Apparent Sporadic Lymphangioleiomyomatosis in a Man as a Result of Extreme Mosaicism for a TSC2 Mutation

To the Editor:

Lymphangioleiomyomatosis (LAM) is a rare, cystic lung disease associated with renal angiomyolipoma, lymphatic involvement, female sex, and metastasis of tuberous sclerosis complex mutation bearing smooth muscle-like cells to the lung. LAM occurs both sporadically and in association with tuberous sclerosis complex (TSC), but is very rare in men. Here we report a normal man who presented as sporadic LAM, but who was found to have extreme mosaicism for a TSC2 mutation.

Case Summary
A 48-year old African-American man with a history of hypertension and diabetes presented to the emergency department with sudden onset of left-sided chest pain. Chest X-ray demonstrated a pneumothorax, which was treated with tube thoracostomy. He was a lifelong nonsmoker with no prior history of lung disease, neurologic disorder, or dermatologic condition. He had fathered two normal children. Physical exam revealed a phenotype normally male with facial hair and two descended testicles and no stigmata of TSC. Chest computed tomography showed hundreds of thin-walled cysts of variable size throughout both lungs, from the apices to the bases, and without nodules or infiltrates (Figure 1). The pneumothorax resolved with chest tube drainage alone, but recurred several weeks later. Mechanical pleurodesis and thoracoscopic lung biopsy were performed. Skin examination by an expert TSC dermatologist (T.N.D.) showed none of the classic skin findings of TSC (hypomelanotic macules, fibrous cephalic plaque, facial angiofibromas, ungl fibromas, or shagreen patch). On closer inspection of the head and neck, multiple subtle, skin-colored, 1-mm perifollicular papules were observed on the posterior ears and lateral neck with no facial involvement. Pulmonary function testing revealed FEV1 63% predicted, TLC 80% predicted, and diffusing capacity of the lung for carbon monoxide 71% predicted. The patient had a normal comprehensive metabolic panel and complete blood count. His VEGF-D level, a biomarker for LAM, was 570 pg/ml (upper limit of the normal range). Chromosome analysis on a peripheral blood sample at 550 band resolution demonstrated a 46, XY normal male karyotype.

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Author Contributions: M.K.H., D.J.K., T.N.D., and J.L.M. all examined the patient; E.A.K. and J.L.M. provided expertise in interpretation of radiologic and pathologic data; M.E.T. performed the genetic studies that were reviewed by D.J.K.; and all authors contributed to scientific discussion of the case and preparation of the manuscript.

Lung biopsy demonstrated infiltrates of smooth muscle-like spindle and epithelioid cells associated with diffuse cystic change. The smooth muscle-like cells were reactive with antibodies against smooth muscle actin, HMB45, and estrogen receptor/ progesterone receptor. The renal lesion was resected and revealed an angiomyolipoma, with cytologically bland spindle cells, epithelioid cells with clear cytoplasm, abundant vessels, and rare foci of adipose tissue. Immunohistochemistry analysis showed strong staining with HMB-45 and smooth muscle actin. Biopsy of a papule on the back of the patient’s right ear showed a perifollicular fibroma, a pathologic finding that can be seen in either TSC or Birt-Hogg-Dubé syndrome.

DNA was prepared from formalin-fixed, paraffin-embedded sections of angiomyolipoma and lung, saliva, blood, dermis of the skin papule, and normal skin. A TSC2 mutation (c.2320delA) was identified in the angiomyolipoma by Sanger sequencing of the exons of TSC2. The allele frequency of this mutation was assessed in all available samples by amplicon next-generation sequencing (Table 1) (2). The c.2320delA mutant allele frequency in the angiomyolipoma was higher than 50% (providing evidence of loss of heterozygosity), 13.5% in the skin lesion, and 8.65% in the lung biopsy, respectively (Table 1). It was also clearly detectable at very low frequency (0.20–0.32% in normal skin, blood, and saliva), in contrast to control DNA samples.

Discussion
Sporadic pulmonary LAM is caused by somatic mutations in the TSC2 gene, with an estimated prevalence of two to five per million women (3). Pneumothorax is a common presentation. Nearly all patients with TSC-LAM and approximately 50% of patients with sporadic pulmonary LAM have renal angiomyolipomas (4). LAM is considered to be a low-grade, destructive neoplasm, and is caused predominantly by mutations in TSC2. The TSC2 protein, in complex with TSC1 and TBC1D7, functions to regulate the activation of mTOR complex 1 (mTORC1), which regulates many anabolic and growth-promoting processes.

TSC is a tumor suppressor gene syndrome in which both neurologic symptoms and tumors are major features and is a result of mutations in either TSC1 or TSC2. Cystic lesions consistent with LAM are seen in up to 80% of women with TSC by age 40 years (5). Both renal angiomyolipoma and LAM are more common in patients with TSC than in patients with TSC2 mutations than in patients with TSC with TSC1 mutations (5). Pulmonary cystic lesions are seen in about 10% of males with TSC (6).

Biopsy-proven LAM has been reported in four men, including three with definite or probable TSC (7–9). The fourth report described a karyotypically normal man with pulmonary LAM, but without clinical features of TSC (10). A TSC2 mutation was not identified in this patient’s lung tissue, which may reflect the limitations of the genetic methods employed at the time. Most cases of TSC occur in patients without a family history and are a result of new mutations in TSC1 or TSC2.

Mosaicism is the occurrence of cells in a single organism with different genetic constitutions. Mosaicism is known to occur in all humans because of random errors in DNA replication, which are fortunately rare, but nonetheless begin to accumulate in the
developing human after fertilization. Mosaicism for TSCI or TSC2 mutations in TSC subjects is increasingly recognized, and can be seen at extremely low levels with mutant allele frequency less than 1%; a genetic profile we term extreme mosaicism (2).

In our patient, we found a TSC2 mutation, c.2320delA, at high frequency with evidence of loss of heterozygosity in his angiomyolipoma (Table 1). The mutation was also seen in both his lung biopsy and his perifollicular fibroma, indicating that mutation-bearing cells likely formed the nidus for proliferation and stromal recruitment in each of those lesions. The TSC2 c.2320delA mutation was seen at extremely low frequency (0.20–0.32%) in several normal tissues of this patient, but was much higher than in control DNA samples (Table 1). Overall, these data are consistent with this patient being an extreme mosaic for the TSC2 c.2320delA mutation, leading to subsequent renal angiomyolipoma and LAM development.

Sporadic LAM is thought to be a result of two postembryonic somatic mutations in TSC2. The genetic findings in our patient suggests that some cases of apparent sporadic LAM in women may be a result of extreme mosaicism for a mutation in TSC2, with at least one hit occurring during embryogenesis. This mechanism could explain the occurrence of sclerotic bone lesions and/or multiple bilateral angiomyolipomas in some patients diagnosed with sporadic LAM who do not have other disease manifestations of TSC (4).

Table 1. Allele frequency of the TSC2 c.2320delA mutation in different samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wild-type reads</th>
<th>Mutant reads</th>
<th>Mutant reads (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney angiomyolipoma</td>
<td>37,705</td>
<td>84,948</td>
<td>69.3</td>
</tr>
<tr>
<td>Perifollicular fibroma</td>
<td>68,171</td>
<td>10,646</td>
<td>13.5</td>
</tr>
<tr>
<td>Lung</td>
<td>45,578</td>
<td>4314</td>
<td>8.65</td>
</tr>
<tr>
<td>Normal skin</td>
<td>277,755</td>
<td>904</td>
<td>0.32</td>
</tr>
<tr>
<td>Blood</td>
<td>106,622</td>
<td>296</td>
<td>0.28</td>
</tr>
<tr>
<td>Saliva</td>
<td>185,310</td>
<td>373</td>
<td>0.20</td>
</tr>
<tr>
<td>Control 3</td>
<td>81,016</td>
<td>8</td>
<td>0.01</td>
</tr>
<tr>
<td>Control 1</td>
<td>81,609</td>
<td>2</td>
<td>0.00</td>
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<tr>
<td>Control 2</td>
<td>90,304</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Note the control samples were blood DNA samples from normal controls.

References

4 Avila NA, Dwyer AJ, Rabel A, Moss J. Sporadic lymphangioleiomyomatosis and tuberous sclerosis complex with multiple bilateral angiomyolipomas in some patients diagnosed with sporadic LAM who do not have other disease manifestations of TSC (4).


Careful Planning Reduces Cryobiopsy Complications

To the Editor:

We read with interest the article recently published by DiBardino and colleagues (1). The authors describe their experience with the introduction of transbronchial lung cryobiopsy at their academic medical center in Philadelphia, Pennsylvania. Their series of 25 patients included 17 patients with interstitial lung disease, six patients evaluated for a lung nodule, and two lung transplant recipients. The authors report a high rate of serious bleeding complications (12%), and a low diagnostic yield (56%). The procedures were performed using flexible bronchoscopy without preplacement of a balloon occlusion catheter, without a standardized protocol, without standardized selection of patients, and with fluoroscopy for only 40% of the procedures.

We have recently reported our experience on the introduction of transbronchial lung cryobiopsy for the diagnosis of interstitial lung disease at Aarhus University Hospital, a tertiary care referral center in Denmark. Our initial experience was more favorable (2).

We started by planning the procedure meticulously. One experienced interventional pulmonologist visited another center with great deal of experience in transbronchial cryobiopsy procedures (O.M., Department of Diseases of the Thorax, Forlì, Italy). On return, that physician trained the two other interventional pulmonologists. All procedures are still performed by these same three physicians.

Our patients are carefully selected to avoid serious complications. We exclude patients with any of the following conditions: body mass index higher than 35 kg/m², FVC lower than 45%, diffusing capacity for carbon monoxide lower than 35%, and significant cardiac comorbidity or a tricuspid gradient higher than 40 mm Hg by echocardiography. Patients with a NT-pro-BNP lower than 95 ng/L are accepted for cryobiopsies without echocardiography (3).

We perform all our cryobiopsy procedures under general anesthesia and fluoroscopy, using a flexible bronchoscope with a balloon occlusion catheter in place for use if needed. With this standardized protocol, we performed 86 procedures to date, without any severe bleeding complications.

Six of the 38 patients included in our published report experienced moderate bleeding, controlled during the procedure by use of the balloon occlusion catheter, installation of ice water, and intravenous administration of tranexamic acid. One patient experienced minor hemoptysis for 10 days afterward. Two patients showed signs of a respiratory infection. Ten (26%) of the 38 patients experienced a pneumothorax, six of whom required placement of a chest tube. Altogether, 20 patients (53%) had no complication.

Plural tissue was present in the biopsies from eight of 38 patients, explaining the incidence of pneumothorax, but also demonstrating that we obtained tissue from peripheral lung areas. Transbronchial cryobiopsy contributed to a confident multidisciplinary diagnosis for 74% of 38 patients. Surgical lung biopsy was recommended for two patients (2).

We recommend that centers introducing transbronchial cryobiopsy send one or more of their interventional pulmonologists to train at an experienced center, and then carefully prepare a standardized protocol before initiating their program. Procedures should be performed under general anesthesia and fluoroscopy, with a balloon occlusion catheter in place to be deployed in the event of active bleeding.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Elisabeth Bendstrup, M.D., Ph.D.
Sissel Kronborg-White, M.D.
Aarhus University Hospital
Aarhus, Denmark

Venerino Poletti, M.D., Ph.D.
Aarhus University Hospital
Aarhus, Denmark

and

Ospedale Morgagni
Forlì, Italy

Birgitt Fokkens, M.D.
Nina Voldby, M.D.
Torben R. Rasmussen, M.D., Ph.D.
Aarhus University Hospital
Aarhus, Denmark

ORCID IDs: 0000-0002-4238-6963 (E.B.); 0000-0002-5526-5079 (S.K.-W.); 0000-0002-8634-2284 (V.P.); 0000-0001-7703-8312 (B.F.); 0000-0002-5757-4107 (N.V.); 0000-0002-7030-9260 (T.R.R.).

References


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