Birt-Hogg-Dubé Syndrome
Clinicopathologic Findings and Genetic Alterations

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• Context.—Birt-Hogg-Dubé (BHD) syndrome is a rare clinicopathologic condition transmitted in an autosomal dominant fashion. This complex entity is characterized by cutaneous fibrofolliculomas, kidney tumors, pulmonary cysts, and spontaneous pneumothorax. Recently, the gene possibly responsible for the clinical manifestations of BHD syndrome has been cloned and characterized. The few reviews of BHD syndrome found in the English literature mostly focus on the skin lesions or genetics, with limited information on other pathologic changes, particularly the kidney lesions.

Objective.—To review the literature on this subject with a special emphasis on BHD syndrome-associated renal pathology as well as recent advances in molecular genetic discovery of the BHD syndrome.

Data Sources.—We used all data available after performing a literature search using MEDLINE and searching under the headings “Birt-Hogg-Dubé,” “hybrid oncocytic tumors,” and “folliculin.”

Conclusions.—The presence of BHD syndrome should be investigated in any patient with multiple bilateral kidney tumors, especially if the predominant histologic type is chromophobe renal cell carcinoma or the so-called hybrid oncocytic tumor. The genetic alteration for BHD syndrome has been mapped to chromosome 17p12q11, and the gene in this region has been cloned and believed to be responsible for the BHD syndrome. The function of the BHD product, called folliculin, is still unknown, although it is speculated to be a tumor suppressor gene. Numerous mutations have been described in the BHD gene. Studies are ongoing to determine the relationship between the BHD gene and development of sporadic renal cell carcinoma and other lesions.

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Although the majority of kidney neoplasms occur sporadically, approximately 4% are associated with heritable syndromes such as Beckwith-Wiedemann syndrome associated with Wilms tumors, von Hippel-Lindau syndrome associated with renal cell carcinoma (RCC), tuberous sclerosis associated with angiomyolipoma, hereditary papillary RCC, and familial renal oncocytoma. Birt-Hogg-Dubé (BHD) syndrome is another rare clinicopathologic complex associated with renal neoplasms.

In 1975, Hornstein and Knickenberg1 described an inherited syndrome in 2 siblings that was characterized by multiple perifollicular fibromas on the face, neck, and trunk that were associated with multiple intestinal polyps. Numerous soft pedunculated fibromas resembling skin tags were also noted in both patients. The patients’ father reportedly had similar skin lesions and a history of kidney cysts. The authors discussed the possibility of a peculiar cutaneous-intestinal syndrome not previously described in the literature.

In 1977, Birt, Hogg, and Dubé2 reported on a Canadian kindred in which 15 of 70 members were reported to have multiple 2-mm to 4-mm yellow-white, dome-shaped papules on the forehead, scalp, face, and neck that were inherited in an autosomal dominant fashion. Multiple small skin tags were intermingled with the facial tumors and also appeared on the upper eyelids and axillary folds. Histologic findings of biopsied papules revealed circumscribed proliferations of specialized connective tissue around the variably dilated hair follicles. The authors designated the term fibrofolliculoma to describe these lesions. In addition, previously described trichodiscomas were present in 3 of the 5 patients who underwent skin biopsies. Skin tumors appeared only after the age of 25 years. The authors proposed the possibility of an autosomally inherited cutaneous syndrome but made no mention of any additional gastrointestinal, pulmonary, or kidney findings in these patients.

The aforementioned syndromes described by Hornstein and Knickenberg1 and Birt et al2, respectively, are now considered to represent the same syndrome designated as the Birt-Hogg-Dubé syndrome,3,4 although it was also referred to as the Hornstein-Knickenberg syndrome in literature in the past. Since the sentinel studies, more than 30 families have been identified with BHD syndrome, and it has been shown that patients with this syndrome also have an increased incidence of renal tumors, lung cysts, and spontaneous pneumothorax.5 In this article we review the literature on BHD syndrome, with special emphasis on associated renal and pulmonary pathology and recent molecular discoveries concerning this interesting entity.
Figure 1. Different types of kidney tumors that occurred in a single patient with Birt-Hogg-Dubeé (BHD) syndrome: renal oncocytoma (A), chromophobe renal cell carcinoma (B), and hybrid oncocytic tumor, which typically has an admixture of eosinophilic cells with round nuclei resembling oncocyes (C), as well as clear cells with prominent nuclear membranes that are more reminiscent of chromophobe renal cell carcinoma (D) (hematoxylin-eosin, original magnifications ×400 [A through D]).

RENAL PATHOLOGY

In the initial reports by Hornstein and Knickenberg1 and Birt et al,2 kidney lesions were not recognized as a part of the BHD syndrome. Only since 1993, 18 years after the first description of BHD syndrome, has the association between BHD syndrome and renal neoplasms become clearer. Birt-Hogg-Dubé syndrome has been reported in association with renal tumors of a variety of histologic types including clear cell, papillary, and chromophobe types of RCC as well as oncocytomas.6–9

In 1993 Roth et al7 described the first documented association of the BHD syndrome with renal pathology in a man with bilateral kidney tumors. Histopathologic examination revealed 1 clear cell RCC and 1 chromophobe RCC with a mixed population of clear and eosinophilic cells in the same tumor. In a study of 13 patients with BHD syndrome, Toro et al10 later reported that 7 of these patients had renal neoplasms, including renal oncocytomas and papillary RCCs. In the largest single study of 98 patients with BHD syndrome, Zbar et al6 determined that the odds ratio for developing renal tumors in BHD syndrome-affected family members adjusted for age was 6.9, with renal tumors found in 15 (15%) of the patients.

Two unique features of renal tumors in patients with BHD syndrome are the diversity of histologic types of tumors seen even in a single kidney (or patient) (Figure 1) and distinct cell populations within individual tumors. Prior analyses have noted the presence of clear cell RCCs, papillary RCCs, chromophobe RCCs, and oncocytomas, as well as so-called hybrid oncocytic tumors (not previously described in other syndromes) in patients with BHD syndrome.10,11 Moreover, chromophobe RCC is the most predominant type of renal tumor.6,10,11 Kidney tumors in BHD syndrome seem to occur earlier than sporadic tumors, and are usually multiple and bilateral.10,11

CHROMOPHobe RCC, ONCOCYTOma, AND HYBRID TUMORS

Oncocytoma and chromophobe RCC are thought to originate from the intercalated cells of renal collecting tubules and share overlapping histologic features.12 In fact, these 2 tumors share strong similarities in gene expression profiles in addition to their overlapping morphologies.13 Numerous case reports have described both oncocytoma (Figure 1, A) and chromophobe RCC (Figure 1, B) occurring in patients with BHD syndrome.7,9–11,14,15 In the pre-
Globally mentioned study of 98 patients with BHD syndrome by Zbar et al, chromophobe RCC was the predominant type of renal cancer found in these patients, seen in 7 of 14 histologically examined tumors.

In addition to diverse histologic subtypes of renal tumors, a single tumor may be composed of multiple distinct cell populations. So-called hybrid oncocytic tumors are frequently observed in patients with BHD syndrome. Hybrid oncocytic tumors are characterized by histologic features similar to both chromophobe RCC and oncocytoma. Furthermore, a mixture of several distinct populations of tumor cells can be seen in hybrid tumors (Figure 1, C and D). Curiously, the case report of the first documented renal tumors in a patient with BHD syndrome gave a peculiar description of "chromophobe adenocarcinoma with a mixed population of clear and eosinophilic cells," which raises the possibility that this tumor was actually a hybrid tumor. Typically, hybrid tumors contain an admixture of areas histologically resembling oncocytoma (Figure 1, C) and areas more reminiscent of chromophobe RCC (Figure 1, D).

Hybrid oncocytic tumors have been reported to be frequent in patients with BHD syndrome. In 2002, Pavlovich et al reviewed 130 renal tumors from 30 patients with BHD syndrome in 19 affected families. Mean patient age was 51 years; there were 25 men, and 5 women, and most patients had multiple and bilateral tumors associated with oncocytosis. The authors designated 50% of tumors as hybrid oncocytic neoplasms, 34% as chromophobe RCCs, 9% as clear cell RCCs, 7% as oncocytomas, and 2% as papillary RCCs. The hybrid tumors contained oncocytes and chromophobe cells but rarely contained cells with perinuclear halos, and they were usually arranged in a plantlike pattern reminiscent of oncocytoma.

The biological behavior of hybrid oncocytic tumors is still not entirely certain. The initial studies by Tickoo et al and Pavlovich et al did not mention any worrisome features such as tumor necrosis, vascular invasion, or distant metastasis in any of the tumors studied. Recently, Mai et al examined 5 hybrid tumors, although none were associated with BHD syndrome, ranging in size from 1.8 to 5 cm and with no tumor necrosis seen. Vascular invasion into medium-sized veins was identified in only one tumor. None of the patients had distant metastasis. From these observations, the authors concluded that sporadic hybrid oncocytic tumors may have a favorable prognosis. To our knowledge, there has not been a report of a hybrid oncocytic tumor, either sporadic or associated with BHD syndrome, behaving in an aggressive or malignant fashion. It may be reasonable to assume that these renal hybrid oncocytic tumors have biological behavior somewhere between oncocytoma and low-grade chromophobe RCC.

CLEAR CELL AND PAPILLARY RCC

There also appears to be an association between BHD syndrome and clear cell RCC and, to a lesser extent, papillary RCC. The initial case report of renal tumors in a patient with BHD syndrome by Roth et al describes one of the 2 lesions as a clear cell RCC. The previously mentioned epidemiologic study by Zbar et al reported 4 of 14 renal tumors as clear cell RCCs in patients with BHD syndrome. Similarly, papillary RCC has also been reported in patients with BHD syndrome, although less commonly than clear cell RCC. In the study by Pavlovich et al, the patients with clear cell RCC often had multiple bilaterally hybrid tumors and/or chromophobe RCCs as well as oncocytosis. The clear cell RCCs tended to be much larger than the other renal tumors. Molecular analysis performed on some of the clear cell RCCs showed 3p loss and von-Hippel Lindau (VHL) gene mutations, raising the question as to whether hybrid tumors and chromophobe RCCs progress to more aggressive clear cell RCCs through additional genetic mutations. Further investigation is required to answer this question definitively.

The clinical behavior of clear cell and papillary RCC in patients with BHD syndrome is not entirely clear because of the relatively small number of cases reported. In 9 patients with BHD syndrome with clear cell RCC studied by Pavlovich et al, 3 had T3 lesions, with one showing sarcomatoid change and another showing lymph node metastasis. In addition, 3 of 9 patients had tumors with grade 3 nuclei; the remainder had grade 2 nuclei. Therefore, it appears that these tumors show behavior similar to that of sporadic clear cell RCC.

PULMONARY PATHOLOGY

Another important and well-recognized component of BHD syndrome is pulmonary cysts and spontaneous pneumothorax. Toro et al, in 1999, originally described the association between pulmonary cysts and BHD syndrome when they observed lung cysts in 4 of 28 patients studied. Since then, numerous patients with BHD syndrome have been reported to have pulmonary cysts and/or spontaneous pneumothorax. Zbar et al determined the odds ratio for pneumothorax in BHD syndrome-affected individuals, adjusted for age, was 50.3, and approximately 32 times higher when adjusted for the other risk variables.

Histologically, pulmonary cysts are characterized by cystic dilatation of alveolar spaces ranging from microscopic foci to a few millimeters in diameter. The thin-walled cysts are lined by cuboidal epithelium (Figure 2) with no obvious fibrous or smooth muscle tissue in the wall. Sometimes cysts may bulge into the pleural surface. When the cysts rupture under pressure of inhalation, pneumothorax may develop. By a strict definition, pneumothorax in BHD syndrome is not spontaneous but rather is secondary to the ruptured pulmonary cysts.

CUTANEOUS PATHOLOGY

The initial article by Birt et al described only skin tumors as part of BHD syndrome. They also coined the term for the most commonly seen lesion in these patients as fibrofolliculoma. This tumor is a circumscribed proliferation of collagen and fibroblasts that surrounds distorted hair follicles from which basaloid cells protrude into the surrounding fibromucinous stroma (Figure 3). Trichodiscomas, sharply defined fibrovascular tumors in the superficial dermis, and acrochordons were also described in these patients. Birt et al also noted that these lesions may be subtle and may increase with age. Most consider the skin findings as the defining pathology of BHD syndrome, and the 3 aforementioned lesions have been extensively reported in BHD syndrome in the literature. In fact, before genetic testing became available, 1 publication defined an individual as having BHD syndrome if he or she had more than 10 lesions clinically consistent with fibrofolliculomas and a minimum of one lesion confirmed as a fibrofolliculoma by examination of a biopsy specimen. However, whether these lesions are neoplastic in nature or a result of hair follicle malformation is still unknown. We will dis-
cuss the lack of BHD gene mutation in these skin lesions later in this article, but the relationship between skin lesions and BHD gene function is far from clear.

Some experts question whether fibrofolliculoma and trichodiscoma represent the same lesion. Ackerman et al\textsuperscript{18} contend that fibrofolliculoma and trichodiscoma are different names for a single pathologic process at different stages of development. In addition, immunohenotypic studies with peril follicular vimentin and CD34 support a similar histogenic precursor of fibrofolliculoma and trichodiscoma.\textsuperscript{18} Other authors have challenged the notion that acrochordons are a part of BHD syndrome. De la Torre et al\textsuperscript{19} reported that 2 patients with BHD syndrome had lesions that were clinically acrochordon-like and proved to correspond to the same histopathologic spectrum as fibrofolliculoma and trichodiscoma on biopsy. They concluded that fibrofolliculoma, trichodiscoma, and the acrochordon-like lesions are histologic variations of a single entity.

OTHER LESIONS WITH UNCERTAIN ASSOCIATION WITH BHD SYNDROME

In the initial study by Hornstein and Knickenberg,\textsuperscript{1} the authors described intestinal polyps in their 2 sibling patients in addition to cutaneous lesions. Almost 15 years later, Rongioletti et al\textsuperscript{20} described a patient with multiple tumors in addition to cutaneous lesions. Almost 15 years after the initial study, the clinical suspicion of an association increased when Zabor et al\textsuperscript{21} reported occurrence of intestinal polyps in a number of patients with BHD syndrome. The authors concluded that patients with BHD syndrome should be examined periodically for intestinal polyps. However, in the large epidemiologic study by Zbar et al,\textsuperscript{6} no association was found between BHD syndrome and colon cancer and colon polyps. The general consensus now is that patients with BHD syndrome are not at increased risk for colon polyps or colonic adenocarcinoma.

Some patients with BHD syndrome also have been reported to have neurothekeomas,\textsuperscript{21} meningiomas,\textsuperscript{21} multiple lipomas,\textsuperscript{22} parathyroid adenomas,\textsuperscript{22} intraoral papules,\textsuperscript{23} flecked chorioretinopathy,\textsuperscript{24} and a parotid oncocytoma,\textsuperscript{25} although a real association of these lesions with BHD syndrome is not proven. Other cutaneous tumors described in patients with BHD syndrome include lipomas,\textsuperscript{9} collagenomas,\textsuperscript{9} perivascular fibromas,\textsuperscript{26} angiofibromas,\textsuperscript{27} and melanomas,\textsuperscript{9,28} although their correlations to BHD syndrome are not as striking or definitive as fibrofolliculoma, trichodiscoma, and acrochordon lesions.

MOLECULAR GENETICS

In 2001, Khoo et al\textsuperscript{29} first reported their finding of the BHD gene location. They used polymorphic microsatellite markers on a large Swedish family with BHD syndrome, and mapped the BHD gene locus to chromosome 17p12q11.2. In addition, folliculin mRNA was expressed strongly in fibrofolliculomas but not in renal tumors from patients with BHD syndrome. Because of loss of folliculin expression in folliculomas but not in renal tumors from patients with BHD syndrome, the lesion that defines the syndrome (H&E, original magnification ×400).

To confirm the identity of the BHD gene, Nickerson et al\textsuperscript{31} found protein-truncating mutations in a panel of families with BHD syndrome, with a 44% frequency of insertion/deletion mutations within a hypermutable 8 cytosine tract. In 2004, Warren et al\textsuperscript{32} measured the expression of folliculin mRNA in healthy and neoplastic human tissues by fluorescent in situ hybridization. They also further defined that folliculin mRNA was expressed in a variety of tissues, including the skin and its appendages, the distal nephron of the kidney, and stromal cells and type 1 pneumocytes of the lung. Tissues with reduced expression of folliculin mRNA included heart, muscle, and liver. In addition, folliculin mRNA was expressed strongly in fibrofolliculomas but not in renal tumors from patients with BHD syndrome. Because of loss of folliculin expression in chromophobe RCCs and hybrid tumors, the authors postulated a tumor suppressor role for the protein.\textsuperscript{32} However, this finding ironically does not explain the role of follic-
ulin in the development of fibrofolliculomas, one of the major defining characteristics of BHD syndrome.

Since its identification, various specific mutations have been identified in the folliculin gene in patients with BHD syndrome, and molecular testing has been developed to identify these mutations. In 2002, Khoob et al identified 2 distinct germline mutations on exon 11 of the folliculin gene (c.1733insC and c.1733delC) in 3 of 4 families with BHD syndrome. In addition, a novel somatic mutation, c.1732delTCinsAC, was detected in a BHD syndrome-related chromophobe renal carcinoma, supposedly providing a “second hit” toward tumor development. Their results confirmed the (C)_n tract in exon 11 as a mutational hot spot in the BHD gene.

In 2005, Schmidt et al identified mutations along the entire length of the coding region of the folliculin gene, including 16 insertion/deletion, 3 nonsense, and 3 splice-site mutations in 51 of 61 families with BHD syndrome. The majority of BHD mutations were predicted to truncate the BHD protein, folliculin. Interestingly, among patients with a mutation in the exon 11 hot spot, significantly fewer renal tumors were observed in patients with the C-deletion than those with the C-insertion mutation. The same group also studied 77 renal tumors from 12 patients with BHD syndrome. Using DNA sequencing, alterations were detected in the majority of renal tumors (41/77 [53%]), with loss of heterozygosity at the BHD locus in a minority of additional tumors (14/77 [18%]). The somatic mutations were distributed across the entire gene, and the majority resulted in frameshifts predicted to lead to truncated BHD proteins.

In addition, mutations truncating folliculin have been described in patients with familial lung cysts and/or spontaneous pneumothorax without skin lesions or kidney tumors. Currently, these patients are not considered to have BHD syndrome because of the lack of defining skin lesions. Perhaps the spectrum of clinical criteria for BHD syndrome should be reexamined using existing genetic data.

Genetic analyses in sporadic renal tumors have shown that folliculin inactivation occurs in a subset of tumors of various histologic types. Another study also suggests that folliculin inactivation may play a role in colorectal carcinogenesis in a subset of tumors, further supporting the role of folliculin as a tumor suppressor gene.

**FUNCTION OF FOLLICULIN**

The full-length BHD cDNA sequence predicted a novel 64 kd protein, folliculin, which was highly conserved across species. As predicted from RNA analysis, there is widespread tissue expression of folliculin, including expression in kidney, lung, and skin.

The studies of folliculin at protein levels are limited by the availability of antibodies specific for folliculin. By immunohistochemical evaluation, Wang et al reported folliculin immunoreactivity occurred in the nucleolus of normal cells and was associated with mitosis. Furthermore, loss of folliculin expression was seen in oncocytoma (1/30 [3.3%]), chromophobe RCC (17/28 [60.7%]), papillary RCC (4/11 [36.4%]), and clear cell RCC (20/95 [21.1%]). Abnormal accumulation in the cytoplasm was also observed in oncocytoma (23/30 [76.7%]), chromophobe RCC (1/28 [3.6%]), and clear cell RCC (14/95 [14.7%]). The authors concluded that loss of BHD tumor suppressor gene expression was a common event in tumorigenesis of different types of sporadic renal cell neoplasms.

In summary, the Birt-Hogg-Dube syndrome is an autosomal dominantly inherited syndrome consisting of skin lesions, lung cysts/spontaneous pneumothorax, and kidney lesions. The presence of BHD syndrome should be investigated in any patient with multiple bilateral kidney tumors, especially if the predominant histologic type is chromophobe RCC or the so-called hybrid oncocytic tumor. The genetic alteration for BHD syndrome has been mapped to chromosome 17p12q11.2, and the gene in this region has been cloned and believed to be responsible for the BHD syndrome. The function of the BHD product, identified as folliculin, is still unknown, although it is speculated to be a tumor suppressor gene. Numerous mutations have been described in the BHD gene, and most have been located within a hypermutable 8 cytosine tract, resulting in a truncated folliculin. Studies are ongoing to determine the relationship between the BHD gene and the development of sporadic RCC and other lesions.

**References**


