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

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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*.

(J Med Genet 2000;37:55–57)

Keywords: *TSC2*; pulmonary lymphangioleiomyomatosis

occurrence of angiomyolipomas in both TSC associated and sporadic LAM has led to the hypothesis that these diseases have a common genetic basis.^{4–7}

There are two TSC genes, *TSC1* on chromosome 9q34⁸ and *TSC2* on chromosome 16p13.⁹ The frequency of *TSC1* or *TSC2* loss of heterozygosity (LOH) in TSC associated angiomyolipomas is approximately 60%.¹⁰ In contrast, *TSC2* LOH occurs in only 10% of angiomyolipomas from patients who do not have either TSC or LAM.¹¹ We recently found that 54% of angiomyolipomas from women with sporadic LAM have LOH in the *TSC2* region of chromosome 16p13.¹² We did not find *TSC1* LOH in the sporadic LAM associated angiomyolipomas.¹²

The *TSC2* LOH in the LAM associated angiomyolipomas led us to test the hypothesis that some patients with sporadic LAM have de novo germline mutations in *TSC2* associated with a mild phenotype affecting only lung and kidney. Phenotypic variability in TSC is common, with some patients having only mild signs or symptoms of disease, and approximately 70% of TSC patients have new germline mutations, with no previous family history of the disease. We therefore analysed DNA from 21 women with sporadic LAM for *TSC2* mutations. The sources of DNA were peripheral blood lymphocytes (12 patients), cultured lung cells obtained at the time of lung transplantation (eight patients), and angiomyolipoma (one patient).

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

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

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Revised version received 19 July 1999
Accepted for publication 30 July 1999

Pulmonary lymphangioleiomyomatosis (LAM) is a rare and often fatal disease of unknown aetiology affecting almost exclusively young women.^{1,2} Microscopically, LAM is characterised by diffuse infiltration of the pulmonary interstitium by smooth muscle cells and cystic distortion of the lung architecture. LAM can occur as an isolated disorder (sporadic LAM) or in association with tuberous sclerosis (TSC). TSC is an autosomal dominant disorder characterised by seizures, mental retardation, and hamartomatous tumours of the brain, heart, kidney, lung, and skin. LAM affects 2.3% of people (or 4.6% of women) with tuberous sclerosis.³ Renal angiomyolipomas, which are benign tumours composed of fat, smooth muscle, and dysmorphic vessels, occur in 70% of TSC patients and in 63% of women with sporadic LAM.^{4–6} The

Figure 1 (A) Detection of variant band in exon 34. Lanes 1 and 2 contain DNA from controls and lane 3 contains DNA from patient 539. The arrow indicates the variant band. (B) Sequencing of exon 34 from patient 539. The arrow indicates the double peak of C and T at nucleotide 4536.

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 *TSC2* LOH.¹² The angiomyolipomas from patients 423, 480, 481, 489, and 491 did not have *TSC1* or *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

Table 2 *TSC2* polymorphisms in LAM patients

Patient	Exon	Nucleotide change	Amino acid
494	12	C1281A	I427I
494	14	C1578T	S526S
539	34	C4536T	D1489D

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 *TSC2* gene. The primers amplifying each of the 41 exons of *TSC2* and the PCR conditions have been previously reported by Au *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 *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.^{4,7} Our recent finding that 54% of angiomyolipomas from sporadic LAM patients have LOH in the *TSC2* region of chromosome 16p13¹² supported this hypothesis. In this study, DNA samples from 21 women with pulmonary LAM, 12 of whom also had renal angiomyolipomas, were examined for *TSC2* mutations in all 41 exons using SSCP. No definite mutations were identified.

Our data indicate that germline mutations in *TSC2* are infrequent in sporadic LAM. In addition, the lack of *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 *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 *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 *TSC2* or for deletions. Large *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 *TSC1* mutations because we had previously found *TSC2* LOH, but not *TSC1* LOH, in angiomyolipomas from women with sporadic LAM.¹²

In summary, this is the first report of *TSC2* mutational analysis in patients with pulmonary lymphangiomyomatosis. We did not find *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.

We are grateful to William A Petri Sr and Drs Ann Petri, Becky Raftogianis, and Warren Kruger for critical review of the manuscript, and to Dr Alfred Knudson, Dr Frank McCormack, Ms Sue Byrnes, and The LAM Foundation for their ongoing support. We also thank the patients who contributed blood and tissue specimens for this research. This work was supported by The LAM Foundation (Cincinnati, OH) and the National Institutes of Health (HL 60746).

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