Nonsense Mutations in Folliculin Presenting as Isolated Familial Spontaneous Pneumothorax in Adults

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Rationale: Approximately 10% of patients who have a spontaneous pneumothorax have a positive family history. Objectives: We sought to identify DNA sequence variations that confer susceptibility to pneumothoraces. Methods: We collected 12 families that had at least 2 first-degree relatives with a spontaneous pneumothorax. All affected family members had no obvious stigmata of known genetic disorders associated with pneumothoraces. We used haplotype analysis, DNA sequencing, and restriction fragment analysis of mutations to evaluate the individuals in these families. Main Results: In 2 of the 12 families the disorder cosegregated with markers flanking a candidate locus, FLCN. Sequencing the linked alleles revealed 2 mutations predicted to introduce premature stop codons in 2 of the 12 families. Most mutations in FLCN cause a rare disease, Birt–Hogg–Dubé syndrome, characterized by autosomal dominant inheritance of multiple benign skin lesions, renal tumors, pulmonary blebs, and pneumothoraces. None of the family members with the nonsense mutations had skin manifestations of Birt–Hogg–Dubé syndrome or renal cancer. Pathologic examination of lung tissue from three affected nonsmokers revealed blebs and underlying emphysema. Conclusion: Isolated familial spontaneous pneumothorax can be caused by mutations of the FLCN gene. Because development of a pneumothorax and/or pulmonary blebs may be the earliest or the only clinical manifestation of FLCN mutations, pulmonologists should be alert to the contribution of this gene toward this familial form of emphysema.

Keywords: BHD (Birt–Hogg–Dubé) protein, human; genetics; pneumothorax, familial; pulmonary emphysema

A spontaneous pneumothorax is generally thought to occur as a consequence of the rupture of peripheral pulmonary blebs (1). The etiology of these blebs is unknown, and has been considered to represent a subtype of emphysema, termed distal acinar emphysema, or paraseptal or subpleural emphysema (2). The incidence of spontaneous pneumothorax is estimated to be about 7.4/100,000 per year for men and about 1.2/100,000 per year for women (3). Familial spontaneous pneumothorax was first reported in 1921 (4) and since then it has been estimated that 11.5% of individuals with a spontaneous pneumothorax have a positive family history (5). Known genetic causes of spontaneous pneumothorax include α1-antitrypsin deficiency, Marfan syndrome, Ehlers–Danlos syndrome, and the rare dominant disorder Birt–Hogg–Dubé syndrome. Case reports of families in which multiple family members have spontaneous pneumothoraces without clinical evidence of the above-cited diseases or syndromes have been described (5–13), but the genetic defects causing this disorder have not been found. The disorder generally segregates in a pattern consistent with an autosomal dominant mode with reduced penetrance, especially in females (5). Other families appear to have an X-linked recessive mode of inheritance.

Birt–Hogg–Dubé (BHD) syndrome was initially described in 1977 as a rare dominant dermatologic syndrome characterized by multiple asymptomatic dome-shaped skin papules involving the head, neck, chest, back, and arms (14). These skin findings were characterized as fibrofolliculomas, or hamartomas of the hair follicles, trichodiscomas (tumors of the hair disk), and acrochordons (skin tags). The disease is associated with hereditary cancer, especially various renal cell carcinomas and possibly colon cancer, and is strongly associated with pneumothoraces (15–17). In one study more than 25% of the BHD-affected individuals had a pneumothorax (17). The defect is in the folliculin gene (FLCN), which encodes a protein of unknown function (18). Most of the FLCN mutations causing BHD syndrome are frameshift mutations, resulting in the production of a truncated protein. The role of folliculin as a tumor suppressor has been demonstrated in human renal cancers as well as in a rat model of this disease (19, 20).

Given the association of BHD with lung cysts and pneumothoraces, we examined the segregation of FLCN alleles in families collected on the basis of a familial spontaneous pneumothorax phenotype. In our collection of 12 families, none of the affected family members have α1-antitrypsin deficiency, a connective tissue disease such as Marfan syndrome or Ehlers–Danlos syndrome, or skin or kidney manifestations of BHD syndrome. In two of these families with isolated familial spontaneous pneumothorax we identified nonsense mutations in FLCN.

METHODS

Familial Spontaneous Pneumothorax Kindreds/Specimens

The University of Texas Southwestern Medical Center (Dallas, TX) Institutional Review Board approved this investigation. Written consent was obtained from the subjects as required. All participants completed a medical questionnaire. Clinical records of affected members were reviewed when available. Photographs taken during surgery were provided by family members. Surgical biopsies of paraffin-embedded lung tissue, if available, were reviewed by a lung pathologist. Genomic DNA was isolated from circulating leukocytes with Puregene kits (Gentra Systems, Minneapolis, MN).

Haplotype Analysis

Haplotypes flanking the FLCN locus at 17p11.2 were created by genotyping the polymorphic microsatellite markers D17S1857, D17S740, D17S783, and D17S1824 (21). Genomic DNA was amplified by polymerase chain reaction (PCR) in 12-μl reactions containing 1× Gold buffer with 1.5 mM MgCl2 (Applied Biosystems, Foster City, CA), 0.4 μM fluorescently tagged oligonucleotides (Applied Biosystems), 1.3 mM dNTPs, and 1 U of AmpliTaq Gold enzyme (Applied Biosystems). Each marker was amplified, pooled, and mixed with marker standards. The markers were separated with an ABI 3100 capillary electrophoresis...
system (Applied Biosystems). Haplotypes were determined by GENEHUNTER, version 2.1 (22).

Sequencing Analysis

The exons and flanking intron sequences of FLCN were amplified from genomic DNA by PCR using oligonucleotides (Integrated DNA Technologies, Coralville, IA) listed in Table 1.

Each PCR was performed as described above in 20-μl reactions with 0.2 mM dNTPs and a 0.6 μM concentration of each oligonucleotide. The PCR was performed as follows: 95°C for 5 minutes; 30 cycles of 94°C for 15 seconds, 60°C for 15 seconds, and 72°C for 30 seconds; followed by 72°C for 10 minutes. Products were purified with a Rapid PCR purification system (Marligen Biosciences, Ijamsville, MD) and both strands were sequenced on an ABI 3730 automated sequencer using BigDye terminator cycle sequencing reagents (Applied Biosystems).

Restriction Fragment Analysis of Mutations

A restriction endonuclease site was engineered about each of the two nonsense mutation by the use of custom PCR primers, which were designed with the program SNPkit (23). For the exon 9 c.943 G>T mutation, the PCR primers were 5'-AGAAGGCTCTGTCTGCA-3' and 5'-ATGACTGCTTCTTCTCTGA-3'. For the exon 12 c.3429 C>T mutation, the PCR primers were 5'-GAGGGGTGTAGCTCTTCGc GAC-3' and 5'-TCAGAGCCGCTTCAATCTTA-3'. A restriction endonuclease site was engineered into the wild-type sequence by a 1-base pair oligonucleotide mismatch (indicated by the lowercase letters in the preceding sequences). Each PCR was performed as described above in 20-μl reactions with 2.5 mM MgCl₂, 0.2 mM dNTPs, and a 0.6 μM concentration of each oligonucleotide. A "touchdown" PCR protocol was used as previously described (23). The PCR product was purified with QIAquick spin columns (Qiagen, Valencia, CA), digested either with 20 units of Hpy188I overnight at 37°C or with 10 units of BspEI for 2 hours at 60°C (exo 12 assay) (New England BioLabs, Beverly, MA), electrophoresed on a 15-cm 14% acrylamide gel, and visualized with ethidium bromide.

RESULTS

Familial Spontaneous Pneumothorax Families

We collected 12 families in which at least 2 first-degree relatives had a spontaneous pneumothorax (data not shown). The average number of individuals who had a spontaneous pneumothorax was 3.5 ± 1.5, with a range of two to six affected individuals in each family. The affected individuals from seven of these families, including three of the affected individuals depicted in Figure 1A, were examined by one of us and lacked any clinical evidence of Marfan syndrome, Ehlers–Danlos syndrome, or Birt–Hogg–Dubé syndrome. None of the affected individuals had α₁-antitrypsin deficiency.

Clinical Features of Two Families with FLCN Mutations

The two families in which nonsense mutations were found are shown in Figure 1 and the clinical features of all affected individuals are provided in Table 2. The family depicted in Figure 1A is of Swedish descent and the family in Figure 1B is Slovenian. Neither family has a history of consanguinity. Individuals II:4 and III:5 of Family A were monitored by dermatologists for cancerous and precancerous lesions of the skin; there was no mention of the appearance of multiple benign skin lesions compatible with BHD syndrome for these two individuals. Family members of both families were asymptomatic for renal tumors; however, abdominal computed tomography scans were not performed.

Between the two families, there were four affected males and seven affected females who had at least one spontaneous pneumothorax. The mean age at onset was 36.2 ± 12 years,

Table 1. Oligonucleotides Used for Amplification and Sequencing of FLCN

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Amplicon Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5'-GGAGGGTTTCACTGAGGATCAA-3'</td>
<td>5'-CTCTCAGTGCTCTCTCTGCA-3'</td>
<td>402</td>
</tr>
<tr>
<td>5</td>
<td>5'-CCGAGCTCAGTATGCTGAGATAAA-3'</td>
<td>5'-AGAAGATGCTCAGACAGCTCA-3'</td>
<td>437</td>
</tr>
<tr>
<td>6</td>
<td>5'-ATTACCGCTGTCGCTGCAAATG-3'</td>
<td>5'-AGCTCTGTCAGTCACTCTCTGCA-3'</td>
<td>486</td>
</tr>
<tr>
<td>7</td>
<td>5'-CTCAAGCCATTCTCTCTGTCG-3'</td>
<td>5'-GCCCAAACTAGATGTCAGTCGACT-3'</td>
<td>446</td>
</tr>
<tr>
<td>8</td>
<td>5'-AAGGACCTTGGCTGCTGCTCCT-3'</td>
<td>5'-GCAAACGAGACAGGAAATCAG-3'</td>
<td>463</td>
</tr>
<tr>
<td>9</td>
<td>5'-CATCTAGCTATGCTGCTGCTGCA-3'</td>
<td>5'-GAGGCTCTGTCAGTCACTCTCTGCA-3'</td>
<td>393</td>
</tr>
<tr>
<td>10</td>
<td>5'-CTTACCGCTTCTTCTGCTCCTA-3'</td>
<td>5'-GCCAAAGAGAAGGAAACTAC-3'</td>
<td>497</td>
</tr>
<tr>
<td>11</td>
<td>5'-GGTCCCTGCCRGGCATTCG-3'</td>
<td>5'-CTCCACAGAGAGATGCTCAG-3'</td>
<td>270</td>
</tr>
<tr>
<td>12</td>
<td>5'-ATTAGCGAGAGAGACAGGACT-3'</td>
<td>5'-CCGCCAAAGAGAAGGAAACTAC-3'</td>
<td>427</td>
</tr>
<tr>
<td>13</td>
<td>5'-ACGATCGAGAGAGACAGGACT-3'</td>
<td>5'-AGGCTCTGTCAGTCACTCTCTGCA-3'</td>
<td>345</td>
</tr>
<tr>
<td>14</td>
<td>5'-GCCGAGGAATTGCTGCTGCTGCA-3'</td>
<td>5'-ATTGCCACAGAGAGAAGGAAACTAC-3'</td>
<td>470</td>
</tr>
</tbody>
</table>
TABLE 2. CLINICAL AND PATHOLOGIC DATA FROM AFFECTED INDIVIDUALS OF FAMILIES A AND B

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Sex</th>
<th>Age at First Pneumothorax (yr)</th>
<th>No. of Pneumothorax Episodes</th>
<th>Pneumothorax Location</th>
<th>Smoking History</th>
<th>Pathologic and Surgical Findings</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>II:2</td>
<td>F</td>
<td>59</td>
<td>1</td>
<td>Left</td>
<td>Never</td>
<td>Left lower lung bullae, largest measuring 15 × 8 cm; left lower lung with multiple blebs and emphysematous changes</td>
</tr>
<tr>
<td></td>
<td>II:6</td>
<td>F</td>
<td>23</td>
<td>2</td>
<td>Left</td>
<td>Never</td>
<td>Entire diaphragmatic surface of the left lower lobe replaced by several large blebs; blebs on the surface of the lingula, the right apex, the inferior surface of the right upper lobe, and the superior surface of the right middle lobe</td>
</tr>
<tr>
<td></td>
<td>III:1</td>
<td>M</td>
<td>30</td>
<td>4</td>
<td>Bilateral</td>
<td>3.5 pack-yr</td>
<td>Left lower lobe, 5-cm bulla; several blebs on the lingula; bullous emphysema of the lung</td>
</tr>
<tr>
<td>B</td>
<td>II:1</td>
<td>F</td>
<td>Unknown</td>
<td>2</td>
<td>Unknown</td>
<td>30–40 yr</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>II:3</td>
<td>F</td>
<td>50</td>
<td>5</td>
<td>Bilateral</td>
<td>Never</td>
<td>No surgical reports available</td>
</tr>
<tr>
<td></td>
<td>II:4</td>
<td>F</td>
<td>38</td>
<td>1</td>
<td>Right</td>
<td>Never</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>II:5</td>
<td>M</td>
<td>44</td>
<td>2</td>
<td>Left</td>
<td>40 yr</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>III:1</td>
<td>M</td>
<td>33</td>
<td>3</td>
<td>Bilateral</td>
<td>Never</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>III:2</td>
<td>F</td>
<td>29</td>
<td>6</td>
<td>Bilateral</td>
<td>2.5 pack-yr</td>
<td>Several blebs on the left lower lobe</td>
</tr>
</tbody>
</table>

*Definition of abbreviations: F = female; M = male; N/A = not available.*

ranging from 23 to 59 years. Each affected individual had a mean of 2.8 ± 2 episodes of pneumothorax. Four of the five affected individuals in Family A and two of the six affected individuals in Family B underwent surgical treatment for the pneumothoraces, which were recurrent in many cases. When radiographic and surgical records were available, these were examined for the description of the location of the pulmonary blebs and bullae. In general, bleb location was not uniform. As seen in the photographs taken at surgery for Individual III:1 of Family A, the anterior surface of the left lung was normal in appearance (Figure 2A), whereas the diaphragmatic surface of the left lower lung was almost completely replaced by blebs (Figure 2B). In other individuals, blebs were noted on the surface of the lung apices, the lingula, the right middle lobe, and the lower lobes. Individual II:3 of Family B was treated for pulmonary tuberculosis at the age of 33 years.

Slides from resected tissue were available for review for three individuals in Family A, II:2, III:2, and III:5 (two different surgical cases). In all four cases, bullae, blebs, pleural changes consistent with pneumothorax, as well as underlying emphysematous changes were seen. The emphysema was significant in that all three individuals had never smoked. Two representative pictures are shown in Figures 2C and 2D from Individuals II:2 and III:5 of Family A, respectively.

Although none of the family members had renal cell cancers, the cancer typically associated with BHD syndrome, a number of affected subjects had other types of cancer. In Family A, the proband has chronic lymphocytic leukemia; malignant melanoma, for which he underwent curative resection; two resected basal cell carcinomas of the skin; multiple actinic keratoses; and two dermatofibromas, a lesion not associated with BHD syndrome. His sister, Individual III:4, who is unaffected, had a resected hepatic sarcoma. Individual I:1 died of colon cancer. In Family B, affected Individual II:1 died of colon cancer.

**Analysis of FLCN Gene**

Genotyping closely linked polymorphic DNA markers flanking the FLCN locus allowed us to follow the segregation of the different parental alleles through the families. For each of the two families presented in this article, all the affected individuals shared a common haplotype that segregated with the pneumothorax phenotype (see Figure E1 in the online supplement). Direct sequencing of the coding exons of the FLCN gene from genomic DNA of the probands of these two families led to the discovery of two novel nonsense mutations (Figure 3A). The proband of Family A was heterozygous for an exon 9 c.943 G>T transversion that changes a glutamic acid at codon 315 to a nonsense codon. The proband of Family B was heterozygous for an exon 12 c.1429 C>T transversion that changes an arginine at codon 477 to a nonsense codon. Both mutations are predicted to cause premature termination of the translated folliculin protein (Figure 3B). The entire coding region of the FLCN gene was sequenced for the probands of the 10 additional families with familial spontaneous pneumothorax and for 10 unrelated individuals who had a spontaneous pneumothorax (data not shown). No other sequence polymorphisms were detected in the coding regions of the gene.

The presence of the mutations was tracked through the two families by restriction fragment analysis (Figure 4). Custom oligonucleotides were used to design a restriction endonuclease site in the wild-type, but not the mutant, sequence. All individuals who had a pneumothorax (as indicated by the shaded symbols) were heterozygous for one of the mutations. One individual, II:4 of Family A, is nonpenetrant for the mutation; she has the mutation but has not had a pneumothorax or skin lesions associated with BHD syndrome.

These two different restriction fragment assays were used to determine the frequency of the two nonsense mutations in the general population. The mutations were not present in genomic DNA samples from 200 unrelated chromosomes from white individuals (data not shown), indicating a frequency of less than 0.005. These sequence variations are not common polymorphisms but rare mutations.

**DISCUSSION**

We report the finding of two nonsense mutations in FLCN in two different families that presented with isolated familial spontaneous pneumothorax. Mutations in this gene had previously

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**Definition of abbreviations:**
- F: female
- M: male
- N/A: not available

**Abbreviations:**
- FLCN: folliculin
- BHD: Birt-Hogg-Dube syndrome
- SNP: single nucleotide polymorphism
- cDNA: complementary DNA
- PCR: polymerase chain reaction
- kb: kilobase
- bp: base pair
- pter: pterion
- GLI: glial cell line-derived neurotrophic factor
- MB: megabase
- N/A: not available

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been described in families with Birt–Hogg–Dube´ syndrome, a rare inherited skin disorder that is associated with renal cancer, lung cysts, and pneumothoraces. We show in this article that two different nonsense mutations in FLCN can cause familial spontaneous pneumothorax. Their segregation through the two kindreds presented in this article is consistent with autosomal dominant inheritance with reduced penetrance, one of the proposed modes of inheritance for familial spontaneous pneumothorax (5). Genomic DNA samples were not available for all family members, so it is not possible to calculate the exact penetrance of these mutations for the pneumothorax phenotype. From the available data for Family A, four of the five individuals who carried the mutation had a pneumothorax (Figure 4A). Because cigarette smoking is known to increase the risk for developing a pneumothorax in a dose-dependent manner (24), the effect of smoking will likely influence the penetrance of the pneumothorax phenotype in these families.

None of the affected family members had the typical skin lesions characteristic of BHD syndrome. It is possible that the presence of skin lesions was missed on some individuals because we have dermatologic reports for only two individuals in Family A and only self-reported medical information for individuals in Family B. The proband of Family A had two resected dermatofibromas; although this type of benign skin tumor is not associated with human BHD syndrome, the skin finding in the canine model of this disease is nodular dermatofibrosis, or numerous

Figure 2. Gross and histologic features of affected individuals. Photographs taken at the time of surgery for Individual III:1 of Family A show that the anterior surface of the left lung appeared normal (A) whereas the entire diaphragmatic surface of the left lower lobe was replaced by blebs (B). Hematoxylin–eosin-stained sections of resected lung tissue from Individuals II:2 (C) and III:5 (D) of Family A. Scale bars correspond to a length of 500 μm.

Figure 3. Genomic structure of FLCN, mutations found in families with familial spontaneous pneumothorax (A), predicted amino acid sequence, and location of mutations in probands (B). FLCN consists of 14 exons (rectangles); the coding regions are indicated by the shaded portions. Two nonsense mutations in FLCN, c.943 G→T and c.1429 C→T, are found in Families A and B, respectively. These mutations change a glutamic acid at codon 315 to a nonsense codon in Family A and change an arginine at codon 477 to a nonsense codon in Family B. Sequence tracings for the probands of each family are shown with the consensus sequence listed beneath each tracing. The sequence differences are listed below the line indicating the triplet codon for the predicted translated amino acid. Schematic representation of folliculin shows the location of the mutations identified in this study (boxed) as well as others identified in family studies (18, 26). Frameshift mutations are indicated at the position of a nucleotide(s) insertion, deletion, or duplication that results in a frameshift and protein truncation. The numbering of the nucleotides and amino acids is based on RefSeq NM_144997.
have a single band. Gous for the mutation have two bands; individuals without the mutation may respond to the pedigree symbol directly above it. Fifty nanograms of genomic DNA from each individual was amplified by PCR, using specific oligonucleotides, as described in Methods. The restriction enzyme HpaI digests the 191-base pair wild-type PCR product but not the mutant product containing the exon 9 c.943 G>T mutation for members of Family A (A). Similarly, the BseI digest of the 158-bp wild-type PCR product, but not of the mutant product containing the exon 12 c.1429 C>T mutation, is shown for Family B (B). Individuals heterozygous for the mutation have two bands; individuals without the mutation have a single band.

![Image](https://example.com/image.png)

**Figure 4.** Restriction fragment analysis of mutations detected in members of Family A (A) and Family B (B). A condensed pedigree of each family is depicted above the restriction fragment analysis. Numbers below the symbols correspond to patient identifiers shown in Figure 1 (circle = female; square = male; open symbol = unaffected by pneumothorax; solid symbol = affected by pneumothorax). Each lane corresponds to the pedigree symbol directly above it. Fifty nanograms of genomic DNA from each individual was amplified by PCR, using specific oligonucleotides, as described in Methods. The restriction enzyme HpaI digests the 191-base pair wild-type PCR product but not the mutant product containing the exon 9 c.943 G>T mutation for members of Family A (A). Similarly, the BseI digest of the 158-bp wild-type PCR product, but not of the mutant product containing the exon 12 c.1429 C>T mutation, is shown for Family B (B). Individuals heterozygous for the mutation have two bands; individuals without the mutation have a single band.

firm nodules of dense collagen fibers in the skin and subcutis (25). Individuals with only the pneumothorax phenotype, or those who were “skin negative,” have previously been reported as disease gene carriers in families with BHD syndrome (17). In addition, while this article was undergoing final preparation, a report appeared that described a four-nucleotide deletion in the 9 bp wild-type PCR product but not the mutant product containing the exon 9 c.943 G>T mutation for members of Family A (A). Similarly, the BseI digest of the 158-bp wild-type PCR product, but not of the mutant product containing the exon 12 c.1429 C>T mutation, is shown for Family B (B). Individuals heterozygous for the mutation have two bands; individuals without the mutation have a single band.

There are no cases of renal cancer in either of the two families. However, one individual in each family had colon cancer, including Individual II:1 of Family B, who also had a pneumothorax.

If colon cancer is an associated phenotype for these mutations, then perhaps the patriarch of Family A also carried the mutation. Other cancers identified in our families in patients with FLCN mutations were chronic lymphocytic leukemia, malignant melanoma, and basal cell carcinoma. Given the known role of folliculin as a tumor suppressor, it may be that mutations in this gene increase the predisposition to multiple different types of cancer, in addition to renal malignancies. Additional family studies will be necessary to determine the full range of cancers associated with FLCN mutations.

The most striking phenotype of the two families with the FLCN nonsense mutations is the associated lung phenotype. Many affected nonsmokers had recurrent pneumothoraces with bilateral disease. In comparison, Zbar and coworkers studied 33 white families with BHD syndrome and cases of pneumothorax occurred only in 13 of the 33 families (43%) (17). It is not clear why pulmonary manifestations predominate in these two families. The distribution of the pulmonary blebs is also distinctive. In general, spontaneous pneumothorax is associated with apical subpleural lung blebs (27), presumably caused by increased physical stresses at the apex in the upright lung (28), which may explain why spontaneous pneumothoraces are more common in tall asthenic males (29). In contrast, lower lobe emphysema is typically associated with α1-antitrypsin deficiency (30). Of the five individuals whose surgical records were available, all had evidence of blebs in extraapical locations. Painter and coworkers similarly reported that high-resolution computed tomography scans of the chest detected the presence of multiple lung bullae randomly situated throughout the lungs in 100% of the affected individuals in one large family (26). Another study reported the presence of pulmonary blebs or cysts as detected by high-resolution thoracic computed tomography scans in 83% of affected individuals as well as in 10% of controls in BHD families (17). The cysts are lined by a smooth, definable wall, with the majority found in basilar, subpleural locations. The distribution of pulmonary cysts in extraapical regions of the lung may be a subtle phenotype of pneumothoraces caused by FLCN mutations.

The predominant subpleural location of the cysts suggests that folliculin may have a unique role in the periphery of the lung. Expression of FLCN mRNA has been shown to be present within the connective tissue, the type I pneumocytes, and the alveolar macrophages of the lung, with no difference in the pattern of expression between normal lung and lung resected from a patient with BHD syndrome for spontaneous pneumothorax (31). Because postnatal alveolar proliferation occurs preferentially at the periphery of the lung in animal models (32), peripheral pulmonary blebs may occur when the regulated process of lung growth is altered.

The full-length FLCN gene encodes folliculin, a protein of 579 amino acids without significant homology to known human proteins. The amino acid sequence of folliculin predicts a short hydrophobic N-terminal sequence, one putative glycosylation site, a glutamic acid–rich coiled coil domain, several potential myristoylation sites, and multiple predicted phosphorylation sites. Organelle localization sites and transmembrane domains are absent. The two nonsense mutations described in this study are shown in the predicted protein structure in Figure 3B. To date, all familial human mutations of FLCN are predicted to cause truncations of the protein, resulting from either frameshift or nonsense mutations. These truncation mutations all raise the possibility that the extreme C-terminal region of the protein has an essential function.

The function of folliculin remains unknown. However, its role as a tumor suppressor gene is supported by evidence of a second hit at the Flcn locus in primary renal cancers in Nihon
rats, which carry a germ line frameshift mutation in the first coding exon of the gene (20) as well as evidence of inactivation of the FLCN locus by loss of heterozygosity, promoter methylation, and somatic mutation in human sporadic renal and colon cancers (19, 33–35). But how do germ line mutations of FLCN give rise to pulmonary or skin manifestations? Further studies of the cellular function of folliculin will be necessary to answer to this question. Understanding the cellular pathway of folliculin action may provide other candidate genes for screening patients with familial spontaneous pneumothorax who do not have FLCN mutations. Besides providing insights into the molecular basis of this rare type of hereditary spontaneous pneumothorax, it may be applicable to the pathogenesis of the more common disease, bullous emphysema.

Conflict of Interest Statement: R.B.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; B.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.K.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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