1	М	158	70	Y	Bilateral, multiple	55	3	55 (TD), 67 (TD + CP)	70 (TD)
II-3	F	154	60	Ν	bilateral, multiple	42	3	42 (TD), 54 (TD + CP)	48 (TD)
II-6	М	160	71	Y	bilateral, multiple	41	4	53 (TD), 57 (VB + MP)	41 (TD), 47 (TD)
II-7	F	153	65	Ν	bilateral, multiple	45	2		45 (TD), 50 (VB + MP)
III-19	М	170	60	Ν	bilateral, multiple	24	1	24 (VB + MP)	
IV-8	М	172	75	Y	bilateral, multiple	25	1	25 (VB + MP)	

CT, computed tomography; CP, chemical pleurodesis; MP, mechanical pleurodesis; TD, tube drainage; VB, VATS bullectomy; M, male; F, female; Y, yes; N, no.

Table 2 Characters of patients with pneumothorax (n=6)

Characters	Value		
Year of first episode [median (IQR)]	41.5 (24.8–47.5)		
BMI [median (IQR)]	26.5 (24.2–27.8)		
No. of pneumothorax [median (IQR)]	2.5 (1.0–3.3)		
Male	4 (66.7%)		
Smoker	3 (50%)		
Generation			
I	4 (66.7%)		
III	1 (16.7%)		
IV	1 (16.7%)		

IQR, interquartile range.

Moreover, none of the members showed any clinical signs or symptoms of Marfan syndrome or Ehlers-Danlos syndrome. Alpha-1 antitrypsin deficiency was also excluded. The median age for the initial onset of pneumothorax was 41.5 (IQR: 24.8-47.5) years, and the male to female ratio was 4:2. The median BMI was 26.5 (IQR: 24.2–27.8) kg/m² and the median number of episodes was 2.5 (IQR: 1.0-3.3). All patients with pneumothorax showed bilateral multiple pulmonary cysts on CT imaging. Although III-2 and III-3 had never developed pneumothorax, chest CT showed multiple cysts in the bilateral lungs. The proband had no renal tumor or skin lesion, as confirmed by abdominal MRI and a thorough examination by a dermatologist. None of the 76 members reported a history of renal cancer. The dead members died of stroke (I-1 and II-2), heart failure (I-2), and breast cancer (III -9).

Sequence analysis of the *FLCN* gene revealed a deletion mutation (c.1285delC) in exon 11 (*Figure 2*). In total, 17 of the 35 family members harbored the same mutation (*Figure 3*). Of all the mutation carriers, nine were male and eight were female. Five of the 17 mutation carriers developed pneumothorax (29.4%). All members with spontaneous pneumothorax, except IV-8, whose blood sample was not obtained, showed positive results for the mutation test. Besides, III-2 and III-3, who showed pulmonary cysts on chest CT but did not develop pneumothorax, were also mutation carriers. None of the 20 unrelated healthy controls harbored this mutation.

Discussion

FSP accounts for approximately 11.5% of all PSP cases. Some small scale genetic studies on FSP have been reported. Jodie *et al.* reported a large single family with FSP from Finland and found a 4-bp deletion in the *FLCN* gene. In addition, all carriers of the mutation in this family had bullous lung lesions, as confirmed by HRCT (15). Ren *et al.* reported a cohort of patients with spontaneous pneumothorax from China and found that out of 10 unrelated patients with FSP, five patients were confirmed to harbor the *FLCN* mutation. They also demonstrated that *FLCN* mutation contributes to not only familial but also apparently sporadic patients with isolated spontaneous pneumothorax (7). Some other single-family reports of FSP from Korea (6), India (16) and the USA (17,18) also found different *FLCN* gene mutations.

The clustering of spontaneous pneumothorax in this family raised a concern regarding BHD syndrome. Using the sequence analysis, we identified a heterozygous C



Figure 2 Sequence analysis revealed a deletion mutation (c.1285delC) in exon 11 of the FLCN gene (A: healthy control; B: mutation carrier).



Figure 3 Distribution of mutation carriers in this family. Individuals positive for the FLCN mutation are highlighted in yellow, while those negative for the mutation are highlighted in blue. Patients who developed pneumothorax are indicated with an arrow on the lower left.

deletion in the poly-C tract in exon 11 cDNA nucleotide position 1285. This mutation was firstly reported in 2002 by Khoo *et al.* (19), and it is one of the most frequently reported mutations in BHD syndrome. This mutation is predicted to cause premature truncation of the FLCN protein 39 missense amino acids downstream. Loss of function of this protein can cause alveolar enlargement and cysts formation, consequently leading to pneumothorax. Recently, Elena *et al.* reported the possible molecular mechanism underlying lung epithelial cell apoptosis and alveolar enlargement, through E-cadherin, LKB1, and AMPK pathway (20).

In this family, we identified 17 members harboring this mutation. Pneumothorax was observed in 5 out of 17 (29.4%) members who carried a mutation in this family, which was similar to a prevalence of 29% in a previous report (21). However, according to Jodie *et al.*, the bullous lung changes observed on chest CT scan should be considered as the pulmonary manifestation of this syndrome (15). The multiple pulmonary cysts observed in mutation carriers III-2 and III-3 support this idea. Therefore, it was difficult to calculate the accurate frequency of pulmonary involvement in this family without CT imaging; several previous reports with different sample sizes showed that the number ranges from 70% to 100% (15,22-24). Late onset may also play a role in the reported difference in pulmonary involvement. None of the healthy controls harbored this mutation, suggesting the rarity of this mutation in the normal population.

The genetic analysis confirmed the diagnosis of BHD syndrome in this family according to the diagnostic criteria (25). However, we did not find any renal tumor or skin lesion in the proband. The incomplete penetrance of BHD syndrome has been widely reported (10,22). Some genotypes are believed to be associated with certain clinical manifestations. For example, Toro et al. reported that FLCN mutation in exon 9 and 12 is associated with a higher number of pulmonary cysts, larger cyst diameter and more episodes of pneumothorax (11). Schmidt et al. reported that the frequency of renal neoplasm is significantly lower in patients with a deleted cytosine in exon 11 than in patients with an inserted cytosine in this exon (26). However, these genotype-phenotype correlations are generally not accepted. Further studies involving more patients are warranted to address this issue.

In the family of this study, 6 members developed

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14 episodes of spontaneous pneumothorax altogether. The median age at initial onset was 41.5 years old and the median BMI was 26.5 kg/m². Although more males developed pneumothorax (4:2), the distribution of mutation carriers in male and female patients was almost equal (9:8). These characteristics are different from those of primary spontaneous pneumothorax, which typically affects tall, thin males aged between 10 and 30 years (27). The treatment options for the 14 episodes of pneumothorax included chest tube drainage, chemical pleurodesis, VATS bullectomy and mechanical pleurodesis. The recurrence rate after chest tube drainage and chemical pleurodesis was 50% (5 in 10 episodes), while no recurrent pneumothorax occurred after VATS bullectomy and mechanical pleurodesis (0 in 4 episodes). Toro et al. reported that for BHD syndrome-related FSP, observation or tube drainage with or without chemical pleurodesis resulted in a significantly higher recurrence rate of 54.7% (35 in 64 episodes), compared with that observed after bullectomy combined with mechanical or chemical pleurodesis, which was 21.6% (8 in 37 episodes) (11). As for primary spontaneous pneumothorax, the average rate of recurrence after observation, needle aspiration or tube drainage is about 30% (28), which is lower than the rate of BHD syndrome-related FSP with these treatments. The difference in the recurrence rate may be explained by the multiple pulmonary cysts in patients with BHD syndrome-related pneumothorax.

The special characteristics of BHD syndrome-related FSP suggest that spontaneous pneumothorax is a heterogeneous disease group and needs to be stratified. Considering that over 10% of patients with spontaneous pneumothorax have a positive family history and the potential risk of developing renal cancer (1), we suggest that patients with spontaneous pneumothorax, especially those with a positive family history, should undergo the FLCN mutation screening, since genetic analysis is gradually becoming a routine test in more and more medical centers. Currently, there is no curative treatment for BHD syndrome. For patients who develop spontaneous pneumothorax, VATS bullectomy combined with pleurodesis seems to be a more effective choice of treatment. Taking into account of our experience and previously published data, early surgical intervention may be advised for BHD syndrome-related FSP. However, further prospective trials are warranted to guide the choice of treatment. Renal cancer is the most life-threatening complication in BHD syndrome, with a reported range of prevalence from 6.5% to 34% (9,26,29). Hence, we recommend annual screening for early detection of renal

neoplasm in such patients.

This study has several limitations. First, we did not obtain the detailed clinical information of all family members, including skin examination, and chest and renal scan. This information could help us better understand the association between phenotype and genotype in BHD syndrome. Second, results from a single family and the retrospective nature of the study make it difficult to draw conclusion. Therefore, a prospective cohort with a larger sample size and detailed clinical information is required.

In conclusion, we reported a large family that presented with FSP. Genetic analysis found a germline mutation of *FLCN* gene in this family, which confirmed the diagnosis of BHD syndrome. To the best of our knowledge, this is the largest single-family report of BHD syndrome-related FSP from China, in which the clinical characteristics were discussed. Further studies are warranted to better understand the nature of this disease.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by the Ethics Committee of Peking University People's Hospital in China (No. 2014PHB061-01) and written informed consent was obtained from all patients.

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