Recurrent primary spontaneous pneumothorax in a large Chinese family: a clinical and genetic investigation

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Abstract

Background: Primary spontaneous pneumothorax (PSP) is a common manifestation of Birt-Hogg-Dubé (BHD) syndrome, which is an autosomal dominant disorder caused by mutation of the folliculin (*FLCN*) gene. This study was established to investigate the mutation of the *FLCN* gene and the phenotype in a family with PSP.

Methods: We investigated the clinical and genetic characteristics of a large Chinese family with recurrent spontaneous pneumothorax. Genetic testing was performed by Sanger sequencing of the coding exons (4–14 exons) of the *FLCN* gene.

Results: Among ten affected members in a multi-generational PSP kindred, with a total of 18 episodes of spontaneous pneumothorax, the median age for the initial onset of pneumothorax was 42.5 years (interquartile range: 28.8–57.2 years). Chest computed tomography scan of the proband showed pulmonary cysts and pneumothorax. A novel nonsense mutation (c.1273C>T) in exon 11 of *FLCN* gene that leads to a pre-mature stop codon (p.Gln425*) was identified in the family. The genetic analysis confirmed the diagnosis of BHD syndrome in this family in the absence of skin lesions or renal tumors.

Conclusions: A novel nonsense mutation of *FLCN* gene was found in a large family with PSP in China. Our results expand the mutational spectrum of *FLCN* gene in patients with BHD syndrome.

Keywords: Primary spontaneous pneumothorax; Birt-Hogg-Dubé syndrome; *FLCN* gene

Introduction

Primary spontaneous pneumothorax (PSP, Online Mendelian Inheritance in Man [OMIM] 173600) is a lung pathology characterized by the spontaneous occurrence of pneumothorax in the absence of obvious underlying lung disease. Since familial spontaneous pneumothorax was first reported in 1921,[1] it has been estimated that 11.5% of individuals with spontaneous pneumothorax have a positive family history.[2] Various genetic causes of spontaneous pneumothorax have been reported, such as α1-anti-trypsin deficiency, lymphangioleiomyomatosis, Langerhans cell histiocytosis, cystic fibrosis, Marfan syndrome, Ehlers-Danlos syndrome, and Birt-Hogg-Dubé (BHD) syndrome (OMIM 135150).[3] BHD syndrome is the most common genetic cause of familial pneumothorax, the accumulating familial cases have been confirmed to be associated with BHD syndrome.[4-9]

BHD syndrome is a rare autosomal dominant disorder, the main three symptoms of which are lung-related symptoms of multiple pulmonary cysts and/or recurrent pneumothorax, skin fibrofolliculoma, and renal cancer. Three symptoms may occur separately and often present in an atypical manner.[10,11] Such lung-related involvement is usually the earliest symptom to appear. Thus patients with BHD syndrome could present a pneumothorax-dominant phenotype with no or reduced penetrance of skin or renal manifestations.[4,5,12,13]

The gene responsible for BHD syndrome, folliculin (*FLCN*) gene, consists of 14 exons, is located at chromosome 17p11.2, and is predicted to encode a 579-amino-acid protein that is highly conserved across species. *FLCN* has a wide expression pattern in various tissues, including the skin and its appendages, the distal nephron of the kidney, stromal cells, and type 1 pneumocytes of the

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lung.[14] Although exact molecular functions of *FLCN* gene are unknown, it is believed to be a tumor suppressor gene which is known to be involved in several signaling pathways, including mammalian target of rapamycin (mTOR) and adenosine monophosphate-activated protein kinase signaling pathways.[15,16] Different types of mutation have been found along the entire *FLCN* gene, such as insertions, deletions, missense, splicing, and nonsense mutations. The majority of *FLCN* germline mutations are predicted to truncate the protein, leading to its dysfunction.[17] Given the association of the *FLCN* gene and phenotype of spontaneous pneumothorax, it is imperative to perform genetic testing in patients with PSP.

Here we reported a large Chinese family affected by recurrent PSP. We investigated the clinical characteristics of BHD-related spontaneous pneumothorax of the affected family members. All coding exons with the flanking sequences of the *FLCN* gene were examined, and a novel nonsense mutation in the *FLCN* gene was identified.

Methods

Ethical approval

This study was approved by the Ethics Committee of the Beijing Chaoyang Hospital, Capital Medical University (ID: 2018-S-285). Written informed consent was obtained from the subjects as required. The clinical records of affected family members were collected when available.

Patient recruitment and characteristic analysis

A large PSP-affected family from Northeastern China was recruited for this study. The proband, a 29-year-old woman with a first episode of left-lung pneumothorax, was treated by video-assisted thoracoscopic surgery, namely bullectomy. Other different diagnoses such as lymphangioleiomyomatosis, Marfan syndrome, Ehlers-Danlos syndrome as well as other known syndromic pulmonary disorders, were carefully ruled out. Detailed clinical information on the affected members, including their medical history, body weight and height, smoking status, chest computed tomography (CT) imaging, and the treatment of pneumothorax was retrospectively collected.

Mutation analysis of the FLCN gene

Peripheral blood samples were collected and genomic DNA was extracted from blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to standard procedures. The coding regions of the *FLCN* gene consisting of exons 4 to 14 and flanking sequences were amplified using the polymerase chain reaction (PCR) method. The primer sequences are listed in Supplementary Table 1, http://links.lww.com/CM9/A95. The PCR products were purified and sequenced bidirectionally using the Sanger method with the ABI 3700 DNA sequencer (Applied Biosystem, Foster City, CA, USA). Mutations were described according to the nomenclature recommended at http://www.HGVS.org/varnomen. Nucleotide numbers are derived from GenBank accession number NM_144997. Mutations were checked in disease databases including ClinVar, OMIM, and the Human Gene Mutation Database. The mutation that had not previously been reported is referred to as a novel mutation in this study.

Statistical analysis

Patient characteristics were analyzed by basic descriptive statistics and data were shown as n or median (interquartile range [IQR]), or otherwise noted. A Mann-Whitney non-parametric test was used to compare the continuous variance between groups. Statistical analysis was performed with SPSS 19.0 (IBM Corporation, Chicago, IL, USA), a value of $P < 0.05$ was considered as statistically significant.

Results

Patient characteristics

The pedigree of this Chinese family with PSP includes four generations and 14 affected family members [Figure 1]. Pedigree analysis revealed an autosomal dominant mode of inheritance. Specific symptoms of inherited diseases were not found and other related lung diseases were excluded among the members of the extended family. The clinical information and characteristics of ten affected family members for whom such information was available are listed in Table 1. Among the ten affected family members, with a total of 18 episodes of spontaneous pneumothorax,

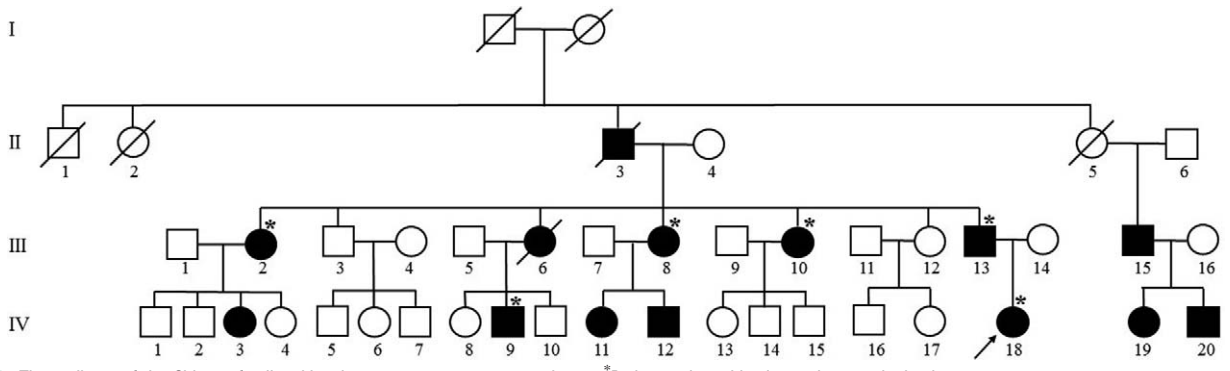

Figure 1: The pedigree of the Chinese family with primary spontaneous pneumothorax. *Patients whose blood sample was obtained.

Table 1: Clinical features of family members with primary spontaneous pneumothorax.

Patient no.	Sex	BMI (kg/m^2)	Smoking history	Age of first episode (years)	No. of pneumothorax*	Treatment	Lung cysts and/or bullae	Skin lesions	Kidney lesions
III-2	F	18.7	Y	56	1 (left), 1 (right)	TD	Y	N	N
III-6	F	22.0	N	69	2 (right)	TD	Y	N	N
III-8	F	19.5	N	48	1 (left)	TD	Y	N	N
III-10	F	23.9	Y	20	2 (left), 3 (right)	TD	Y	N	N
III-13	M	27.8	Y	46	1 (left)	TD	Y	N	N
III-15	M	25.4	N	61	1 (left)	TD	Y	N	N
IV-9	M	26.4	Y	39	3 (left)	TD	Y	N	N
IV-18	F	21.8	N	29	1 (left)	VB+MP	Y	N	N
IV-19	F	20.0	N	31	1 (left)	TD	Y	N	N
IV-20	M	23.7	Y	28	1 (left)	VB+MP	Y	N	N

* Location of pneumothorax in left or right lung is indicated in parentheses. BMI: Body mass index; F: Female; M: Male; Y: Yes; N: No; TD: Tube drainage; VB: Video-assisted thoracoscopic surgery, namely bullectomy; MP: Mechanical pleurodesis.

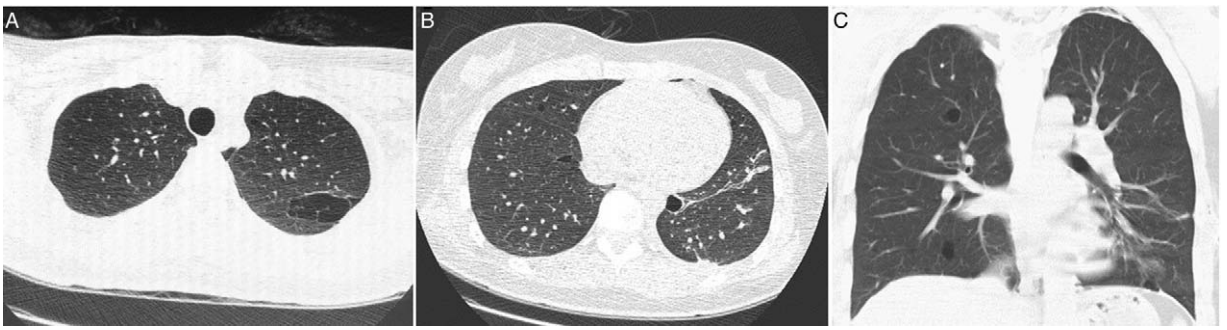

Figure 2: Chest computed tomography scan of the proband. (A) One dominant cyst was in the left lung. (B) There were multiple cysts in the bilateral lung. (C) Computed tomography image showed a left-side pneumothorax.

the median age for the initial onset of pneumothorax was 42.5 years (IQR: 28.8–57.2 years). There were no significant differences in repeated episodes of pneumothorax and the age at the first episode when the patients were grouped by either sex or smoking history [Supplementary Table 2, http://links.lww.com/CM9/A95]. As shown in Figure 2, the proband had bilateral multiple pulmonary cysts and pneumothorax on CT imaging. Patient II-3 and III-6 died of stroke and lung cancer, respectively. No clinical evidence of skin fibrofolliculoma or renal abnormalities was discovered in the family.

Germline mutation of the FLCN gene

Direct sequencing of the coding exons of the *FLCN* gene from genomic DNA of the proband led to the discovery of a novel nonsense mutation in the pedigree. The proband was heterozygous for a c.1273C>T transversion that changes a glutamic acid at codon 425 to a nonsense codon (p.Gln425*) in exon 11 [Figure 3]. Another five affected family members who consented to genetic testing were positive for the *FLCN* c.1273C>T mutation. This novel mutation was predicted to cause pre-mature termination of the translated FLCN protein and/or to trigger nonsense-mediated mRNA decay, leading to a loss-of-function effect. No other sequence variants were detected in the coding regions of this gene.

Discussion

This study presented the clinical and genetic characteristics of a large Chinese family with spontaneous pneumothorax. A novel nonsense mutation c.1273C>T (p.Gln425*) of *FLCN* gene in exon 11 was identified, all patients who had undergone genetic testing for *FLCN* gene carried this mutation. According to the diagnostic criteria for BHD syndrome proposed by Menko *et al*,[11] the genetic analysis confirmed the diagnosis of BHD syndrome in this family in the absence of skin lesions or renal tumors.

BHD syndrome typically exhibits clinical heterogeneity and patients do not always have the three characteristic manifestations (skin, kidney, and lung involvement). Pulmonary symptoms are often among the most common manifestations.[18] Similar clinical patterns were found in the present study. PSP was the early onset symptom of our patients with BHD syndrome, while no clinical evidence of skin fibrofolliculoma or renal abnormalities has yet been discovered. Such incomplete penetrance of the disease may exist especially in Asian populations. Japanese researchers suggested that recurrent episodes of pneumothorax and

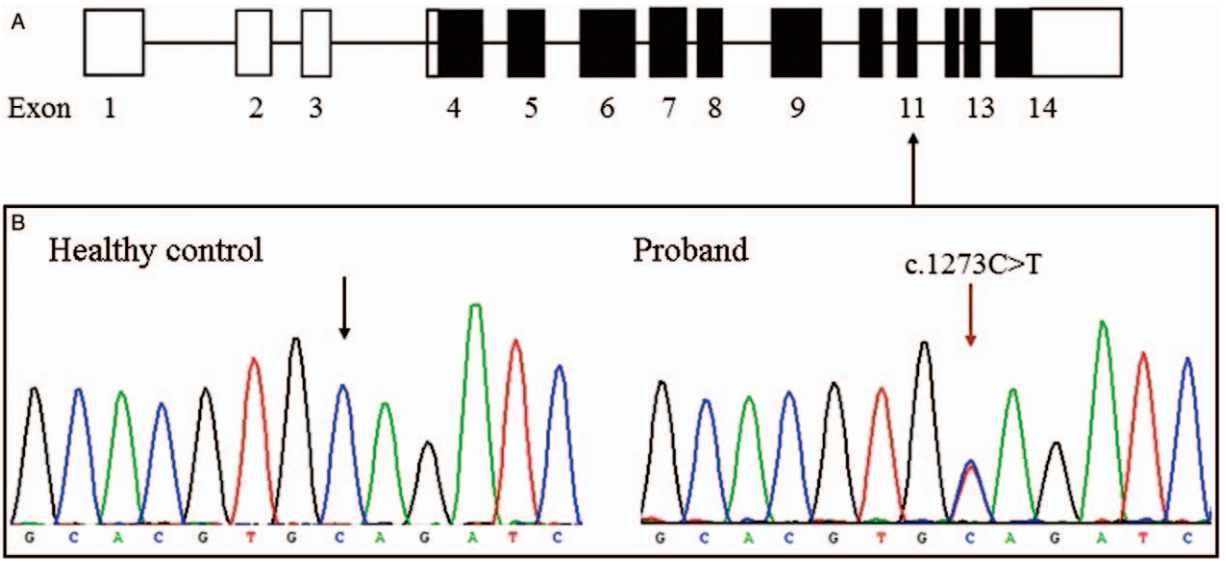

Figure 3: Genomic structure of *FLCN* gene and the mutation identified in the family. (A) Exon structure of the *FLCN* gene. Boxed symbols indicate exons, lines indicate introns, filled boxes indicate coding exons, unfilled boxes indicate the 5′ and 3′ untranslated region. (B) Sanger sequencing showing the novel *FLCN* mutation in exon 11 of the proband compared with a healthy control. Red arrow indicates the site of mutation. *FLCN*: Folliculin.

characteristic features of lung lesions were more informative as diagnostic criteria for BHD syndrome in the Japanese population.[19,20] In the Chinese population, Ren *et al*[8] reported that the *FLCN* gene mutations contribute to both familial and sporadic PSP patients, but none of the mutation carriers had skin or renal features of BHD syndrome. The present findings, along with several previous studies confirmed that for familial or sporadic PSP individuals, may harbor mutations of *FLCN* gene even in the absence of the renal and skin lesions typical of BHD syndrome.

In the affected family members, there were similar numbers of men and women, and the median age of onset of pneumothorax was 42.5 years, which was consistent with the results from two recent studies of the large families with BHD syndrome. Skolnik *et al*[21] reported that the mean age at diagnosis was 42 years, and Xing *et al*[9] suggested that the median age at initial onset was 41.5 years from another large Chinese family with BHD syndrome. Smoking history is not the risk factor for the disease, which was also indicated in this family. Recurrent pneumothorax ratio of our study was 40%, which was similar to a recurrent pneumothorax rate of 42% in the largest single-family that has been reported.[21]

Over 100 patterns of mutation in the *FLCN* gene have been reported in the Leiden Open Variation Database. Geographic variation in these mutations has been noted from the findings of several large cohort studies. Analysis of Caucasian data demonstrated that a cytosine deletion or duplication within a poly-C tract (c.1285dupC or delC) in exon 11 of *FLCN* gene was common.[22] In Japan, a mutational analysis of BHD including 312 patients with BHD syndrome from 120 different families was performed, which identified 31 *FLCN* sequence variants. Such results led to a conclusion that BHD syndrome in Japanese was associated with three *FLCN* mutational hotspots. Besides the C8 tract of exon 11, the other two hotspots are c.1533_1536delGATG in exon 13 and c.1347_1353dup CCACCCT in exon 12.[20] A recent genetic study of Chinese patients with BHD syndrome also identified the C8 tract in exon 11 of *FLCN* gene as a mutation hotspot, suggesting the consistency of this finding among different ethnic populations. Although no other significant mutation hotspots were found in this report, the results indicated that the mutation spectrum in the Chinese population is even more extensively distributed over the entire *FLCN* gene than that in Caucasians.[23] This is exemplified by the novel nonsense mutation (c.1273C>T) discovered in the present study, which is located in exon 11 near the C8 tract mutation hotspot.

The majority of *FLCN* mutations are predicted to truncate the protein, indicating that BHD syndrome arises through a haploinsufficiency mechanism. Emerging evidence has linked *FLCN* gene with a number of molecular pathways and cellular processes. For example, studies of *FLCN*-deficient models suggested that *FLCN* gene may modulate AKT-mTOR signaling in a context-dependent manner.[24,25] Down-regulation of FLCN leads to increased cell-cell adhesion and loss of cell polarity, which may result in increased vulnerability to physical forces induced by respiration.[26] Loss of function of FLCN may lead to epithelial apoptosis, alveolar enlargement and impaired pulmonary function through E-cadherin, liver kinase B1, and the AMP-activated protein kinase signaling pathway, consequently leading to pneumothorax.[27] However, the exact mechanism by which the *FLCN* gene and involved pathways contribute to this syndrome still needs to be further understood.

This study has demonstrated the importance of considering BHD syndrome when patients have recurrent pneumothorax and/or positive family history. Genetic testing of the *FLCN* gene is the most reliable method for the clinical

molecular diagnosis of BHD syndrome, especially in Asian patients who do not have skin and renal manifestations. Our results suggested that *FLCN* mutation screening should be conducted in patients with spontaneous pneumothorax, particularly those with a positive family history. PSP is often the earliest phenotypic manifestation in BHD syndrome, while the skin and renal symptoms are associated with advancing age.[22,28] It has been reported that *FLCN* mutation carriers have an increased risk of developing renal cell carcinoma.[29] Hence, early detection of renal neoplasm in such patients is recommended.

There are several limitations to this study. First, affected family members in this study were mostly identified from consultations at the Department of Thoracic Surgery and respiratory clinic, so few of them underwent skin examinations or renal screening. As such, it is possible that diagnoses of skin and renal lesions were missed. Second, the genetic results from this single-family and the function of the novel mutation were only predicted here, although this co-segregating nonsense mutation might be strongly pathogenic. Therefore, more studies with larger populations of Chinese patients with BHD syndrome accompanied by detailed clinical information and further functional analysis of *FLCN* gene are warranted.

In conclusion, this study reported a large Chinese family with spontaneous pneumothorax caused by a novel nonsense mutation in exon 11 of *FLCN* gene, in which the diagnosis of BHD was confirmed. Our finding of the novel mutant locus in this family expands the mutation spectrum of BHD syndrome in the Chinese population.

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Conflicts of interest

None.

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