

Birt-Hogg-Dubé Syndrome: Clinical and Genetic Studies of 20 Families

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Birt-Hogg-Dubé syndrome (BHD) is an autosomal-dominant genodermatosis characterized by skin fibrofolliculomas and an increased risk of spontaneous pneumothorax, renal and possibly other tumors. A causative gene (*FLCN*) on chromosome 17p has recently been identified. We here report clinical and genetic studies of 20 BHD families ascertained by the presence of multiple fibrofolliculomas or trichodiscomas in the proband. Pathogenic *FLCN* germline mutations were found in 11 (69%) of 16 probands tested and in 14 family members. Six different *FLCN* germline mutations were detected, four of which have not been reported previously. The clinical features were variable. None and less than 10 skin lesions were observed in two mutation carriers at the age of 67 and 29 years, respectively. Spontaneous pneumothorax was reported in four and renal carcinoma of mixed histological types in two of 36 BHD-affected individuals and/or *FLCN* mutation carriers. Both the prevalence of spontaneous pneumothorax and renal tumors appeared to be relatively low compared with previously reported data. Various other extracutaneous tumors were observed in 11 of 36 BHD-affected individuals and/or *FLCN* mutation carriers. This study of the second largest cohort to date contributes to the expanding data on the variable phenotype and underlying gene defects in BHD.

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INTRODUCTION

Birt-Hogg-Dubé syndrome (BHD (OMIM #135150)) was first described in 1977 by three Canadian physicians who studied a family whose members were affected with multiple skin fibrofolliculomas, trichodiscomas, and acrochordons on the face, neck, and upper torso (Birt *et al.*, 1977). The genetic predisposition for this triad of cutaneous lesions became known as BHD. The syndrome is now known to be an autosomal-dominant genodermatosis of unknown incidence. In addition to fibrofolliculomas, which tend to appear in the third or fourth decade of life, affected individuals may have pulmonary air filled cysts (Toro *et al.*, 1999; Zbar *et al.*,

2002), spontaneous pneumothorax (Toro *et al.*, 1999; Zbar *et al.*, 2002), and renal tumors (Roth *et al.*, 1993; Toro *et al.*, 1999; Zbar *et al.*, 2002). In a recent study, Zbar *et al.* (2002) found that the odds ratios for developing spontaneous pneumothorax and renal tumors in BHD-affected individuals were 50.3 and 6.9, respectively. In the past, an association between BHD and colorectal polyps and cancer was also suggested (Rongioletti *et al.*, 1989). However, recent studies indicate that colorectal tumors are not associated with the syndrome (Zbar *et al.*, 2002) or, alternatively, occur only in a small subset of families (Khoo *et al.*, 2002).

In 2001, evidence for linkage of BHD on chromosome 17p11 was demonstrated (Khoo *et al.*, 2001; Schmidt *et al.*, 2001). In 2002, the *FLCN* gene at this locus was identified (Nickerson *et al.*, 2002). The inactivating nature of the majority of *FLCN* mutations reported to date (Khoo *et al.*, 2002; Nickerson *et al.*, 2002; Schmidt *et al.*, 2005; Van Steensel *et al.*, 2007), biallelic inactivation of *FLCN* in BHD-associated renal tumors (Vocke *et al.*, 2005) and animal studies of BHD (Lingaas *et al.*, 2003; Okimoto *et al.*, 2004) indicate a tumor-suppressor role of *FLCN*. A recent report suggests that the corresponding protein folliculin (FLCN) and its interacting partner FNIP1 may be involved in energy and/or nutrient-sensing signaling pathways (Baba *et al.*, 2006). Initial reports on germline mutation analysis in BHD patients have identified the poly (C) tract in exon 11 of the *FLCN* gene as a mutational hotspot (Khoo *et al.*, 2002; Nickerson *et al.*, 2002), but mutations located along the entire length of the coding region of the gene have been identified (Schmidt *et al.*, 2005).

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Abbreviations: BHD, Birt-Hogg-Dubé; RCC, renal cell carcinoma

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We here describe our observations of 20 BHD families, ascertained by the department of dermatology at a university hospital, a referral center for adnexal tumors of the skin. Family studies and DNA mutation analysis were aimed at a detailed evaluation of the clinical and genetic variability of the syndrome.

RESULTS

The clinical and genetic features of BHD-affected individuals and/or *FLCN* mutation carriers are summarized in Table 1. Twenty clinically affected probands, ascertained from different regions in the Netherlands, were investigated. Family studies revealed 12 clinically affected family members. The mean age at clinical diagnosis was 43.3 ± 14.2 years (mean \pm SD).

Germline mutation analysis was performed in 16 of the 20 probands. A pathogenic *FLCN* mutation was found in 11 (69%) of these 16 probands. The *FLCN* mutation found in the proband was identified in a total of 14 family members, that is, 10 clinically affected individuals, one individual without any and one with less than 10 fibrofolliculomas or trichodiscomas at the age of 67 and 29 years, respectively, and two individuals who were not examined dermatologically. Six different pathogenic germline mutations (Figure 1) were found, the majority of which are inactivating frameshift and nonsense mutations. Only c.1740dupC, also referred to as c.1733insC, and c.875delC have previously been reported as germline mutations. The latter mutation has recently been reported as 420delC by another Dutch group (Van Steensel *et al.*, 2007). Through personal communication we have learnt that our patient IV-9 from family 3 and patient MB61 from the Van Steensel *et al.* (2007) study represent the same subject. One additional frameshift mutation was found in the remaining families. The nonsense mutation c.1065_1066delGCinsTA (Figure 2) was detected in three probands. The c.1074-1G>A and c.[1756-7del11; 1778del-CinsGA] mutations that were detected both affect splice acceptor sites.

Two probands had a history of spontaneous pneumothorax. At the time of diagnosis, BHD was considered in neither of these two patients. Family studies revealed two additional cases of pneumothorax. In family 1, the two cases were diagnosed at the age of 44 and 30 years, respectively. One patient in family 14 experienced spontaneous pneumothorax at the age of 36 years and the proband from family 18 had an unconfirmed case at the age of 22 years. None of the four patients had bilateral or recurrent spontaneous pneumothorax.

Two patients, both from family 6, had a history of renal cell carcinoma (RCC). Through family studies we learnt that subject III-23, the son of the proband, had been diagnosed with a symptomatic unilateral focus of mixed papillary and clear cell RCC at the age of 39 years. He died at the age of 40 years due to metastatic disease. Dermatological examination was not performed, but the patient had no reported relevant skin lesions. *FLCN* mutation analysis in histologically normal tissue from tumor slides revealed that he was a carrier of the mutation c.1740dupC, previously identified in his mother.

Independently, in another hospital, BHD was clinically confirmed in subject III-30 from the same family after several years of follow-up by the dermatologist. Subsequently, she was referred for renal imaging and was diagnosed with an occult unilateral bifocal RCC with elements of oncocytoma. After surgery, she was referred for genetic counseling. Mutation analysis revealed that she also carries the c.1740dupC mutation.

Following genetic counseling, clinically affected patients and/or *FLCN* mutation carriers were referred for renal tumor screening by a combination of magnetic resonance imaging investigation and baseline ultrasound. To date, no renal tumors were detected in the baseline screening round in 21 subjects with a mean age of 48.0 ± 12.1 years. The first annual follow-up screening round by renal ultrasound also revealed no tumors.

Other tumors were reported in 11 clinically affected BHD patients and/or *FLCN* mutation carriers: benign breast disease, colorectal adenoma, oral fibroma, lipomas, inverted papilloma of the nose, fibrosarcoma of the leg, skin basal cell carcinoma, skin squamous cell carcinoma, human immunodeficiency virus-related B-cell non-Hodgkin lymphoma, breast cancer, and an unconfirmed case of skin melanoma.

DISCUSSION

The clinical and genetic data of our 20 BHD families, the second largest cohort to date, add in several ways to our insight into the syndrome. Both the prevalence of renal tumors and spontaneous pneumothorax appeared relatively low compared with previously reported data. In the recent study of BHD by Schmidt *et al.* (2005), renal tumors were found in 38 (20%) of 187 affected individuals. Among the 36 clinically affected BHD patients and/or *FLCN* mutation carriers in the 20 families we studied, 2 (6%) had a history of RCC. As yet, screening of subjects did not reveal additional renal tumors. In BHD, the histology of renal tumors is unusual: various and/or mixed histological types are observed not only within families but also in individual patients (Pavlovich *et al.*, 2002), which concurs with the histological findings in our two RCC cases.

In the study by Schmidt *et al.* (2005), 64 (32%) of 198 BHD patients had a history of spontaneous pneumothorax. Of the 129 BHD patients who were screened by pulmonary CT scan, 110 (85%) were found to have one or more lung cysts consistent with the BHD phenotype. We found spontaneous pneumothorax in 4 (11%) of 36 clinically affected BHD patients and/or *FLCN* mutation carriers. We did not systematically subject mutation carriers to CT scans of the lungs, as there would be no direct medical consequences of the demonstration of pulmonary cysts. Different ascertainment of patients, different patient characteristics, environmental and/or additional genetic factors may be the cause of the apparent relatively low prevalence of renal tumors and spontaneous pneumothorax in our study group. Because of these small numbers possible genotype-phenotype correlations cannot be speculated on.

Three families were diagnosed with trichodiscomas only. The findings in these families are similar to those reported

Table 1. Clinical and genetic features of 20 BHD families

Family	Subject	Sex	Skin lesions (age at diagnosis (years))	Medical history			FLCN germline mutation analysis
				Pneu (age (years))	RCC (age (years))	Extracutaneous tumors (age (years))	Nucleotide change (effect)
BHD1	III-10	m	Td(58)	0	0	0	c.1065_1066delGCinsTA (p.Ala204X)
	III-11	f	Ff(57)	0	0	0	c.1065_1066delGCinsTA
	III-12	f	Ff(49)	1 (44)	0	BBD(53)	c.1065_1066delGCinsTA
	IV-4	m	Td(34)	1 (30)	0	0	c.1065_1066delGCinsTA
	IV-9	f	Ff(28)	0	0	Fs (14)	c.1065_1066delGCinsTA
BHD2	III-3	m	Ff(38)	0	0	0	No mutation detected
BHD3	III-6	f	No Ff/Td(67)	0	0	0	c.875delC (p.Ile141fs)
	IV-9	m	Ff(40)	0	0	CrA(40)	c.875delC
BHD4	III-1	m	Ff(56)	0	0	BCC(54-64),SCC(51-61),NHL(62)	c.1740dupC (p.His429fs)
BHD5	III-19	m	Ff(33)	0	0	OF(34),L(31-36)	No mutation detected
BHD6	II-9	f	Ff(56)	0	0	IP(50),BCC(54/70),BC(60)	c.1740dupC (p.His429fs)
	III-23	m	U [‡] (40)	0	1 (39)	0	c.1740dupC
	III-25	f	Td(40)	0	0	0	c.1740dupC
	III-30	f	Td(40)	0	1 (40)	0	c.1740dupC
BHD7		m	Ff(62)				DGC
BHD8	IV-5	f	Ff(45)	0	0	BC(44)	c.1110dupG (p.Ala219fs)
	IV-6	f	Ff(38)	0	0	0	c.1110dupG
BHD9		m	Ff(34)	0	0	0	No mutation detected
BHD10		m	Ff(55)				DGC
BHD11	IV-3	f	Td(38)	0	0	0	Not tested yet
BHD12	III-1	m	Ff(46)	0	0	CrA(42/47/49)	c.1740dupC (p.His429fs)
BHD13		m	Ff(41)			CrA(41)	DGC
BHD14	II-6	m	Ff(74)	0	0	0	c.1065_1066delGCinsTA (p.Ala204X)
	III-15	m	Ff(44)	1 (36)	0	0	c.1065_1066delGCinsTA
	III-17	m	Ff(37)	0	0	0	c.1065_1066delGCinsTA
BHD15	III-1	m	Ff(55)	0	0	0	c.1074-1G>A (SS)
	IV-1	f	<10 Ff(29)	0	0	0	c.1074-1G>A
	II-7	f	U [‡] (88)	0	0	CrA(83)	c.1074-1G>A
BHD16	III-28	f	Ff(57)	0	0	0	c.1065_1066delGCinsTA (p.Ala204X)
	III-29	f	Ff(55)	0	0	0	c.1065_1066delGCinsTA
BHD17	III-6	m	Td(34)	0	0	0	No mutation detected
	IV-1	m	Td(6)	0	0	0	Not tested
BHD18	III-12	m	Ff(56)	1 (22)	0	M(38)	c.[1756-7del11; 1778delCinsGA] (SS)
BHD19	III-7	m	Ff(36)	0	0	0	c.1074-1G>A (SS)
BHD20	III-10	f	Td(33)	0	0	0	No mutation detected
	IV-6	m	Td(10)	0	0	0	Not tested

BBD, benign breast disease; BC, breast cancer; BCC, basal cell cancer of the skin; CrA, colorectal adenoma; DGC, declined genetic counseling (i.e., family studies and mutation analysis); f, female; Ff, fibrofolliculomas; Fs, fibrosarcoma of the leg; IP, inverted papilloma of the nose; L, lipomas; m, male; M, melanoma; NHL, non-Hodgkin lymphoma of the stomach; OF, oral fibroma; Pneu, spontaneous pneumothorax; RCC, renal cell carcinoma; SCC, squamous cell cancer of the skin; SS, splice-site mutation predicted to cause exon skipping; Td, trichodiscomas; U, unknown.

[‡]Deceased (age at death in years).

Subjects in bold represent the proband. Mutation numbering according to GenBank sequence AF_517523.

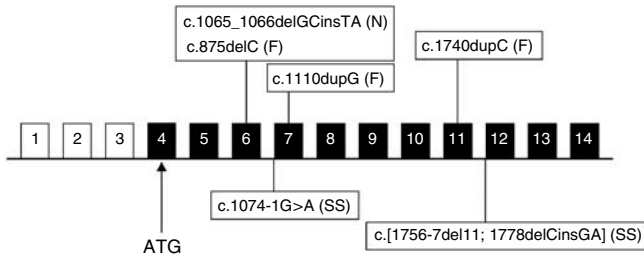


Figure 1. Genomic structure of *FLCN* showing the location of the six different germline mutations identified in 20 BHD families. In the coding region of the gene (exons 4–14), three frameshift (F) and one nonsense (N) mutation, all predicted to prematurely truncate the *FLCN* protein, were detected. Two splice-site (SS) mutations predicted to cause exon skipping were identified in introns 6 and 11.

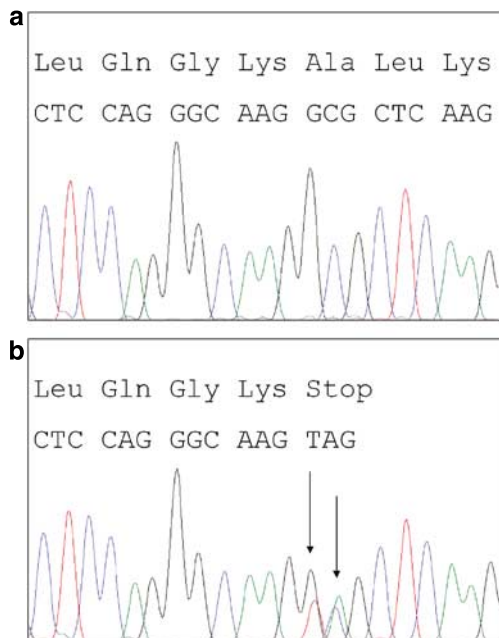


Figure 2. A novel *FLCN* germline mutation found in three BHD families. *FLCN* sequence showing the (a) wild-type and (b) c.1065_1066delGCinsTA allele.

two decades ago by one of the authors (Starink *et al.*, 1985) in another family, which was not available for inclusion in this study. *FLCN* mutations were identified in neither of our two trichodiscoma-only families tested. The young age at clinical diagnosis, 6 and 10 years respectively, in two subjects from the two trichodiscoma-only families tested for *FLCN* mutations is atypical for BHD. However, only one lesion was investigated histologically in each family member and mutation analysis by DNA sequencing was performed in one subject only from both families. These families might represent a subtype of BHD or, alternatively, a distinct syndrome.

The molecular analysis in this study has yielded several novel *FLCN* germline mutations. The identification of mainly inactivating frameshift and nonsense mutations in our study group concurs with previous reports (Khoo *et al.*, 2002;

Nickerson *et al.*, 2002; Schmidt *et al.*, 2005; Van Steensel *et al.*, 2007). In a recent update of molecular analysis, Schmidt *et al.* (2005) found mutations in 51 (84%) of 61 BHD families. More than half of all mutations consisted of an insertion or deletion of a cytosine in a C8 tract within exon 11 of the gene, a mutational hotspot known from previous studies (Khoo *et al.*, 2002; Nickerson *et al.*, 2002). We identified six different germline *FLCN* mutations in 11 (69%) of 16 probands tested of which 1740dupC, also referred to as 1733insC, has frequently been reported previously and involves the hypermutable poly (C) tract in exon 11. Except for c.875delC, none of the other germline mutations have been reported previously. The c.875delC mutation has previously been reported as a somatic mutation in a BHD-associated renal tumor (Vocke *et al.*, 2005) and as 420delC by another Dutch group (Van Steensel *et al.*, 2007) in a blood sample, which they had received for research purposes from the family 3 index patient. Two of the remaining mutations in our study, c.1074-1G>A and c.[1756-7del11; 1778delCinsGA], were intronic involving the splice acceptor site and are predicted to cause exon skipping. Of note, referred to as IVS6-1G>A, the former has previously been reported as a somatic mutation in a BHD-associated renal tumor (Vocke *et al.*, 2005). In addition to 1740dupC, c.1074-1G>A and c.1065_1066delGCinsTA were found in more than one family and may represent founder mutations.

Among the patients we studied, several had undergone colonoscopies due to the previously presumed association with colorectal tumors (Rongioletti *et al.*, 1989). Although some small adenomatous polyps were found, we did not observe colorectal cancer in our BHD families. In fact, the only individual who has had bowel cancer was shown not to be carrier of the *FLCN* mutation running in his family. Our current data therefore do not suggest an increased risk for colorectal tumors.

In conclusion, BHD should be suspected not only in patients with the classical dermatological manifestations but also in patients with few or even without fibrofolliculomas and/or trichodiscomas who may present with (familial) renal tumors and/or spontaneous pneumothorax. However, the prevalence of renal tumors and spontaneous pneumothorax in BHD families may vary due to yet unknown reasons. Additional insight will be gained from future clinical and molecular studies.

MATERIALS AND METHODS

Patients and clinical studies

Patients with BHD referred by the department of dermatology of the VU University Medical Center in Amsterdam, The Netherlands, were invited for family studies and *FLCN* germline mutation analysis. We defined an individual as clinically affected with BHD when the individual showed 10 or more skin lesions clinically compatible with fibrofolliculomas or trichodiscomas (Figure 3) of which one was histologically confirmed as such. Family studies were performed and medical and histopathological data of patients and family members were reviewed. Written informed consent was obtained from all patients for all studies in accordance with institutional requirements and the Declaration of Helsinki Principles.

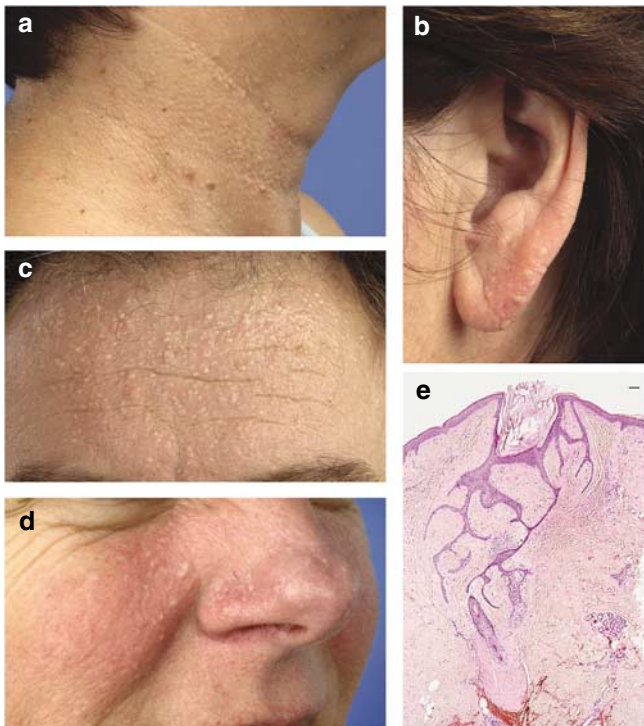


Figure 3. Skin manifestations in four BHD patients. Multiple small skin-colored papules clinically compatible with fibrofolliculomas or trichodiscomas (a) in the neck area and (b) on the ear of patient III-12 from family 1, (c) on the forehead of patient III-3 from family 2, and (d) on the nose and paranasal area of patient IV-6 from family 8. (e) Biopsy of one of the papules of the proband from family 13 shows the characteristic histological picture of a fibrofolliculoma. The hair follicle is dilated, its basal layer forms fine anastomosing strands of basaloid cells and is surrounded by a sharply demarcated layer of fibromucinous stroma. Bar = 250 μ m.

Mutation analysis

Genomic DNA was extracted from blood samples. Histologically normal tissue from a renal carcinoma slide of a deceased member of family 6 was also included for DNA extraction. Primers for the amplification and sequencing of the 14 exons were as detailed by Nickerson *et al.* (2002). Amplification by PCR was performed using a PE 9700 thermocycler (Applied Biosystems, Foster City, CA). Sequencing reactions were performed using Big Dye Terminator (Applied Biosystems) and run on an ABI 3100 genetic analyzer (Applied Biosystems).

Imaging studies

Following genetic counseling, clinically affected patients and/or *FLCN* mutation carriers were referred for renal tumor screening by a combination of magnetic resonance imaging investigation (Siemens Sonata 1.5 T) and baseline ultrasound (Siemens Acuson Sequoia 512) starting at the age of 25 years. The magnetic resonance imaging scan was carried out with the fast imaging technique (TrueFisp), T1 and T2 pulse sequences and T2 Haste with prospective acquisition correction (PACE) in order to readily distinguish solid from small cystic lesions. Intravenous gadolinium diethylenetriamine penta-acetic acid (DTPA) was administered when necessary. Annual follow-up was performed by renal ultrasound.

No imaging studies were performed to evaluate the presence of lung cysts.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

- Baba M, Hong SB, Sharma N, Warren MB, Nickerson ML, Iwamatsu A *et al.* (2006) 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 USA* 103:15552–7
- Birt AR, Hogg GR, Dubé WJ (1977) Hereditary multiple fibrofolliculomas with trichodiscomas and acrochordons. *Arch Dermatol* 113:1674–7
- Khoo SK, Bradley M, Wong FK, Hedblad MA, Nordenskjöld M, Teh BT (2001) Birt-Hogg-Dubé syndrome: mapping of a novel hereditary neoplasia gene to chromosome 17p12-q11.2. *Oncogene* 20:5239–42
- Khoo SK, Giraud S, Kahnoski K, Chen J, Motorna O, Nickolov R *et al.* (2002) Clinical and genetic studies of Birt-Hogg-Dubé syndrome. *J Med Genet* 39:906–12
- Lingaas F, Comstock KE, Kirkness EF, Sørensen A, Aarskaug T, Hitte C *et al.* (2003) A mutation in the canine BHD gene is associated with hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis in the German Shepherd dog. *Hum Mol Genet* 12:3043–53
- Nickerson ML, Warren MB, Toro JR, Matrosova V, Glenn G, Turner ML *et al.* (2002) 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-Dubé syndrome. *Cancer Cell* 2:157–64
- Okimoto K, Sakurai J, Kobayashi T, Mitani H, Hirayama Y, Nickerson ML *et al.* (2004) A germ-line insertion in the Birt-Hogg-Dubé (BHD) gene gives rise to the Nihon rat model of inherited renal cancer. *Proc Natl Acad Sci USA* 101:2023–7
- Pavlovich CP, Walther MM, Eyer RA, Hewitt SM, Zbar B, Linehan WM *et al.* (2002) Renal tumors in the Birt-Hogg-Dubé syndrome. *Am J Surg Pathol* 26:1542–52
- Rongioletti F, Hazini R, Gianotti G, Rebora A (1989) Fibrofolliculomas, trichodiscomas and acrochordons (Birt-Hogg-Dubé) associated with intestinal polyposis. *Clin Exp Dermatol* 14:72–4
- Roth JS, Rabinowitz AD, Benson M, Grossman ME (1993) Bilateral renal cell carcinoma in the Birt-Hogg-Dubé syndrome. *J Am Acad Dermatol* 29:1055–6
- Schmidt LS, Nickerson ML, Warren MB, Glenn GM, Toro JR, Merino MJ *et al.* (2005) Germline BHD-mutation spectrum and phenotype analysis of a large cohort of families with Birt-Hogg-Dubé syndrome. *Am J Hum Genet* 76:1023–33
- Schmidt LS, Warren MB, Nickerson ML, Weirich G, Matrosova V, Toro JR *et al.* (2001) Birt-Hogg-Dubé syndrome, a genodermatosis associated with spontaneous pneumothorax and kidney neoplasia, maps to chromosome 17p11.2. *Am J Hum Genet* 69:876–82
- Starink TM, Kisch LS, Meijer CJ (1985) Familial multiple trichodiscomas. A clinicopathologic study. *Arch Dermatol* 121:888–91
- Toro JR, Glenn G, Duray P, Darling T, Weirich G, Zbar B *et al.* (1999) Birt-Hogg-Dubé syndrome: a novel marker of kidney neoplasia. *Arch Dermatol* 135:1195–202
- Van Steensel MA, Verstraeten VL, Frank J, Kelleners-Smeets NW, Poblete-Gutiérrez P, Marcus-Soekarman D *et al.* (2007) Novel mutations in the BHD gene and absence of loss of heterozygosity in fibrofolliculomas of Birt-Hogg-Dubé patients. *J Invest Dermatol* 127: 588–93
- Vocke CD, Yang Y, Pavlovich CP, Schmidt LS, Nickerson ML, Torres-Cabala CA *et al.* (2005) High frequency of somatic frameshift BHD gene mutations in Birt-Hogg-Dubé-associated renal tumors. *J Natl Cancer Inst* 97:931–5
- Zbar B, Alvord WG, Glenn G, Turner M, Pavlovich CP, Schmidt L *et al.* (2002) Risk of renal and colonic neoplasms and spontaneous pneumothorax in the Birt-Hogg-Dubé syndrome. *Cancer Epidemiol Biomarkers Prev* 11:393–400