Mutational and Radiographic Analysis of Pulmonary Disease Consistent with Lymphangioleiomyomatosis and Micronodular Pneumocyte Hyperplasia in Women with Tuberous Sclerosis

DAVID NEAL FRANZ, ALAN BRODY, CRISTOPHER MEYER, JENNIFER LEONARD, GAIL CHUCK, SANDRA DABORA, GOPALAN SETHURAMAN, THOMAS V. COLBY, DAVID J. KWIATKOWSKI, and FRANCIS X. McCORMACK

Departments of Pediatrics, Neurology, Radiology, and Medicine, Division of Pulmonary and Critical Care Medicine, and the Center for Biostatistical Services, University of Cincinnati College of Medicine, Cincinnati, Ohio; Department of Pathology and Laboratory Medicine, Mayo Clinic, Scottsdale, Arizona; and Department of Medicine, Division of Hematology, Brigham and Women's Hospital, Boston, Massachussetts

Lymphangioleiomyomatosis (LAM) and multifocal micronodular pneumocyte hyperplasia (MMPH) produce cystic and nodular disease, respectively, in the lungs of patients with tuberous sclerosis. The objective of this study was to prospectively characterize the prevalence, clinical presentation, and genetic basis of lung disease in TSC. We performed genotyping and computerized tomographic (CT) scanning of the chest on 23 asymptomatic women with tuberous sclerosis complex (TSC). Cystic pulmonary parenchymal changes consistent with LAM were found in nine patients (39%). These patients tended to be older than cyst-negative patients (31.9 ± 7.6 yr versus 24.8 ± 11.6 yr, $p = 0.09$). There was no correlation between presence of cysts and tobacco use, age at menarche, history of pregnancy, or estrogen-containing medications. Three of the cyst-positive patients had a prior history of pneumothorax. Pulmonary function studies revealed evidence of gas trapping but normal spirometric indices in the cyst-positive group. All nine cyst-positive patients had angiomyolipomas (AML), which were larger ($p < 0.05$) and more frequently required intervention ($p = 0.08$) than cyst-negative patients (8 of 14 with AMLs, $p < 0.05$). Ten patients (43%) had pulmonary parenchymal nodules. Pulmonary nodules were more common in women with cysts (78% versus 21%, $p < 0.05$), and 52% of all patients had either cystic or nodular changes. TSC2 mutations were identified in all cyst-positive patients who were tested ($n = 8$), whereas both TSC1 and TSC2 mutations were found in patients with nodular disease. Correlation of the mutational and radiographic data revealed one pair of sisters who were discordant for cystic disease, two mother–daughter pairs who were discordant for nodular disease, and no clear association between cyst development and a specific mutational type. This prospective analysis demonstrates that cystic and nodular pulmonary changes consistent with LAM and MMPH are common in women with TSC.

Keywords: lymphangioleiomyomatosis; multifocal micronodular pneumocyte hyperplasia; cystic lung disease; tuberous sclerosis; angiomyolipoma

Lymphangioleiomyomatosis (LAM) is a rare lung disease that is characterized by cystic change and infiltration of the pulmonary parenchyma with histologically benign cells resembling smooth muscle cells (1). LAM occurs almost exclusively in women, but consistent clinical presentations (2) and biopsy-confirmed LAM (3) have also rarely been reported in men. Registry-based efforts to locate LAM patients in France (4) and the United Kingdom (5) have identified 70 and 60 patients, respectively, suggesting a minimum prevalence of 2–6 per million women in each of those countries (based on United Nation's 1998 census [6]). Patients with LAM suffer a variably progressive course of dyspnea on exertion, frequently punctuated by recurrent pneumothoraces and chylous pleural effusions. After an average age of onset of 35 yr, the average survival at 8.5 yr has been variably reported at 38% (7), 58% (4), and 78% (8). LAM is empirically managed with antiestrogen therapies, based largely on the observed gender predilection and reports that birth control pills and pregnancy can worsen the disease (9, 10), but there is no conclusive evidence that hormonal fluxes are a pathogenic influence (11) or that hormonal suppression is an effective treatment. The majority of LAM patients in retrospective clinical series presented with dyspnea with routine activities and advanced, irreversible disease (4, 5, 7, 8, 12, 13). The design of definitive clinical trials to determine the effectiveness of empiric and experimental therapies would be greatly facilitated by the identification of LAM patients in greater numbers and in earlier stages of their illness.

LAM occurs without evidence of other genetic disease (sporadic or S-LAM), and also in patients with tuberous sclerosis (TSC-LAM) (14). Tuberous sclerosis complex (TSC) is an autosomal dominant syndrome characterized by hamartomatous growths in the skin, eyes, kidney, and central nervous system. TSC is known to result from mutations in the tumor suppressor genes TSC1, encoding hamartin, on chromosome 9q34 (15), and TSC2, encoding tuberin, on chromosome 16p13.3 (16). The functions of hamartin and tuberin are uncertain, but the two proteins bind each other with high affinity, tuberin has GTPase activity for rap1 (17), and hamartin may play a role in adhesion and cytoskeletal organization (18). TSC-LAM occurs in patients with germline mutations in TSC1 or TSC2 (19), which are inherited in one-third of patients and which arise *de novo* in two-thirds (20). Recent findings indicate that S-LAM can be caused by somatic TSC2 mutations, which occur in patients who do not have germline mutations in TSC genes (21). The prevalence of TSC-LAM has been reported to be between 0.1 and 2.3% of TSC patients (2, 22, 23). However, a recent retrospective analysis of computed tomography (CT) scans and medical records of 78 women (ages > 21) with TSC identified 20 (26%) with cystic pulmonary changes consistent with LAM (26), including seven with biopsy or autopsy confirmed disease, suggesting a much higher prevalence (24). Mother-to-daughter transmission of TSC-LAM, but not S-LAM, has been documented (19, 25). TSC-LAM but not S-LAM has been re-

(*Received in original form November 6, 2000 and in revised form March 7, 2001*)

This work was supported by NIH NINDS NS31535, NIH NCI CA71445, NIH NCI CA86248, and the National Tuberous Sclerosis Association.

Correspondence and requests for reprints should be addressed to Dr. David Neal Franz, Neurology/OSB-5, Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039. E-mail: d.franz@chmcc.org

Am J Respir Crit Care Med Vol 164. pp 661–668, 2001
Internet address: www.atsjournals.org

ported in males (3). Chest X-ray and lung function abnormalities are similar in TSC-LAM and S-LAM series, as are the occurrence of associated manifestations of chylous pleural effusions, pneumothoraces, and hemoptysis (22). Renal angiomyolipomas, which occur in approximately 66% of patients with TSC, are found in up to 47–60% of patients with S-LAM (12, 26). In both TSC-LAM and S-LAM, smooth muscle cell infiltration of the lung parenchyma, airways, lymphatics, and blood vessels is associated with multiple thin-walled cysts. Collectively, the data suggest that S-LAM and TSC-LAM share a common pathophysiological basis.

Multifocal micronodular pneumocyte hyperplasia (MMPH) is another pulmonary manifestation of TSC, which was described by Popper and coworkers in 1991 (27). MMPH presents with multiple, diffuse pulmonary nodules on chest roentgenogram or high-resolution CT scan, due to nodular proliferation of alveolar type II cells (28, 29). MMPH has been reported in males and females with and without TSC (29), in males and females with TSC and LAM (3, 29), and in women with S-LAM (29). The frequency of concurrence of LAM and MMPH in patients with TSC is not clear. Less than 20 cases of biopsy-confirmed MMPH have been published in the literature, and the physiological and prognostic consequences of MMPH are not known.

The purpose of this study was to prospectively define the prevalence of pulmonary abnormalities in women followed in a University TSC clinic who were asymptomatic at the time of enrollment, and to examine the correlation of pulmonary changes with mutational analysis and other clinical features.

METHODS

Study Subjects

Patients for the study were consecutively recruited from the Tuberous Sclerosis Clinic at the University of Cincinnati Children's Hospital Medical Center during the period August 1998–April 2000. Patients were included if they were postmenarchal, nonpregnant females, met diagnostic criteria for TSC based on modified Gomez criteria (30), and were able to cooperate with thoracic CT scanning. Mutational analysis was approved by the Institutional Review Board of the Children's Hospital Medical Center and the Brigham and Women's Hospital, and was performed with the informed consent of the participants. Patients were evaluated with complete history, physical exam, and clinically indicated imaging and pulmonary function studies. The University TSC clinic is staffed by a multidisciplinary team that includes a neurologist, social worker, nurse practitioner, cardiologist, nephrologist, and pulmonologist.

Imaging

High-resolution computed tomography (HRCT) scans of the chest were obtained on all subjects, except for one patient who had a conventional CT performed at her local institution. Subjects were studied with General Electric Lightspeed and CTi scanners (Milwaukee, WI), using an HRCT protocol that included both inspiratory and expiratory images. Data were acquired with 1 mm slice thickness, 1 s scan time, 200 mA at 120 kVp. Images were obtained at 10-mm intervals during voluntary inspiration and 20-mm intervals during voluntary expiration. Images were blindly reviewed for the presence of cysts and nodules by two radiologists, using a window of 1500 and level of −600. It is important to note that this method provides only a qualitative estimate of the number of parenchymal abnormalities, because HRCTs are noncontiguous and survey only about 10% of the lung parenchyma. CT scans were judged to be consistent with LAM if both radiologists agreed that thin-walled cysts with clear margins were present. Cysts were evaluated for number, average and largest size, shape, and wall thickness. Nodules were evaluated for size, number, and calcification. The craniocaudal distribution (upper lung, mid lung, lower lung) and radial distribution (central, midlung, or peripheral) of both cysts and nodules were evaluated. Adenopathy, pleural effusions, and air trapping apparent on comparison of inspiratory and expiratory views were also recorded. The presence and size of renal angiomyolipomas were assessed by abdominal CT or renal ultrasound as part of the routine evaluation of all patients in the clinic.

Pulmonary Function Testing

Pulmonary function tests were obtained only on patients with cystic changes on HRCT scan using a SensorMedic Vmax 22. Spirometry and lung volume data were obtained in the sitting position using standard techniques recommended by the American Thoracic Society (31).

Mutational Analysis

Mutations in the TSC1 and TSC2 genes were identified by denaturing high-performance liquid chromatography (DHPLC) of amplified TSC1 and TSC2 exons, long-range polymerase chain reaction (PCR), and quantitative PCR, as described in detail elsewhere (32, 33). DNA was extracted from leukocytes using standard methods. Mutations were identified by double-stranded sequencing using Big Dye dideoxy nucleotides and the ABI 377 (32), and confirmed by family analysis. Mutation detection efficiency was 82%.

Statistical Analysis

The cyst-positive group and the cyst-negative group were compared using a chi-square test of independence. The variables compared were nodules, multinodular pulmonary disease (>10 nodules), pneumothoraces, angiomyolipomas, angiomyolipomas > 4 cm, and embolization or resection of angiomyolipomas. The cyst-positive group and the cyst-negative group were also compared for age using a Student's *t* test.

RESULTS

Patient Characteristics

A total of 24 women with TSC were enrolled. One subject with severe scoliosis and limited ability to cooperate was excluded from the study, and only the 23 with interpretable CT scans were included in the analysis. None of the patients had active pulmonary symptoms or a suspected diagnosis of LAM on initial presentation to the clinic. The cohort ranged in age from 12 to 51 yr, with a mean of 27.6 ± 10.7 yr (Table 1). A family history of TSC was present in 13 patients (57%), and the cohort included two mother–daughter pairs and one pair of sisters. Of the 22 patients for whom an accurate smoking history was available, 5 patients (22%) were active smokers with an average consumption of 9.8 ± 9.1 pack-years, and 17 patients (74%) had never smoked. The average age at menarche was 12.6 ± 3.4 yr. Half of the patients (11 of 22) had used birth control pills, for an average period of 3.7 ± 3.6 yr. None of the patients had received therapy specifically for LAM, but four patients had been treated with intramuscular medroxyprogesterone for an average duration of 2.7 ± 2.1 yr for birth control. Eleven of 22 patients (50%) had been pregnant once (three patients), twice (five patients), three times (two patients), and seven times (one patient), resulting in a total of 22 term births. The patient with seven pregnancies had three term births, one spontaneous abortion, and three elective abortions. Angiomyolipomas had been documented in 17 patients, including seven patients who had tumors > 4 cm in diameter (Table 2).

The pulmonary history of the patients was reviewed (Table 3). One patient carried a diagnosis of asthma from childhood, and was using rescue inhalers only. Three patients reported a remote history of four spontaneous pneumothoraces. The pneumothoraces had developed 16 yr and 1.5 yr prior to the study in the two single occurrence patients, and 2 and 3 yr earlier in the patient who had had two occurrences. Two of the three patients with pneumothoraces were active smokers at the time of the event; the other patient had never smoked. Three of the pneumothoraces had required chest tube placement and

TABLE 1. PATIENT CHARACTERISTICS

Clinical Feature	All Patients (*n = 23*)	Cyst-negative (*n = 14*)	Cyst-positive (*n = 9*)
Age*	27.6 ± 10.7	24.8 ± 11.6	31.9 ± 7.6
Familial/sporadic	13/10	8/6	5/4
Smoking			
Never	17	11	6
Current, average pack-years	5 (9.8 ± 9.1)	2 (5.5 ± 6.4)	3 (12.7 ± 10.8)
Unknown	1	1	0
Age at menarche, yr	12.6 ± 3.4	12.5 ± 4.4	12.9 ± 1.2
Depoprovera, average yr	4 (2.7 ± 2.1 yr)	3 (2.1 ± 2.1 yr)	1 (4.5 yr)
Pregnancy, average/patient	11 (2)	6 (1.8)	5 (2.2)
BCP use, average yr	11 (3.7 ± 3.6 yr)	7 (2.9 ± 1.8 yr)	4 (4.1 ± 4.4 yr)

Definition of abbreviation: BCP = birth control pill.
* Comparison of cyst versus no cysts, $p < 0.09$ (according to text).

one patient had undergone pleurodesis. Although two of the three patients with pneumothoraces had used birth control pills and two had been pregnant, the onset of pneumothorax did not correlate with estrogen therapy or pregnancy in any case. No other active pulmonary symptoms or complications were reported, including dyspnea on exertion, chest pain, chronic cough, chyloptysis, or hemoptysis.

TSC1 and TSC2 Mutations

TSC gene mutations were identified in 15 patients (Table 4, Figure 1), five were unknown and three have not been tested. Two patients had TSC1 mutations, a mother and daughter, and 13 patients had TSC2 mutations, including one mother–daughter pair and one set of sisters. The TSC1 mutation was a nonsense point mutation in exon 15 (not shown in Figure 1). The TSC2 mutations consisted of five small deletions or insertions (1–34 bp), one large deletion of exon 9, one large duplication, three missense point mutations, and two splice mutations.

Radiographic Findings

HRCT was performed in 22 patients and conventional CT was performed in one patient (Tables 3 and 4). Abnormal chest CTs, defined by the presence of cysts or nodules or both, were found in 12 patients (52%). The most striking abnormality, seen in nine patients (39%), was the presence of variable numbers of cysts without fibrotic changes (Figure 2). Five patients had more than 30 cysts counted on the available HRCT cuts, two patients had 11 to 30 cysts, and two patients had less than 10 cysts. Cysts were either mid to upper lung zone predominant or randomly distributed in patients with more than 30 cysts, and either peripheral or upper lobe predominant in patients with less than 30 cysts. The average cyst size in each subject ranged from 4 to 10 mm (average 6 ± 2 mm) and the largest cysts in individual patients ranged in diameter from 3 to 25 mm (average 12 ± 7 mm.). The walls of most cysts were thin (< 1 mm) and well defined, but in two patients they were barely perceptible.

Ten patients were found to have noncalcified pulmonary nodules in two distinct patterns; seven patients had scattered nodules from 3 to 10 mm in size, and three patients had numerous miliary 1 to 3-mm nodules (Figure 3). Five patients had 11 to 30 or more nodules counted, and five patients had fewer than 10 nodules. The distribution of the nodules was upper lung zone predominant (two patients) or peripheral (three patients) or both (five patients) (Figure 4). Seven of the nine patients with cysts also had nodules (78%), whereas only 3 of 14 (21%) patients without cysts had nodules ($p < 0.05$). Multinodular pulmonary disease, arbitrarily defined as > 10 nodules counted, was more common in the cyst-positive patients (4 of 9) than in the cyst-negative patients (1 of 14) ($p < 0.05$). Air trapping was noted in four patients in the cyst-positive group, including two smokers and one patient with asthma, and in two nonsmoking patients in the cyst-negative group. All but one of the six patients with air trapping also had pulmonary nodules. Chylous effusions were not found. Finally, four patients, including two in the cyst-positive group, were found to have evidence of hilar or mediastinal adenopathy on CT.

Comparison of Clinical Features of the Cyst-positive and Cyst-negative Groups

The clinical features of the cyst-positive and cyst-negative patients were compared (Table 1). The cyst-positive patients tended to be older (31.9 ± 7.6 yr) than the cyst-negative patients (24.8 ± 11.6 yr), but the difference was not significant ($p = 0.09$). Three of the patients with pulmonary cysts were active smokers (average 12.7 ± 10.8 pack-years), compared with two cyst-negative patients (average 5.5 pack-years). Age at menarche, history of pregnancy, use of estrogen supplements, and use of depoprovera for birth control were similar in both groups.

Three patients with cysts experienced a total of four pneumothoraces, compared with none of the patients without cysts (Tables 3 and 4) ($p < 0.05$). The patient with mild childhood onset asthma was in the cyst-positive group. Pulmonary spirometry was attempted on all patients in the cyst-positive group, but only five patients were able to cooperate. Normal values were obtained for forced expiratory volume in 1 s (FEV_1) (91.0 ± 5.5% predicted) and forced vital capacity (FVC) (95.3 ± 8.1% predicted), FEV_1/FVC ratios (0.89 ± 0.12) and diffusion capacity for carbon monoxide (86.4 ± 25% of predicted). Although the total lung capacity was normal (95.0 ± 12.4% of predicted), elevations of the functional residual capacity (117.8 ± 1.3% of predicted) and the residual volume (129.5 ± 54.8% of predicted) suggested air trapping. Pulmonary func-

TABLE 2. RENAL MANIFESTATIONS IN PATIENTS WITH AND WITHOUT PULMONARY CYSTS

Clinical Feature	All Patients (*n = 23*)	Cyst-negative (*n = 14*)	Cyst-positive (*n = 9*)
AML*	17 (74)†	8 (57)	9 (100)
AML > 4 cm*	7 (30)	2 (14)	5 (56)
AML embolized	5 (22)	1 (7)	4 (44)
AML resected	3 (9)	1 (7)	2 (22)

Definition of abbreviation: AML = renal angiomyolipoma.
* Comparison of cyst-positive and cyst-negative, $p < 0.05$.
† Percentages in parentheses.

TABLE 3. PULMONARY MANIFESTATIONS IN PATIENTS WITH AND WITHOUT PULMONARY CYSTS

Clinical Feature	All Patients (*n = 23*)	Cyst-negative (*n = 14*)	Cyst-positive (*n = 9*)
Nodules*	10 (43)†	3 (14)	7 (78)
Nodules >10*	5 (22)	1 (7)	4 (44)
Air trapping	6 (26)	2 (14)	4 (44)
Thoracic adenopathy	4 (17)	2 (14)	2 (22)
Pneumothorax*	3 (13)	0	3‡ (33)

* Comparison of cyst-positive and cyst-negative, $p < 0.05$.
† All values = number of patients (percentages in parentheses).
‡ One patient had two pneumothoraces for a total of 4.

tion tests were not obtained on patients in the cyst-negative group.

Renal angiomyolipomas were found in all nine of the cyst-positive patients, compared with 8 of 14 (57%) of the patients in the cyst-negative group ($p < 0.05$) (Table 2). Angiomyolipomas were also larger in the cyst-positive group (56% of angiomyolipomas > 4 cm) than in the cyst-negative group (14% of angiomyolipomas > 4 cm) ($p < 0.05$). Four patients in the cyst-positive group had undergone six interventions (embolization or resections) for their angiomyolipomas, compared with one patient in the cyst-negative group who had undergone resection and embolization for angiomyolipomas ($p = 0.08$).

Family History and Mutational Analyses

Five patients in the cyst-positive group had a family history of TSC and four were sporadic cases, compared with eight familial and six sporadic patients in the cyst-negative group (Table 4). TSC2 mutations were found in eight patients in the cyst-positive group and five patients in the cyst-negative group. TSC1 mutations were found in two patients in the cyst-negative group, in a mother–daughter pair. The TSC genotype was not determined in one cyst-positive patient and seven cyst-negative patients.

Mutations identified in both cyst-positive and cyst-negative patients were distributed throughout the TSC2 gene, and were of several types (Table 4, Figure 1). In three cyst-positive patients, but none of the cyst-negative patients, mutations were identified near the 3′ end of the coding region of TSC2. Due to the small sample size, the significance of this observation is uncertain.

All three familial pairs in the study were discordant for TSC-related pulmonary abnormalities. One of the cyst-positive patients with a protein truncating TSC2 splice mutation had a sister in the cyst-negative group who was 4 yr younger. In addition, two mother–daughter pairs in the cyst-negative group were discordant for the presence of nodular pulmonary changes, including the family with a protein-truncating splice mutation in TSC1. When the clinical features of family members with and without pulmonary abnormalities were compared (Table 4), there were no obvious differences.

DISCUSSION

HRCT screening of 23 postmenarchal female TSC patients identified cystic and/or nodular radiographic abnormalities in 52% (12 of 23). Cystic lesions consistent with LAM were found in 39% (9 of 23), and nodular changes were seen in 43% (10 of 23). Both findings were present in 30% of patients (7 of 23) and multinodular changes consistent with MMPH were more common in patients with cysts. The cyst-positive patients, on average, were older and had large renal angiomyolipomas that more commonly required intervention. All three pairs of patients from TSC families were discordant for pulmonary disease including two for nodular change and one for

TABLE 4. MUTATION FINDINGS IN CYST-POSITIVE AND CYST-NEGATIVE TSC PATIENTS

Mutation	Mut Type	Age	F/S	Tob (*py*)	BCP (*yr*)	Preg (*No.*)	Cyst (*No.*)	Nod (*No.*)	Ptx (*No.*)	AML (*cm*)	AML Proc
Cyst-positive											
2:E27, 3214delA	del	44	F	–	1	2	> 30	> 10	1	< 4	–
2:E9, 903-922del20	del	23	F	–	–	–	> 30	< 10	2	> 4	2
2:E39, 5126C>T,P1709L	mis	37	S	–	–	–	> 30	–	–	> 4	1
2:E9, 465 bp del	lgdel	39	F	25	5	2	> 30	> 10	–	> 4	2
2:I18, 2098-1G>A	spl	28	F-Sis1	8	2	7	> 10	< 10	–	< 4	–
Not tested*		25	S	–	–	–	< 10	< 10	–	> 4	1
2:E18, 1960-61del GG	del	31	S	–	–	–	< 10	–	–	> 4	–
2:E38, 5024C>T,P1675L	mis	35	F	–	–	2	> 10	> 10	–	< 4	–
2:E38, 5051-5068,+16del34	del	21	S	5	3.5	1	> 30	> 10	1	< 4	–
Cyst-negative											
Unknown		26	F-D2	–	5	–	–	> 10	–	–	–
Unknown		51	F-M2	1	.75	2	–		–	–	–
2:duplication exons 16-25	lgdup	13	S	–	–	–	–	–	–	–	–
2:I7, 774+2T>A	spl	14	S	–	–	–	–	–	–	< 4	–
2:E16, 1832G>A, R611Q	mis	26	S	–	–	–	–	–	–	> 4	2
1:E15, 1746C>T, R509X	non	43	F-M3	–	6	3	–	< 10	–	< 4	–
1:E15, 1746C>T, R509X	non	20	F-D3	–	–	–	–	–	–	–	–
Not tested		13	S	–	–	–	–	–	–	–	–
2:E9, 871insC	ins	16	S	–	1	–	–	–	–	> 4	–
Not tested		16	S		–	1	–	–	–	–	–
Unknown		34	F	10	13	2	–	–	–	< 4	–
Unknown		30	F	–	2	3	–	< 10	–	< 4	–
2:I18, 2098-1G>A	spl	24	F-Sis1	–	1	1	–	–	–	< 4	–
Unknown		15	F	–	–	–	–	–	–	< 4	–

Definition of abbreviations: Standard nomenclature is used to designate mutations (39); del = deletion; mis = missense; lgdel = large deletion; spl = splice; lgdup = large duplication; non = nonsense; ins = insertion; F = familial; S = sporadic; Sis1 = sister-family 1; M2 = mother-family 2; D2 = daughter-family 2; Nod = pulmonary nodule; Tob = tobacco; py = pack year; preg = pregnancy; AML = angiomyolipoma; AML Proc = number of AML procedures of nephrectomy or embolectomy; BCP = birth control pills; Ptx = number of pneumothoraces.
* Unknown–mutational analysis attempted but not successful.

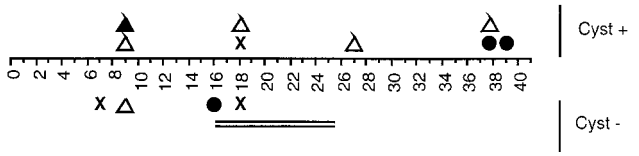

Figure 1. Distribution of TSC2 mutations in cyst-positive and cyst-negative patients. The locations of mutations including small deletions and insertions (*open triangle*), missense point mutations (*filled circles*), large deletions (*closed triangle*), splice mutations (x), and duplications (*double lines*) are plotted with respect to the 41 exons of the TSC2 gene.

cystic changes. TSC2 mutations were more common than TSC1 mutations in all patients, and were distributed throughout the TSC2 gene without clear clustering. This prospective analysis demonstrates that cystic and nodular pulmonary changes consistent with LAM and MMPH are common in women with TSC.

Similar to mutational analyses in large cohorts of TSC patients (19, 32, 34), mutations identified in this cohort of patients with pulmonary nodular and cystic changes were distributed throughout TSC1 and TSC2. TSC2 mutations were found in all cyst-positive patients from whom DNA samples were available, which suggests that TSC2 mutations are more commonly associated with this clinical feature than mutations in TSC1. However, TSC2 mutations account for about five times as many TSC cases as TSC1 mutations, in general (19, 32, 34). Three particularly interesting mutations were found at the 3′ end of the TSC2 gene in the cyst-positive group only. The missense mutation and small deletion in exon 38 are located in the region of homology to rap1GAP, encoded by exons 34–38 (16, 17), which has previously been identified as a minor hotspot for TSC2 mutations (34). The missense mutation in exon 39 is located in a region that may interact with the rab 5 adaptor, rabaptin 5, encoded by exons 38–41 (35). TSC mutation information is available from 14 TSC patients with clinical LAM (19, 36–39), and there are three TSC-LAM patients with missense mutations or small deletions in exons 40 and 41 (39). Collectively, these data suggest that LAM can be caused by any of the diverse mutations that inactivate the TSC2 gene and cause TSC.

Pulmonary parenchymal cysts are very rarely seen in the normal population or in patients with asthma (40). The occurrence of multiple lung cysts in nonsmoking women with tuberous sclerosis is most consistent with LAM, as is the thin-walled appearance of the cysts in our patients. Cysts or cyst-like lesions can also occur in pulmonary emphysema and pulmonary eosinophilic granuloma, but in these diseases the cyst walls are imperceptible and thick walled, respectively. In addition, emphysema is rare in young patients and both diseases are usually associated with heavy smoking, which characterizes only one of the cyst-positive patients in this study.

The frequency of lung involvement in TSC was originally estimated to be less than 1% by Dwyer and coworkers (2) and Jao and coworkers (23), and later by Castro and coworkers to be approximately 2.3% of all TSC patients (22). Our results are consistent with the prevalence data that was recently reported by Costello and coworkers (41), who found that cystic pulmonary parenchymal changes are much more common in TSC than previous series suggest. They performed a retrospective chart review of 478 patients who were referred to the Mayo clinic over a 23-yr period and identified 78 adult women (age > 21) who met the modified Gomez criteria for the "definite" diagnosis of TSC (30). Of those patients, 26 had images of the chest on abdominal or chest CT scans that were available for evaluation, including seven patients who also had pathological specimens of the lung available from surgical biopsy or autopsy. Twenty patients were found to have cystic changes on CT and/or biopsy documented LAM ($n = 7$), including 12 patients who had pulmonary symptoms that originally prompted their evaluation. Their 26% value for the prevalence of cystic pulmonary change in TSC is a minimum estimate, as 52 of their 78 patients did not have a CT scan, lung biopsy, or autopsy. The 39% prevalence of cystic change in TSC from the current study is an estimate of true prevalence based on the prospective analysis of an unselected, currently asymptomatic TSC patient population.

We also observed a strong association between the presence of renal angiomyolipomas and pulmonary cystic changes. The angiomyolipomas in the cyst-positive patients were large and more often required resection or embolization. This association is especially interesting in light of recent genetic findings suggesting a possible metastatic mechanism for the devel-

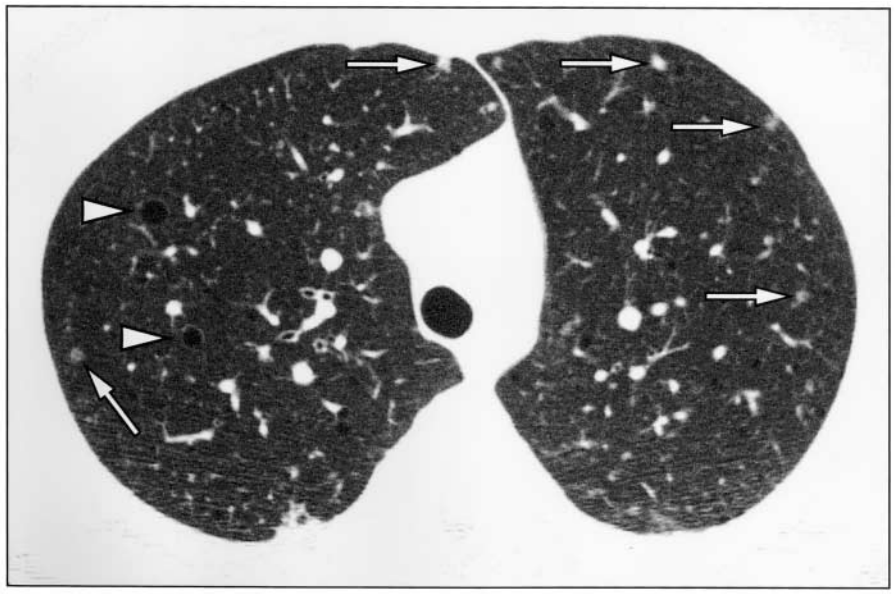

Figure 2. HRCT image of a 21-yr-old woman with both cysts (*arrowheads*) and nodules (*arrows*). Round cysts from a few millimeters 10 mm in diameter with thin walls are distributed throughout both lungs. Multiple 3 to 5-mm nodules, with an upper lung and peripheral predominance, are present bilaterally.

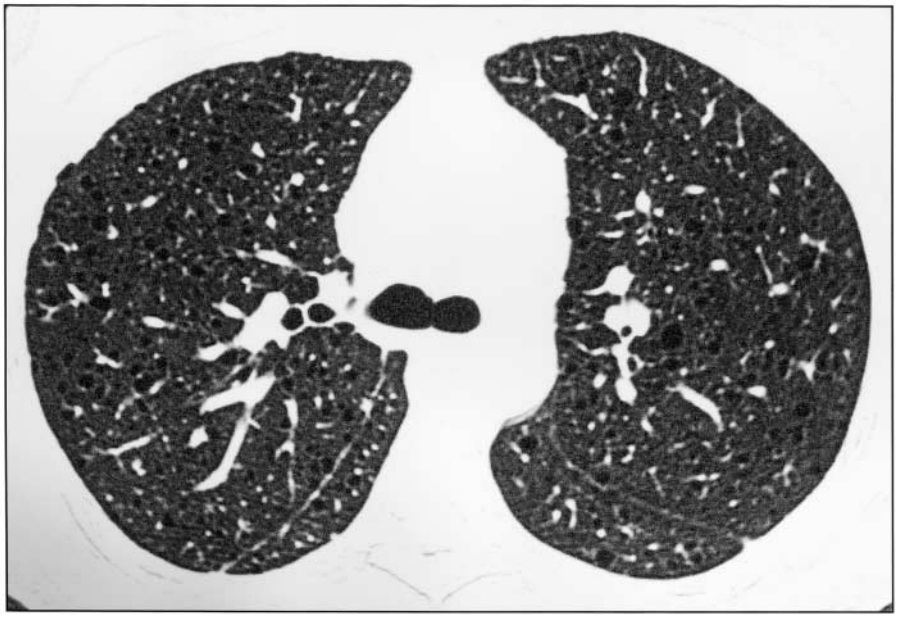

Figure 3. HRCT image of a 23-yr-old woman with extensive cysts and miliary nodules. Innumerable round cysts with imperceptible walls are present throughout both lungs; 1 to 2-mm nodules are scattered throughout both lungs.

opment of S-LAM (21). Specifically, matching TSC2 mutations were identified in the kidney and lung lesions from four patients with S-LAM, and these mutations were not present in normal lung, kidney, or blood cells from the same patient (21). These observations fit a model in which smooth muscle cells migrate from renal angiomyolipoma to lung, or arise from a disseminated common precursor cell. Our observations support this model for development of TSC-LAM, and suggest that a large angiomyolipoma tumor burden may enhance seeding of the lung with cells that result in cystic changes in the pulmonary parenchyma. The recurrence of LAM lesions in the donor lung of three LAM patients who have undergone lung transplantation is also consistent with this metastatic theory (42, 43). However, some evidence suggests that recurrent LAM after lung transplant may be due to proliferation of donor lung cells, suggesting a circulating factor model for LAM pathogenesis (44).

The prevalence of pulmonary nodules in this TSC patient cohort is also much higher than previously recognized (29). Nodular changes in the lungs occurred in most of the TSC patients who had cysts, but they also occurred in some cyst-negative patients. Given that HRCT is not as sensitive as histological examination for detection of nodules, micronodular disease may be even more common in TSC than the radiographic data suggest. We have identified a number of patients with TSC-LAM who have cystic disease without nodular change on histological examination (unpublished observation, T.V. Colby), however, indicating that MMPH does not always coexist with LAM in this patient population. This area requires further study, as the patchy nature of MMPH and LAM that is evident on HRCT of many TSC-LAM patients may result in a sampling error that limits their histological identification. Although MMPH is the most likely etiology for the nodular radiographic findings, a definitive diagnosis cannot be made on

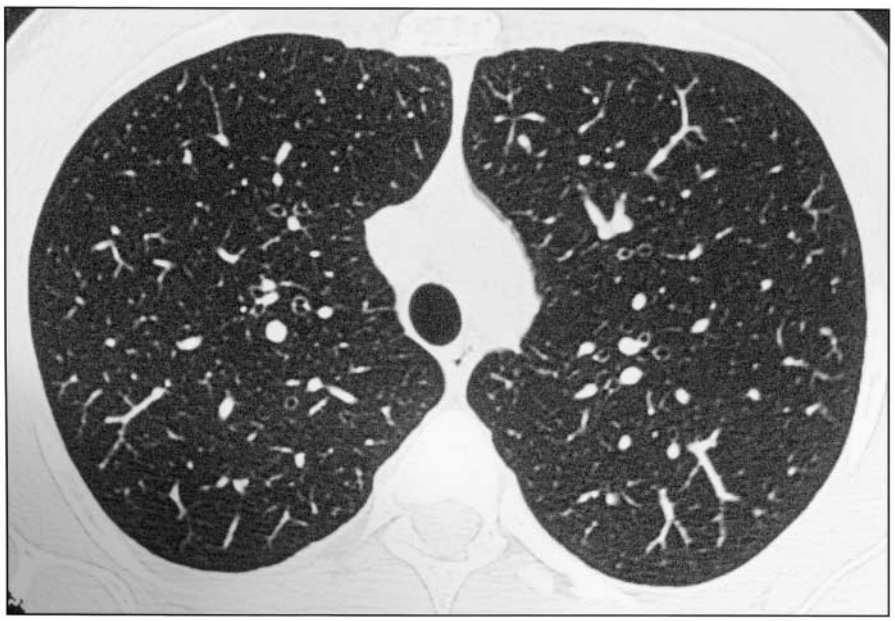

Figure 4. HRCT image of a 26-yr-old woman with miliary nodules; 1 to 2-mm nodules are present with an upper lobe predominance. No cysts are present.

the imaging appearance alone. Histoplasmosis, which is endemic in southwest Ohio, could potentially produce multiple, diffuse, pulmonary nodules that could be confused with MMPH. However, the absence of pulmonary calcifications, which are present in more than 90% of patients with inactive histoplasmosis, makes this diagnosis unlikely (45). Other granulomatous disorders, such as sarcoidosis and mycobacterial disease, and a variety of other nodular lung diseases, such as tumorlets or metastases, could also produce this radiographic pattern. Given the clinical context and the absence of other constitutional symptoms, we are reasonably confident that the nodular disease in the majority of our patients represents MMPH.

There are several important differences between previous reports of S-LAM and TSC-LAM and the present population, many of which can be attributed to early identification. Other than the three patients who had prior pneumothoraces and the one patient with longstanding mild asthma, none of our patients had pulmonary symptoms or signs. Specifically, our patients denied dyspnea at rest or on exertion, frequent cough, chest pain, chyloptysis, or hemoptysis. None of our patients had historical or radiographic evidence of chylous effusions, which are common in other LAM series. The HRCT manifestations of pulmonary cystic disease in our patients were much more limited than in clinically diagnosed LAM, and were peripheral, and mid-to-upper zone predominant rather than diffusely distributed. The pulmonary function abnormalities in the present series were very minor, suggesting only a mild degree of air trapping. Nonetheless, these radiographic features and the concordance with pneumothoraces in three patients, a feature typical of LAM, suggests that HRCT screening of TSC patients identifies a LAM patient population early in the natural history of the disease. Based on the prevalence of TSC and these findings, we estimate that there may be 8,000 TSC patients with early LAM in North America. Further study of these patients, including definition of the natural history of this early phase of LAM, is clearly required. The diagnosis of LAM should be considered in all adult females with TSC to enhance vigilance for complications of dyspnea, effusions, and pneumothoraces, and for counseling regarding potential risk factors for progression of disease including estrogen exposure, cigarette smoking, and pregnancy.

Acknowledgment: The authors wish to thank the LAM Foundation for their assistance.

References

1. Sullivan EJ. Lymphangioleiomyomatosis: a review. *Chest* 1998;114:1689–1703.
2. Dwyer JM, Hickie JB, Garvan J. Pulmonary tuberous sclerosis. Report of three patients and a review of the literature. *Q J Med* 1971;40:115–125.
3. Aubry MC, Myers JL, Ryu JH, Henske EP, Logginidou H, Jalal SM, Tazelaar HD. Pulmonary lymphangioleiomyomatosis in a man. *Am J Respir Crit Care Med* 2000;162:749–752.
4. Urban T, Lazor R, Lacronique J, Murris M, Labrune S, Valeyre D, Cordier JF. Pulmonary lymphangioleiomyomatosis: a study of 69 patients. *Medicine (Baltimore)* 1999;78:321–337.
5. Johnson S. Rare diseases. 1. Lymphangioleiomyomatosis: clinical features, management and basic mechanisms. *Thorax* 1999;54:254–264.
6. *World population*, 1998 ed: Department of Economic and Social Affairs, Population Division; 1998.
7. Kitaichi M, Nishimura K, Itoh H, Izumi T. Pulmonary lymphangioleiomyomatosis: a report of 46 patients including a clinicopathologic study of prognostic factors. *Am J Respir Crit Care Med* 1995;151:527–533.
8. Taylor JR, Ryu J, Colby TV, Raffin TA. Lymphangioleiomyomatosis: clinical course in 32 patients. *N Engl J Med* 1990;323:1254–1260.
9. Shen A, Iseman MD, Waldron JA, King TE. Exacerbation of pulmonary lymphangioleiomyomatosis by exogenous estrogens. *Chest* 1987;91:782–785.
10. Warren SE, Lee D, Martin V, Messink W. Pulmonary lymphangiomyomatosis causing bilateral pneumothorax during pregnancy. *Ann Thorac Surg* 1993;55:998–1000.
11. Wahedna I, Cooper S, Williams J, Paterson IC, Britton JR, Tattersfield AE. Relation of pulmonary lymphangio-leiomyomatosis to use of the oral contraceptive pill and fertility in the UK: a national case control study. *Thorax* 1994;49:910–914.
12. Chu SC, Horiba K, Usuki J, Avila NA, Chen CC, Travis WD, Ferrans VJ, Moss J. Comprehensive evaluation of 35 patients with lymphangioleiomyomatosis. *Chest* 1999;115:1041–1052.
13. Oh YM, Mo EK, Jang SH, Yoo CG, Kim YW, Seo JW, Han SK, Im JG, Shim YS. Pulmonary lymphangioleiomyomatosis in Korea. *Thorax* 1999;54:618–621.
14. Lie JT. Pulmonary tuberous sclerosis, in tuberous sclerosis complex. In: Gomez MR, Sampson JR, Whittemore VH, editors. New York: Oxford University Press, 1999; p. 207–217.
15. van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, Lindhout D, van den Ouweland A, Halley D, Young J, *et al.* Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science* 1997;277:805–808.
16. Consortium ECTS. Identification and characterization of the tuberous sclerosis gene on chromosome 16: The European Chromosome 16 Tuberous Sclerosis Consortium. *Cell* 1993;75:1305–1315.
17. Wienecke R, Konig A, DeClue JE. Identification of tuberin, the tuberous sclerosis-2 product: tuberin possesses specific Rap1GAP activity. *J Biol Chem* 1995;270:16409–16414.
18. Lamb RF, Roy C, Diefenbach TJ, Vinters HV, Johnson MW, Jay DG, Hall A. The TSC1 tumour suppressor hamartin regulates cell adhesion through ERM proteins and the GTPase Rho [see comments]. *Nat Cell Biol* 2000;2:281–287.
19. Jones AC, Shyamsundar MM, Thomas MW, Maynard J, Idziaszczyk S, Tomkins S, Sampson JR, Cheadle JP. Comprehensive mutation analysis of TSC1 and TSC2-and phenotypic correlations in 150 families with tuberous sclerosis. *Am J Hum Genet* 1999;64:1305–1315.
20. Sampson JR, Scahill SJ, Stephenson JB, Mann L, Connor JM. Genetic aspects of tuberous sclerosis in the west of Scotland. *J Med Genet* 1989;26:28–31.
21. Carsillo T, Astrinidis A, Henske EP. Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. *Proc Natl Acad Sci USA* 2000;97:6085–6090.
22. Castro M, Shepherd CW, Gomez MR, Lie JT, Ryu JH. Pulmonary tuberous sclerosis. *Chest* 1995;107:189–195.
23. Jao J, Gilbert S, Messer R. Lymphangiomyoma and tuberous sclerosis. *Cancer* 1972;29:1188–1192.
24. Costello LC, Hartman TE, Ryu JH. High frequency of pulmonary lymphangioleiomyomatosis in women with tuberous sclerosis complex. *Mayo Clin Proc* 2000;75:591–594.
25. Slingerland JM, Grossman RF, Chamberlain D, Trembly CE. Pulmonary manifestations of tuberous sclerosis in first degree relatives. *Thorax* 1989;44:212–214.
26. Bernstein SM, Newell Jr. JD, Adamczyk D, Mortensen R, King Jr. TE, Lynch DA. How common are renal angiomyolipomas in patients with pulmonary lymphangiomyomatosis? *Am J Respir Crit Care Med* 1995;152:2138–2143.
27. Popper HH, Juettner-Smolle FM, Pongratz MG. Micronodular hyperplasia of type II pneumocytes—a new lung lesion associated with tuberous sclerosis. *Histopathology* 1991;18:347–354.
28. Lantuejoul S, Ferretti G, Negoescu A, Parent B, Brambilla E. Multifocal alveolar hyperplasia associated with lymphangioleiomyomatosis in tuberous sclerosis. *Histopathology* 1997;30:570–575.
29. Muir TE, Leslie KO, Popper H, Kitaichi M, Gagne E, Emelin JK, Vinters HV, Colby TV. Micronodular pneumocyte hyperplasia. *Am J Surg Pathol* 1998;22:465–472.
30. Roach ES, Gomez MR, Northrup H. Tuberous sclerosis complex consensus conference: revised clinical diagnostic criteria. *J Child Neurol* 1998;13:624–628.
31. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152:1107–1136.
32. Dabora SL, Jozwiak S, Franz DN, Roberts PS, Nieto A, Chung J, Choy YS, Reeve MP, Thiele E, Egelhoff JC, Kasprzyk-Obara J, Domanska-Pakiela D, Kwiatkowski DJ. Mutational analysis in a cohort of 224 tuberous sclerosis patients indicates increased severity of TSC2, compared with TSC1, disease in multiple organs. *Am J Hum Genet* 2001; 68:64–80.
33. Tang NL, Pang CP, Yeo W, Choy KW, Lam PK, Suen M, Law LK, King WW, Johnson P, Hjelm M. Prevalence of mutations in the BRCA1 gene among Chinese patients with breast cancer. *J Natl Cancer Inst* 1999;91:882–885.

34. Cheadle JP, Reeve MP, Sampson JR, Kwiatkowski DJ. Molecular genetic advances in tuberous sclerosis [In Process Citation]. *Hum Genet* 2000;107:97–114.
35. Xiao GH, Shoarinejad F, Jin F, Golemis EA, Yeung RS. The tuberous sclerosis 2 gene product, tuberin, functions as a Rab5 GTPase activating protein (GAP) in modulating endocytosis. *J Biol Chem* 1997;272: 6097–6100.
36. Beauchamp RL, Banwell A, McNamara P, Jacobsen M, Higgins E, Northrup H, Short P, Sims K, Ozelius L, Ramesh V. Exon scanning of the entire TSC2 gene for germline mutations in 40 unrelated patients with tuberous sclerosis. *Hum Mutat* 1998;12:408–416.
37. Niida Y, Lawrence-Smith N, Banwell A, Hammer E, Lewis J, Beauchamp RL, Sims K, Ramesh V, Ozelius L. Analysis of both TSC1 and TSC2 for germline mutations in 126 unrelated patients with tuberous sclerosis. *Hum Mutat* 1999;14:412–422.
38. Zhang H, Yamamoto T, Nanba E, Kitamura Y, Terada T, Akaboshi S, Yuasa I, Ohtani K, Nakamoto S, Takeshita K, *et al*. Novel TSC2 mutation in a patient with pulmonary tuberous sclerosis: lack of loss of heterozygosity in a lung cyst. *Am J Med Genet* 1999;82:368–370.
39. Strizheva GD, Carsillo T, Kruger WD, Sullivan EJ, Ryu JH, Henske EP. The spectrum of mutations in TSC1 and TSC2 in women with tuberous sclerosis and lymphangiomyomatosis. *Am J Respir Crit Care Med* 2001;163:253–258.
40. Laurent F, Latrabe V, Raherison C, Marthan R, Tunon-de-Lara JM. Functional significance of air trapping detected in moderate asthma [In Process Citation]. *Eur Radiol* 2000;10:1404–1410.
41. Costello LC, Hartman TE, Ryu JH. High frequency of pulmonary lymphangioleiomyomatosis in women with tuberous sclerosis complex. *Mayo Clin Proc* 2000;75:591–594.
42. Bittmann I, Dose TB, Muller C, Dienemann H, Vogelmeir C. Lymphangioleiomyomatosis: recurrence after single lung transplantations. *Hum Pathol* 1997;26:1420–1423.
43. O'Brien JD, Lium JH, Parosa JF, Deyoung BR, Wick MR, Trulock EP. Lymphangioleiomyomatosis recurrence in the allograft after single lung transplantation. *Am J Respir Crit Care* 1995;151:2033–2036.
44. Hayashi T, Fleming MV, Stetler-Stevenson WG, Liotta LA, Moss J, Ferrans VJ, Travis WD. Immunohistochemical study of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in pulmonary lymphangioleiomyomatosis. *Hum Pathol* 1997;28:1071–1078.
45. Gurney JW, Conces DJ. Pulmonary histoplasmosis. *Radiology* 1996;199: 297–306.