Mutational analysis of the tuberous sclerosis gene TSC2 in patients with pulmonary lymphangioleiomyomatosis

Aristotelis Astrinidis, Leena Khare, Thomas Carsillo, Teresa Smolarek, Kit-Sing Au, Hope Northrup, Elizabeth Petri Henske

Abstract
Pulmonary lymphangioleiomyomatosis (LAM) is a rare disorder limited almost exclusively to women of reproductive age. LAM affects about 5% of women with tuberous sclerosis complex (TSC). LAM also occurs in women who do not have TSC (sporadic LAM). TSC is a tumour suppressor gene syndrome characterised by seizures, mental retardation, and tumours in the brain, heart, and kidney. Angiomyolipomas, which are benign tumours with smooth muscle, fat, and dysplastic vascular components, are the most common renal tumour in TSC. Renal angiomyolipomas also occur in 63% of sporadic LAM patients. We recently found that 54% of these angiomyolipomas have TSC2 loss of heterozygosity, leading to the hypothesis that sporadic LAM is genetically related to TSC. In this study, we screened DNA from 21 women with sporadic LAM for mutations in all 41 exons of TSC2. Twelve of the patients had known renal angiomyolipomas. No TSC2 mutations were detected. We did find three silent TSC2 polymorphisms. We conclude that patients with sporadic LAM, including those with renal angiomyolipomas, do not have a high frequency of germline mutations in the coding region of TSC2.

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Table 1
Presence or absence of angiomyolipomas in patient, and source of DNA for mutational analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Angiomyolipoma(s) present?</th>
<th>DNA source</th>
</tr>
</thead>
<tbody>
<tr>
<td>421</td>
<td>+</td>
<td>Peripheral blood lymphocytes</td>
</tr>
<tr>
<td>422</td>
<td>–</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>423</td>
<td>+</td>
<td>Angiomyolipoma</td>
</tr>
<tr>
<td>476</td>
<td>–</td>
<td>Peripheral blood lymphocytes</td>
</tr>
<tr>
<td>478</td>
<td>–</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>480</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>481</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>489</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>491</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>492</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>494</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>496</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>505</td>
<td>+</td>
<td>Lymphoblastoid cell line</td>
</tr>
<tr>
<td>537</td>
<td>–</td>
<td>Cultured lung cells</td>
</tr>
<tr>
<td>538</td>
<td>+</td>
<td>Cultured lung cells</td>
</tr>
<tr>
<td>539</td>
<td>–</td>
<td>Cultured lung cells</td>
</tr>
<tr>
<td>540</td>
<td>+</td>
<td>Cultured lung cells</td>
</tr>
<tr>
<td>541</td>
<td>–</td>
<td>Cultured lung cells</td>
</tr>
<tr>
<td>542</td>
<td>–</td>
<td>Cultured lung cells</td>
</tr>
<tr>
<td>543</td>
<td>–</td>
<td>Cultured lung cells</td>
</tr>
<tr>
<td>547</td>
<td>–</td>
<td>Cultured lung cells</td>
</tr>
</tbody>
</table>

Keywords: TSC2; pulmonary lymphangioleiomyomatosis
Patients, methods, and results

This study was approved by the Institutional Review Board of Fox Chase Cancer Center. None of the patients in this study had dermatological or neurological signs or symptoms of TSC. Twelve of the patients had known angiomyolipomas (Table 1). The angiomyolipoma from patient 492 had \textit{TSC2} LOH.12 The angiomyolipomas from patients 423, 480, 481, 489, and 491 did not have \textit{TSC1} or \textit{TSC2} LOH.12 Tissue from the remaining six angiomyolipomas was not available for LOH analysis. The source of DNA was either peripheral blood lymphocytes or a lymphoblastoid cell line for 12 patients (Table 1). For one patient (423) DNA was isolated directly from fresh angiomyolipoma tissue. Fresh or frozen angiomyolipoma tissue from which genomic DNA could be prepared was not available from the other patients. For eight patients, DNA was isolated from primary cultures of lung tissue established at the time of lung transplantation for LAM.

Single strand conformation analysis (SSCP) was used to search for mutations in the coding regions of the \textit{TSC2} gene. The primers amplifying each of the 41 exons of \textit{TSC2} and the PCR conditions have been previously reported by Au \textit{et al}.13 The PCR products were run on MDE gels (AT Biochem). To maximise the detection of variant bands, each PCR product was run on two gels: one without glycerol and one with 5% glycerol. Samples in which variant bands were detected were reamplified and sequenced.

We found three variant bands in exons 12, 14, and 34, all of which were found by sequencing to represent silent polymorphisms (Table 2). All three polymorphisms have been previously reported and are present in the TSC Variation Database (http://expmed.bwh.harvard.edu/ts/). No \textit{TSC2} alterations resulting in amino acid changes were detected.

Discussion

The clinical and pathological similarities between TSC and sporadic LAM have led to the hypothesis that LAM is a form of TSC.47 Our recent finding that 54% of angiomyolipomas from sporadic LAM patients have LOH in the \textit{TSC2} region of chromosome 16p13.12 supported this hypothesis. In this study, DNA samples from 21 women with pulmonary LAM, 12 of whom also had renal angiomyolipomas, were examined for \textit{TSC2} mutations in all 41 exons using SSCP. No definite mutations were identified.

Our data indicate that germline mutations in \textit{TSC2} are infrequent in sporadic LAM. In addition, the lack of \textit{TSC2} mutations in the eight DNA samples from primary cell cultures established from LAM lung tissue at the time of lung transplantation suggests that somatic mosaicism for \textit{TSC2} mutations is not a frequent cause of sporadic LAM. It is possible that in some patients, however, mutations were missed. The sensitivity of SSCP in \textit{TSC2} analysis is not known, but for other genes the sensitivity for a single gel condition has been estimated at 75-98%.14 Each PCR product in our study was run under two gel conditions (with and without glycerol). We also did not screen for mutations in the non-coding regions of \textit{TSC2} or for deletions. Large \textit{TSC2} deletions are often associated with renal cysts,15 which were not present in any of the patients in this study. We did not analyse these samples for \textit{TSC1} mutations because we had previously found \textit{TSC2} LOH, but not \textit{TSC1} LOH, in angiomyolipomas from women with sporadic LAM.12

In summary, this is the first report of \textit{TSC2} mutational analysis in patients with pulmonary lymphangiomyomatosis. We did not find \textit{TSC2}
mutations in DNA isolated from lymphocytes (12 patients), primary cultures of lung tissue (eight patients), or angiomyolipoma (one patient). We conclude that germline mutations in the coding regions of TSC2 are uncommon in sporadic LAM despite the striking clinical and pathological overlap between these two diseases. Additional studies will be required to determine whether other types of TSC2 mutations not detected by SSCP occur in sporadic LAM, whether some patients have somatic mosaicism for TSC2 mutations, or whether mutations in other genes are involved.

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