



Lymphoplasmacytic lymphoma in a patient with Birt-Hogg-Dube syndrome

Kurata, Keiji ; Matsumoto, Hisayuki ; Jimbo, Naoe ; Yakushijin, Kimikazu ; Yamamoto, Katsuya ; Ito, Mitsuhiro ; Nakamachi, Yuji ;...

(Citation)

International Journal of Hematology, 112(6):864-870

(Issue Date)

2020-12

(Resource Type)

journal article

(Version)

Accepted Manuscript

(Rights)

© Japanese Society of Hematology 2020. The original publication is available at www.springerlink.com

(URL)

<https://hdl.handle.net/20.500.14094/90007629>



Lymphoplasmacytic lymphoma in a patient with Birt-Hogg-Dubé syndrome

Keiji Kurata^{1*}, Hisayuki Matsumoto², Naoe Jimbo³, Kimikazu Yakushijin¹, Katsuya Yamamoto¹, Mitsuhiro Ito¹, Yuji Nakamachi², Hiroshi Matsuoka¹, Jun Saegusa², Kuniaki Seyama⁴, Tomoo Itoh³, Hironobu Minami¹

Affiliations:

1. Department of Medical Oncology and Hematology, Kobe University Hospital, Japan
2. Department of Clinical Laboratory, Kobe University Hospital, Japan
3. Department of Diagnostic Pathology, Kobe University Hospital, Japan
4. Divisions of Respiratory Medicine, Juntendo University Faculty of Medicine and Graduate School of Medicine, Japan

***Corresponding author:** Keiji Kurata

Department of Medical Oncology and Hematology, Kobe University Hospital, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe, 650-0017, Japan

E-mail: kkurata@med.kobe-u.ac.jp

TEL: +81-78-382-5820

FAX: +81-78-382-5821

Running Head: LPL in a patient with BHD syndrome

Type of Manuscript: Case report

Compliance with Ethical Standards

Conflicts of interest: All of the authors, except one, report that they have no conflict of interest to disclose. Dr. Minami reports the following grants outside the submitted work

during the period of the study: personal fees, and other from Chugai; grants and personal fees from Eisai.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional board on ethics, at Kobe University Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The patient provided written consent for all the investigations carried out in this study and for the publication of this manuscript.

Abstract

Birt-Hogg-Dubé (BHD) syndrome is an autosomal dominant disease characterized by benign skin hamartomas, pulmonary cysts leading to spontaneous pneumothorax, and an increased risk of renal cancer. BHD syndrome is caused by germline mutations in the folliculin (*FLCN*) gene, a putative tumor suppressor, which result in loss of function of the folliculin protein and may cause cancer predisposition. In a 45-year-old woman with anemia, lymphadenopathy, and a history of recurrent spontaneous pneumothorax, ¹⁸F-FDG PET/CT detected diffuse and slight ¹⁸F-FDG accumulation in the bone marrow, enlarged spleen, and systemic multiple enlarged lymph nodes. Genetic examination identified a germline nonsense mutation [c.998C>G (p.Ser333*)] on exon 9 of *FLCN*. Pathological examination of the lymph node revealed a diffuse neoplastic proliferation of plasmacytoid lymphocytes. The neoplastic lymphoid cells were positive for CD20, CD138, and light chain kappa as per immunohistochemistry and mRNA *in situ* hybridization, and a *MYD88* gene mutation [c.755T>C (p.L252P)] was identified. Accordingly, she was diagnosed with lymphoplasmacytic lymphoma concomitant with BHD syndrome. To the best of our knowledge, this is the first report describing the development of hematological malignancy in a patient with BHD syndrome. The *FLCN* mutation might contribute lymphomagenesis as an additional mutation cooperating with the *MYD88* mutation.

Keywords

Birt-Hogg-Dubé syndrome, lymphoplasmacytic lymphoma, hereditary tumor syndrome, folliculin, gene mutation

Introduction

Birt-Hogg-Dubé (BHD) syndrome is an autosomal dominant disease, first described in 1977, characterized by benign skin hamartomas, pulmonary cysts resulting in spontaneous pneumothorax, and an increased risk of renal cancer (1). BHD syndrome is caused by germline mutations in the folliculin (*FLCN*) gene, which encodes the protein folliculin, and is a putative tumor suppressor gene whose function is still under investigation (2-8). *FLCN* gene mutations have been suggested to result in the loss of function of folliculin protein and may cause cancer predisposition (7, 9). Although a wide spectrum of tumors has been reported in association with BHD syndrome, there is no report on hematological malignancies. We herein describe, possibly the first patient with BHD syndrome, who developed lymphoplasmacytic lymphoma (LPL).

Case report

A 45-year-old woman presented to our hospital with fatigue. She had experienced several episodes of spontaneous pneumothorax and had undergone lower pleural covering techniques within the last 10 years. Although there was no family history of pulmonary cysts/spontaneous pneumothorax and renal tumors, she was diagnosed with BHD syndrome by *FLCN* gene mutation analysis 10 years ago. At the first visit, her vitals were stable although physical examination revealed conjunctival pallor, systolic murmur, and lymphadenopathy of the neck and axilla. There were no skin lesions of tumor such as fibrofolliculomas. Her laboratory tests showed pancytopenia, with a white blood cell count of 2,900/ μ L (neutrophils 36%, eosinophils 7%, basophils 1%, monocytes 10%, lymphocytes 41%, and abnormal lymphocytes 5%), hemoglobin level of 4.8 g/dL, hematocrit 16.2%, and a platelet count of 106,000/ μ L (Fig. 1A). Monoclonal gammopathy of IgM kappa type was also detected by immunofixation electrophoresis, although serum IgM was not remarkably

increased with a level of 299 mg/dL. The laboratory findings are summarized in the Table 1. The ^{18}F -fluorodeoxyglucose positron emission tomography/computed tomography presented diffuse and slight accumulation in the bone marrow, enlarged spleen, and systemic multiple enlarged lymph nodes; there was no obvious tumor or abnormal accumulation in the kidney (Fig. 1B). Moreover, chronic pneumothorax was found, with emphysematous changes and typical cysts in the lungs. Axillary lymph node and bone marrow biopsies were performed to confirm the diagnosis. Pathological examination revealed LPL, and chemotherapy with bendamustine plus rituximab was initiated.

Materials and methods

Histopathological and immunohistochemical examination, and RNA *in situ*

hybridization

The resected lymph node was fixed in 10% buffered formalin, embedded in paraffin after routine processing, and stained with hematoxylin and eosin (H&E). Immunostaining was performed with antibodies against the following: CD3 (Dako, clone: M7254), CD5 (Leica Biosystems, clone: PA0168), CD10 (Leica Biosystems, clone: RTU-CD10-270), CD20 (L-26) (Dako, clone: IR604), CD138 (Dako, clone: M7228), and folliculin (Abcam, ab176707). mRNA *in situ* hybridization was performed with antibodies against the following: L-chain kappa (DB Biotech, clone: H16-E) and L-chain lambda (DB Biotech, clone: K22.Y).

Mutation analyses of *FLCN* and *MYD88*, and loss of heterozygosity (LOH) analysis of the *FLCN* gene-associated region

To identify a *FLCN* mutation, genomic DNA isolated from peripheral blood mononuclear cells or lymph nodes were used according to the method described previously (6, 10). Briefly, each exon of *FLCN* was separately amplified and then screened by denaturing high-performance liquid chromatography. When a mobility shift was detected, sequencing of the exon of concern was performed using an automated sequencer (ABI Prism BigDye Terminator v1.1 Cycle Sequencing kit and ABI 3130 Genetic Analyzer; both Applied Biosystems, Foster City, CA).

For *MYD88* gene mutation analysis, genomic DNA was isolated from the lymph node at diagnosis and from the buccal mucosa using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Each DNA sample was examined to identify a somatic *MYD88* gene mutation according to the method described previously (11). The sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed on a 3500 Dx Genetic Analyzer (Applied Biosystems, Foster City, CA).

For LOH analysis of the *FLCN* gene-associated region (chromosome 17p12.2), each genomic DNA sample, extracted from the lymph node at diagnosis and from the buccal mucosa, was utilized. Two microsatellite markers, D17S740 and D17S2196, were examined for LOH analysis according to the method described previously (12); *FLCN* is located between the two markers. The PCR products were electrophoresed using an Applied Biosystems 3130/3130xl Genetic Analyzer. LOH analysis was performed using Gene Mapper 4.0 (both from Applied Biosystems, Foster City, CA). To confirm reproducibility, all lesions were examined at least twice. A > 50% reduction of signal intensity was defined as LOH.

Results

Mutation analysis identified a germline nonsense mutation (c.998C>G) on exon 9 of *FLCN* (NM_144997.5) (Fig. 2). The expected result would be premature termination of protein translation p.Ser333*, thereby establishing the diagnosis of BHD syndrome (13).

Immunohistochemistry of axillary lymph node revealed a diffuse neoplastic proliferation of small lymphocytes, plasma cells, and plasmacytoid lymphocytes partly with Dutcher body. The neoplastic lymphoid cells were positive for CD20, CD138, and light chain kappa and negative for CD3, CD5, CD10, and light chain lambda as seen by immunohistochemistry and mRNA *in situ* hybridization (Fig. 1C). Folliculin was slightly positive in the neoplastic lymphoid cells (Fig. 2B). Bone marrow examination also detected diffuse infiltration of neoplastic lymphoid cells (Fig. 1A). Further, the *MYD88* (NM_002468.5) mutation [c.755T>C (p.L252P)] on exon 5 was identified in the lymph node, but not in buccal cells, thus being consistent with the diagnosis of LPL (stage IV) (Fig. 1D).

Since more than half of the BHD syndrome-associated renal cell carcinomas show either a somatic *FLCN* mutation or LOH, thereby inactivating the second *FLCN* allele, we performed somatic *FLCN* gene mutation analysis and LOH analysis with lymph node involved in LPL. There were no somatic second hit mutations in the remaining wild type *FLCN* allele (data not shown), and the microsatellite markers on the *FLCN* loci showed no evidence of LOH (Fig. 2B).

Discussion

To the best of our knowledge, this is the first report describing the development of hematological malignancy in a patient with BHD syndrome. In addition to the high risk of developing renal tumors, an increased incidence of other tumor entities has also been suggested in BHD syndrome. In this context, there are isolated reports of a variety of

cutaneous and internal tumors in patients with BHD syndrome, including lipomas, cutaneous cysts, melanoma, and breast, lung, parotid, thyroid, and parathyroid tumors (14, 15). Unlike other cases, however, the patient presented in this report developed LPL.

MYD88 is known to play an important part in Toll-like receptor and interleukin-1 receptor signaling, leading to enhanced B cell survival. *MYD88* gene mutation has been suggested to play a central role in the pathogenesis of LPL by enhancing Janus kinase/signal transducer and activator of transcription (JAK-STAT) and nuclear factor κ B (NF- κ B) signaling pathways (16, 17). Mutated MYD88 also triggers transcription and activation of the Src Family Tyrosine Kinase HCK, which triggers several pro-growth and survival signaling pathways including AKT-mTOR pathway (18). However, it has been shown in mouse models that MYD88 L252P mutation alone is not sufficient to induce tumor formation and requires a second somatic mutation, including TNFAIP3, CXCR4, ARID1A/B, and CD79A/B in LPL, to provide an advantage for continuous B-cell clonal selection (19-21).

The *FLCN* gene, located on chromosome 17p11.2, encodes the protein folliculin and belongs to the tumor suppressor gene family. Although folliculin is thought to function as a tumor suppressor, its exact mechanism of action is not completely understood (9). Folliculin interacts with folliculin-interacting proteins 1 and 2 (FNIP1 and FNIP2, respectively), and folliculin and FNIP1 interact with 5'-AMP-activated protein kinase (AMPK), a negative regulator of mechanistic target of rapamycin (mTOR). Thus, it is suggested that folliculin may act as a tumor suppressor by controlling the interactions between FNIP1/FNIP2 and the mTOR signaling pathway (9). This is also supported by several reports demonstrated mTOR signal activation following to folliculin depletion (22-26). Furthermore, a previous report had suggested the folliculin protein to play a key role as a negative regulator of mTOR and may have small GTPase RAGA/B and RAGC/D interactions in B-lymphoma cell lines (27). Thus, we hypothesize that under co-existence of the both *MYD88* and *FLCN* mutation, activated

mTOR pathway via HCK upregulation could be further accelerated by the lack of negative regulation exerted by folliculin protein. The role of folliculin dysfunction upon mTOR pathway may be more complex and depend on cell types and/or nutritional status as there are several conflicting data (9). A number of published reports also support roles for folliculin in other signaling pathways and cellular processes including TGF- β signaling, regulation of HIF1 α transcriptional activity, cell cycle progression, regulation of rRNA synthesis, and interaction with HSP90 as a client with co-chaperones FNIP1/2 (9). More interestingly, the folliculin-FNIP complex is also necessary for B lymphocyte development (28-31). Although BHD syndrome has not previously been associated with B lymphocyte defects, suggesting that biallelic *FLCN* mutations in B lymphocyte precursors are required for the development defect, the monoallelic *FLCN* mutation in the present case might provide an advantageous condition for lymphomagenesis.

In the present case, somatic second hit mutations in the remaining wild type *FLCN* allele or LOH were not observed in LPL. In a study of 77 renal tumors from BHD patients with germline *FLCN* mutations, LOH on the short arm of chromosome 17 or somatic second hit mutations was identified in 70% of the BHD-associated renal tumors (32). On the other hand, it is reported that no evidence of somatic second hit mutations or LOH was found in fibrofolliculomas (33). It is also considered that *FLCN* transcripts containing premature termination codon are degraded by nonsense-mediated mRNA decay and not translated to produce truncated proteins, and the majority of mutant folliculin proteins, even if translated, are unstable and degraded (34, 35). Furthermore, the *FLCN* exon 1 deletion, including a putative promoter region, ablates the transcription of the *FLCN* allele (36, 37). These reports suggest that *FLCN* may be haploinsufficient in some contexts. On the contrary, it is also reported that some mutations can produce a truncated folliculin protein, which may have altered or dominant negative function (38-40). In fact, this is supported by the studies

reporting that some mutant proteins act as preventing the function of the wild-type folliculin protein (40, 41). Thus, it may well be true that the monoallelic *FLCN* mutation in the present case contributes lymphomagenesis cooperating with the *MYD88* mutation.

Regarding the current patient, no family member had been identified with either *FLCN* gene mutation or clinical history associated with BHD syndrome. However, it is possible that older family members of the presented patient carried the undiagnosed BHD syndrome. While approximately 200 families have been identified worldwide, *de novo* cases are very rare; there is only one reported case (38, 42). Alternatively, almost 200 unique *FLCN* germline mutations spanning all 14 exons have been identified till date and are listed in the Leiden Open Variation Database (LOVD, version 3.0) (43). The mutation (c.998C>G) on exon 9 of *FLCN*, identified in the present case, was a novel germline mutation.

Till date, unequivocal evidence in support of the association between BHD syndrome and malignancies, except for renal tumors, is lacking (15). However, considering the wide spectrum of tumors reported in association with BHD syndrome, more attention should be given to it as a hereditary tumor syndrome. Further, all patients with BHD syndrome should be screened for renal and other tumors, which may have occurred not just by chance.

Figure Legends

Fig 1. Histological, imaging, and genetic examinations for lymphoplasmacytic lymphoma.

(A) Peripheral blood (a) and bone marrow smear (b) with May-Giemsa stain show small and round plasmacytoid cells containing basophilic cytoplasm and condensed chromatin. Scale bar = 10 μ m. **(B)** The 18 F-FDG PET/CT scans of the patient. (a, b) Axial CT image in the lung window and axial PET/CT fusion image at the level of the carina. Bilateral enlarged lymph nodes of axilla with slight accumulation, and left pneumothorax. (c, d) Axial CT

image in the abdominal window and axial PET/CT fusion image at the level below the hepatic portal. Splenomegaly with slight accumulation. (e, f) Axial CT image in the abdominal window and axial PET/CT fusion image at the level of renal pelvis. Multiple enlarged lymph nodes with slight accumulation. (g) Maximum intensity projection image of ^{18}F -FDG PET showed diffuse accumulation in the systemic bone marrow and lymph nodes. Abbreviations: CT, computed tomography; FDG, fluorodeoxyglucose; PET, positron emission tomography. **(C)** Pathological findings of the axillary lymph node. The nodule consisted of diffuse infiltration of small and round shaped plasmacytoid lymphocytes with a Dutcher body (a, b) (Hematoxylin-Eosin Stain). Immunohistochemistry and mRNA *in situ* hybridization revealed that abnormal lymphocytes were focally positive for CD20 (c), diffusely positive for CD138 (d), and light chain kappa (e), and negative for CD3 (f), CD5 (g), CD10 (h), and light chain lambda (i). **(D)** Mutation analysis of *MYD88*. (a) Sequencing of exon 5 of *MYD88* from peripheral blood mononuclear cells demonstrates a missense mutation. The T to C transition at position 794 in exon 5, results in an amino acid substitution from leucine to proline at position 252. (b) The mutation was also detected by agarose gel electrophoresis of allele-specific polymerase chain reaction products. Lane M; the DNA of 100 bp ladder as a size marker; lane 1; lymph node, lane 2; buccal mucosa, lane 3; H₂O, lane A; wild type sequencing primer, lane B; mutated sequencing primer.

Fig 2. Genetic and histological examinations for folliculin. **(A)** Mutation analysis of *FLCN*. Sequencing of exon 9 of *FLCN* from peripheral blood mononuclear cells demonstrates a nonsense mutation. The C to G mutation, a transition at position 998 in exon 9, results in a premature stop codon at serine 333. **(B)** Immunohistochemistry staining for folliculin. (a) The neoplastic lymphoid cells of the axillary lymph node are slightly positive for folliculin. (b, c) The folliculin staining is strongly positive in *de novo* LPL cases. (d) The normal lymph node

is positive for folliculin. (C) Results of loss-of-heterozygosity (LOH) analysis.

Representative results of LOH analysis at D17S740 and D17S2196. Genomic DNA was isolated from both buccal mucosa and lymph node. The microsatellite markers on *FLCN* loci showed no evidence of LOH. The upper bar with numerals shows an electrophoretic position of DNA fragment size (bases).

Table 1. Laboratory findings at the diagnosis

References

1. Birt AR, Hogg GR, Dube WJ. Hereditary multiple fibrofolliculomas with trichodiscomas and acrochordons. *Archives of dermatology*. 1977;113(12):1674-7.
2. Cash TP, Gruber JJ, Hartman TR, Henske EP, Simon MC. Loss of the Birt-Hogg-Dube tumor suppressor results in apoptotic resistance due to aberrant TGFbeta-mediated transcription. *Oncogene*. 2011;30(22):2534-46.
3. Furuya M, Hong SB, Tanaka R, Kuroda N, Nagashima Y, Nagahama K, et al. Distinctive expression patterns of glycoprotein non-metastatic B and folliculin in renal tumors in patients with Birt-Hogg-Dube syndrome. *Cancer science*. 2015;106(3):315-23.
4. Hong SB, Oh H, Valera VA, Baba M, Schmidt LS, Linehan WM. Inactivation of the FLCN tumor suppressor gene induces TFE3 transcriptional activity by increasing its nuclear localization. *PloS one*. 2010;5(12):e15793.
5. Hudon V, Sabourin S, Dydensborg AB, Kottis V, Ghazi A, Paquet M, et al. Renal tumour suppressor function of the Birt-Hogg-Dube syndrome gene product folliculin. *Journal of medical genetics*. 2010;47(3):182-9.
6. Kunogi M, Kurihara M, Ikegami TS, Kobayashi T, Shindo N, Kumasaka T, et al. Clinical and genetic spectrum of Birt-Hogg-Dube syndrome patients in whom pneumothorax and/or multiple lung cysts are the presenting feature. *Journal of medical genetics*. 2010;47(4):281-7.
7. Lim DH, Rehal PK, Nahorski MS, Macdonald F, Claessens T, Van Geel M, et al. A new locus-specific database (LSDB) for mutations in the folliculin (FLCN) gene. *Human mutation*. 2010;31(1):E1043-51.
8. Nickerson ML, Warren MB, Toro JR, Matrosova V, Glenn G, Turner ML, et al. Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. *Cancer cell*. 2002;2(2):157-64.

9. Schmidt LS, Linehan WM. FLCN: The causative gene for Birt-Hogg-Dube syndrome. *Gene*. 2018;640:28-42.
10. Gunji Y, Akiyoshi T, Sato T, Kurihara M, Tominaga S, Takahashi K, et al. Mutations of the Birt Hogg Dube gene in patients with multiple lung cysts and recurrent pneumothorax. *Journal of medical genetics*. 2007;44(9):588-93.
11. Varettoni M, Arcaini L, Zibellini S, Boveri E, Rattotti S, Riboni R, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. *Blood*. 2013;121(13):2522-8.
12. Khoo SK, Kahnoski K, Sugimura J, Petillo D, Chen J, Shockley K, et al. Inactivation of BHD in sporadic renal tumors. *Cancer research*. 2003;63(15):4583-7.
13. Schmidt LS, Linehan WM. Molecular genetics and clinical features of Birt-Hogg-Dube syndrome. *Nature reviews Urology*. 2015;12(10):558-69.
14. Palmirotta R, Savonarola A, Ludovici G, Donati P, Cavaliere F, ML DEM, et al. Association between Birt Hogg Dube syndrome and cancer predisposition. *Anticancer research*. 2010;30(3):751-7.
15. Steinlein OK, Ertl-Wagner B, Ruzicka T, Sattler EC. Birt-Hogg-Dube syndrome: an underdiagnosed genetic tumor syndrome. *Journal der Deutschen Dermatologischen Gesellschaft*. 2018;16(3):278-83.
16. Poulain S, Roumier C, Decambron A, Renneville A, Herbaux C, Bertrand E, et al. MYD88 L265P mutation in Waldenstrom macroglobulinemia. *Blood*. 2013;121(22):4504-11.
17. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. *The New England journal of medicine*. 2012;367(9):826-33.
18. Yang G, Buhrlage SJ, Tan L, Liu X, Chen J, Xu L, et al. HCK is a survival determinant transactivated by mutated MYD88, and a direct target of ibrutinib. *Blood*. 2016;127(25):3237-52.
19. Wang JQ, Jeelall YS, Beutler B, Horikawa K, Goodnow CC. Consequences of the recurrent MYD88(L265P) somatic mutation for B cell tolerance. *J Exp Med*. 2014;211(3):413-26.
20. Hunter ZR, Yang G, Xu L, Liu X, Castillo JJ, Treon SP. Genomics, Signaling, and Treatment of Waldenstrom Macroglobulinemia. *Journal of Clinical Oncology*. 2017;35(9):994-1001.
21. Yu X, Li W, Deng Q, Li L, Hsi ED, Young KH, et al. MYD88 L265P Mutation in Lymphoid Malignancies. *Cancer research*. 2018;78(10):2457-62.
22. Baba M, Furihata M, Hong SB, Tessarollo L, Haines DC, Southon E, et al. Kidney-targeted Birt-Hogg-Dube gene inactivation in a mouse model: Erk1/2 and Akt-mTOR activation, cell hyperproliferation, and polycystic kidneys. *J Natl Cancer Inst*. 2008;100(2):140-54.
23. Chen J, Futami K, Petillo D, Peng J, Wang P, Knol J, et al. Deficiency of FLCN in mouse kidney led to development of polycystic kidneys and renal neoplasia. *PloS one*. 2008;3(10):e3581.
24. Hasumi Y, Baba M, Ajima R, Hasumi H, Valera VA, Klein ME, et al. Homozygous loss of BHD causes early embryonic lethality and kidney tumor development with activation of mTORC1 and mTORC2. *Proc Natl Acad Sci U S A*. 2009;106(44):18722-7.
25. Khabibullin D, Medvetz DA, Pinilla M, Hariharan V, Li C, Hergrueter A, et al. Folliculin regulates cell-cell adhesion, AMPK, and mTORC1 in a cell-type-specific manner in lung-derived cells. *Physiol Rep*. 2014;2(8):e12107.
26. Chen J, Huang D, Rubera I, Futami K, Wang P, Zickert P, et al. Disruption of tubular Fln expression as a mouse model for renal tumor induction. *Kidney Int*. 2015;88(5):1057-69.

27. Ying ZX, Jin M, Peterson LF, Bernard D, Saiya-Cork K, Yildiz M, et al. Recurrent Mutations in the MTOR Regulator RRAGC in Follicular Lymphoma. *Clinical cancer research*. 2016;22(21):5383-93.
28. Baba M, Hong SB, Sharma N, Warren MB, Nickerson ML, Iwamatsu A, et al. Folliculin encoded by the BHD gene interacts with a binding protein, FNIP1, and AMPK, and is involved in AMPK and mTOR signaling. *Proc Natl Acad Sci U S A*. 2006;103(42):15552-7.
29. Baba M, Keller JR, Sun HW, Resch W, Kuchen S, Suh HC, et al. The folliculin-FNIP1 pathway deleted in human Birt-Hogg-Dubé syndrome is required for murine B-cell development. *Blood*. 2012;120(6):1254-61.
30. Park H, Staehling K, Tsang M, Appleby MW, Brunkow ME, Margineantu D, et al. Disruption of Fnip1 reveals a metabolic checkpoint controlling B lymphocyte development. *Immunity*. 2012;36(5):769-81.
31. Ramírez JA, Iwata T, Park H, Tsang M, Kang J, Cui K, et al. Folliculin Interacting Protein 1 Maintains Metabolic Homeostasis during B Cell Development by Modulating AMPK, mTORC1, and TFE3. *J Immunol*. 2019;203(11):2899-908.
32. Vocke CD, Yang Y, Pavlovich CP, Schmidt LS, Nickerson ML, Torres-Cabala CA, et al. High frequency of somatic frameshift BHD gene mutations in Birt-Hogg-Dubé-associated renal tumors. *J Natl Cancer Inst*. 2005;97(12):931-5.
33. van Steensel MA, Verstraeten VL, Frank J, Kelleners-Smeets NW, Poblete-Gutiérrez P, Marcus-Soekarman D, et al. Novel mutations in the BHD gene and absence of loss of heterozygosity in fibrofolliculomas of Birt-Hogg-Dubé patients. *J Invest Dermatol*. 2007;127(3):588-93.
34. Fröhlich BA, Zeitz C, Mátyás G, Alkadhi H, Tuor C, Berger W, et al. Novel mutations in the folliculin gene associated with spontaneous pneumothorax. *Eur Respir J*. 2008;32(5):1316-20.
35. Nahorski MS, Reiman A, Lim DH, Nookala RK, Seabra L, Lu X, et al. Birt Hogg-Dubé syndrome-associated FLCN mutations disrupt protein stability. *Human mutation*. 2011;32(8):921-9.
36. Benhammou JN, Vocke CD, Santani A, Schmidt LS, Baba M, Seyama K, et al. Identification of intragenic deletions and duplication in the FLCN gene in Birt-Hogg-Dubé syndrome. *Genes Chromosomes Cancer*. 2011;50(6):466-77.
37. Enomoto Y, Namba Y, Hoshika Y, Komemushi Y, Mitani K, Kume H, et al. A case of Birt-Hogg-Dubé syndrome implying reduced or no wild-type folliculin without mutated protein is pathogenic. *Eur J Med Genet*. 2020;63(4):103820.
38. Menko FH, Johannesma PC, van Moorselaar RJ, Reinhard R, van Waesberghe JH, Thunnissen E, et al. A de novo FLCN mutation in a patient with spontaneous pneumothorax and renal cancer; a clinical and molecular evaluation. *Familial cancer*. 2013;12(3):373-9.
39. Laviolette LA, Wilson J, Koller J, Neil C, Hulick P, Rejtar T, et al. Human folliculin delays cell cycle progression through late S and G2/M-phases: effect of phosphorylation and tumor associated mutations. *PloS one*. 2013;8(7):e66775.
40. Luijten MN, Basten SG, Claessens T, Vernooij M, Scott CL, Janssen R, et al. Birt-Hogg-Dubé syndrome is a novel ciliopathy. *Hum Mol Genet*. 2013;22(21):4383-97.
41. Hasumi H, Hasumi Y, Baba M, Nishi H, Furuya M, Vocke CD, et al. H255Y and K508R missense mutations in tumour suppressor folliculin (FLCN) promote kidney cell proliferation. *Hum Mol Genet*. 2017;26(2):354-66.
42. Menko FH, van Steensel MA, Giraud S, Friis-Hansen L, Richard S, Ungari S, et al. Birt-Hogg-Dubé syndrome: diagnosis and management. *The Lancet Oncology*. 2009;10(12):1199-206.

43. LOVD. The Leiden Open variation Database,
<https://databases.lovd.nl/shared/genes/FLCN>.

Table 1. Laboratory findings at the diagnosis

Variable			Variable		
WBC	2900	/μl	TP	10.1	g/dl
Neutrophil	36	%	Alb	42.2	%
Lymphocyte	41	%	α1-Glb	1.9	%
Monocyte	10	%	α2-Glb	5.1	%
Eosinophil	7	%	β-Glb	3.5	%
Basophil	1	%	γ-Glb	47.3	%
Abnormal Lymphocytes	5	%	Alb	3.2	g/dl
RBC	154	×10 ⁴ /μl	IgG	110	mg/dl
Hb	4.8	g/dl	IgA	5	mg/dl
Ht	16.2	%	IgM	299	mg/dl
Plt	10.6	×10 ⁴ /μl	M-protein (IFE)	IgM-κ	
APTT	49.3	sec	BJP (urine)	κ-type	
PT-INR	1.19				
AST	15	U/l	Bone Marrow		
ALT	7	U/l	NCC	1.9	×10 ⁴ /μl
γ-GTP	10	U/l	Mgk	0	/μl
ALP	158	U/l	M/E	3.88	
T-Bil	0.6	mg/dl	Abnormal Lymphocytes	89.4	%
LDH	163	U/l			
Glu	90	mg/dl			
Na	139	mmol/l			
K	3.7	mmol/l			
Cl	105	mmol/l			
Ca	8.9	mg/dl			
BUN	15	mg/dl			
Cre	0.76	mg/dl			
CRP	0.02	mg/dl			

WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; Plt, platelet; APTT, activated partial thromboplastin time; PT-INR, prothrombin time-international normalized ratio; AST, Aspartate transaminase; ALT, Alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; T-Bil, total bilirubin; LDH, lactate dehydrogenase; Glu, glucose; BUN, blood urea nitrogen; Cre, creatinine; CRP, C-reactive protein; TP, total protein; Alb, albumin; Glb, globulin; Ig, Immunoglobulin; IFE, immunofixation; BJP, Bence Jones protein; NCC, nucleated cell count; Mgk, megakaryocyte; M/E, myeloid/erythroid ratio



