Respiratory Medicine (2011) 105, 768–774

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Pulmonary features of Birt-Hogg-Dubé syndrome: Cystic lesions and pulmonary histiocytoma

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Received 4 November 2010; accepted 4 January 2011
Available online 26 February 2011

KEYWORDS
Genetic skin diseases;
Lung diseases;
Kidney neoplasms;
Histiocytoma;
Pneumothorax

Summary
Background: to describe clinical, radiologic and pathologic features of lung lesions in Birt-Hogg-Dubè syndrome (BHDS) (MIM 135150).
Method: review of 12 patients of BHDS from 3 unrelated Italian families evaluated at GB Morgagni Hospital, Forlì, from 2005 to 2010.
Results: mean age (±SD) at diagnosis was 44.6 (±16) years, 8 (66%) were male. All three index cases presented with a history of recurrent pneumothorax and/or cystic lung lesions evaluated by CT scan request by referring pulmonary physicians, none were diagnosed to have BHDS at the time of initial pulmonary evaluation. One of the three cases was a middle-aged female patient with a clinical phenotype indistinguishable from lymphangioleiomyomatosis (LAM), characterized by cystic lung lesions and kidney angiomyolipoma. In one case of BHDS presenting with recurrent pneumothorax and a solitary lung nodule, surgical lung resection revealed a pulmonary histiocytoma. In one case a novel mutation of BHD gene was detected (c.771 del, exon 7).
Conclusions: BHDS is associated with cystic lung disease largely under-recognized by pulmonary physicians and can mimic LAM and may be associated with lung tumor, pulmonary histiocytoma. In one case we found a novel mutation in exon 7, c.771 del (ref.seq. NM_144997.5) never reported before.

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doi:10.1016/j.rmed.2011.01.002

Introduction

Birt-Hogg-Dubè syndrome (BHDS) (MIM 135150) is an autosomal dominant genodermatosis, predisposing to the development of follicular hamartomas of the skin, cystic lung lesions, pneumothorax and kidney neoplasms. BHDS was described for the first time in 1977, lung features of this syndrome have been described only in recent years and are limited. BHDS is caused by germline mutations in the BHD (FLCN) gene that is located in the short arm of chromosome 17 (17p11.2) and encodes for folliculin, a tumor suppressor protein.[1] Characteristic skin lesions described in BHDS are fibrofolliculomas (FF) and trichodiscomas (TD) of the skin, presenting as small, multiple papules on the face, neck and upper torso, characterized by strands of proliferating epithelial cells surrounded by a thick layer of connective tissue.[2] Pulmonary cysts are reported in 89% of affected members of BHDS families, and approximately 24% of subjects have a history of spontaneous pneumothorax.[3] Cystic lung disease in BHDS varies in severity, smoking has been described to be associated with a more severe lung disease in one study.[4] BHDS is associated with renal tumors such as chromophobe renal cell carcinoma (33%), hybrid oncocytic renal cell carcinoma (50%) clear cell renal carcinoma (9%) or oncocytoma (5%). The association of kidney angiomyolipoma is unusual in BHD but common in LAM, a cystic lung disease that shares with BHDS many features and common pathogenetic mechanisms involving the m-TOR pathway.[5,6] Extra-renal neoplasm such as colorectal adenomas, parotid oncocytomas, parathyroid adenomas, neural tissue tumors, lipomas, angiolipomas and connective tissue abnormalities, have been occasionally observed.[7,8] In this study we describe additional pulmonary features of this under-recognized entity, including the clinical presentation, radiologic, functional and histopathologic aspects.

Materials and methods

Study population

By referral from pulmonary physicians we evaluated three index cases and consecutively nine affected family members from three unrelated BHDS families at the GB Morgagni Hospital in Forlì and at the S. Maria Hospital in Terni — Italy, from January 2005 to June 2010. Molecular analysis of the BHD gene was performed after patient informed consent. Ten patients underwent genetic testing and all were found positive for the heterozygous FLCN mutation (Table 1). Six cases presented with skin lesions histological proven FF and one case with a histologically proven trichodiscoma, all were associated with cystic lung disease and/or kidney neoplasm and were considered affected with BHDS. Among the remaining five cases, all positive for BHDS gene mutation, two cases presented solely cystic lung lesion on HRTC and three cases did not show any clinical features of the disease. Histologically an FF was characterized according to previously published criteria.[2] This study was notified to the institutional review boards of the local ethic committee in accordance to our national legislation.

Clinical data collection

Data collection included demographic data, clinical presentation, laboratory results, radiologic findings, pulmonary function studies and pathologic analysis. CT scans of the chest were reviewed by two Radiologists (A.C. and N.S.). All 12 affected members had both high-resolution and standard CT scans of the chest available. All tissue specimens, including seven skin biopsies, one kidney resection, one surgical lung biopsy and one surgical lung resection, were reviewed by a lung pathologist (M.C.). Pulmonary function measurements included plethysmographically determined total lung capacity, residual volume and diffusing capacity for carbon monoxide were performed in seven cases. Pulmonary function data were expressed as percentage of predicted normal values. Additional information was obtained from patients interview and outside medical records review.

Mutation analysis

Genotype profile of family members was evaluated by mutation analysis. DNA extraction and mutation analysis of BHD gene were performed at Ospedale S. Croce e Carle, Cuneo — Italy. Genomic DNA was extracted and analyzed as previously reported by our group.[9] Genetic profile of the cases here presented (Table 1, family # 1 and family # 2) have already been discussed in our previously published paper.[9] In one case (Table 1, family # 3) we found a novel mutation in exon 7, c.771 del (ref.seq. NM_144997.5) never reported before and confirmed to be a mutation instead a polymorphism on 100 health donors. This is a frameshift mutation causing premature stop at protein level (p.Phe258LeufsX5).

Results

Clinical characteristics

Three index cases were referred to our institution, two for a history of recurrent pneumothorax and one for cystic lung disease. One of them was a 57 female patient with a unilateral kidney angiomyolipoma diagnosed by abdomen CT, suspected to be a case of pulmonary lymphangioleiomyomatosis (LAM). All three cases had cystic lung lesions documented by HRTC, none of them had a diagnosis of BHDS at the time of the initial evaluation by referring pulmonary physicians. At the time of evaluation at our center we documented skin lesions in seven cases, renal mass in two patients, cystic lung lesions in nine patients. Mean age (±SD) at diagnosis was 44.6 years (±16), and 8 (66%) were male. Histological examination of skin lesions confirmed six fibrofolliculomas and one trichodiscoma. Two patients were current smokers (mean, 11 pack-years) and two were former smokers (mean, 20 pack-years). None of them had a significant environmental exposure. Four patients (age range, 47—57 years) reported a history of recurrent pneumothorax (between 1 and 3 pneumothoraces per patient), two were former smokers. All cases of recurrent pneumothorax were treated with pleurodesis. Two patients were symptomatic and reported mild exertional dyspnea and cough, one was a never smoker patient with innumerable bilateral cysts and normal PFTs (Table 1,

Table 1 Clinical and CT findings.

Geder/age	FLNC gene mutation	Smoking	Clinical presentation	CT of the chest	Pulmonary function
Family # 1					
F/57 INDEX CASE	ND	Never	Fibrofolliculomas of the face and neck. Recurrent pneumothorax. Bilateral pulmonary cysts monolateral renal angiomyolipoma.	Innumerable bilateral cysts.	ND
M/50	c.1127 G > A exon 10	Former, pack-yr 20	Fibrofolliculomas of the face and neck. Recurrent pneumothorax. Bilateral pulmonary cysts on HRCT. Pulmonary nodule 12 mm (hystiocytoma). No renal tumor.	2 to 7 cysts (8—16 mm in diameter) in each lung	Normal
M/52	c.1127 G > A exon 10	Former, pack-yr 20	Fibrofolliculomas of the face and neck. Recurrent pneumothorax. Bilateral pulmonary cysts on HRCT. Kidney cancer involving the renal vein (mixed chromophobe, clear cell and oncocytic).	3 to 6 cysts (5—31 mm in diameter) in each lung (Fig. 2)	ND
M/37	c.1127 G > A exon 10	Never	No skin lesions. No respiratory symptoms. No renal tumor.	Normal	ND
F/31	c.1127 G > A exon 10	Never	No skin lesions. No respiratory symptoms. No renal tumor.	Normal	ND
F/85	ND	Never	Fibrofolliculomas of the face and neck. Dyspnea. Bilateral pulmonary cysts and emphysema on HRCT. No renal tumor.	Innumerable bilateral cysts and emphysema	ND
Family # 2					
F/47 INDEX CASE	c.1492C > T exon 12	Never	Fibrofolliculomas of the face. Bilateral pneumothorax. Bilateral pulmonary cysts on HRCT. No renal lesions.	Innumerable bilateral cysts (4—57 mm in diameter)	Normal PFTs.Mild reduction of DLco 62%pred.
M/42	c.1492C > T exon 12	Never	No respiratory symptoms. No skin lesions. No renal tumor. Bilateral pulmonary cysts on HRCT.	6 to 9 cysts (4—26 mm in diameter) in each lung	Normal
M/44	c.1492C > T exon 12	Current, pack-yr 20	Trichodiscomas of the face. No respiratory symptoms. Bilateral pulmonary cysts on HRCT. No renal tumor.	4 cysts (43—13 mm in diameter) in each lung	Normal
M/20	c.1492C > T exon 12	Current, pack-yr 2,5	No respiratory symptoms. No skin lesions. No renal tumor.	Normal	Normal
M/24	c.1492C > T exon 12	Never	No respiratory symptoms. Bilateral pulmonary cysts on HRCT. No skin lesions. No renal tumor.	4 to 7 cysts (3—8 mm in diameter) in each lung (Fig. 2)	Normal
Family # 3					
M/47	c.771 del exon 7	Never	Cough. Bilateral pulmonary cysts on HRCT. Fibrofolliculomas of the face. No renal lesions.	Innumerable bilateral cysts (5—30 mm in diameter) in each lung.	Mild restriction and mild reduction in DLco (TLC 62%; DLco 60%).

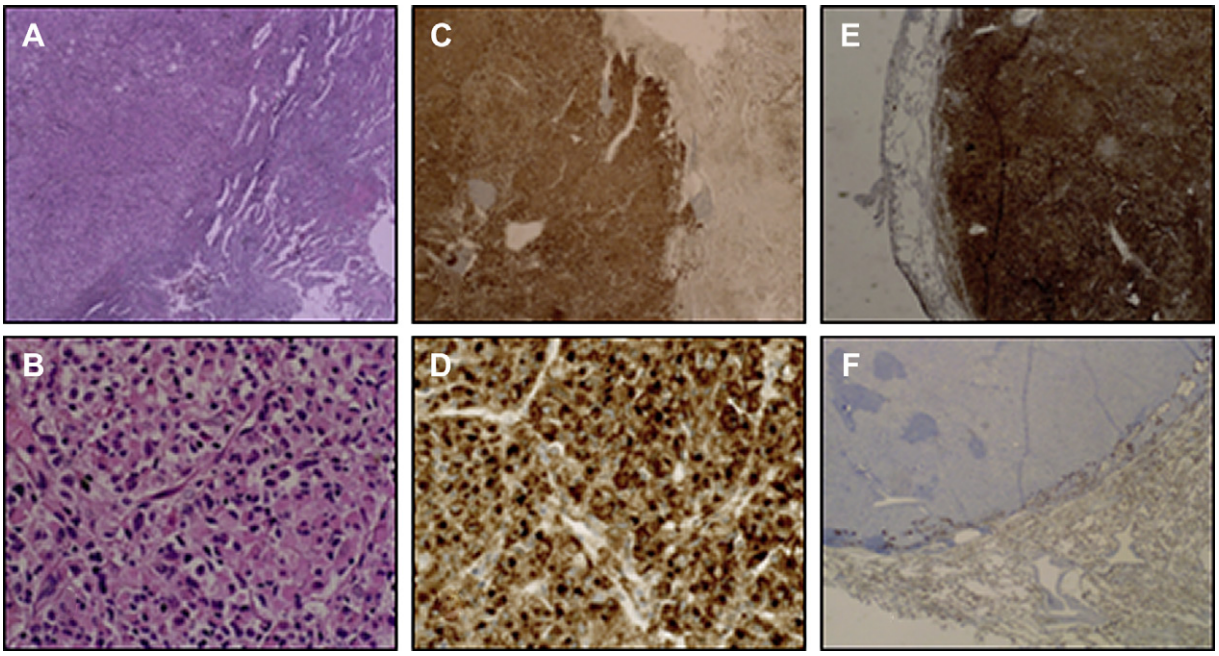

Figure 1 The morphologic and immunophenotypic characterization of 12 mm solitary lung nodule in the left lower lobe **A-B**) Hematoxylin — Eosin: morphologic features were consistent with pulmonary histiocytoma; **C-D**) Cathepsin — k was strongly expressed, **E**) CD16 was positive; **F**) CK8 was negative.

case M/47) and the other one was a 85 year-old female non smoker with cystic lung disease and emphysema.

Indication for CT scan was recurrent pneumothorax in two index cases, persistent cough in one index case and screening for BHDS in all other cases evaluated at our institution. Cystic lung lesions were detectable on high-resolution CT scan of the thorax in nine patients (75%), age ranging from 24 to 85 years. The cysts were variably demarcated by a thin-wall which ranged from invisible to nearly 2 mm thick, round or oval in shape with a surrounding normal parenchyma; they were scattered throughout the lungs with a slightly higher frequencies in the lower zones, the size of the cysts tending to be larger in the lower lobes, ranging between 3 and 57 mm. Lung cysts in patients presenting with bilateral pneumothorax were significantly more profuse and larger than those of other family members.

Pulmonary function tests were performed in seven patients and one patient had mild restriction (total lung capacity 62% of predicted value) with a mild reduction of DLco in two patients (DLco 62% and 60% of predicted value) (Table 1).

Surgical lung biopsy specimens were available in two patients who underwent pleurodesis and subsegmental lung resection (Table 1, case F/47 and case M/50). Histologic sections showed pulmonary cysts characterized by cystic dilatation of alveolar spaces ranging from microscopic foci to a few millimeters in diameter. The thin walled cysts were lined by cuboidal epithelium with no fibrous or smooth muscle tissue in the wall.

One patient underwent a surgical lung resection for a pulmonary solitary nodule measuring 12 mm in the left lower lobe. The morphologic and immunophenotypic characterization of this lesion were consistent with diagnosis of histiocytoma. Immunohistochemical staining with TTF1, HMB45, S-100, KERATIN 8, 18 and19, CD1a and CD208 were all negative. CD16, CD68 were positive and cathepsin-K was strongly expressed (Fig. 1). The surrounding lung parenchyma and lung cysts were negative for cathepsin-k expression. Histological review of the only case (Table 1, case M/52) of kidney neoplasm (right kidney, 3 cm of diameter with renal vein involvement) revealed a mixed pattern of clear cell, cromophobe and oncocytic renal cancer, with no expression of Cathepsin-K.

Discussion

This study reports 12 cases of BHDS, nine (75%) of whom presented with characteristic cystic lung lesions and four (33%) with a history of recurrent pneumothorax. Cystic lung lesions associated with BHD syndrome can appear at varying ages, in this case series ranging between 20 and 85 years. Severity of cystic lung disease appeared related to age but not to smoking history. Recent reports have well described radiologic and clinical features of cystic lung disease in BHDS and failed to document a possible relation between the severity of cystic lung disease and smoking history,[3] that is in accordance with our data. Most patients were asymptomatic, older patients and those with more severe lung involvement, tended to be more likely to have symptoms, particularly dyspnea, cough and recurrent pneumothorax. Pulmonary function was normal in most cases and only mildly impaired in two cases.

BHDS has become more recognized in recent years, but awareness of pulmonary physicians is still low. In the present study none of three index cases evaluated with CT scan prior to referral to our center was suspected to have BHDS. Differential diagnosis for cystic lung disease of BHDS has been discussed in

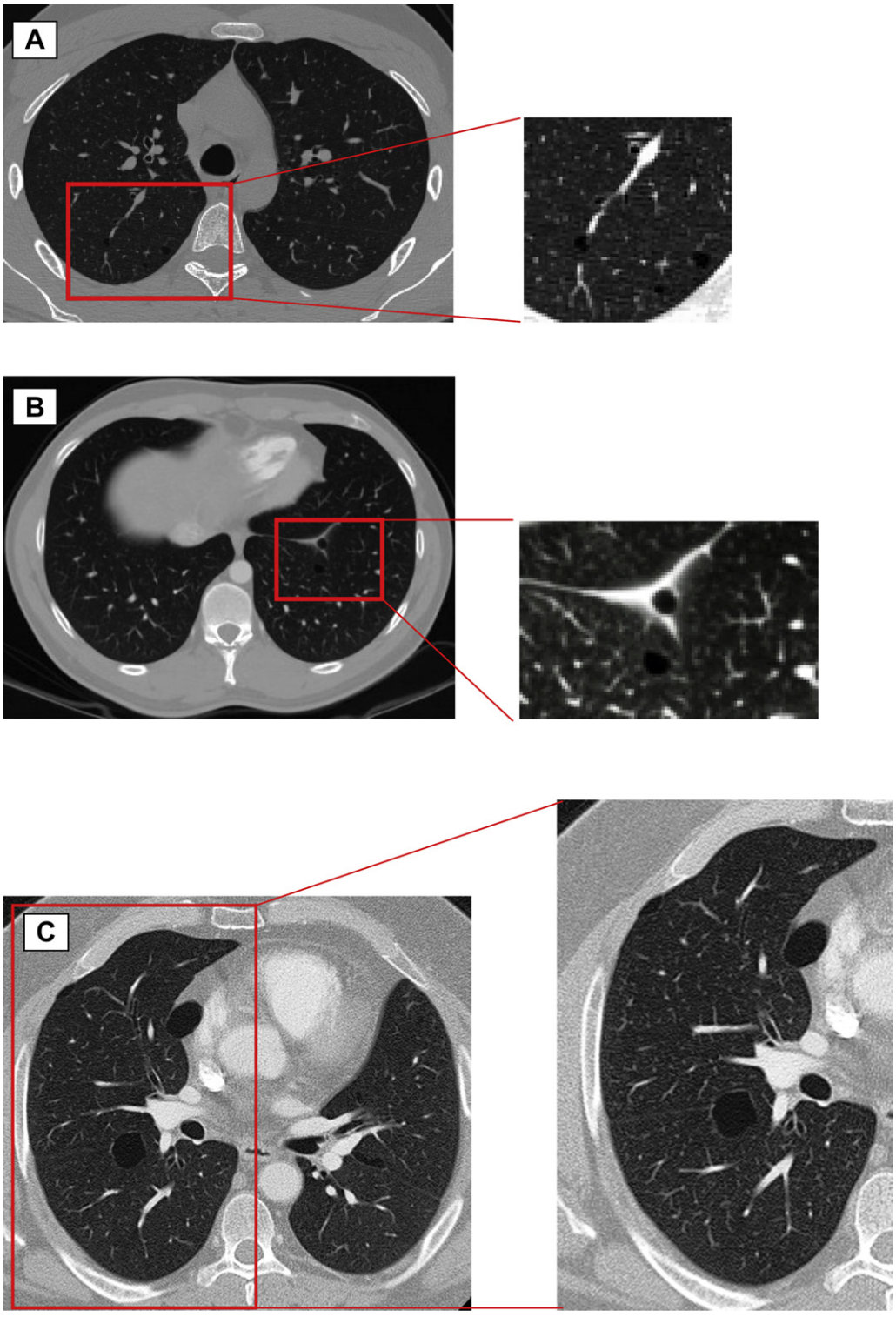

Figure 2 The high-resolution CT scan of two cases of BHDS. CT scan of upper lobes (**A**) and lower lobes (**B**) of the patient M/24, family #2. This case is representative of an **early case of BHDS**. Cysts were variably demarcated by a thin-wall and surrounded by normal parenchyma. Cysts were 4 in right lung and 7 in the left lung with diameter ranging from 3 to 8 mm. (**C**) CT scan of case M/52, family #1. This patient had a history of recurrent bilateral pneumothorax. The HRCT documented 3 to 6 cysts (5—31 mm in diameter) in each lung.

previous studies[3,4,14,15] and include pulmonary LAM, pulmonary Langerhans cell histiocytosis, Pneumocystis pneumonia, lymphocytic interstitial pneumonia and metastatic neoplasms including adenocarcinomas and low-grade sarcomas.

In particular, distinguishing BHDS from LAM can be very difficult. In the present report we describe a case of a 57 year-old woman presenting with FF of the face and a history of recurrent pneumothoraces. The CT scan documented

cystic lung lesions and a unilateral angiomyolipoma of the kidney. FF has been considered to be clinically characteristic of BHDS, but a recent report described FF of the skin in a patient with tuberous sclerosis complex (TSC). Therefore, the presence of FF is a useful but not a sufficient clinical element in the diagnosis of BHDS.[10,11] The renal angiomyolipoma is a type of kidney tumor that has never been observed in BHDS and is characteristically associated with sporadic LAM or tuberous sclerosis complex (TSC). This lesion represents another previously unreported overlap in clinical features between LAM and BHDS. The patient denied her consent for genetic testing and the final diagnosis of BHDS in this patient was based on family history of BHDS: four siblings (Table 1) were affected and positive for BHD gene mutation (c.1127 G > A mutation, exon 10). To exclude the presence of TSC one brother (Table 1, case M/50) was tested for both BHDS gene mutation and TSC1 and 2 and was found positive only for BHDS.

Clinical similarities between BHDS and TSC have induced a search for a common molecular pathogenesis for BHD and TSC and have led to the discovery that both BHD and TSC1/TSC2 are tumor suppressor genes that function through the common pathway of target of rapamycin proteins (m-TOR), associated with renal tumorigenesis. Kidney tumors are present in one third of patients with BHDS, are often multiple, and can present different histologic types, including oncocytome, chromophobe carcinoma, renal cell carcinoma and hybrid oncocytic/chromophobe carcinoma. Other neoplastic lesions of BHDS include intestinal colorectal adenomas, parotid oncocytomas, parathyroid adenomas, and neural tissue tumors.[7,8]

Association between lung tumor and BHDS has never been previously reported. Herein, we describe a case of pulmonary histiocytoma in a 50 year-old male, former smoker (PY 20), presenting with fibrofolliculomas of the face and neck, a history of recurrent pneumothorax and cystic lung lesions (Table 1, case M/50). The patient underwent surgical lung resection of a left basal solitary lung nodule of 12 mm. Pathologic analysis documented a pulmonary histiocytoma in the context of lung parenchyma disseminated with cystic lesions characterized by enlargement of alveolar spaces lined by cuboidal epithelium with no fibrous or smooth muscle tissue in the wall. Histiocytoma is a tumor composed of true histiocytes. It belongs to the large and controversial family of fibrohistiocytic tumors characterized by a dual cellular composition: cell with a fibroblastic appearance mixed with other cells having histiocytic features. Fibrohistiocytic tumors in the lung can present with bilateral cystic lesions or thin walled cavities causing hemoptysis, dyspnea or pneumothorax.[12] In the present case, histologic features of lung cysts were found to be consistent with the typical cystic lesions of BHDS and the research of spindle cells underlying the cystic wall was negative. This allowed a clear distinction from cystic fibrohistiocytic tumor. The lung nodule here reported presented the classical immunohistochemical profile and morphologic characteristics of histiocytoma. Interestingly cathepsin-K was strongly expressed in both spindle and epithelioid-shaped cells of the histiocytoma, but negative both in the normal parenchyma and in the cystic lesions. Lesions with a strong expression of this marker have never been described in BHDS. Given that cathepsin-K is known to be highly expressed in LAM[13] (particularly in the pulmonary foci of LAM cells and in renal angiomyolipoma cells) and that the m-TOR pathway regulates cathepsin-K expression, it is possible to speculate that also in BHDS the genetic mutation causing deregulation in m-TOR pathway leads to the production of cathepsin-K by spindle cells in the pulmonary histiocytoma here described. However, the complete lack of cathepsin-K expression documented in both lung cysts of this BHDS case and the kidney neoplasm of one brother (Table 1, case M/52) militates against this hypothesis. This is the first report of a true histiocytoma in the lung of a patient affected by BHDS. Whether there is or not a correlation between the BHD gene mutation and the development of this lesion remains to be addressed. This family, presenting with two peculiar cases among the six affected members, carries a novel mutation involving exon 10, whether this peculiar clinical phenotype is related to this novel mutation remains to be clarified.

The two main limitations of this study are that it is retrospective and conducted on a small number of patients.

It appears that cystic lung disease in BHDS may vary in severity and is mainly affected by age in accordance with previous studies.[3,14,15] The identification of early cases (Fig. 2) and the differential diagnosis from other cystic lung disorders is still problematic. The collection of an accurate family history and performing genetic investigations are two key steps in reaching a differential diagnosis with other diffuse cystic lung disorders that can mimic BHDS (particularly LAM and TSC). Lung tumor in BHDS has never been previously reported and further studies are needed to clarify whether the pulmonary histiocytoma here described could be linked to this rare disease or if it is just an incidental finding.

Conflict of interest

None of the authors have conflicts of interest to declare. Any author has current funding from the tobacco industry, or has any perceived links with the tobacco industry. The study was approved by the local ethic committee according to the current legislation.

Acknowledgments

We thank the families with BHDS for their cooperation. We thank Prof.J.H. Ryu, Mayo Clinic Rochester, USA, for the unique contribution he gave revising this manuscript. We also want to thank Prof. Migone, Torino University, Italy, for his contribution in genetic analysis of TSC1/TSC2 mutations. This publication has been supported by AMMP (Associazione Morgagni per le Malattie Polmonari).

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