Chapter 7
Birt-Hogg-Dubé Syndrome

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Introduction

Birt-Hogg-Dubé (BHD) syndrome is a rare, autosomal dominant inherited disorder that predisposes affected individuals to develop benign cutaneous papules called fibrofolliculomas, pulmonary cysts, recurrent spontaneous pneumothoraces, and kidney tumors. Germline inactivating mutations in the *folliculin* (*FLCN*) gene located on the short arm of chromosome 17 are responsible for BHD syndrome [1]. Hornstein and Knickenberg [2] were the first to describe "cutaneous perifollicular fibromatosis cutis" that appeared to be inherited in a small two-generation family. Two years later, Canadian physicians Birt, Hogg, and Dubé reported a large multi-generation family in which 15 adult members presented with facial papules histologically consistent with fibrofolliculomas that were nearly indistinguishable clinically and histologically from the perifollicular fibromas described by Hornstein and Knickenberg [3]. The segregation patterns in both of these kindreds supported an autosomal dominant mode of inheritance for the cutaneous lesions, and the disease was subsequently named Birt-Hogg-Dubé (BHD) syndrome. An association of recurrent spontaneous pneumothoraces [4, 5] and/or bilateral renal tumors [6, 7] with BHD cutaneous lesions was suggested by several early case studies. A risk assessment performed by Zbar and colleagues in a cohort of BHD-affected family members and their unaffected siblings confirmed that renal tumors and spontaneous

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pneumothoraces were part of the phenotypic spectrum of BHD [8]. This chapter reviews the clinical manifestations, diagnostic criteria, molecular genetics, and management of BHD syndrome.

Clinical Manifestations of BHD Syndrome

BHD syndrome is characterized by phenotypic variability. Individuals affected with BHD may present with skin, pulmonary, or renal manifestations singly or in any combination. The clinical presentation may vary among affected individuals from the same BHD family or among BHD families that inherit the identical *FLCN* mutation. Familial spontaneous pneumothorax families with germline *FLCN* mutations have been reported in which pulmonary cysts and pneumothorax were the only presenting features [9–11], and BHD families presenting with renal cancer without pulmonary or cutaneous manifestations have also been described [12].

Cutaneous

Fibrofolliculomas, trichodiscomas, and acrochordons were described by Birt and colleagues as the hallmark cutaneous features of BHD syndrome [3]. Fibrofolliculomas, the most frequently observed manifestation, will develop in over 85% of individuals affected with BHD, usually in the third decade of life. They appear as white to flesh-colored papules, 2–4 mm in size, on the face, neck, ear lobes, and upper trunk (Fig. 7.1a). They can occur singly or in numbers >100, and are neither painful nor pruritic [13]. It has been suggested that fibrofolliculomas and trichodiscomas are in

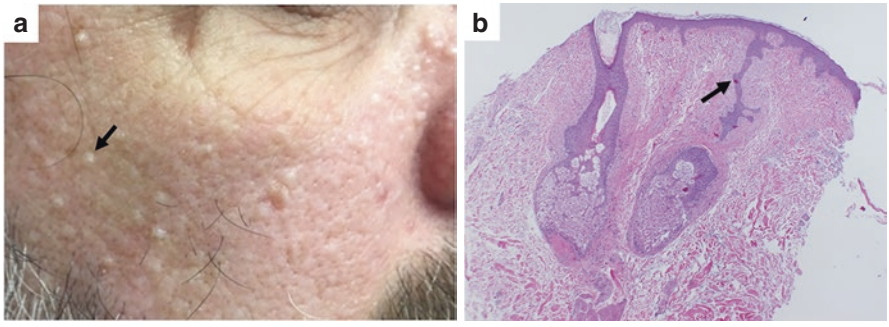

Fig. 7.1 (**a**) Multiple white, dome-shaped fibrofolliculomas (representative lesion indicated by arrow) on the face of a patient with BHD syndrome. (**b**) Hematoxylin and eosin staining of a fibrofolliculoma showing strands of epithelial cells surrounded by fibrous stroma (arrow) with adjacent hair follicle. (Adapted from Schmidt LS, Linehan WM [13])

fact the same lesion, but that different sectioning planes lead to the appearance of different histologies [14]. Acrochordons are common in the general population and, therefore, not specific for BHD syndrome. Other cutaneous manifestations reported in BHD patients include fibrous papules/angiofibromas, which are characteristically associated with tuberous sclerosis complex [15], lipomas [5, 7], perifollicular fibromas [16, 17], and papules of the oral mucosa [7].

Pulmonary

Cystic lung disease is present in approximately 90% of patients with BHD syndrome [11, 18, 19], typically appearing by the fourth decade of life, and in some cases representing the sole manifestation of the syndrome. Patients may note nonspecific respiratory symptoms including episodic chest pain, mild dyspnea, and cough [18], but it is uncertain whether symptoms are truly a consequence of the presence of cysts. Limited data on pulmonary function testing in BHD syndrome patients suggest that lung function is usually normal, though longitudinal assessment of lung function is lacking [20–23]. To date, there are no published reports of respiratory failure developing as a consequence of BHD syndrome.

As expected in the presence of cystic lung disease, spontaneous pneumothorax is relatively common in patients with BHD syndrome and is often the event that first brings them to medical attention. It has been estimated that BHD syndrome accounts for 5–10% of patients presenting with "primary" spontaneous pneumothorax [24]. The reported frequency of pneumothorax varies widely and is influenced by the particular characteristics of the study population. For example, 24% of patients at the National Institutes of Health recruited principally on the basis of dermatological manifestations had experienced at least one spontaneous pneumothorax compared to 76% of patients surveyed from the Rare Lung Diseases Clinic Network [18, 19]. The risk of pneumothorax is 50-fold higher among patients with BHD syndrome compared to unaffected family members [8].

Among BHD syndrome patients, the average age at the time of the first pneumothorax approximates 38 years [11, 18, 19], but this complication has been reported in individuals as young as 7 years of age. Total lung cyst volume, largest cyst diameter and volume, and extent of lower lung zone disease have been identified as risk factors for spontaneous pneumothorax [19, 21]. A family history of pneumothorax is elicited in up to 50% of BHD syndrome patients presenting with pneumothorax. Patients with a positive family history appear to be at greater risk for developing a spontaneous pneumothorax than those BHD syndrome patients without such a history [25]. In contrast, age, gender, smoking history, and specific *FLCN* gene mutation have not been associated with increased risk [19, 25]. For those patients experiencing an initial spontaneous pneumothorax, recurrence rates in the absence of pleurodesis are high, ranging from 42% to 86% in published series [11, 18, 19, 21].

Renal

Bilateral, multifocal renal tumors develop in up to one-third of BHD syndrome patients (range 12–35%) with a median age at diagnosis of 50–52 years [12, 25–28]. Zbar and colleagues found that BHD syndrome-affected family members had a sevenfold increased risk for developing kidney tumors when compared to their unaffected siblings [8]. BHD syndrome-affected individuals may present with a variety of histologic subtypes of renal tumors, most frequently chromophobe renal cancer and hybrid oncocytic tumors with features of chromophobe renal cancer and oncocytoma [29]. Multiple tumors with different histologies may be present in a single kidney of a BHD syndrome patient. Although quite rare, renal tumor metastases can develop in the setting of BHD syndrome [12, 27, 28].

Other Clinical Manifestations

A number of different clinical manifestations have been reported in patients with BHD syndrome including parotid gland tumors and parotid oncocytomas [26, 28, 30–32], parathyroid adenomas [5], thyroid nodules, and thyroid cancer [11, 28, 33], but whether these clinical features are part of the BHD syndrome phenotype remains to be determined.

Several early reports suggested a link between the BHD syndrome-associated cutaneous lesions and colonic polyps/cancer in BHD syndrome-affected individuals [2, 4, 34, 35]. However, a risk assessment conducted by Zbar et al. [8] in a BHD syndrome cohort from the United States did not find an increased risk for colon manifestations in BHD syndrome patients compared to their unaffected siblings. On the other hand, Nahorski et al. compared two groups of BHD syndrome patients from the United Kingdom with two different *FLCN* mutations and found a significantly greater risk for colon neoplasia in individuals with the c.1285dupC mutation relative to those with the c.610delGCinsTA mutation [36], suggesting the possibility that different *FLCN* variants may predispose to a greater or lesser risk of colon neoplasia in BHD syndrome. The lifetime risk of developing colon neoplasia by the age of 80 in the BHD syndrome cohort from the United Kingdom (n = 149) was 20% compared to a baseline of 4.9% in the unaffected population, which may suggest that additional factors affect colon polyp development in the setting of BHD syndrome including environment, population ethnicity, or *FLCN* genotypes.

Radiographic and Histologic Features of BHD Syndrome

Cutaneous

Fibrofolliculomas are characterized by centrally located, aberrant hair follicles surrounded by a moderately well-circumscribed proliferation of loose connective tissue with fine collagen fibers embedded in mucin-rich stroma (Fig. 7.1b). Anastomosing strands of epithelial cells, 2–4 cells thick, typically extend from the infundibulum of the hair follicle into the connective tissue mantle [3]. Small sebocytes are found within these epithelial structures [7]. In fact, many of the epithelial strands are continuous with the sebaceous glands, which often display hyperplasia, suggesting that fibrofolliculomas may be caused by abnormal growth and differentiation arising from the "mantle," which represents a stage in sebaceous gland morphogenesis [37].

Pulmonary

Several radiographic features visualized on high resolution computed tomography (HRCT) of the lungs may be helpful in distinguishing BHD syndrome from other etiologies of diffuse cystic lung disease [38, 39]. The cysts are thin walled, have a lower lung zone predominance, and frequently abut the pleural surface. They range in size from a few millimeters to over 2 centimeters. While smaller cysts tend to be circular, the larger cysts are often irregular or lentiform in shape and can be multiseptated (Fig. 7.2a, b). At least some of the cysts abut or incorporate proximal

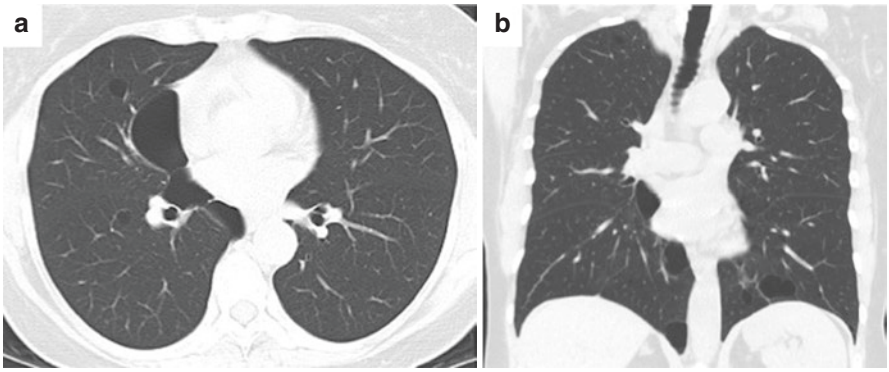

Fig. 7.2 Pulmonary manifestations of BHD syndrome. Axial (**a**) and coronal (**b**) HRCT images demonstrating the characteristic radiographic appearance of BHD lung cysts. (Note the subpleural and basilar distribution of the cysts and the subtle septations of the large cyst abutting the mediastinum on the axial image)

segments of the lower lobe pulmonary artery or vein [39]. Attesting to the distinct radiographic features of BHD syndrome, three expert thoracic radiologists presented with HRCT images from 89 patients with various cystic lung diseases were able to diagnose BHD syndrome with an accuracy of 93% [40].

In contrast, the histopathology of BHD syndrome lung cysts is not particularly distinctive and has been reported to closely resemble that of emphysematous blebs [41]. Several investigators have challenged this claim, arguing that this conclusion was based on studies of ruptured cysts in the setting of pneumothorax and that unruptured cysts must be closely scrutinized in order to appreciate the subtle differences [42, 43]. These investigators cite the absence of inflammatory infiltrates in the portion of the cyst wall remote from the subpleural region as the major feature that distinguishes BHD syndrome cysts from blebs. Because there are no pathognomonic features, however, lung biopsy cannot be used as a means to establish a diagnosis of BHD syndrome.

Renal

BHD syndrome patients present with bilateral, multifocal renal tumors that can be detected by magnetic resonance imaging (MRI) or computed tomography (CT) of the abdomen (Fig. 7.3a, b). Histologically, BHD syndrome-associated renal tumors

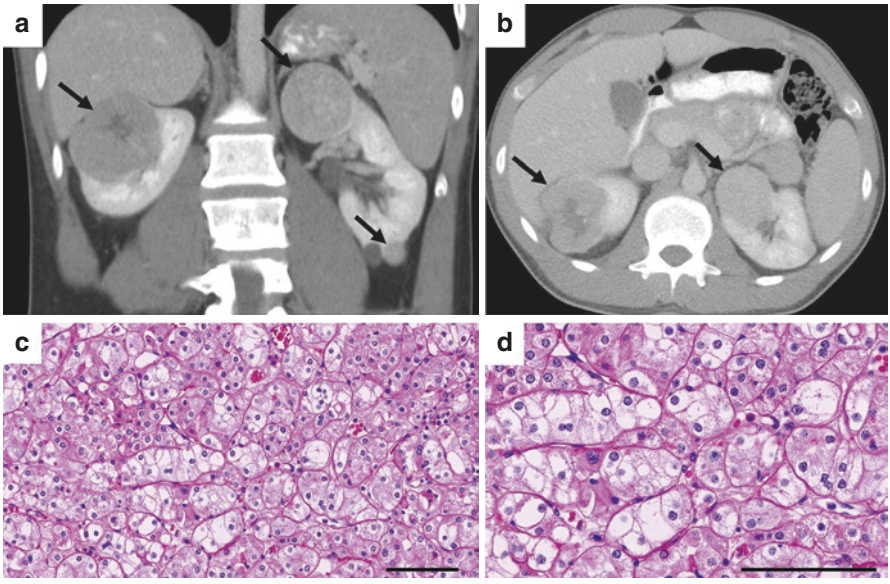

Fig. 7.3 Renal manifestations of BHD syndrome. Abdominal coronal (**a**) and axial (**b**) CT scans showing bilateral, multifocal renal tumors in a BHD patient (*arrows*). (**c**, **d**) Histology of hybrid oncocytic renal tumors that present most frequently in BHD patients. Scale bar, 100 μm. (Adapted from Schmidt LS, Linehan WM [13])

are most frequently chromophobe renal carcinoma (34%) or hybrid oncocytic tumors with features of both chromophobe renal carcinoma and oncocytoma (50%) (Fig. 7.3c, d), but clear cell renal carcinoma (9%), oncocytoma (5%) and, although rare, papillary renal cell carcinoma (2%) have also been reported [29]. Renal cysts have been documented in the setting of BHD syndrome, but no comparisons to general population-based frequencies have been performed [28, 33, 44]. Kidneys of BHD syndrome patients frequently harbor regions within the apparently normal renal parenchyma that contain microscopic foci of oncocytic cells termed "oncocytosis." Since oncocytosis develops in the kidneys of BHD syndrome patients who present with different histologic tumor subtypes, it has been suggested that renal oncocytic cells are precursors to all subtypes of renal cancer [29].

Diagnostic Criteria for BHD Syndrome

It is important to be aware of the phenotypic variability associated with BHD syndrome when evaluating the manifestations of a patient to confirm a diagnosis. Physicians should consider BHD syndrome in the differential diagnosis of a patient who presents with cutaneous papules clinically consistent with fibrofolliculomas or has a family history of these lesions. Additionally, pulmonary cysts, especially when located in the lung bases, or history of pneumothorax in a patient or as part of his/her family history, should raise suspicion for BHD syndrome. Finally, a personal or family history of bilateral or multifocal renal cancer with early age of onset (<50 years), especially with hybrid oncocytic or chromophobe histology, may be suggestive of BHD syndrome [13, 45, 46]. These characteristic features of BHD syndrome may be present singly or in any combination in a patient and his/her affected family members.

Several groups have proposed diagnostic criteria for BHD syndrome [13, 45, 46]. Two examples are presented in Tables 7.1 and 7.2; the latter is a "pulmonary-centric"

Table 7.1 Diagnostic criteria for Birt-Hogg-Dubé syndrome

Diagnosis requires one major or two minor criteria:
Major criteria
At least five fibrofolliculomas or trichodiscomas, with at least one histologically confirmed; adult onset
Pathogenic *FLCN* germline mutation
Minor criteria
Multiple lung cysts: bilateral basally located lung cysts with no other apparent cause, with or without spontaneous pneumothorax
Renal cancer: early onset (<50 years) or multifocal or bilateral renal cancer, or renal cancer of mixed chromophobe and oncocytic histology
A first-degree relative with BHD

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Table 7.2 Alternative diagnostic criteria for Birt-Hogg-Dubé syndrome

Characteristic[a] or compatible[b] lung HRCT with one of the following:
Skin biopsy positive for fibrofolliculoma or trichodiscoma
Confirmed family history of BHD in first- or second-degree family member
Histologic confirmation of chromophobe renal carcinoma or hybrid oncocytic tumor
Genetic testing positive for *FLCN* mutation

Modified from [46]

[a]Multiple thin-walled, round, elliptical, or lentiform well-defined air-filled cysts without internal structure, in a basilar, medial, and subpleural predominant distribution, with preserved or increased lung volume, and no other significant pulmonary involvement (specifically no interstitial lung disease)

[b]Thin-walled cysts without the more typical elliptical shape or subpleural distribution

schema that is applicable in the commonly encountered clinical context of the patient presenting with cystic lung disease. All the proposed criteria consider demonstration of a pathogenic *FLCN* mutation by genetic testing to be one means of confirming the diagnosis. In contrast to other cystic lung diseases (see below), lung biopsy does not provide diagnostic confirmation and should not be employed unless an alternative diagnosis is being strongly considered.

For the patient presenting with cystic lung disease, major diagnostic considerations other than BHD syndrome include lymphangioleiomyomatosis, pulmonary Langerhans cell histiocytosis, and lymphoid interstitial pneumonia. Clinical, radiographic, and laboratory features of each of these entities are summarized in Table 7.3.

Genetics of BHD Syndrome: *FLCN* Gene

FLCN Gene Discovery and Mutation Spectrum

Genetic linkage analysis in families with the cutaneous features of BHD syndrome localized the disease gene to the short arm of chromosome 17 [47, 48]. Using positional cloning strategies, Nickerson and colleagues narrowed the BHD genetic locus to chromosome 17p11.2, and subsequently identified germline protein-truncating mutations in a novel gene, *folliculin* (*FLCN*), which encodes a protein that is conserved across species [1]. In the 16 years since the *FLCN* gene was discovered, the mutation spectrum as reported in a number of large BHD cohorts [25–28, 33,

Table 7.3 Clinical, radiographic, and laboratory features of the common diffuse cystic lung diseases

Cystic lung disease	Clinical features	Typical HRCT features	Laboratory features
LAM	Almost exclusively in females (occasionally in males with TSC) Renal angiomyolipomas in up to 50% of cases Retroperitoneal or pelvic lymphangioleiomyomas Chylous effusion May be accompanied by clinical features of TSC	Small thin-walled round cysts, varying little in size or shape, uniformly distributed throughout the lungs	Serum VEGF-D > 800 pg/ml Presence of *TSC1* or *TSC2* mutation (in patients with underlying TSC) Airflow obstruction on spirometry Lung biopsy demonstrating characteristic histology with HMB-45 positive smooth muscle cells
LCH	Young adults between 20 and 40 years of age Nearly all affected adults are current or former smokers Diabetes insipidus in 15% Bone lesions in up to 15%	Early disease: nodules, some cavitary ("cheerio sign") Advanced disease: Cysts of varying size and irregular shape, with prominent walls Nodules and cysts have an upper and mid-lung zone distribution, often sparing the costophrenic angles	Bronchoalveolar lavage with ≥5% CD1a-positive cells Lung biopsy demonstrating characteristic histology with Langerhans cells staining positive for S-100 protein and CD1a
LIP	Underlying autoimmune or immunodeficiency disorder in majority of cases Sjogen's syndrome is most common underlying disorder; other disorders include lupus, HIV (typically in children), common variable immunodeficiency	Few thin-walled cysts, typically arising in areas of ground-glass opacity Often accompanied by centrilobular or subpleural nodules	Positive ANA, anti-Ro/SS-A, and/or anti-La/SS-B antibody Positive serologic test for HIV Hypogammaglobulinemia Lung biopsy demonstrating polyclonal infiltration of interstitium and alveolar air spaces with mature lymphocytes and plasma cells
BHD	Fibrofolliculomas Renal neoplasms, typically multifocal hybrid oncocytic tumors or chromophobe renal cell carcinomas Family history of pneumothorax or renal neoplasms	Bilateral thin-walled round or lentiform cysts of variable sizes, often subpleural, with a lower lung zone predominance	Genetic testing positive for *FLCN* mutation No distinctive features on lung biopsy

Abbreviations: *LAM* lymphangioleiomyomatosis; *LCH* Langerhans cell histiocytosis; *LIP* lymphoid interstitial pneumonia; *BHD* Birt-Hogg-Dubé; *TSC* tuberous sclerosis complex

49–51] now encompasses nearly 150 unique *FLCN* mutations located in all coding exons and includes all mutation types (insertion/deletion, nonsense, missense, splice site, intragenic deletions) [52]. Frameshift insertion/deletion mutations are the most frequent mutation type in BHD syndrome patients [52], and a mutation "hot spot" (c.1285dup/delC) has been reported in exon 11 in a mononucleotide run of 8 cytosines [1, 12, 26–28, 33, 51]. Due to the development of reliable methods to detect intragenic deletions/duplications (e.g., multiplex ligation-dependent probe amplification [MLPA], comparative genomic hybridization [CGH]) and more accurate DNA sequencing technologies, the mutation detection rate for *FLCN* reported in multiple BHD syndrome cohort studies is approaching 90% (range 69–88%) [25–28, 33].

Genotype-Phenotype Associations

To date, no clear association between *FLCN* mutation type or location within the gene and any of the phenotypic features of BHD has been reported; however, some interesting genotype-phenotype trends have emerged. Furuya and colleagues found the highest incidence of renal tumors in BHD syndrome patients who carried the c.1285dupC *FLCN* "hot spot" mutation (40.5%) [28], and Schmidt and coworkers saw significantly more renal cancer in c.1285dupC carriers relative to c.1285delC carriers [26]. Toro et al. reported a trend toward more pneumothoraces in individuals with *FLCN* mutations in exons 9 and 12. In addition, individuals with mutations in exons 9 and 12 had significantly more cysts, and larger cyst diameters and volumes than individuals with *FLCN* mutations in other exons [19]. Kunogi and coworkers [11] found more mutations in the 3′ end of the *FLCN* gene (exons 12 and 13; 13/25, 52%) in Japanese BHD patients, but this observation was not supported in another Japanese BHD cohort ($n = 120$) evaluated by Furuya and colleagues who found equal distribution of *FLCN* mutations along the entire length of the gene [28].

FLCN as a Tumor Suppressor Gene

Multiple lines of evidence support a role for *FLCN* as a tumor suppressor gene. The majority of *FLCN* mutations are insertion/deletion and nonsense mutations predicted to truncate the FLCN protein and lead to loss of function. *Flcn*-deficient mouse, rat, and canine models that carry a mutant or inactivated copy of the *Flcn* gene develop kidney tumors or multi-cystic kidneys subsequent to loss of the remaining wild-type *Flcn* allele [53–57]. Kidney tumors that develop in BHD syndrome patients show loss of the second copy of *FLCN* by mutation or loss of chromosome 17p sequences (loss of heterozygosity, LOH) (Fig. 7.4) [58]. Furthermore, a *FLCN*-null renal tumor cell line established from a BHD syndrome patient was tumorigenic in immunocompromised mice, but lost its oncogenic properties when wild-type *FLCN*

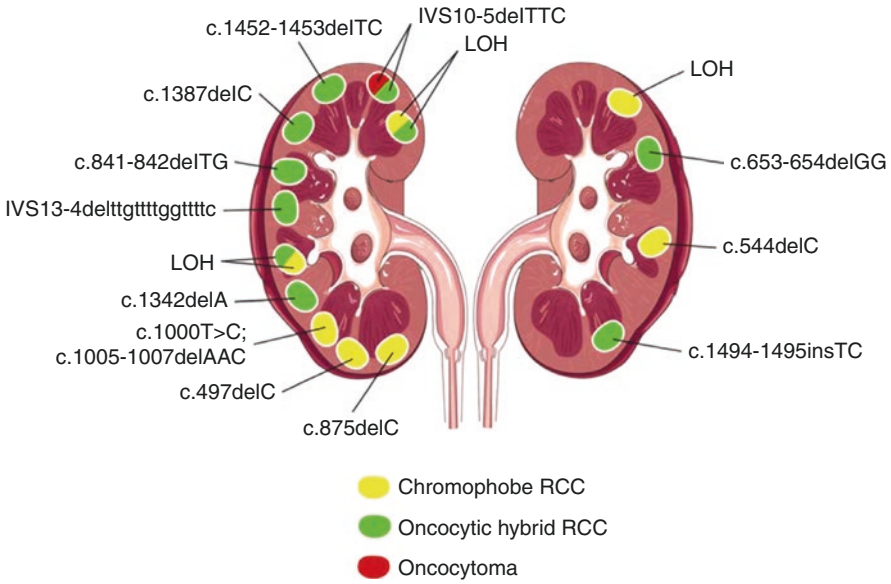

Fig. 7.4 *FLCN* is a tumor suppressor gene. Different somatic second hit *FLCN* mutations in multiple tumors that developed in the kidneys of a BHD patient with a germline *FLCN* mutation demonstrate that *FLCN* is a tumor suppressor gene. Histologic subtypes are color coded. Chromophobe renal cell carcinoma (RCC), yellow; oncocytic hybrid tumor, green; renal oncocytoma, red. LOH, loss of heterozygosity. (Reprinted with permission from Vocke CD et al. [58])

expression was restored [59, 60]. Interestingly, BHD pulmonary cysts [61] and fibrofolliculomas [62] stain positively for FLCN by immunohistochemistry raising the possibility that *FLCN* haploinsufficiency may be sufficient to trigger the aberrant cutaneous and pulmonary changes that lead to the BHD syndrome-associated phenotypes in these tissues.

Potential Functions of the FLCN Protein

The *FLCN* gene encodes a 64 kDa protein that is highly conserved across species but does not contain classical protein domains to suggest a functional role in cells. FLCN was shown to interact through its carboxy-terminus with two novel proteins, folliculin-interacting protein 1 (FNIP1) [63] and folliculin-interacting protein 2 (FNIP2) [64, 65], and with AMP-activated protein kinase (AMPK). AMPK is a critical energy and nutrient sensor in cells and an important negative regulator of mechanistic target of rapamycin (mTOR), which in turn is the master controller of cellular protein synthesis and cell growth [66]. The interaction of AMPK with FLCN and FNIP1/FNIP2 led researchers to investigate a potential role of FLCN in modulating mTOR activity.

Role of FLCN in Modulating mTOR Activity

Early work to elucidate FLCN function relied upon the development and study of *Flcn*-deficient in vivo models. Genetically engineered mouse models in which *Flcn* inactivation was targeted to the kidney developed polycystic kidneys and cystic renal tumors, which displayed activation of mTORC1 and reduced tumor/cyst formation in response to the mTOR inhibitor, rapamycin [55, 56, 67]. Renal tumors that developed in a heterozygous *Flcn* +/− mouse model subsequent to loss of the wild-type *Flcn* allele showed both mTORC1 and mTORC2 activation [57]. However, Hartman and colleagues generated a *Flcn* +/− mouse model that was also subjected to ENU mutagenesis, and the kidney cysts/tumors that developed in this model displayed mTORC1 inhibition (reduced phospho-S6 staining, a readout for mTORC1 activity) [68]. Hudon and coworkers developed another *Flcn* +/− mouse model in which phospho-S6 staining was negative in small single cysts but positive in large complex cysts, leading to the suggestion that the role of Flcn in modulating mTORC1 is complex and may be cell and context dependent [69].

Role of FLCN in Amino Acid-Dependent Regulation of mTOR Activation on the Lysosome

Functional insight has been gained from work by Nookala and colleagues who resolved the crystal structure of the carboxy-terminal half of FLCN and identified structural similarity to the differentially expressed in normal cells and neoplasia (DENN) domain proteins. DENN domain proteins are a family of Rab guanine nucleotide exchange factors (GEFs) involved in GDP-GTP exchange and an essential part of vesicle membrane transport [70]. Recent work from two independent laboratories has expanded this idea further by demonstrating that FLCN coordinates cellular responses to changes in amino acid availability through regulation of another Ras-related family of GTP-binding proteins, the heterodimers RagA/B and Rag C/D [71, 72]. mTORC1 activation by amino acids requires its recruitment to the lysosome surface, which is facilitated by a complex of lysosome-associated proteins including vacuolar adenosine triphosphatase, the Ragulator complex, RagA/B and RagC/D [73]. Petit and colleagues found that when amino acids are low, the FLCN-FNIP complex bound to RagA/B [72]; Tsun and coworkers showed that FLCN displayed GTPase activating protein (GAP) activity toward RagC/D placing it in its GDP-bound form, which is necessary for mTORC1 recruitment to the lysosome [71]. Meng and coworkers [74] have demonstrated that FLCN (in association with FNIP) binds to RagA/B only when it is in its GDP-bound state, which is achieved through the GTPase activating protein (GAP) activity of the GATOR1 complex [74]. These studies underscore a role of FLCN in amino acid-dependent regulation of mTOR activation, but the details remain to be fully elucidated.

Role of FLCN in Other Pathways Involved in Cellular Homeostasis

Experimental evidence has been presented to support a role for FLCN in regulating other important pathways and cellular processes including TFE3/TFEB transcriptional activation [72, 75, 76], canonical WNT signaling [77], regulation of PGC1α and mitochondrial biogenesis [78, 79], and autophagy [80, 81].

Role of FLCN in Maintenance of Proper Cell-Cell Adhesion and Cell Polarity

The mechanisms by which FLCN inactivation can lead to the development of pulmonary cysts and subsequent spontaneous pneumothorax remain under active investigation. Using a yeast two-hybrid approach, two independent laboratories discovered a physical interaction between FLCN and the adherens junction protein p0071 (also known as plakophilin-4), which is characterized by armadillo repeats and is a positive regulator of the small GTPase RhoA [82, 83]. However, their reports differ on whether FLCN positively or negatively affects p0071 function. Medvetz and coworkers [82] reported that downregulation of FLCN led to increased cell-cell adhesion and disruption of cell polarity, which was phenocopied by downregulation of p0071, and demonstrated that FLCN was a positive regulator of RhoA and Rho-associated kinase (ROCK) activity. Nahorski and colleagues [83] showed that FLCN colocalized with p0071 at cell junctions and the midbody during cytokinesis leading to disordered cytokinesis under conditions of FLCN deficiency. In contrast to Medvetz et al., they found that FLCN was a negative regulator of RhoA activity, and they showed that treatment of *FLCN*-deficient cells with a downstream inhibitor of ROCK activity reversed the increased cell migration phenotype [83]. Regardless of the disparate results that demonstrate both negative and positive regulation of RhoA activity by FLCN, both studies provide convincing evidence that the FLCN-p0071 interaction is important for proper cell polarity and intercellular junctions. Mice with *Flcn* inactivation targeted to the epidermal layer of the skin show a striking phenotype including skin and hair abnormalities, epidermal hyperplasia, and increased mTOR activation, providing further support for the in vitro studies that suggest that FLCN deficiency leads to deregulated cell-cell adhesion [82].

AMPK is required for cell survival and maintenance of epithelial cell junctions, and its activity is regulated through phosphorylation by the serine/threonine kinase LKB1. Localization of LKB1 to epithelial cell junctions is regulated by E-cadherin [84]. In the studies described above, Nahorski and colleagues observed a mislocalization of E-cadherin in a mouse inner medullary collecting duct-3 cell model with FLCN or p0071 knockdown [83]. A role for FLCN in regulating localization of E-cadherin was also supported by studies in a mouse model in which *Flcn*

inactivation was targeted to surfactant protein-C expressing alveolar epithelial cells (AECs) [85]. In this study, *Flcn*-deficient AECs displayed reduced AMPK phosphorylation, lowered LKB1 levels, and marked reduction of E-cadherin in the adherens junctions of cell membranes, which were reversed by Flcn expression. *Flcn* loss in AECs resulted in increased cell permeability and elevated apoptosis. Taken together these studies support a role for Flcn in the maintenance of epithelial cell-cell junctions and AEC survival, potentially through the E-cadherin-LKB1-AMPK axis, and demonstrate that *Flcn* loss may lead to alveolar airspace enlargement and cyst formation through dysregulation of this cascade.

The "stretch hypothesis" [82, 86, 87] has been proposed to explain how alveolar enlargements that produce BHD syndrome-associated pulmonary cysts develop. During respiration, cell-cell junctions allow the lung to expand and then "snap back" to its original shape and cellular structure. Defects in cell-cell adhesion under FLCN deficiency may lead to stretch-induced stress at regions with weaker cell-cell adhesion forces including interlobular septa and attachments to visceral pleura, resulting in airspace enlargement. Continued research efforts will be necessary to validate this proposed mechanism for pulmonary cyst development in BHD syndrome.

Management of BHD Syndrome

Cutaneous

Although fibrofolliculomas are not painful or pruritic, BHD syndrome patients often pursue treatment for removal of these lesions because they can be quite disfiguring. Surgical intervention using curettage and hyfrecation has provided satisfactory results in some cases [88]. Laser ablation (erbium:YAG or CO2) is another treatment option for removal of fibrofolliculomas without scarring, but partial relapse and occurrence of new lesions were noted after a period of 6 months [89]. Vernooij et al. have suggested that fibrofolliculomas may arise from abnormal growth of the sebaceous gland mantle, and since sebaceous gland growth can be inhibited by certain retinoids, topical treatment with these compounds may represent a potential therapy for the cutaneous lesions in BHD syndrome [37].

Pulmonary

HRCT is often performed as part of the diagnostic evaluation, particularly for patients whose initial presentation involved a spontaneous pneumothorax. For those patients for whom the diagnosis of BHD syndrome was based on renal or cutaneous manifestations or *FLCN* genetic testing, we recommend obtaining a baseline-HRCT, as the presence of cystic lung disease would prompt counseling the patient on the possible future occurrence of pneumothorax. We also recommend obtaining

baseline pulmonary function testing in those patients with cystic lung disease and those who report dyspnea on exertion since our current understanding of the impact of BHD syndrome on lung function is incomplete. CT scans and pulmonary function testing should be repeated only as warranted by new or worsening respiratory symptoms; there is currently no indication to perform serial testing for surveillance purposes alone.

Pulmonary management of BHD syndrome principally centers on treatment and secondary prevention of pneumothoraces. Because of the high recurrence rate with conservative management alone (i.e., chest tube thoracostomy), performance of pleurodesis following an initial spontaneous pneumothorax is generally recommended. Data on the efficacy of pleurodesis in this setting are scant, but one survey-based study suggested an ipsilateral pneumothorax recurrence rate of 33% following pleurodesis compared to 63% when pneumothorax was managed conservatively [18]. A Japanese group has developed a novel thoracoscopic approach to pneumothorax prevention, covering the entire visceral pleura with an absorbable cellulose mesh applied with fibrin glue. This not only provides an immediate seal at the site of the pleural rent but also induces fibrous thickening of the visceral pleura, without producing visceral to parietal pleural adhesions and presumably without compromising lung expansion and lung function. Among 52 BHD syndrome patients undergoing total pleural covering, none experienced a subsequent recurrent pneumothorax, with a mean follow-up period of 38 +/− 22 months [90]. However, this procedure is not widely available, and additional studies will be required before it can be recommended as an alternative to pleurodesis.

An area of uncertainty in managing BHD syndrome patients with cystic lung disease is the risk of pneumothorax associated with commercial air travel. As ambient pressure falls in a partially pressurized commercial jet flying at cruising altitude, the trapped gas within non-communicating lung cysts expands according to Boyle's Law, thus risking cyst rupture and pneumothorax. The actual risk of pneumothorax with air travel for the BHD syndrome patient is uncertain but likely small, with two survey-based studies estimating the risk to be 0.12–0.63% per flight [18, 91]. The risk is likely further mitigated following pleurodesis. Although patients should be counseled about the potential risk, air travel is not contraindicated in the absence of significantly compromised lung function (which would not be anticipated from BHD syndrome alone). Similar uncertainties exist about the risk of pneumothorax with diving, but at least one professional society considers cystic lung disease of any cause to represent an absolute contraindication [92].

Renal

Individuals with a confirmed diagnosis of BHD syndrome are at lifelong risk for the development of bilateral, multifocal renal tumors. Since renal tumors have been reported in BHD syndrome patients as young as 20 years of age [12], it is recommended that at-risk individuals undergo serial abdominal imaging every 36 months

starting at 20–21 years of age [45, 93]. CT or magnetic resonance imaging with intravenous contrast is preferred to give the best anatomic detail of the kidneys. Renal ultrasonography is not recommended because it may not detect some tumors due to small size or because of similar echogenicity of BHD-associated hybrid oncocytic or chromophobe renal tumors to the surrounding renal parenchyma. Once a renal tumor is identified, the patient is monitored closely until the tumor reaches 3 cm in maximum dimension, at which point surgical intervention is recommended. Since BHD syndrome patients are at risk for developing multiple tumors, and therefore, may undergo repeat surgeries during their lifetime, nephron sparing surgical procedures are optimal to conserve as much normal kidney parenchyma as possible. Cryotherapy or radiofrequency ablation can complicate future surgical procedures; hence, these approaches are not recommended for BHD syndrome patient management unless the patient is a poor surgical candidate [93].

Conclusion

In the 16 years since germline mutations in the novel tumor suppressor gene *FLCN* were identified as causative for BHD syndrome, we have expanded our knowledge of the BHD clinical phenotype and our understanding of the *FLCN* mutation spectrum. Through the development and study of a number of *FLCN*-deficient in vitro and in vivo models, significant advancements have been made in our understanding of the cellular pathways and processes in which FLCN may have a functional role (Fig. 7.5; extensively reviewed in Schmidt et al. [94]).

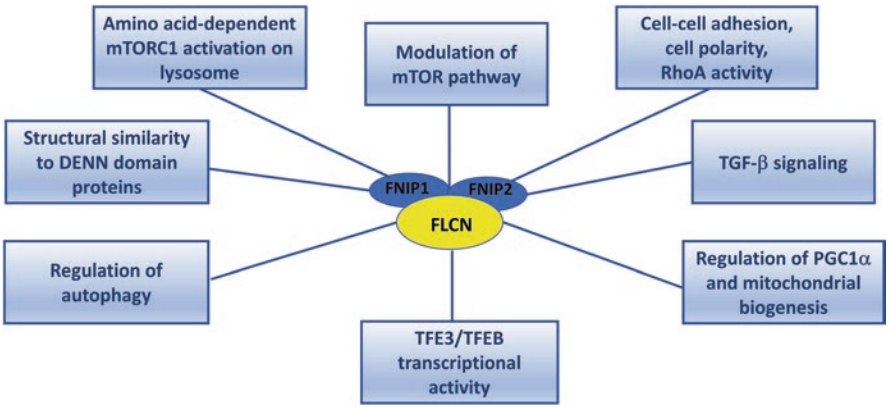

Fig. 7.5 Molecular pathways and cellular processes in which FLCN may have a functional role. (Adapted from Schmidt LS, Linehan WM [77])

However, details remain elusive as to the exact mechanisms underlying pulmonary cyst formation and kidney tumor development. Further studies are needed to identify biomarkers prognostic for more aggressive kidney cancer and to inform therapeutic drug design, and to predict those BHD syndrome-affected individuals who will be most at risk for developing pneumothoraces. Physicians need to be on high alert for the clinical manifestations and patient/family histories that would lead to suspicion for BHD syndrome. If warranted, they should recommend *FLCN* genetic diagnostic testing, and if positive, set up appropriate routine surveillance for kidney tumors and advise patients on ways to reduce recurrence of pneumothorax.

Key Learning Points

- Birt-Hogg-Dubé (BHD) syndrome is an autosomal dominant inherited disorder that predisposes at-risk individuals to develop benign cutaneous fibrofolliculomas, pulmonary cysts, recurrent spontaneous pneumothoraces, and bilateral multifocal kidney tumors.
- Germline loss-of-function mutations in the *folliculin* (*FLCN*) gene are responsible for BHD syndrome.
- Loss of heterozygosity or somatic mutation of the remaining copy of the *FLCN* gene was demonstrated in kidney tumors that develop in animal models of BHD syndrome and in BHD syndrome patients confirming a tumor suppressor role for *FLCN*.
- Evidence is emerging that FLCN may function in a number of energy and nutrient sensing pathways including the AMPK-mTOR axis, PGC1α regulation and control of mitochondrial biogenesis, amino acid-dependent activation of mTOR on the lysosomes through Rag GTPases, and cell-cell adhesion through RhoA signaling.
- Pulmonary management of patients with BHD syndrome principally centers on treatment and secondary prevention of spontaneous pneumothorax; pleurodesis following an initial episode is recommended due to the high rate of ipsilateral recurrence.
- Individuals who inherit a pathogenic *FLCN* mutation are at lifelong risk for developing kidney tumors and therefore should undergo routine abdominal imaging to monitor for tumor development.

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