Recurrence 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
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. 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. 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,
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., 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. 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

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

| Patient no. | Sex | BMI (kg/m²) | 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.
characteristic features of lung lesions were more informative as diagnostic criteria for BHD syndrome in the Japanese population. In the Chinese population, Ren et al. 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. reported that the mean age at diagnosis was 42 years, and Xing et al. 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.

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. 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 CCACCT in exon 12. 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. 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. 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. 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. 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 populations of Chinese patients with BHD syndrome accompanied by detailed clinical information and further functional analysis of FLNC 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 FLNC 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.

**References**
1. Faber E. Spontaneous pneumothorax in 2 siblings. Hospital Stidende 1921;6:573–574.
2. Abolnik Iz, Lossos IS, Zlotogora J, Brauer R. On the inheritance of primary spontaneous pneumothorax, Am J Med Genet 1991; 40:155–158. doi: 10.1002/ajmg.1320400207.
3. Chiu HT, Garcia CK. Familial spontaneous pneumothorax. Curr Opin Pulm Med 2006;12:268–272. doi: 10.1097/01.mcp.0000230630.73139.f0.
4. Painter JN, Tapanainen H, Somer M, Tukiainen P, Aittomaki K. A 4-bp deletion in the Birt-Hogg-Dube gene (FLCN) causes dominantly inherited spontaneous pneumothorax. Am J Hum Genet 2005;76:522–527. doi: 10.1086/428455.
5. Graham RB, Nolasco M, Peterlin B, Garcia CK. Nonsense mutations in folliculin presenting as isolated familial spontaneous pneumothorax in adults. Am J Respir Crit Care Med 2005;172:39–44. doi: 10.1164/cccm.200501-147OC.
6. Kim J, Yoo JH, Kang DY, Cho NJ, Lee KA. Novel in-frame deletion mutation in FLCN gene in a Korean family with recurrent primary spontaneous pneumothorax. Gene 2012;499:339–342. doi: 10.1016/j.gene.2012.03.037.
7. Ray A, Paul S, Chattopadhyay E, Kundu S, Roy B. Genetic analysis of familial spontaneous pneumothorax in an Indian family. Lung 2015;193:433–438. doi: 10.1007/s00408-015-9723-9.
8. Ren HZ, Zhu CC, Yang C, Chen SL, Xie J, Hou YY, et al. Mutation analysis of the FLNC gene in Chinese patients with sporadic and familial isolated primary spontaneous pneumothorax. Clin Genet 2008;74:178–183. doi: 10.1111/j.1399-0004.2008.01030.x.
9. Xing H, Liu Y, Jiang G, Li X, Hou Y, Yang F, et al. Clinical and genetic study of a large Chinese family presented with familial spontaneous pneumothorax. J Thorac Dis 2017;9:1967–1972. doi: 10.21037/jtd.2017.06.69.
10. Maffe A, Toschi B, Circo G, Gaschino D, Giglio S, Rizzo A, et al. Constitutional FLNC mutations in patients with suspected Birt-Hogg-Dube syndrome ascertained for non-cutaneous manifestations. Clin Genet 2011;79:345–354. doi: 10.1111/j.1399-0004.2010.01480.x.
11. Menko FH, van Steenew MA, Giraud S, Frise-Hansen L, Richard S, Ungari S, et al. Birt-Hogg-Dube syndrome: diagnosis and management. Lancet Oncol 2009;10:1199–1206. doi: 10.1016/S1470-2045(09)70188-3.
12. Frohlich BA, Zeitz C, Matyas G, Alkadhi H, Tuer C, Berger W, et al. Novel mutations in the folliculin gene associated with spontaneous pneumothorax. Eur Respir J 2008;32:1316–1320. doi: 10.1183/09031936.00132707.
13. Kunogi M, Kurihara M, Ikegami TS, Kobayashi T, Shindo N, Kumasaka T, et al. Clinical and genetic spectrum of Birt-Hogg-Dube syndrome patients in whom pneumothorax and/or multiple lung cysts are the presenting feature. J Med Genet 2010;47:281–287. doi: 10.1136/jmg.2009.070563.
14. Warren MB, Torres-Cabala CA, Turner ML, Merino MJ, Matrosova VV, Nickerson ML, et al. Expression of Birt-Hogg-Dube gene mRNA in normal and neoplastic human tissues. Mod Pathol 2004;17:998–1011. doi: 10.1038/modpathol.3800152.
15. Nickerson ML, Warren MB, Toro JR, Matrosova V, Glenn G, Turner ML, et al. Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. Cancer Cell 2002;2:157–164. doi: 10.1016/S1535-6108(02)00104-6.
16. Kennedy JC, Kahabibulin D, Henske EP. Mechanisms of pulmonary cyst pathogenesis in Birt-Hogg-Dube syndrome: the stretch hypothesis. Semin Cell Dev Biol 2015;24:47–52. doi: 10.1016/j.semcdb.2015.02.014.
17. Wei MH, Blake PW, Shevchenko J, Toro JR. The folliculin mutation database: an online database of mutations associated with Birt-Hogg-Dube syndrome. Hum Mutat 2009;30:E880–E890. doi: 10.1002/humu.21075.
18. Schmidt LS, Linehan WM. Molecular genetics and clinical features of Birt-Hogg-Dube syndrome. Nat Rev Urol 2015;12:558–569. doi: 10.1038/nruro.2015.206.
19. Gunji Y, Akiyoshi T, Sato T, Kurihara M, Tominaga S, Takahashi K, et al. Mutations of the Birt Hogg-Dube Gene in patients with multiple lung cysts and recurrent pneumothorax. J Med Genet 2007;44:588–593. doi: 10.1136/jmg.2007.049874.
20. Furuya M, Yao M, Tanaka R, Nagashima Y, Kuroda N, Hasumi H, et al. Genetic, epidemiologic and clinicoepidemiologic studies of Japanese Asian patients with Birt-Hogg-Dube syndrome. Clin Genet 2016;90:403–412. doi: 10.1111/age.12807.
21. Skolnik K, Tsai WH, Dornan K, Perrier R, Burrows PW, Davidson WJ. Birt-Hogg-Dube syndrome: a large single family cohort. Respir Res 2016;17:22. doi: 10.1186/s12931-016-0339-2.
22. Khoo SK, Giraud S, Kahanoski K, Chen J, Motorna O, Nickolov R, et al. Clinical and genetic characteristics of Chinese patients with Birt-Hogg-Dube syndrome. Orphanet J Rare Dis 2017;12:104. doi: 10.1186/s13023-017-0656-7.
23. Liu Y, Xu Z, Feng R, Zhan Y, Wang J, Li G, et al. Clinical and genetic characteristics of Chinese patients with Birt-Hogg-Dube syndrome. Orphanet J Rare Dis 2017;12:104. doi: 10.1186/s13023-017-0656-7.
24. Baba M, Furutara M, Hong SB, Tannassollo L, Haines DC, Southon E, et al. Kidney-targeted Birt-Hogg-Dube gene inactivation in a mouse model: Erk1/2 and Akt-mTOR activation, cell hyperproliferation, and polycystic kidneys. J Natl Cancer Inst 2008;100:140–154. doi: 10.1093/jnci/djn288.
25. Hong SR, Oh H, Valera VA, Baba M, Schmidt LS, Linehan WM. Inactivation of the FLCN tumor suppressor gene induces TFE3 transcriptional activity by increasing its nuclear localization. PloS One 2010;5:e15793. doi: 10.1371/journal.pone.0015793.
26. Medvetz DA, Khabibullin D, Hariharan V, Ongusaha PP, Goncharova EA, Schlechter T, et al. Folliculin, the product of the Birt-Hogg-Dubé tumor suppressor gene, interacts with the adherens junction protein p0071 to regulate cell-cell adhesion. PloS One 2012;7:e47842. doi: 10.1371/journal.pone.0047842.

27. Goncharova EA, Goncharov DA, James ML, Atochina-Vasserman EN, Stepanova V, Hong SB, et al. Folliculin controls lung alveolar enlargement and epithelial cell survival through E-cadherin, LKB1, and AMPK. Cell Rep 2014;7:412–423. doi: 10.1016/j.celrep.2014.03.025.

28. Schmidt LS, Nickerson ML, Warren MB, Glenn GM, Toro JR, Merino MJ, et al. Germline BHD-mutation spectrum and phenotype analysis of a large cohort of families with Birt-Hogg-Dubé syndrome. Am J Hum Genet 2005;76:1023–1033. doi: 10.1086/430842.

29. Houweling AC, Gijezen LM, Jonker MA, van Doorn MB, Oldenburg RA, van Spandonck-Zwarts KY, et al. Renal cancer and pneumothorax risk in Birt-Hogg-Dubé syndrome; an analysis of 115 FLCN mutation carriers from 35 BHD families. Br J Cancer 2011;105:1912–1919. doi: 10.1038/bjc.2011.463.

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