Review

Association Between Birt Hogg Dubé Syndrome and Cancer Predisposition

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Abstract. The Birt Hogg Dubé syndrome (BHD) is a rare autosomal dominant genodermatosis predisposing patients to developing fibrofolliculoma, trichodiscoma and acrochordon. The syndrome is caused by germline mutations in the folliculin (FLCN) gene, encoding the folliculin tumor-suppressor protein. Numerous mutations have been described in the FLCN gene, the most frequent occurring within a C8 tract of exon 11. This hypermutability is probably due to a slippage in DNA polymerase during replication, resulting in gains/losses of repeat units, causing cancer predisposition. The main phenotypic manifestations related to this disease are lung cysts, leading to pneumothorax, and a 7-fold increased risk for renal neoplasia, although other neoplastic manifestations have been described in BHD-affected individuals. Of particular interest is the often reported genotype/phenotype correlation between FLCN mutations and risk of colon or breast cancer. This paper describes our current knowledge on the association between BHD and cancer predisposition and briefly summarizes our experience in this field.

The Birt Hogg Dubé syndrome (BHD) (OMIM 135150) is a rare dominantly inherited autosomal genodermatosis, first described as an inherited skin disorder in 1977 and characterized by a predisposition to the development of a triad of cutaneous signs including acrochords and multiple hamartomas of the hair follicle such as fibrofolliculoma and trichodiscoma (1).

Clinically, the BHD syndrome exhibits numerous asymptomatic, skin colored, dome-shaped papules over the face, neck, and upper trunk. These lesions represent benign proliferations of the ectodermal and mesodermal components of the pilar apparatus (2). Fibrofolliculomas are benign tumors of the perifollicular connective tissue, occurring as one or more yellowish dome-shaped papules, usually on the face. Trichodiscomas are parafollicular mesenchymal hamartomas of the mesodermal portion of the hair disk, usually occurring as multiple small papules. Acrochordons are small outgrowths of epidermal and dermal tissue, which may be pedunculated, smooth or irregular, flesh-colored and benign; they usually appear as pedunculated skin tags, occurring principally on the neck, eyelids, upper chest, and axillae in older women (3).

There are numerous reports that describe a wide spectrum of neoplastic phenotypic manifestations in BHD-affected individuals, including for example lipoma, melanoma, parathyroid adenoma (3) or thyroid gland alterations, as found in the original family described by Birt, Hogg and...
Dubre (1), but the significance of these associations has not been statistically substantiated. The association with colonic polyps and colonic adenocarcinoma still remains uncertain (8, 9). This paper describes our current knowledge on the association between BHD and cancer predisposition and briefly summarize, our own experience in this field (10), with a particular emphasis on breast cancer predisposition in BHD patients.

The BHD Gene

The gene responsible for BHD was firstly identified in 2001, simultaneously in two different laboratories using polymorphic microsatellite markers to perform a genome-wide linkage analysis on large BHD families and mapping the BHD gene locus to chromosome 17p12q11.2 (11, 12). The gene sequence was identified in 2002 by positional cloning (13). The gene, named folliculin and approved with the symbol FLCN (OMIM# 607273, GenBank accession number AF517523 - NM_144997.5) contains 14 exons, 11 of which are codifying.

The FLCN transcript mRNA, starting from the first ATG of exon 4, encodes a 579 amino acid protein, highly conserved between humans and homologs in mice, Drosophila, and Caenorhabditis elegans.

Numerous mutations have been described in the FLCN gene, the most frequent being a hot spot mutation occurring within a polycytosine C8 tract of exon 11 and resulting in truncated folliculin; this has been found in the germ line of 44% of BHD patients (13). The mutation is probably due to a slippage in DNA polymerase during DNA replication, leading to gains or losses of repeat units, as happens for other genes causing cancer predisposition (5, 9). However, mutations located elsewhere in the coding sequence are heterogeneous and several reports suggest that all translated exons might be mutated (14, 15).

The first online database (http://www.skingenedatabase.com) detailing all FLCN variants reported in the literature was developed during the past year. To date, the FCLN database describes 53 unique germ line mutations, including a hot spot mutation (1285dup-delC), and 31 single nucleotide polymorphisms (SNPs) (16). However, the fact that a significant proportion of FLCN mutations is described in should be taken into account in the mutational screening of the gene, and should lead to study of the overall coding region (17).

FLCN Protein

The BHD gene encodes a 64-kDa protein called folliculin (FLCN), whose functions are not completely understood. Recent studies have identified two FLCN-interacting proteins, FLCN-interacting protein 1 (FNIP1) (18) and FLCN-interacting protein 2 (FNIP2) (19, 20). FLCN, through its interaction with FNIP1 and FNIP2, might represent a downstream effector of the mammalian target of rapamycin (mTOR), or adenosine monophosphate-activated protein kinase (AMPK) and a modulator of energy/nutrient-sensing signaling pathways by some as-yet-unknown molecular mechanisms (21).

Using fluorescent in situ hybridization in healthy and neoplastic human tissues, it was demonstrated that FLCN mRNA was predominantly expressed in various tissues, including the skin and its appendages, the distal nephron of the kidney, stromal cells and type 1 pneumocytes of the lung, epithelial ducts of the breast, acinar cells of the pancreas, serous gland of parotid and ovary. No expression was seen in colon mucinous glands and epithelium (22). Another study in which FLCN mRNA levels in 20 different normal human tissues were compared showed the highest levels of transcript to be in testis, ovary, brain and lung (21).

As for its distribution in neoplastic tissues, FLCN was found to be overexpressed in proliferating epithelial fibrofolliculomas, but significantly reduced in renal tumor tissues from BHD patients regardless of histological type. Although the significance of FLCN expression remains to be established, these data certainly suggest some function of the FLCN protein in many tissues, including the target organs skin, lung and kidney, characterizing the BHD phenotype, and confirm the tumor suppressor function of FLCN in kidney cancer (22).

Lung Cysts and Pneumothorax

As stated above, chief components of the BHD syndrome phenotype are pulmonary cysts, leading to emphysema and spontaneous recurring pneumothorax. The presence of lung cysts in association with BHD syndrome was first confirmed in 1999, in a study of three extended families affected by renal tumors and characteristic skin manifestations (23). The risk of BHD patients developing spontaneous pneumothorax, a condition in which rupture of a cyst causes air to enter the pleural space with resultant lung collapse, is increased 50-fold compared with control populations (5, 8). Histologically, pulmonary cysts appear as cystic dilatation of alveolar spaces that can reach the size of few millimeters in diameter (3). In several cases, the presence of lung cysts and subsequent spontaneous pneumothorax is the only phenotypic manifestation of the disease and is not associated with other events, such as classical involvement of skin and kidney tumors (7, 6, 14).

It is noteworthy that FLCN mRNA has been shown to be strongly expressed within the connective tissue of the normal lung and lung blebs resected from a BHD patient with spontaneous pneumothorax (22). It was also assumed that the predominant subpleural location of the lung cysts is caused by a distinctive role of FLCN in the periphery of the lung (6).
The frequent occurrence of lung cysts and pneumothorax in BHD patients and the evidence that pulmonary features may precede the development of fibrofolliculomas and renal tumors has been confirmed by several studies (5-8). For this reason, many researchers today suggest that BHD syndrome be considered in the differential diagnosis of spontaneous pneumothorax, even in the absence of characteristic skin lesions and renal tumors, possibly using molecular analysis (5).

Renal Tumors

The renal neoplasms associated with BHD are bilateral and multifocal rather than unilateral and solitary. Tumors occur in a variety of histological types, with different percentages, according to various papers (9, 16, 24-26). The most frequent BHD-associated renal tumors are chromophobe renal-cell carcinomas (34%) and hybrid chromophobe oncocytopomas (50%) (24). Other renal tumors include clear-cell carcinomas (9%), oncocytomas (5%), and, rarely, papillary tumors (2%) (16, 24-26). Renal cancer associated with BHD syndrome occurs at a mean age of 50.7 years (24), although other cases have been reported with a much lower age of onset (9).

The search for a second somatic alteration of the \textit{FLCN} gene in the renal tumor tissue from BHD patients carrying germline \textit{FLCN} mutations has confirmed the presence of a second mutation or loss of heterozygosity (LOH) in 70% of tumors (27). Inactivation of both copies of the \textit{FLCN} gene occurred in all histological types of renal tumors, suggesting that the second \textit{FLCN} inactivation rises at an early stage of renal tumorigenesis and supporting the hypothesis that \textit{FLCN} is a tumor suppressor gene. Somatic \textit{FLCN} gene mutations were also found in cases of sporadic renal cancer, indicating that \textit{FLCN} is intimately involved in kidney tumorigenesis (28, 29). These data were also confirmed in animal models in which the biallelic inactivation of the respective \textit{FLCN} gene homolog determines a phenotype corresponding to human BHD-associated renal cancer (30, 31). Consistent with these results, \textit{FLCN} mRNA expression was elevated in distal nephron and collecting duct of normal kidney; conversely mRNA expression was hardly detectable in BHD-associated renal tumors, including clear cell, papillary, oncocytoma, chromophobe and oncocytic hybrid histotypes (22).

Colon Polyps and Tumors

Early reports, even before the syndrome was clinically characterized, suggested that BHD was associated with a predisposition for the development of intestinal adenomatous polyps with histological features of marked dysplasia and colon carcinoma (32, 33). Over the years, thanks to increased knowledge of the clinical features of the disease, several groups have reported numerous cases of colorectal polyps and neoplasia in families with BHD (34-38), findings that have led to the hypothesis that, relying on clinical evidence, colon cancer is an associated phenotypic manifestation of BHD (9). However, an epidemiological study published in 2002 showed no association between BHD syndrome and colon cancer or polyps (8).

Despite the statistical data, there is evidence of a close link between colon cancer and \textit{FLCN} gene alterations. Indeed, a mutational study performed on sporadic colorectal cancer showed that the poly C8 tract of the \textit{FLCN} gene is an effective target of microsatellite instability (MSI), with a frequency comparable with that of other target genes. These data give reason to believe that \textit{FLCN} is a putative target gene involved in the development of colorectal carcinomas with MSI (39). The involvement of the \textit{FLCN} gene in colorectal tumor progression is also confirmed by the presence of LOH at the chromosomal region surrounding the \textit{FLCN} locus in sporadic colorectal tumors without MSI (40).

No mRNA expression of \textit{FLCN} was seen in epithelium and mucinous glands of colon and data regarding expression in colorectal tumoral tissues are not yet available (21, 22). These observations in colorectal cancer do not indicate a tumor suppressor role for \textit{FLCN}, such as for renal tumors, but suggest a different pathway of carcinogenesis may involve other mechanisms of genomic instability.

Functional studies are still required in order for us to understand the biological role of \textit{FLCN} in colorectal carcinogenesis. Nonetheless, recent topic reviews (41), and extensive screening studies carried out on families with BHD continue to report the presence of colon cancer, or colonic polyposis associated with BHD (10, 42). Indeed, in recent work, Kluger \textit{et al.} reported colorectal polyps (including a tubulovillous adenoma with high-grade dysplasia and a benign gastric polyp) in 50% of BHD patients analyzed (43).

Breast Cancer

The first evidence of a possible association between breast cancer and BHD syndrome was found in a large six-generation Swedish family with BHD (Table I). A genome-wide linkage analysis using polymorphic microsatellite markers on this family led to the identification of the \textit{FLCN} locus on chromosome 17p12q11.2. The authors described two cases of breast fibroadenomatosis in a mother and a daughter of the third and fourth generations, respectively, commenting that since fibroadenoma of the breast is very common in the general population, its association with BHD could have been unrelated and needed to be further explored (11). Subsequently, the same authors reported that mutational analysis of the same family did not identify any germline sequence variant of \textit{FLCN}. However, in this study, they identified a sporadic case with clinical evidence of
fibrofolliculomas, pneumothorax, colon polyps and breast cancer but without any germline FLCN mutation (9), probably due to the presence of a sequence variant in a non-coding region.

Breast cancer was also identified by Ishii et al. in a 29-year-old Japanese woman with radiological evidence of multiple cysts of different sizes in the lung, who belonged to a family with BHD with a deletion of 10 nucleotides from intron 5 to exon 6 of the FLCN gene (44). Similarly, family history of breast carcinoma was reported in another Japanese case of a 38-year-old female BHD patient, carrying an FLCN frameshift mutation in exon 12, with recurrent pneumothorax episodes, vocal cord nodules, myoma of the uterus, carcinoma of thyroid (5). Others reported the occurrence of breast cancer in BHD patients. Leter et al., in a study performed on 20 families with BHD, observed a series of extracutaneous tumors including colorectal adenoma, oral fibroma, lipoma, fibrosarcoma, skin basal cell carcinoma, skin squamous cell carcinoma, non-Hodgkin lymphoma, and two cases of breast cancer, at the age of 44 (1110dupG) and of 60 (1740dupC) years, and a case of benign breast disease at the age of 53 years (15). Moreover, in an extensive study of 50 families, Toro et al. described a series of neoplastic events, such as squamous cell carcinoma, Hodgkin’s disease, uterine cancer, prostate cancer and rhabdomyoma, rarely associated with classical BHD phenotype. In this context, an isolated case of breast cancer without any clinical or molecular findings was also reported (45). More recently, Kluger et al. analyzed 22 BHD-affected patients from 10 unrelated French families, reporting a history of breast cancer in some members of a family, but no medical or molecular data of these individuals were analyzed (43). Two women with multiple breast nodules and cysts were also described in two other families with no evidence of breast cancer (43). In these cases, authors accurately described the presence of benign and malignant mammary tumors and suggested, for the first time in literature, that benign breast nodules and cysts examined needed specific clinical surveillance (43).

Our experience on breast cancer in BHD patients is related to the mutational screening performed on two families from central Italy who were referred to our institutions following a request for dermatological medical advice. In this study, we demonstrated the occurrence of two novel frameshift

Table I. Spectrum of germline FLCN mutations and phenotypic features of Birt Hogg Dubé syndrome patients with breast tumours reported in the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>FLCN mutation</th>
<th>Age at cancer diagnosis (years)</th>
<th>Breast disease</th>
<th>Other cancer</th>
<th>Renal tumor</th>
<th>Lung cysts/pneumothorax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khoo et al. 2001 (11)</td>
<td>NA</td>
<td>58</td>
<td>Fibroadenomatosis of the breast</td>
<td>-</td>
<td>Cysts</td>
<td>No</td>
</tr>
<tr>
<td>Khoo et al. 2002 (9)</td>
<td>NA</td>
<td>81</td>
<td>Fibroadenomatosis of the breast</td>
<td>Sarcoma</td>
<td>Cysts</td>
<td>No</td>
</tr>
<tr>
<td>Gunji et al. 2007 (5)</td>
<td>at 1795 ins</td>
<td>61</td>
<td>Sarcoma</td>
<td>Colon polyps</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>CCACCCT</td>
<td></td>
<td>Familiality*</td>
<td>Vocal cord nodules, myoma of the uterus, carcinoma of thyroid</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Leter et al. 2008 (15)</td>
<td>1110dupG (Ala219fs)</td>
<td>44</td>
<td>Breast cancer</td>
<td>Basal cell cancer of the skin; inverted papilloma of the nose.</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>1740dupC (His429fs)</td>
<td>60</td>
<td>Breast cancer</td>
<td>-</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Toro et al. 2008 (45)</td>
<td>1065_1066delGCinsTA</td>
<td>53</td>
<td>Benign breast disease</td>
<td>Breast cancer</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td>Palmirotta et al. 2008 (10)</td>
<td>ND</td>
<td>44</td>
<td>Breast cancer</td>
<td>Colon cancer</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ishii et al. 2009 (44)</td>
<td>1345delAAAG</td>
<td>29</td>
<td>Breast cancer</td>
<td>Benign breast disease</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td>Kluger et al. 2009 (43)</td>
<td>Exon 11 1285dup (1733insC)</td>
<td>ND</td>
<td>Familiality for melanoma, breast and jaw cancer*</td>
<td>Benign breast disease</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td></td>
<td></td>
<td>Benign breast disease</td>
<td>ND</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Exon 4 -3del (458delIG)</td>
<td>49</td>
<td>Breast cysts and nodules</td>
<td>Thyroid nodule</td>
<td>Cysts</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Exon 9 890_893del (1345_1348del)</td>
<td>50</td>
<td>Breast cysts</td>
<td>Colonic adenomatous polyps with mild dysplasia</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NA: Not available; *familiality for breast tumor; †clinical features observed in other family members; ND: not determined.
mutations located respectively in exons 5 (802insA) and 9 (1345delAAAG) of the FLCN gene, along with a homozygous sequence variant intron 9 (IVS9 +5C>T). The potential relevance of exon 9 mutations in cancer predisposition for BHD kindreds was also discussed, as the proband of the first family had a history of breast cancer at the age of 44 years and of colon cancer at the age of 56 years. This patient was a carrier of the 1345delAAAG frameshift mutation, and the latter was associated with a wide variety of tumors, including stomach, colon and parotid cancer found in other family members. Given the early onset of breast cancer and the lack of a positive family history, we hypothesized that breast cancer might be another rare component of the spectrum of tumors associated with BHD (10). Moreover, we should consider that FLCN mRNA is highly expressed in the epithelial mammary gland ductal cells of normal breast tissue, whereas a lower expression is found at the level of fibroblasts and surrounding stromal tissue. A strong expression of FLCN in tissues with a secretory function, such as pancreas, tonsil germinal center, spleen white pulp, parotid gland and, in particular, breast tissue ductal cells, suggests a potential secretory role of this protein. Of interest, different breast tumors, including ductal, lobular and mucinous carcinoma, as well as normal breast tissue, show a high expression of FLCN mRNA (22). These findings are markedly divergent from those observed in renal cancer. Indeed, in the latter, the reduced expression of the protein is consistent with the molecular data of a second somatic gene mutation, suggesting a tumor suppressor role for FLCN protein (27-29). While genotype–phenotype correlations remain to be elucidated for BHD, given the high frequency of breast cancer in the general female population, additional studies are needed to clarify this point.

**Other Lesions with Uncertain Association**

Several reports have described other additional cutaneous phenotypic manifestations in BHD-affected individuals, including perivascular fibroma (46), angiofibroma (47), oral papules (48, 23) lipoma (23), collagenoma (23) and melanoma (23, 49). Due to the sporadic occurrence of these lesions, a real association such as for fibrofolliculoma, trichodiscoma and acrochordon, with BHD syndrome has not been definitively proven (3).

Furthermore, several other tumor types have occasionally been reported in association with BHD, such as the thyroid gland alterations of the original family described by Birt, Hogg, and Dubé (1), ovarian cysts (42), cutaneous neurothekeoma and meningioma (50), multiple lipomas (38), parathyroid adenoma (38), angiolipoma and collagenoma (23), flecked chorioretinopathy (51), chorioretinal lesions (51, 42) and parotid oncocytoma (14, 52, 4). However, their real association with BHD syndrome has not yet been clinically validated and additional studies specifically addressing this issue are required.

**Conclusion**

From the data reported here, it is possible to deduce that the small number of cases and the limited clinical information reported in the literature do not allow a clear interpretation of the results not a clear association of cancer with BHD. One limitation of previous studies is that certain forms of cancer, such as colorectal or breast tumors, have been described to a lesser extent, putting greater emphasis on the classic clinically overlapping disorders, such as cutaneous manifestations of the syndrome, spontaneous pneumothorax and renal tumors.

Regarding breast cancer, it should also be considered that, given the high frequency of this malignancy in the general female population, the cases described may simply be phenocopies. Nonetheless, additional studies are needed to clarify this latter point because despite the small number of cases described, breast cancer is also often reported as part of the phenotypic spectrum of BHD at official and institutional web sites (e.g.: http://www.ncbi.nlm.nih.gov/sites/GeneTests and http://www.bhdsyndrome.org/). The genes so far clearly associated with hereditary forms of breast cancer are BRCA1 and BRCA2, while a small fraction of risk can currently be attributed to germline mutations in other genes, such as p53, ataxia-telangiectasia mutated gene (ATM), cadherin 1 (CDH1), phosphatase and tensin homolog (PTEN), serine/threonine protein kinase 11 (STK11) and mismatch repair genes (MMRs) (15). The data so far reported, often associated with an early age of onset of breast cancer in patients with mutations in the FLCN gene, suggest a possible association between germline FLCN mutation and the risk of breast cancer, although environmental or additional genetic factors may be involved, such as related genes co-segregating with FLCN or the possible presence of modifying genes in some of the BHD family members. Regarding polyps and colorectal tumors, the molecular data suggest a probable carcinogenetic mechanism involving DNA mismatch repair defects, chromosomal rearrangements and other epigenetic events, including mutation in oncogenes and oncosuppressors related to FLCN gene.

Given all these considerations, the opportunity for early diagnosis makes it imperative to define phenotypic features of disease, with the aim of improving management for possible future cancer and associated disorder development in patients with BHD syndrome. However, whilst awaiting future characterization of mutations associated with BHD and well-defined genotype–phenotype correlations, it is important that all patients with BHD syndrome are screened not only for classical related pathologies, such as for renal tumors and pneumothorax, but also for disorders that may be more than just chance associations.
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