Lung Cysts, Spontaneous Pneumothorax, and Genetic Associations in 89 Families with Birt-Hogg-Dubé Syndrome

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***Rationale*: Birt-Hogg-Dubé syndrome (BHDS) is an autosomal, dominantly inherited genodermatosis that predisposes to fibrofolliculomas, kidney neoplasms, lung cysts, and spontaneous pneumothorax.**

***Objectives*: We evaluated 198 patients from 89 families with BHDS to characterize the risk factors for pneumothorax and genotype–pulmonary associations.**

***Methods*: Helical computed tomography scans of the chest were used to screen for pulmonary abnormalities. *BHD* mutation data were used for genotype–pulmonary associations. We examined the relationship of pneumothorax with categorical parameters (sex, smoking history, and lung cysts) and continuous parameters (number of cysts, lung cyst volume, and largest cyst diameter and volume). Logistic regression analyses were used to identify the risk factors associated with pneumothorax.**

***Measurements and Main Results*: Twenty-four percent (48/198) of patients with BHDS had a history of pneumothorax. The presence of lung cysts was significantly associated with pneumothorax ($p = 0.006$). Total lung cyst volume, largest cyst diameter and volume, and every parameter related to the number of lung cysts were significantly associated ($p < 0.0001$) with pneumothorax. A logistic regression analysis showed that only the total number of cysts in the right parenchymal lower lobe and the total number of cysts located on the pleural surface in the right middle lobe were needed to classify a patient as to whether or not he or she was likely to have a pneumothorax. Exon location of the *BHD* mutation was associated with the numbers of cysts ($p = 0.0002$).**

***Conclusions*: This study indicates that patients with BHDS have a significant association between lung cysts and spontaneous pneumothorax.**

Keywords: Birt-Hogg-Dubé syndrome; familial spontaneous pneumothorax; lung cysts; fibrofolliculomas; renal neoplasms

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Birt-Hogg-Dubé syndrome (BHDS) is an autosomal, dominantly inherited genodermatosis that predisposes to skin lesions (fibrofolliculomas), kidney cancer, lung cysts, and spontaneous pneumothorax. Germline mutation in the *BHD* gene predisposes to BHDS.

What This Study Adds to the Field

Patients with BHDS have a significant association between lung cysts and spontaneous pneumothorax.

Birt-Hogg-Dubé syndrome (BHDS; OMIM [Online Mendelian Inheritance in Man] no. 135150) is the autosomal, dominantly inherited genodermatosis that predisposes to the development of fibrofolliculomas, kidney cancer, lung pneumatocysts, and spontaneous pneumothorax (1, 2). In our first article, we identified three kindreds in whom renal neoplasms and fibrofolliculomas cosegregated (2). In that report, we also identified two individuals with pulmonary cysts and one individual who had a history of spontaneous pneumothorax. These preliminary observations and isolated reports in the literature suggested that pulmonary manifestations were a major feature of BHDS (2, 3). In our studies of the first reported large kindred with BHDS (1), we mapped the *BHD* locus to chromosome 17p11.2 (4). Subsequently, we identified germline mutations in a novel gene, *BHD* (also known as *FLCN*), in BHDS kindreds (5). Most mutations in *BHD* are frameshift or nonsense mutations that are predicted to truncate the BHD protein folliculin (5, 6). The biologic significance of the discovery of the *BHD* gene is supported by the recent identification of germline mutations in *BHD* homologs responsible for naturally occurring inherited kidney cancer syndromes in animals (7, 8). Recently, a novel folliculin-interacting protein, FNIP1, was identified that binds to 5′-AMP activated protein kinase, a negative regulator of mammalian target of rapamycin (mTOR), suggesting that folliculin and its interacting partner may be involved in energy and/or nutrient sensing through the AMPK and mTOR signaling pathways (9).

BHD mRNA is expressed in stromal cells and type I pneumocytes of the lung, suggesting that folliculin plays an important role in lung tissues (10). We have also reported that patients with BHDS are associated with the development of spontaneous

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pneumothorax (11). Recently, families with isolated familial spontaneous pneumothorax (SP) and germline *BHD* mutations have been described (12, 13).

SP is a rare disorder. A history of smoking, height, male sex, and family history are known risk factors for sporadic SP (14). Most cases of SP are sporadic but familial cases have been reported (15–18). Familial SP is genetically heterogeneous and various patterns of inheritance have been reported, including autosomal dominant (16, 18), X-linked recessive (15) and autosomal recessive patterns (17). However, most cases of familial SP are inherited in an autosomal dominant pattern with incomplete penetrance (19, 20). Familial SP may be a complication of various inherited disorders, such as α_1–antitrypsin deficiency (21), Marfan syndrome (22), Ehlers-Danlos syndrome (23), primary lymphangioleiomyomatosis (LAM) (24), tuberous sclerosis (TSC) (25), Langerhans cell histiocytosis (LCH) (26), cystic fibrosis (CF) (27), and BHDS. Therefore, understanding and defining the pulmonary features of BHDS are important for the diagnosis as well as for the treatment of patients. To date, no study has investigated in detail the pulmonary features of BHDS. In this study, we conducted a family-based investigation of the pulmonary features, genetic characteristics, and risk factors for pneumothorax in 198 patients from 89 families with BHDS.

METHODS

Patient Recruitment and Evaluation

We recruited families and individuals to screen for BHDS by mailing four cycles of letters over a 3-year period seeking referrals from the 11,000 members of the American Academy of Dermatology for patients with cutaneous signs of BHDS. Patients were evaluated in consecutive order in a protocol approved by the National Cancer Institute's Institutional Review Board. All members of families screened for BHDS who participated in this study gave written, informed consent.

Families with BHDS were evaluated at the Clinical Center of the National Institutes of Health. Medical histories (fibrofolliculomas, spontaneous pneumothorax, and renal tumors) were obtained and physical examinations were performed. A detailed dermatologic examination was conducted and skin biopsies were obtained of selected lesions suspicious for fibrofolliculoma. The presence of fibrofolliculomas was designated as the sole criterion for the diagnosis of BHDS because fibrofolliculomas are rare and specific for BHDS (2). We defined a family as affected with BHDS when it contained one or more members with BHDS cutaneous lesions and a minimum of one lesion histologically confirmed as a fibrofolliculoma. Helical computed tomography (CT) scans of the chest were used to screen for pulmonary abnormalities in our patient population. The chest was scanned before and after intravenous administration of 120 cm^3 of Ioxilan 300 (Cook Imaging Corp., Bloomington, IN). High-resolution 1-mm sections were obtained through the chest at 10-mm intervals. Pulmonary cysts were diagnosed on the basis of CT scans, and the numbers, location, and size of cysts were recorded. Pneumothorax was documented by history and a review of medical records. We defined recurrence of a pneumothorax as one occurring on the ipsilateral side more than 7 days after the most recent prior pneumothorax has resolved. α_1-Antitrypsin serum levels were obtained in 50 family members evaluated in the National Institutes of Health (NIH) Clinical Center. In addition, capillary oxygen saturation was routinely measured, together with vital signs for each person screened.

The genomic sequence analysis of the *BHD* gene was performed as previously reported (5). The first reported BHDS family (1), in whom a *BHD* germline mutation was not identified (6), although haplotype analysis showed linkage to chromosome 17p11.2 (4), was included in the present study.

Statistical Analysis

Data collected from the 198 patients with BHDS consisted of pack-years of cigarette smoking, number of fibrofolliculomas, sex, number of pneumothoraces, number of cysts in each lung compartment, and the total volume of cysts in the left lung and right lung. Volumes were derived using an estimated formula under an assumption that the cysts were approximately the shape of prolate spheroids: volume $= 4/3\ \pi\ ab^2$, where a is one-half the length of the longest semi-axis and b is one-half the length of the shorter semi-axis. The sum of the individual cyst volumes was used to calculate the total cystic space volume in each lung.

Analyses of dichotomized parameters and their relationship to presence or absence of a pneumothorax were done using a chi-square test. Associations between categorical parameters and presence or absence of a pneumothorax were done using an exact Cochran-Armitage test (28), as were associations between the number of pneumothoraces and dichotomous parameters. Associations between the number of pneumothoraces and ordered categorical parameters were performed using an exact Jonckheere-Terpstra test (29). Continuously measured parameters were compared between subjects with and without a pneumothorax using the Wilcoxon rank sum test. The Jonckheere-Terpstra test was used to assess the statistical significance of the trend in continuously measured parameters across increasing numbers of pneumothoraces. A Kruskal-Wallis test was used to determine whether continuously measured parameters differed according to mutation exon location, and to determine the association between exon location of the mutation and the number of pneumothoraces. Mehta and Patel's version of Fisher's exact test was used to evaluate the significance of the association between continuous parameters and the presence of one or more pneumothoraces (30).

Logistic regression analysis was used to identify whether a combination of factors could be jointly associated with either the presence or absence of a pneumothorax or with increasing numbers of pneumothoraces. Many of the variables related to the numbers of cysts were derived from one another (e.g., the number of lung cysts on the right lung is the sum of the cysts on the right lower and upper lobes). Therefore, the variables included in the model only consisted of parameters that were not directly calculated from one another. In the final regression modeling for classification of "pneumothorax or not," all possible classifications were examined, and a threshold for classification was selected that simultaneously provided high sensitivity and specificity. The Kaplan-Meier method was used to describe the association between age and the probability of development of a first spontaneous pneumothorax (31). All p values are two-sided and were not adjusted for multiple comparisons.

RESULTS

General Clinical and Pulmonary Characteristics

Patients' clinical characteristics and the frequencies are listed in Table 1. Our cohort included 101 men and 97 women with a median age of 49 years. The age range was 22 to 77 years, with only eight patients younger than 30 years. Eighty-nine percent (177/198) of patients with BHDS had lung cysts on CT scans of the chest. Approximately 24% (48/198) of patients and 35% (31/89) of BHDS families screened for lung cysts had a history of spontaneous pneumothorax. In our cohort of patients affected with BHDS, we found a relatively equal distribution of pneumothorax for men and women. Of the men affected with BHDS, 20% (20/101) had a history of a pneumothorax and, of the women with BHDS, 29% (28/97) had a history of a pneumothorax. All patients with a history of pneumothorax had multiple lung cysts identified by chest CT imaging (Figure 1). The earliest reported age of initial pneumothorax was 22 years, the median age of occurrence was 38 years (range, 22–71 yr), and the median age of the last pneumothorax was 42 years (range, 22–75 yr). Seventy-five percent (36/48) of patients had a second pneumothorax, the average number of pneumothoraces per patient was two, and four patients experienced five separate pneumothoraces. Differences in recurrence among patients may reflect efficacy of treatment used.

Ninety-three percent of patients with a diagnosis of BHDS had fibrofolliculomas with a distinctive clinical presentation characterized by multiple white or skin-colored papules distributed over

TABLE 1. GENERAL CLINICAL CHARACTERISTICS AND FREQUENCIES OF LUNG PNEUMATOCYSTS AND PNEUMOTHORACES OF 198 PATIENTS WITH BIRT-HOGG-DUBÉ SYNDROME

			Mean Total No. (*range*)		
	No.	%	All Lung Cysts	Right Lung Cysts	Left Lung Cysts
Total number of patients	198		16 (0–166)	9 (0–83)	7 (0–83)
Sex					
Males	101	51	16 (0–166)	8 (0–83)	7 (0–83)
Females	97	49	16.6 (0–135)	9 (0–71)	7 (0–64)
Smoking					
Yes	75	38	20 (0–166)	11 (0–83)	9 (0–83)
No	123	62	14 (0–135)	7 (0–71)	6 (0–64)
Pneumothorax					
Positive	48	24	29 (0–166)	16 (0–83)	13 (0–83)
Negative	150	76	12 (0–85)	6 (0–47)	6 (0–41)
Lung cysts					
Yes	177	89			
No	21	11			
No. of fibrofolliculomas					
< 10	14	7			
10–100	73	37			
> 100	111	56			

the face, neck, and upper trunk. However, 14 patients in our cohort had fewer than 10 skin lesions suspicious for fibrofolliculoma, but all 14 patients had at least one biopsy-confirmed lesion. Forty-five patients with BHDS had kidney tumors. Of these, 27% (14/45) had a history of pneumothorax and 93% (42/45) had lung cysts.

Table 2 shows the pneumothorax history and treatment of the 48 patients affected by spontaneous pneumothorax. Of these

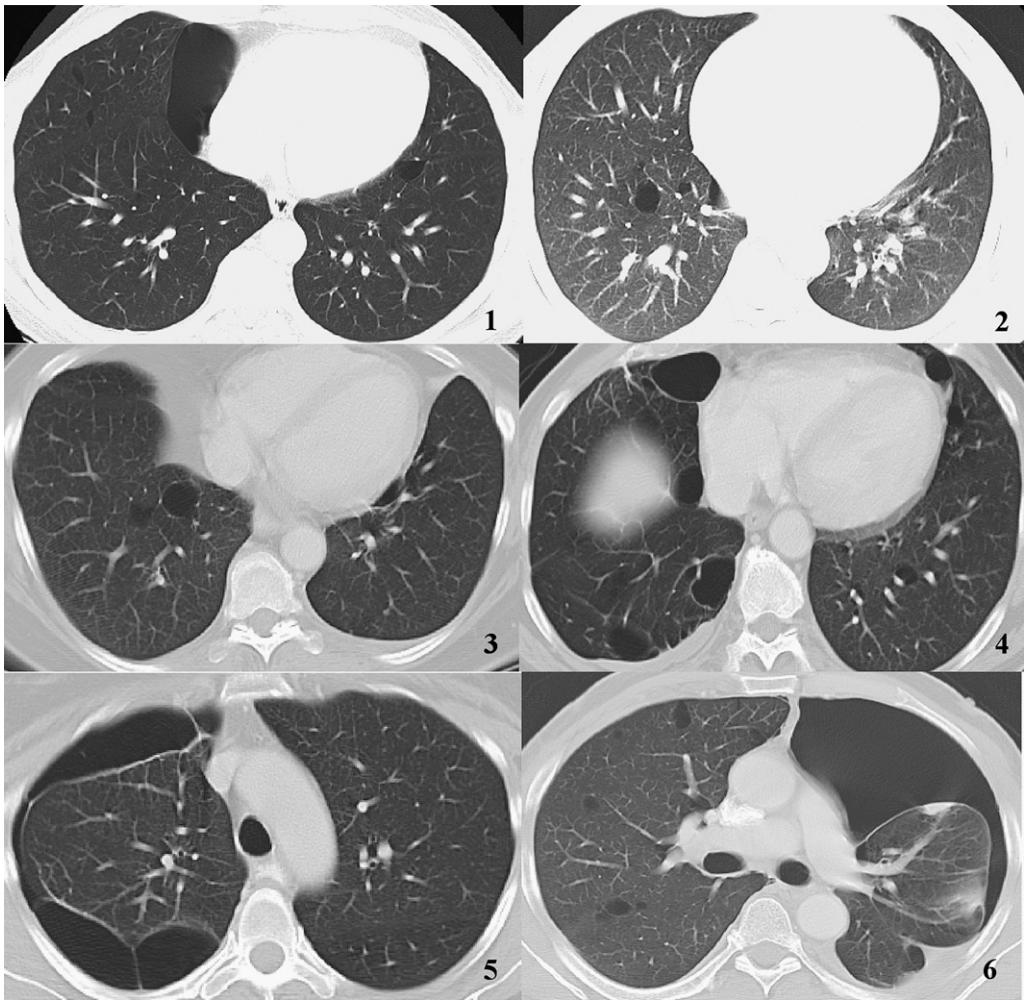

Figure 1. Pulmonary manifestations of patients with Birt-Hogg-Dubé syndrome on conventional helical (5 mm) chest computed tomography imaging. (*1*) Loculated pnemothorax on the right lung as well as multiple bilateral smaller cysts in the lung bases. (*2*) Cluster of small cysts in the right lung base. (*3*) Bilateral basilar lung cysts. (*4*) Bilateral, multiple, peripheral lung cysts more pronounced on the right side. (*5*) Loculated pneumothorax in right upper lobe. (*6*) Pneumothorax on the left with collapse of the left upper lobe and multiple right-sided cysts.

TABLE 2. CLINICAL CHARACTERISTICS OF THE 48 PATIENTS WITH BIRT-HOGG-DUBÉ SYNDROME WITH PNEUMOTHORAX

No.	Sex	Smoking History	No. of Pneumothoraces	Age(s) at Pneumothorax (Treatment)	
				Right Lung Pneumothorax	Left Lung Pneumothorax
1	M	N	5	23 (TT), 25 (TT), 25 (TT/TH/MP)	29 (TT), 29 (TH/MP)
2	M	Y	1	52 (TT/TH/LR)	
3	F	Y	1		30 (TT)
4	F	N	1	27 (TT)	
5	F	N	4	24(TT), 28(TT/TH/CP), 38(TT/TH/MP)	26 (TT/TH/MP)
6	F	N	2		38 (N), 43 (TT/TH/LR)
7	M	Y	4	56 (TT/TH/LR/MP)	43 (TT), 48 (TH/LR/MP), 60 (TT/TH/MP)
8	M	N	2	42 (N), 46 (TH/MP)	
9	M	Y	3	50 (N), 51 (N)	49 (N)
10	F	N	1	43 (N)	
11	M	N	1	45 (TT/TH/LR/MP)	
12	F	Y	2		34 (N), 38 (TH/MP)
13	M	N	1	31 (TT/TH/MP)	
14	F	N	1	41 (TT)	
15	M	N	3	39 (TT), 50 (TH/LR), 52 (TH/MP)	
16	F	N	2	42 (TT), 42 (TT/CP)	
17	F	Y	1	44 (TT)	
18	M	N	1	49 (TT/TH/LR)	
19	M	Y	5	40 (TT), 46 (TT), 52 (TT/CP)	44 (TT), 48 (TH/MP)
20	F	Y	1	54 (N)	
21	M	Y	3		36 (TT), 36 (TT/TH/CP), 38 (TH/MP)
22	M	Y	2	35 (TT), 38 (TT)	
23	M	Y	2		22 (TT), 22 (TT)
24	F	N	2	47 (TH/MP)	37 (TT/CP)
25	M	N	2	41 (N), 42 (N)	
26	F	N	2	37 (N), 41 (TT)	
27	F	N	1	44 (TT)	
28	F	Y	5	22 (TT/TH/LR), 22 (TT/TH/LR), 22 (TH/LR)	29 (N), 39 (N)
29	M	N	1		38 (N)
30	M	N	1		27 (N)
31	F	N	3	31 (TT), 41 (TT/CP), 42 (TT/CP)	
32	M	N	1	35(N)	
33	F	N	2	58 (TT), 58 (TH/MP)	
34	F	N	1		26 (N)
35	M	Y	1	32 (TH/MP)	32 (TH/MP)
36	F	N	2		43 (TT), 45 (TT/TH/LR)
37	M	N	4	30 (TT), 30 (TH/CP), 30 (TH/CP)	32 (N)
38	F	N	1	45 (TT)	
39	F	Y	1	46 (TT)	
40	F	N	4	39 (TT), 39 (TT), 39 (TT/TH/MP)	39 (N)
41	F	N	1	23 (TT)	
42	F	Y	5		24 (N), 25 (N), 25 (N), 26 (N), 27 (TT/TH/LR/MP)
43	F	N	1		36 (TH/LR)
44	F	N	3	50 (TT), 60 (TT/TH/LR/MP)	52 (TT)
45	M	N	2	55 (TT), 55 (TH/PM)	
46	F	Y	2		36 (TT/TH/LR/CP), 36(TT/TH/MP)
47	F	N	2	37 (TT), 38 (TT/CP)	
48	F	N	2		71 (TT), 75 (TT/TH/LR/MP)

Definition of abbreviations: CP = chemical pleurodesis; LR = lung resection; MP = mechanical pleurodesis; N = no treatment; TH = thoracostomy; TT = tube thoracostomy.

patients, 58% (28/48) were women and 41% (20/48) were men. Sixty-seven percent (32/48) of patients were nonsmokers. There were 101 episodes of pneumothoraces and no patient had simultaneous bilateral pneumothoraces. The right lung had the highest frequency of pneumothorax. Approximately 48% (23/48) of patients had a pneumothorax in the right lung only, 29% (14/48) had a pneumothorax in the left lung only, and 23% (11/48) of patients had a pneumothorax in both the right and the left lungs at different times.

Twenty-three percent of pneumothoraces were managed with observation alone. These patients were in stable condition and only minimally compromised by the pneumothorax. Of the pneumothoraces managed by observation alone, only 39% (9/23) completely resolved, and the remaining 61% (14/23) recurred, requiring medical treatment. Of the 101 pneumothoraces, 77% required medical intervention and were treated by various methods. Thirty-five percent (35/101) were treated with tube thoracostomy (chest tube) only. Six pneumothoraces were managed with a combination of tube thoracostomy and chemical pleurodesis (quinacrine, tetracycline, silver nitrate, or talc). Approximately 14% (15/101) of pneumothoraces were treated by open thoracotomy and a second treatment, including mechanical pleurodesis (abrasion to produce adhesion between parietal and visceral pleura) in 10% (10/101), chemical pleurodesis in 2% (2/101),

and lung resection in 3% (3/101). Approximately 13% (13/101) of pneumothoraces were treated with combined tube thoracostomy, thoracotomy, and a third treatment, including mechanical pleurodesis in 7% (7/101), lung resection in 6% (6/101), and chemical pleurodesis in 2% (2/101), respectively. In addition, seven pneumothoraces were treated with a combination of tube and open thoracostomies, lung resection, and mechanical pleurodesis. Another pneumothorax was treated with both a combination of tube and open thoracostomy, lung resection, and chemical pleurodesis. Three patients with BHDS treated at the NIH underwent video-assisted thoracoscopic surgery. Pneumothoraces were not related to a particular calendar period. After serum α_1-antitrypsin levels in 50 patients were within normal limits, we no longer performed the test. Capillary O_2 saturation showed no significant abnormality in the measurements performed during routine screening for BHDS.

Risk Factor Analysis

We examined the association between the history of pneumothorax and the following categorical parameters: sex, smoking history, severity of fibrofolliculomas, and lung cysts. The results of the univariate analysis of categorical parameters are summarized in Table 3. The only categorical parameter that was significantly associated with pneumothorax was the presence of lung cysts ($p = 0.006$).

In a second analysis, we investigated the association of continuous parameters (the total number of cysts per lung lobe, total number of intraparenchymal cysts, total number of subpleural cysts, number of lung lobes with cysts, smoking history, age when scanned, and lung cyst total volume) with pneumothorax. The results of tests for significance from the univariate analysis of continuous parameters are summarized in Table 4. We found that every parameter related to the number of lung cysts was significantly associated with history of pneumothorax. No association was found between age at scan or smoking history and presence or frequency of pneumothoraces. In addition, total lung cyst volume and largest cyst diameter and volume were significantly associated ($p < 0.0001$) with history of pneumothorax (Table 5). In addition, we found an association between the number of pneumothoraces and the total cyst volume ($p < 0.0001$) (Table 5). There was an increase in median total cyst volume (0.8, 5.1, 8.5, and 10.3 cm^3) with increasing number of pneumothoraces (0, 1, 2, +3, respectively). Similarly, we found that the largest cyst diameter and volume were significantly associated with the number of pneumothoraces (Table 5).

The relationship between history of pneumothorax and different parameters related to lung volume and cysts was investigated using a logistic regression model. The logistic regression model determined that only the total number of cysts in the right parenchymal lower lobe ($p = 0.050$) and the total number of cysts in the right pleura-based middle lobe ($p = 0.002$) were needed to classify a patient as to whether he or she was likely to have a pneumothorax or not. These same parameters were also associated with increasing numbers of pneumothoraces.

We used the Kaplan-Meier method to describe the association between age and the first spontaneous pneumothorax among all patients in the BHDS cohort (Figure 2). No pneumothoraces were diagnosed before age 22 years in this cohort, with the oldest patient experiencing her first pneumothorax at 71 years. By age 30 years, the probability of having the first pneumothorax is 6% (95% confidence interval [CI], 3–10%), 14% (95% CI, 10–20%) by 40 years, and 75% (95% CI, 19–32%) by age 50. Of the 48 patients who had a pneumothorax, 88% (42/48) of them had his or her first pneumothorax before age 50, 10% (5/48) had one between the ages of 51 and 60 years, whereas only 1 patient had a pneumothorax that occurred after age 60, at 71 years.

TABLE 3. RESULT OF UNIVARIATE ANALYSIS INVESTIGATING ASSOCIATION BETWEEN CATEGORICAL VARIABLES AND PNEUMOTHORAX PRESENCE AND NUMBERS

	Pneumothorax			No. of Pneumothoraces				
Variable	No	Yes	p Value	0	1	2	3+	p Value
Sex								
M	81	20	0.14	81	6	7	7	0.83
F	69	28	(C)	69	11	12	5	(C-A)
No. of fibrofolliculomas								
< 10	9	5	1.00	9	1	2	2	
10–100	9	14		59	4	6	4	0.83
> 100	82	29	(C-A)	82	12	11	6	(J-T)
Kidney tumors								
Yes	119	34	0.22	119	14	12	8	0.13
No	31	14	(C)	31	3	7	4	(C-A)
Smoking history								
Yes	59	16	0.46	59	5	5	6	0.87
No	91	32	(C)	91	12	14	6	(C-A)
Smoking status								
Nonsmoker	91	32		91	12	14	6	
Quit > 10 yr ago	21	8		21	2	3	3	0.47
Quit < 10 yr ago	9	2	0.33	9	0	1	1	(J-T)
Current	24	5	(C-A)	24	3	0	2	
Lung cysts								
Yes	129	48	0.006	129	17	19	12	0.012
No	21	0	(C)	21	0	0	0	(C-A)

Definition of abbreviations: C = chi-square test; C-A = exact Cochran-Armitage test; J-T = exact Jonckheere-Terpstra test.

BHD Mutation Detection

BHD mutations were present in 81% (154/190) of patients and 85% (68/80) of BHDS families tested. The cytosine mononucleotide tract in exon 11 of *BHD* was most frequently mutated, accounting for 48% (70/154) of patients and 47% (32/68) of BHDS families who had a mutation detected. Thirteen patients from one large BHDS kindred that previously showed linkage to chromosome 17p11.2 by haplotype (4) had no sequence variation in the *BHD* gene (6). Nine families were not screened for *BHD* mutations by direct sequencing.

Patients with a history of pneumothorax share a similar BHDS mutation spectrum with all the patients with BHDS in this study, but with some exceptions. *BHD* mutations were present in 87% (41/47) of individuals and 90% (27/30) of BHDS families with a history of pneumothorax tested. Similar to previous studies, the "hot spot" mutation, c.1733ins/delC, in exon 11 was the most frequent site of mutation, accounting for 38% (18/47) of individuals with a history of a pneumothorax and 44% (18/41) of all the mutations detected. We were unable to detect a sequence variation in *BHD* by direct sequence in six individuals, but four of these individuals were part of a family that showed linkage to chromosome 17p11.2 by haplotype analysis (4). In addition, one individual was not screened for *BHD* mutations.

Analyses of Genotype–Phenotype Correlation

There was no association between *BHD* mutation status (no mutation vs. mutation and/or linkage to chromosome 17p11.2), mutation types (insertion, deletion, nonsense, and splice site, and frameshift vs. nonsense and splice site; splice site vs. exon mutation, or hot-spot mutation vs. all other *BHD* mutations, or splice site vs. all other *BHD* mutations), or location, and lung cyst parameters or pneumothorax. Our analysis showed a trend for differences in pneumothoraces according to exon location ($p = 0.01$), with individuals with *BHD* mutations in exon 9 and

TABLE 4. RESULTS OF UNIVARIATE ANALYSIS INVESTIGATING ASSOCIATION BETWEEN CONTINUOUS PARAMETERS AND PNEUMOTHORAX PRESENCE AND NUMBER

Variable	Pneumothorax (Yes/No) p-Value	No. of Pneumothoraces (0, 1, 2, 3+) p-Value
Pack-years	0.31	0.36
Total no. lung cysts/person	< 0.0001	< 0.0001
Total no. lung cysts, right lobe	< 0.0001	< 0.0001
Right PUL	0.0045	0.0046
Right PML	0.0006	0.0005
Right PLL	< 0.0001	< 0.0001
Right PBUL	0.0005	0.0005
Right PBML	< 0.0001	< 0.0001
Right PBLL	0.0001	< 0.0001
Total left lung	< 0.0001	< 0.0001
Left PUL	0.0011	0.0010
Left PLL	0.0071	0.0047
Left PBUL	< 0.0001	< 0.0001
Left PBLL	0.0026	0.0017
Left PB (LPPUL + LPBLL)	< 0.0001	< 0.0001
Left parenchymal (LPUL + LPLL)	0.0003	0.0003
Right PB (PBUL + PBML + PBLL)	< 0.0001	< 0.0001
Right parenchymal (PUL + PML + PLL)	< 0.0001	< 0.0001
Lower right (PLL + PBLL)	< 0.0001	< 0.0001
Upper right (PUL + PML + PBML + PBUL)	< 0.0001	< 0.0001
Lower left (LPLL + LPBLL)	0.0014	0.0008
Upper left (LPUL + LPBUL)	< 0.0001	< 0.0001
Upper right + upper left	< 0.0001	< 0.0001
Lower left + lower right	0.0001	< 0.0001
PB (left and right)	< 0.0001	< 0.0001
Parenchymal (left and right)	< 0.0001	< 0.0001
No. of compartments	< 0.0001	< 0.0001
Scan age	0.90	0.93

Definition of abbreviations: PBLL = pleura-based lower lobe; PBML = pleura-based middle lobe; PBUL = pleura-based upper lobe; PLL = parenchymal lower lobe; PML = parenchymal middle lobe; PUL = parenchymal upper lobe.

exon 12 having more pnemothoraces than individuals with *BHD* mutations in other exons (Table 6). In addition, our analysis revealed that mutation exon location was associated with the numbers of cysts ($p = 0.0002$), with individuals with *BHD* mutations in exon 9 having more cysts (median = 32) than individuals with mutation in other exons (Table 7). Similarly, we found that the *BHD* mutation exon location was associated with the size ($p < 0.005$) and volume ($p < 0.01$) of the largest cysts. Individuals with mutations in exons 9 and 12 had the largest cyst diameters (1.9 and 2.9 cm) and volumes (2.7 cm^3) (Table 7).

DISCUSSION

This is the largest and most comprehensive study to date of individuals and families affected with BHDS relating the pulmonary risk factors, CT screening, and genotype–pulmonary associations. In this study, for the first time, we showed that lung cysts and every parameter related to the number of lung cysts were significantly associated with spontaneous pneumothorax. Univariate analysis revealed that total lung cyst volume, largest cyst diameter, and largest cyst volume were statistically associated with pneumothorax in patients with BHDS. In addition, logistic regression analysis showed that only the total number of cysts in the right parenchymal lower lobe and in the right pleura-based middle lobe were needed to classify a patient as to whether he or she was likely to have a pneumothorax. The role of lung cysts in the mechanism leading to a spontaneous pneumothorax in BHDS has not been established. One explanation is that lung cysts may be a precursor lesion; a second possibility is that rupture of subpleural blebs on the visceral pleura may lead to a spontaneous pneumothorax. Furthermore, the pathophysiology of lung cysts in BHDS is unknown. Although inactivation of the *BHD* wild-type allele by loss of heterozygosity (LOH) or somatic mutation may explain the BHDS-associated kidney tumors (32), it is possible that haploinsufficiency alone may be responsible for the development of lung cysts. Age of onset of the first reported pneumothorax is also important. Approximately 90% of patients with BHDS had their first pneumothorax by age 50, suggesting that pneumothorax tends to occur during adulthood in patients with BHDS.

Screening of 198 patients with BHDS at the NIH Clinical Center using high-resolution plus standard CT of the chest revealed that most (89%) patients with BHDS have multiple pulmonary cysts. Twenty-four percent of patients with BHDS had a history of one or more pneumothoraces, all of whom had multiple lung cysts identified by chest CT imaging. This study revealed a relatively equal distribution of pneumothorax among men and women. In contrast, previous studies have identified male sex as a risk factor for primary spontaneous pneumothorax (33). In this study, 67% of patients with BHDS who had a history of pneumothorax were nonsmokers, and 61% of those without a pneumothorax were also nonsmokers, supporting the view that smoking is not a risk factor for pneumothorax in our cohort of patients with BHDS. However, in lung studies of other populations, smoking has been shown to be a risk factor for pneumothorax (34). It is important to recognize that smoking can lead to emphysematous cystic and bullous changes in the lung in the general population. However, the location and characteristics of the cystic lesions are usually different from BHDS. The effects of smoking in exacerbating or worsening the pulmonary lung disease (lung cyst and spontaneous pneumothorax) in the setting of BHDS is unknown. In this study, we also found that severity of cutaneous involvement or kidney tumors was not a risk factor for pneumothorax. Sporadic spontaneous pneumothorax is

TABLE 5. RESULT OF UNIVARIATE ANALYSIS INVESTIGATING THE ASSOCIATION BETWEEN TOTAL CYST VOLUME, DIAMETER, AND VOLUME OF LARGEST CYST, AND PRESENCE AND NUMBER OF PNEUMOTHORACES

	n*	Mean	SEM	Min.	Med.	Max.	p Value
Total cyst volume, cm^3							
Pneumothorax							
No	150	8.1	2.0	0	0.8	195	
Yes	48	29.2	8.0	0	7.6	270.2	< 0.0001 (W)
No. of pneumothoraces							
0	150	8.1	2.0	0	0.8	195	
1	17	9.1	3.9	0	5.1	68.1	< 0.0001
2	19	24.5	7.3	0.2	8.5	125.4	(J-T)
3+	12	65.0	27.4	1.2	10.3	270.2	
Volume of largest cyst, cm^3							
Pneumothorax							
No	129†	5.2	1.7	0	0.5	182.2	
Yes	48	12.0	3.5	0	3.0	117.6	< 0.0001 (W)
No. of pneumothoraces							
0	129	5.2	1.7	0	0.5	182.2	
1	17	5.8	3.5	0	1.5	61.0	< 0.0001
2	19	12.7	5.5	0.1	2.7	98.5	(J-T)
3+	12	19.9	9.5	0.7	5.9	117.6	
Diameter of largest cyst, cm							
Pneumothorax							
No	129	1.8	0.1	0.3	1.3	9.5	
Yes	48	2.7	0.2	0.3	2.4	7.7	< 0.0001 (W)
No. of pneumothoraces							
0	129	1.8	0.1	0.3	1.3	9.5	
1	17	2.1	0.3	0.3	1.8	6.6	< 0.0001
2	19	2.7	0.4	0.8	2.3	6.0	(J-T)
3+	12	3.6	0.5	1.3	3.6	7.7	

Definition of abbreviations: J-T = exact Jonckheere-Terpstra test; n = number of patients; W = Wilcoxon rank sum test;
* Based on 177 patients with lung cysts.

associated with apical subpleural lung blebs, whereas individuals with BHDS all have extraapical blebs or cysts (12, 13, 35). The cysts are lined by a smooth wall, with most found in the basilar subpleural region of the lung (12).

In this study, the *BHD* mutation detection rate and spectrum of mutation among patients with BHDS were very similar to our previous reports (5, 6). Similarly, the hot-spot mutation (c.1733ins/delC) in exon 11 was the most common mutation in

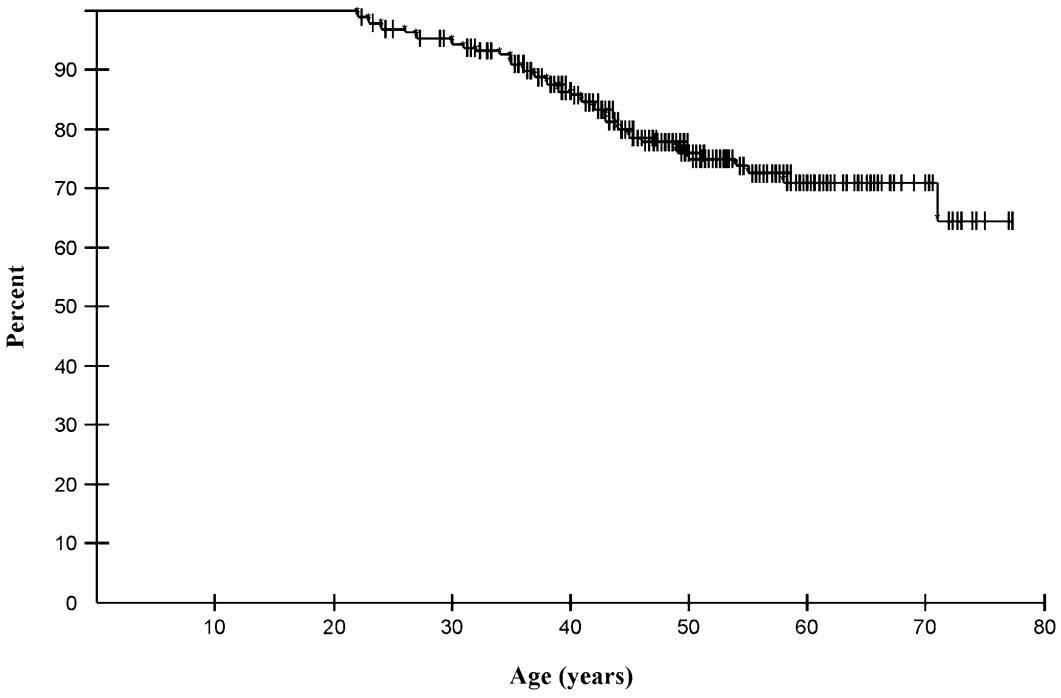

Figure 2. Kaplan-Meier curve showing the association between age and the first spontaneous pneumothorax among patients with Birt-Hogg-Dubé syndrome.

TABLE 6. ANALYSIS INVESTIGATING THE ASSOCIATION BETWEEN EXON LOCATION OF THE *BHD* MUTATION AND PRESENCE AND NUMBER OF PNEUMOTHORACES

	Pneumothorax			No. of Pneumothoraces				
Exon	No	Yes	p Value	0	1	2	3+	p Value
5	7	0		7	0	0	0	
6	4	2		4	2	0	0	
7	10	1		10	1	0	0	
9	9	8	0.01	9	2	3	3	0.012
11	57	18	(M)	57	4	10	4	(K-W OC)
12	4	5		4	1	3	1	
13	0	2		0	1	1	0	
14	2	0		2	0	0	0	

Definition of abbreviations: K-W OC = Kruskal-Wallis test for ordered columns M = Mehta's modification of Fisher's exact test.

this study. In this report, the hot-spot mutation was present in 48% of BHDS families, whereas this mutation in exon 11 was reported in 53% of BHDS families previously (6). One major difference from our previous report is that all patients with BHDS included in this study were only evaluated at the NIH Clinical Center and patients seen on field trips were excluded. This criterion was needed for a systematic chest screening evaluation of lung cysts of all patients. Recently, Painter and colleagues (13) reported on a large Finnish family with primary spontaneous pneumothorax in 8 members and 14 family members who had lung bullae (cysts) on high-resolution CT examination. Direct sequencing of genomic DNA from affected individuals revealed a 4-bp deletion in the first exon of the *BHD* gene. This mutation was not present among our patients with BHDS. Similarly, Graham and coworkers reported two different nonsense mutations (E315X and R477X) in two different families with primary spontaneous pneumothorax (12). These nonsense *BHD* mutations were not identified in our cohort of patients. Painter and colleagues and Graham and coworkers reported that these families lack dermatologic findings. However, dermatologic examinations were not conducted in both studies. On the other hand, we recognized that BHDS can occur in the absence of skin lesions, although it is uncommon.

In this study, we also investigated potential genotype–pulmonary relationships in our patients with BHDS. In general, we found no associations between *BHD* mutation status, or mutation types, and lung cysts parameters and pneumothorax. However, an analysis showed that individuals with *BHD* mutations in exon 9 were associated with more lung cysts than individuals with mutations in other exons. In addition, we found that the size and volume of the largest lung cyst differed significantly by exon, and that these were greater in individuals with *BHD* mutation in exons 9 and 12 than in those with mutations in other exons. These findings suggest that there may be an association between mutation location and lung cyst number and size. It is of interest that recently we also reported that 40% (7/17) of patients with BHDS with putative splice-site mutations in intron 9 (predicted to cause exon 9 skipping) developed renal tumors (6). This is a significantly higher frequency of renal tumors than the overall frequency in all mutation carriers. These two independent observations suggest that exon 9 may have functional importance. These findings need to be confirmed in a future study with a larger number of patients with BHDS. We also found variability of expression of lung cysts and spontaneous pneumothorax both between and within families. These findings suggest that the existence of other genetic and/or environmental factors may also influence the pulmonary phenotype. In addition, the number of lung cysts and pneumothoraces was not a good predictor for kidney cancer status.

The differential diagnosis for a patient with a history of familial spontaneous pneumothorax and diffuse pulmonary cystic changes includes TSC (25), α_1-antitrypsin deficiency (21), Marfan syndrome (22), Ehlers-Danlos syndrome (23), LAM (24), LCH (26), CF (27), primary spontaneous pneumothorax (15), and

TABLE 7. ANALYSIS INVESTIGATING THE ASSOCIATION BETWEEN EXON LOCATION OF THE *BHD* MUTATION AND TOTAL NUMBER OF CYSTS, LARGEST CYST VOLUME, AND DIAMETER

Variable	Exon	n	Mean	SEM	Med.	Min.	Max.	p Value
Total no. of cysts	5	7	4	1.6	2	0	12	
	6	6	18	5.8	19	2	38	
	7	11	8.7	2.6	6	0	30	
	9	17	34.2	7.2	32	4	135	0.0002 (K-W)
	11	75	12.8	1.8	7	0	66	
	12	9	13.7	4.7	9	0	45	
	13	2	34		34	31	37	
	14	2	1.5		1.5	1	2.0	
Diameter of largest cysts, cm	5	6	0.8	0.1	0.7	0.6	1.2	
	6	6	2.4	1.0	1.6	0.9	7.1	
	7	10	1.2	0.2	1.1	0.5	2.1	
	9	17	2.6	0.4	1.9	0.8	5.8	0.005 (K-W)
	11	68	1.7	0.1	1.3	0.3	6.4	
	12	8	3.5	1.0	2.6	0.6	8.0	
	13	2	1.7		1.7	1.6	1.8	
	14	2	1.0		1.0	1	1.1	
Volume of largest cysts, cm^3	5	6	0.3	0.1	0.2	0.1	0.6	
	6	6	31.5	30.1	0.7	0.4	182.2	
	7	10	0.7	0.3	0.4	0.02	2.5	
	9	17	10.4	4.6	2.7	0.04	72.9	0.01 (K-W)
	11	68	2.7	0.7	0.6	0.01	29.5	
	12	8	14.0	7.0	2.7	0.03	52.8	
	13	2	1.7		1.7	1.6	1.8	
	14	2	0.35		0.35	0.34	0.37	

Definition of abbreviation: K-W = Kruskal-Wallis test; n = number of patients.
* Volume units.

BHDS. The distribution of cystic lung changes in radiologic studies may be helpful in distinguishing these diseases. Relative sparing of lung bases from cystic changes is seen in LCH but not in BHDS and LAM. Obstructive findings in a patient with diffuse lung infiltrates are uncommon but can be seen in LAM and LCH. Pulmonary conditions in the general population, including idiopathic pulmonary fibrosis, *Pneumocystis carinii*, lymphocytic interstitial pneumonia, and septic emboli, are also part of the differential diagnosis of cystic lung lesions. Integrating the clinical context is critical in the differential diagnosis of familial spontaneous pneumothorax. Patients' family history and physical examination may provide clues to the nature of the diffuse lung cystic disease. Pulmonary LAM is almost exclusively in women of reproductive age (24). Family history of inheritable skin disorders include TSC, BHDS, LCH, Marfan syndrome (22), and Ehlers-Danlos syndrome (23). The dermatologic manifestations in these syndromes may be helpful in distinguishing these disorders. Patients with BHDS have multiple fibrofolliculomas and/or trichodiscoma, whereas patients with LCH present with scaly patches that histologically show an infiltrate of lymphocytes, eosinophils, and Langerhans cells in the skin. Dermatologically, BHDS and LCH are very distinct. However, TCS and BHDS may be difficult to distinguish. Patients with TSC usually show angiofibromas, hypopigmented macules, shagreen patch, and/or periungual fibromas, and patients with Ehlers-Danlos syndrome typically have fragile thin skin, easy bruising, scarring, and/or hyperextensibility.

Treatment of spontaneous pneumothorax in our patients with BHDS varied from simple observation to open thoracotomy with pleurodesis and lung resection. Seven primary treatment approaches were reported as being used over the study period, including the following: observation alone, tube thoracostomy alone, tube thoracostomy with chemical pleurodesis, thoracotomy with mechanical pleurodesis, and thoracotomy with lung resection. Because different physicians at different hospitals treated patients with a variety of treatment modalities, we cannot exclude that these variables are confounding the risk for recurrence of pneumothorax.

The treatment of pneumothorax in patients with BHDS is similar to the approach taken for any patient with spontaneous pneumothorax. It ranges from observation with repeated radiographic examinations in asymptomatic patients to urgent intervention to evacuate air from the intrapleural space and to prevent recurrence. The mode of therapy is dictated by the clinical presentation of the patient, the chronicity of the condition, and the underlying lung conditions that induced the development of pneumothorax. Placement of a tube thoracostomy enables evacuation of pleural air, and reexpansion of the compressed portion of the lung, and provides a means for chemical pleurodesis. For patients with discreet lung bullae or blebs, or those with recurrent pneumothoraces, treatment may include surgical intervention (thoracotomy or video-assisted thoracoscopy) in combination with mechanical pleurodesis and resection of lung bullae when present. Prospective treatment trials are needed to investigate the best treatment of BHDS-associated pneumothoraces.

The clinical presentation of spontaneous pneumothorax in patients with BHDS is variable. Furthermore, a spontaneous pneumothorax may not be detected on a plain chest X-ray; therefore, it may be overlooked. We advised our patients to inform medical examiners that they have a condition that predisposes them to spontaneous pneumothorax. Although in BHDS it is unknown how to prevent pneumothoraces, certain measures can decrease the risk of developing one. Patients should be cautioned about the increased risk of pneumothorax with scuba diving and air travel due to ambient pressure effects, especially if they have chest symptoms such as pain, discomfort, and/or shortness of breath. We have not observed fatalities or chronic debilitation associated with BHDS lung cysts or pneumothoraces.

In conclusion, this study describes the unique pulmonary features, genetic characteristics, and risk factors for pneumothorax in 198 patients with BHDS. It is important to recognize that, based on the temporality limitations of the study, we cannot clearly determine the true relationship between the number of lung cysts and the risk for spontaneous pneumothorax because, in most cases, the pneumothorax was documented and confirmed before the initiation of study. However, our study has shown a significant association between the lung cysts (number and location) and pneumothorax. Our study contributes to the understanding of the genetic basis of hereditary spontaneous pneumothorax. A prospective study following a cohort of patients should be conducted to validate our present findings. Recognition of the pulmonary features associated with BHDS will improve the diagnosis and treatment of patients with BHDS. Furthermore, recognition of the diagnosis of BHDS will also provide awareness to patients and health care providers of the need for screening and surveillance for renal neoplasms. Future molecular studies may be able to demonstrate if the *BHD* gene is involved in the etiology of sporadic spontaneous pneumothorax and/or emphysema.

***Conflict of Interest Statement*:** J.R.T. is an inventor on a patent application that has been filed for the *BHD* gene. S.E.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.M.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. O.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.-H.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.S.S. is an inventor on a patent application that has been filed for the *BHD* gene. L.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.Z. is an inventor on a patent application that has been filed for the *BHD* gene. P.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.M.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. W.M.L. is an inventor on a patent application that has been filed for the *BHD* gene.

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