CASE REPORT

Chromosome typing in lymphangioleiomyomatosis of the lung with and without tuberous sclerosis

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ABSTRACT: Two cases of lymphangioleiomyomatosis (LLM) of the lungs are compared, one with tuberous sclerosis and the other representing an isolated lung involvement.

An increased frequency of complex chromosomal rearrangements was found in peripheral lymphocytes of the patient with tuberous sclerosis, in comparison to the patient with isolated lymphangioleiomyomatosis. Telomeric associations were found in cultured pulmonary smooth muscle cells from the patient with isolated LLM.
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Tuberous sclerosis is a rare, autosomal dominantly-transmitted disease, the incidence (children under 10 yrs of age) reported so far varies between 1:12–15,000 [1]. Tuberous sclerosis shows a high but incomplete penetrance, and great variability in expression, even within families [2, 3]. It comprises both dysplastic development and heterotopia of the ectodermal cells of the central nervous system. Clinically, it is often accompanied by other developmental abnormalities, such as sebaceous adenoma of the skin, fibrovascular subungual nodules, renal tumours (angiomyolipofibromas) [4], mental retardation, and epilepsy [5]. Pulmonary involvement, such as lymphangioleiomyomatosis (LLM), fibrosis and a variety of hamartomas, is rarely seen [5–8]. So far, it is not clear whether LLM of the lung and draining lymph nodes is a separate entity, sometimes incidentally associated with tuberous sclerosis, or a "forme fruste" thereof [9, 10].

Patients report

Case 1

In 1974, a 34 year old woman was admitted to hospital for cough and dyspnoea, which had developed shortly after delivery of her first child. The routine chest X-ray showed a discrete, bilateral, parahilar infiltration, as well as a pneumothorax and pleural effusion on the right side. The only pathological findings were an elevated blood sedimentation rate (BSR) (50/70 mm) and hypochromic anaemia.

Although infections, allergic, or autoimmunological diseases had been ruled out, corticosteroids and antibiotics (tetracyclines) were administrated. The pneumothorax was successfully treated by aspiration. Six months later a recurrent pneumothorax on the right side was again treated conservatively. During the following years the patient was readmitted several times for dyspnoea and the infiltrate slowly increased. The first pulmonary function tests were not performed until 1982; both static and dynamic lung volumes were within the normal range (table 1), but an increase of compliance was found. In the blood gas analysis, hypocapnia existed during rest and after exertion. In frozen sections, the open lung biopsy specimen obtained at that time was interpreted as fibrosis.

A computed tomographic (CT) scan of the brain was performed because of epilepsia, and revealed subependymal calcifications. Subperiostal ossifications and new bone formations were detected on roentgenograms of both hands. Further investigations confirmed the tentative diagnosis of tuberous sclerosis, documenting angiomyolipomas of the kidney and multiple fibromas of the skin, especially in head and neck areas. The open lung biopsy specimen was re-examined and the diagnosis of LLM was established.

Table 1. – Lung function data of both patients

		Patient No. 1		Patient No. 2		
		At rest	After exertion	April 7, 1986	August 27, 1986	October 23, 1987
FEV₁	l	2.31		2.40	2.65	1.1
FEV₁	% pred	102		82	90	38
VC	l	2.88		3.35	4.05	2.75
VC	% pred	94		89	108	74
FEV₁	%VC*	80		72	65	40
TLC	l	4.99		4.85	5.85	6.83
TLC	% pred*	108		91	109	125
Pao₂	kPa	10.27	10.13		9.73	7.07
Paco₂	kPa	4.53	4.40		4.0	4.8
pH		7.46	7.38		7.41	7.40

*: EGKS - reference (mean - 29) (Europäische Gemeinschaft für Kohle und Stahl). FEV_1: forced expiratory volume in one second; VC: vital capacity; TLC: total lung capacity; Pao_2: arterial oxygen tension; $Paco_2$: carbon dioxide tension.

Case 2

In 1986, a 29 year old woman was admitted for dyspnoea and tachycardia, which she had progressively noticed since the delivery of her first child 2 yrs previously. Routine chest X-rays showed bilateral reticular patterns in the parahilar region. Laboratory parameters and pulmonary function tests were normal.

Five months later, marked dyspnoea had developed. The reticular shadows in both lungs had progressed. Routine laboratory tests were again normal. Blood gas analysis revealed light hypoxaemia and hypocapnia. Pulmonary function tests showed a mild obstructive pattern (table 1), which was reversible (by physiotherapy). At bronchoscopy, the segmental bronchi collapsed totally during forced expiration. Transbronchial biopsy was interpreted as nonspecific interstitial inflammation, with an abundance of histiocytic cells and discrete micronodular proliferations. Antibodies reactive with thermoactinomycetes and *Micropolyspora faeni* were found by crossed immunoelectrophoresis. This led to a diagnosis of hypersensitivity pneumonia and treatment with corticosteroids was started.

One year later, severe dyspnoea and peripheral cyanosis developed, requiring nasal oxygen therapy for 8–12 h·day⁻¹. The radiological features had changed to a distinct striation, suggesting severe fibrosis. The pulmonary artery segment was prominent and lung fields also appeared increased radiologically. Pulmonary function tests showed severe obstruction and blood gas analysis revealed marked hypoxaemia, due to respiratory insufficiency. Because of the prominent right pulmonary artery seen in the chest X-ray, and electrocardiographic signs of right ventricular hypertrophy, a Swan-Ganz catheter investigation was performed. Pulmonary hypertension was confirmed, mean pulmonary artery pressure (PAP) at rest was 5.2 kPa (39 mmHg), and increased to 8 kPa (60 mmHg) during exercise. After breathing 100% oxygen, mean PAP fell to 3.6 kPa (27 mmHg) within 3 min. In the CT scan of both lungs, thin-walled cystic structures with irregular distribution were seen and severe honey-combing, possibly caused by LLM, was diagnosed. Open lung biopsy was performed and LLM confirmed. Due to the rapid progression of the disease a combined heart-lung transplantation was planned and performed one year later.

Methods

Histology

Lung tissue was processed as usual and sections were stained with haematoxylin-eosin (HE), van Gieson's elastica stain, trichrome stain, and the periodic acid-Schiff stain (PAS).

Immunohistochemistry

Sections from the open lung biopsy or LLM cells cultured on slides were processed for routine immunohistochemistry, according to the method described by CORDELL *et al.* [11], using antibodies against vimentin (clone V9, diluted 1:10; Dakopatts), desmin (against 56 kD protein, diluted 1:50; Monosan), actin (anti smooth muscle α-actin, diluted 1:2000; Dr. G. Gabbiani, Geneva, Switzerland), human factor-VIII (F-VIII)-associated antigen (diluted 1:50; Dakopatts), rat antibodies against α₁-antichymotrypsin (diluted 1:1000; Dakopatts), lysozyme (diluted 1:300; Dakopatts), rat antibodies to α-chain and mouse antibodies to β-chain of human chorionic gonadotropin (α- and β-HCG, diluted 1:7500 and 1:2000; NIAMDD and Biogenex), and antibodies to oestrogen and progesterone receptors (ER-ICA, PgR-ICA, Abbott). We included normal lung tissue as positive and negative controls, including bronchi and cases of intra-alveolar and interstitial pneumonias and frozen sections from cases of oestrogen- and progesterone-receptor positive and negative breast cancers.

Electron microscopy

Paraffin-embedded material from the second case was deparaffinized, post-fixed in osmium tetroxide and embedded in Epon 812. Ultrathin sections were contrasted with uranylacetate and lead citrate.

Cell culture

Lung biopsies from patient No. 2 were stored in liquid nitrogen. The tissue was thawed rapidly and dissociated in sterile saline. After centrifugation, one part of the cell suspension was resuspended in growth medium and set up in collagen-coated culture vessels. The other part was further dissociated by cold trypsination (+4°C) for 12 h. For both preparations, Ham's nutrient mixture F12 (PAA Labs, Austria) was used, supplemented with 10% fetal calf serum, penicillin 100 IU·ml^{-1} and streptomycin 100 µg·ml^{-1} medium. The cultures were maintained at 37°C in a 5% CO_2 enriched atmosphere, and subcultured in collagen-coated vessels. At passages 4–6 immunohistochemical reactions for oestrogen and progesterone receptors were performed.

Fig. 1. – Sections through the explanted lungs from patient No. 2 (at the time of combined heart-lung transplantation; isolated lymphangioleiomyomatosis (LLM)). Multiple cystic spaces, diffusely distributed in both lungs, are clearly visible. These cysts correspond to dilated alveoli and lymphatic vessles. A differential diagnosis of LLM, but also severe panacinar emphysema, might be considered.

Cytogenetic preparations

Chromosome slides were prepared from peripheral blood lymphocytes of both patients [12] and, in addition, from cell cultures (passages 1 and 2) derived from the lung lesion of patient No. 2. Subsequently, the metaphase cells were stained by the G-banding technique [13].

Results

Pathological report

Macroscopically, multiple round cysts were focally distributed in the lungs of case 1 and diffuse in the lungs of case 2. In some areas, there was a thickening of the alveolar walls mimicking interstitial fibrosis. Case 2 looked like a severe panacinar emphysema (fig. 1). Histologically, the pleura was focally fibrotic, but the most striking features were dilated lymph vessels. A mild, chronic, nonspecific inflammatory infiltrate was present in both cases. In some areas, the lobular, interlobular and alveolar septa were thickened by proliferating smooth muscle cells and fibroblasts (fig. 2a). Occasionally, a few lymphocytes and histiocytes were seen. In the same areas, dilated, sometimes cystic, lymph vessels were seen in proximity to blood vessels. The endothelial cells were often swollen and protruded into the lumina. In these areas also, the alveoli were dilated, the alveolar septa were reduced in numbers, and some of them were filled with oedematous fluid, erythrocytes and a few detached pneumocytes and macrophages. In other areas, quite normal alveoli could be seen.
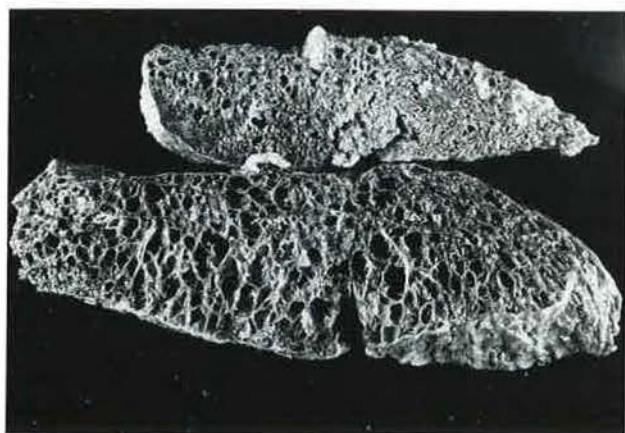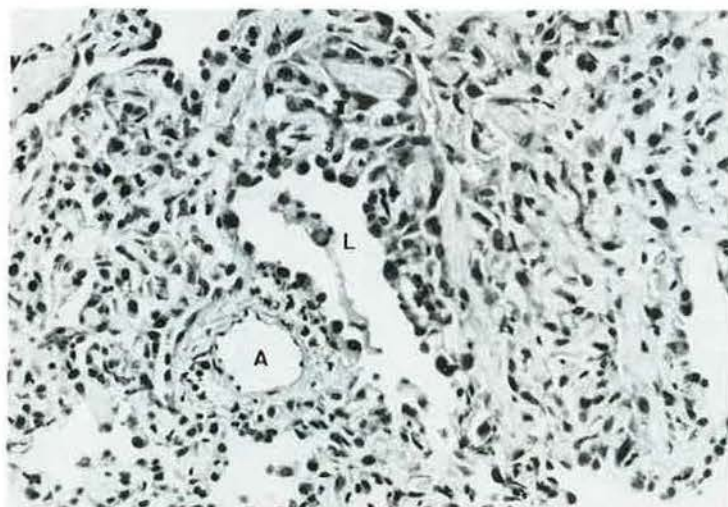
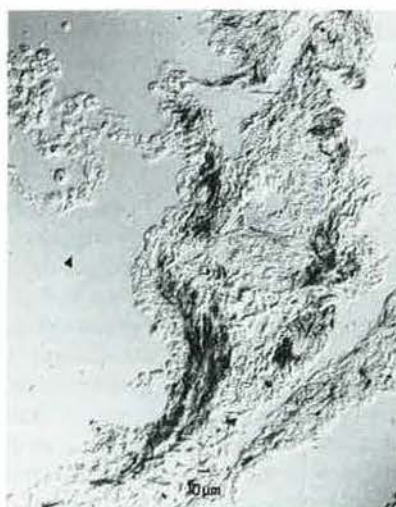a   b

Fig. 2. – Case 2. a) Proliferating cells showing characteristics of smooth muscle cells with elongated cigar-like nuclei. Most cystic spaces are now defined as lymphatic vessels (L), one lying near a small artery (A) (Haematoxylin and eosin, ×100). b) Immunohistochemical reaction with antibodies directed against desmin. The proliferating cells within the alveolar septa are positively stained. (Bar 10 µm, ×50).

Immunohistochemistry

In the lung sections, proliferating smooth muscle cells within alveolar septa were stained with antibodies directed against smooth muscle specific α-actin, desmin (fig. 2b), and vimentin; whereas histiocytic cells, fibroblasts and fibrocytes were stained with vimentin antibodies only. The endothelial cells of the lymph vessels reacted with antibodies against F-VIII-associated antigen. Sometimes, a few F-VIII-associated antigen positive cells could be found within the muscle cell proliferation also. Most cells within the alveolar lumina were stained with ysozyme, but not with α₁-antichymotrypsin antibodies, indicating that most of them were pneumocytes type II. The reactions with α- and β-HCG antibodies were negative in the muscle cell proliferations. Antibody reactions for progesterone and oestrogen receptors, performed with cultured LLM cells from case 2, were also negative.

Electron microscopy

There was an irregular thickening of most alveolar septa, either induced by abundant collagen and elastin deposition, or by proliferating cells composed of fibroblasts, occasionally histiocytes, smooth muscle cells and myofibroblasts. The latter two cell types were characterized by many or few myofilaments in their cytoplasm, a rough endoplasmic reticulum, and long ovoid or spindle-shaped nuclei. The capillaries in the alveolar septa were mostly centrally located and, in addition, lymph capillaries were dilated. At the alveolar site, there was a focal hyperplasia of pneumocytes type II.

Chromosome analysis and familial disposition

Analysis of 70 metaphases from patient No. 1 (LLM with tuberous sclerosis, and peripheral blood lymphocytes) revealed a normal karyotype (1–22, XX). However, an increased rate (8.6%) of abnormal metaphases was found in the same patient. Two cells showed additional chromosomes (one chromosome 15 and one X chromosome, respectively). Complex chromosome rearrangements were present in four cells (fig. 3a–d). M1 was derived from the long arm of chromosome 6 and the distal part of the long arm of chromosome 2. Subsequent non-disjunction resulted in two identical M1 chromosomes. M2 was a dicentric chromosome, composed of the short arms of chromosome 2 and 6, and the proximal part of the long arm of chromosome 2. M3 represented the long arm of chromosome 11, which was translocated to chromosome 20. M4 was the short arm of chromosome 11. M5 and M6 arose by reciprocal translocation between chromosomes 5 and 22. M7 was the short arm of chromosome 1, translocated to the long arm of chromosome 16. M8 resembled the long arm of chromosome 1.

In patient No. 2 (isolated LLM), 110 metaphases were analysed from peripheral blood lymphocytes. No structural rearrangements could be detected. From the

cultured pulmonary smooth muscle cells, 45 G-banded metaphases were obtained. The majority of cells showed a normal diploid karyotype. In several of them (6.3%) telomeric associations or fusions between different chromosomes, including chromosome 14, were present. A long arm deletion of chromosome 14, with breakpoint in q24, was found in two metaphases (fig. 4).

The examination of the relatives from patient No. 2, particularly with respect to causes of death, did not reveal genetic transmission of LLM. Both mother and father are alive, without evidence of pulmonary diseases, both grandmothers died from coronary heart disease, one grandfather died from lung cancer, the other had no lung disease.
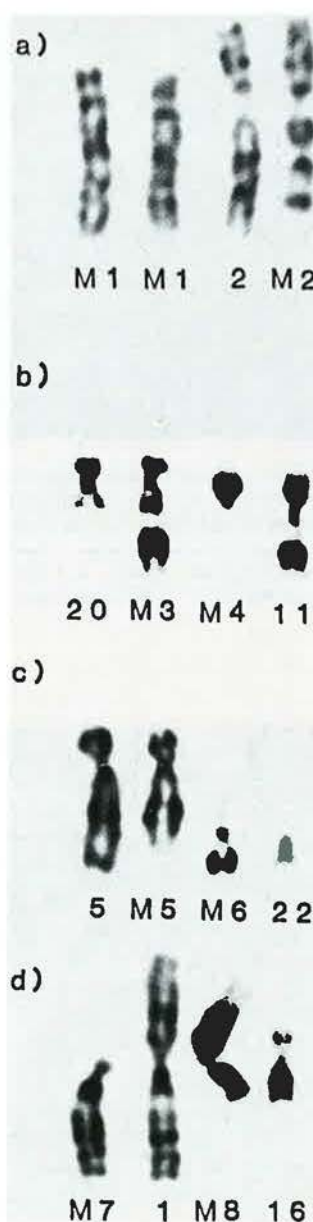

Fig. 3. – Case 1. Chromosomes from cultured blood lymphocytes; a, b, c and d: parts of karyotypes from four lymphocytes with abnormal chromosomes.
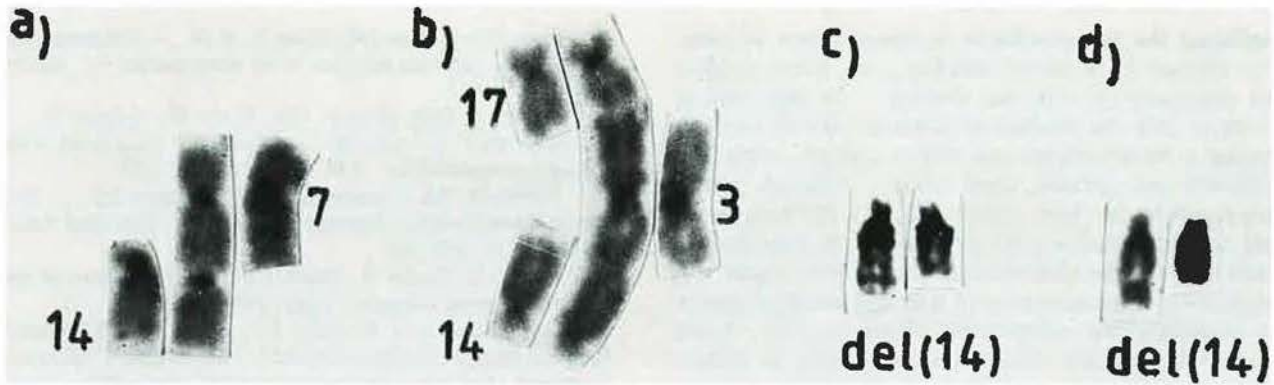
Fig 4. – Case 2. Chromosomes from cultured smooth muscle cells showing partial karyotypes with telomeric associattions (a, b), and long arm deletions of chromosome 14 (c, d). Fusion chromosomes are shown in the middle, normal counterparts are added on both sides (a, b), or on the left side (c, d).

Discussion

LLM is a rare disease, which in some cases is associated with tuberous sclerosis, in others restricted to the lungs, with or without regional lymph node involvement [14]. VALENSI [10] suggested that the isolated form might be a "forme fruste" of the disease. SILVERSTEIN et al. [15] divided LLM into three types: type 1 with involvement of lung parenchyma and mediastinal or retroperitoneal lymph nodes, in most reported cases with severe progressive course; type 2 with focal lymph node and without lung involvement; and type 3 with lung and lymph node involvement, associated with tuberous sclerosis. Our first case belongs to type 3, and the second to type 1 of LLM.

The proliferating cells in LLM were originally misinterpreted as lymphangiopericytes [16], and as glomus-like structures [10], but this was corrected to myoblasts by light [17], and electron microscopy [18]. We were able to confirm these data immunohistochemically on the basis of the positive reaction with desmin and muscle specific α-actin antibodies, and also electron microscopically by the demonstration of myofilaments in the proliferating cells. In our cases, the proliferating smooth muscle cells were well-differentiated, we cannot find a reason why they should be called myoblasts. Myoblasts are defined as immature myogenic cells with un- or underdeveloped myofilaments and other cell organelles, whereas all reports so far published have described proliferating cells with well-developed and structured myofilaments and other organelles. Therefore, the term myoblast should be avoided.

We were unable to detect proliferation of lymph vessels. The proliferating smooth muscle cells occlude the lymph vessels, and by this, secondarily, cause lymphangiectasis of intrapulmonal and pleural lymph vessels, which often rupture causing a chylothorax [7]. The histological changes of LLM in tuberous sclerosis and in the isolated form are identical. We were unable to find differences between the lung lesions in our cases and in the published reports [5–7, 9–10, 15–27].

In the differential diagnosis, a few entities should be considered: eosinophilic granuloma of the lung in most cases is a focal patchy process, characterized by a proliferation of Langerhan's cells, which can be stained by S-100 protein antibodies. Muscular cirrhosis is a focal proliferation of smooth muscle cells, often seen in severe emphysema and some fibrotic lung diseases, but is restricted to the scarred areas, and lymphangiectasis is not found in this disease. Least likely are metastases of a well-differentiated leiomyosarcoma. But leiomyosarcomas form well-circumscribed nodules, and include cysts with epithelial layer, i.e. remnants of bronchioli and alveolar ducts. In the misinterpretations of LLM, the most frequent incorrect diagnosis is interstitial pneumonia with fibrosis [14]. Because of the presence of myofibroblasts in normal lung tissue, and the proliferation of these cells in interstitial pneumonias, the nature of the additional smooth muscle cell proliferation in LLM should be confirmed by immunohistochemistry, using specific antibodies for desmin and smooth muscle specific α-actin: mature smooth muscle cells are positively stained by desmin and α-actin antibodies, whereas myofibroblasts and immature smooth muscle cells are only stained by α-actin antibodies.

It has been suggested [19–21], that LLM is a genetic defect with an underlying abnormal muscular response to female sex hormones. This could also be deduced from the few attempts to improve prognosis of these patients either by oophorectomy [22–24], or progesterone therapy [25, 26] or a combination of both [21]. In our cases, no staining with antibodies directed to the α- and β-chain of human chorionic gonadotrophin (HCG) was found. Abnormal expression of oestrogen and progesterone receptors on proliferating smooth muscle cells of LLM have been reported by COLLEY et al. [27]. Other abnormal receptor expressions were not found, and biochemical receptor studies were unsuccessful, although the onset of LLM (starting after or during pregnancy) suggests an abnormal response to HCG. In case 2 progesterone and oestrogen receptors were not expressed by the LLM smooth muscle cells (cultured cells). This is in accordance with the report by HAUCK et al. [28], who also found progesterone and oestrogen receptor negative LLM cases. This would explain why some cases do, and others do not respond to hormonal treatment.

Tuberous sclerosis is an autosomal dominant disease with incomplete penetrance. RUSHTON and SHAYWITZ [29]

explained the non-penetrance in heterozygotes of tuberous sclerosis by a second unlinked gene, which modifies the expression of tuberous sclerosis. As suggested by COMINGS [30], the situation in tuberous sclerosis might be similar to retinoblastoma and related tumours, where both inherited and sporadic cases occur. Although genetic heterogeneity has been reported [3, 31], the basic defect and the gene itself are not yet known. A constitutional deficiency of deoxyribonucleic acid (DNA) repair may explain the hypersensitivity of tuberous sclerosis patients to irradiation and radiomimetic chemicals [32]. Recent studies on elevated chromosome instability in cultures from angiofibromas, as well as in fibroblasts and lymphocytes from patients with tuberous sclerosis, support this assumption [33]. Similar complex aberrations were described as we found in case 1 (tuberous sclerosis with LLM).

Our studies on lymphocytes from both patients revealed a normal karyotype in both, but an increased rate of nonclonal complex and unstable (dicentric) chromosome aberrations in patient No. 1, similar to those recently described in other patients with tuberous sclerosis [33]. LLM cells were available only from patient No. 2, with the isolated form of LLM. The main abnormalities were end-to-end (telomeric) associations or translocations, which are rarely found in normal cells, but are very common in virus-infected cell lines, and in some tumours [34]. It has been speculated that loss of telomeric repeats (5'-AGGGTT-3'), observed during ageing of cells, and in tumour cells might reduce chromosome stability and give rise to fused chromosomes [34]. The resulting dicentric chromosomes are known to be unstable, and tend to pull apart during mitosis, so that one daughter cell might receive a deleted chromosome and, thus, predispose to generating a malignant clone. One is tempted to speculate that the 14q deletion, present in two metaphases in our LLM sample from patient No. 2, arose in this manner. On the basis of our second case, it seems that isolated LLM is not related to tuberous sclerosis. LLM and tuberous sclerosis are probably incidentally combined. There are no findings indicating a genetic transmission of LLM in our second patient.

In conclusion, isolated LLM seems to be a separate entity and not a "forme fruste" of tuberous sclerosis. There are no complex chromosomal aberrations in the lymphocytes of isolated LLM, as in lymphocytes, adenomas and adenofibromas of tuberous sclerosis patients [33]. LLM can be incidentally combined with tuberous sclerosis, probably as one of the somatic mutations associated with this disease. LLM, with or without tuberous sclerosis, cannot be differentiated by morphological or immunohistochemical investigations, but can be separated by clinical and, probably, by genetic methods.

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