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Case Report of Birt–Hogg–Dubé Syndrome

Germline Mutations of FLCN Detected in Patients With Renal Cancer and Thyroid Cancer

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Abstract: Birt–Hogg–Dubé (BHD) is a rare autosomal dominant inherited syndrome that is characterized by the presence of fibrofolliculomas and/or trichodiscomas, pulmonary cysts, spontaneous pneumothorax, and renal tumors. Here, the 2 patients we reported with renal cell carcinomas and dermatological features were suspected to be suffering from BHD syndrome. Blood samples of these patients were sent for whole exon sequencing performed by Sanger sequencing. Eight mutations, including 5 mutations, which were mapped in noncoding region, 1 synonymous mutation, and 2 missense mutations, were detected in the *FLCN* gene in both patients. The 2 missense mutations, predicted to be disease-causing mutation or affecting protein function by MutationTaster and SIFT, confirmed the diagnosis. In addition, the 2 patients were also affected with papillary thyroid cancer. Total thyroidectomy and prophylactic bilateral central lymph node dissection were performed for them and the BHD-2 also received lateral lymph node dissection. Pathology reports showed that the patients had lymph node metastasis in spite of small size of thyroid lesions.

The 2 missense mutations, not reported previously, expand the mutation spectrum of *FLCN* gene associated with BHD syndrome. For the thyroid cancer patients with BHD syndrome, total thyroidectomy and prophylactic bilateral central lymph node dissection may be suitable and the neck ultrasound may benefit BHD patients and their family members.

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Abbreviations: BHD = Birt–Hogg–Dubé, FAP = familial adenomatous polyposis, MEN2A = multiple endocrine neoplasia

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type 2A, PTC = papillary thyroid carcinoma, RCC = renal cell carcinoma.

INTRODUCTION

Birt–Hogg–Dubé (BHD) syndrome is a rare autosomal dominant inherited disorder, described firstly in 1977.¹ Its clinical characteristics include cutaneous lesions (such as fibrofolliculomas, trichodiscomas), lung cysts, and spontaneous pneumothorax. In 1993, the first case of renal cell carcinoma (RCC) associated with BHD syndrome was reported.² Subsequently, RCC was also described as an important clinical manifestation of BHD syndrome.³ This syndrome is caused by the germline mutations in *FLCN* gene, which is located on chromosome 17p11.2.⁴ *FLCN* gene includes 14 exons and encodes folliculin protein consisting of 579 amino acids. Although this protein was expressed in most tissues, its function has not been elucidated. The *FLCN* gene expression was found in normal renal tissues and the loss of *FLCN* expression was found in all histological subtypes of RCC, suggesting that *FLCN* gene may be a tumor suppressor gene for RCC.⁵ In addition, the association between BHD syndrome and tumors of other organ systems was also repeatedly described, such as colorectal polyps/cancer, lung cancer, breast cancer, parotid oncocyctomas, and so on.⁶

In this study, we report 2 BHD syndrome patients with RCC and papillary thyroid cancer (PTC).

MATERIALS AND METHODS

Two patients with head and neck skin lesions were diagnosed with PTC in our hospital in 2011 and 2014, respectively. Total thyroidectomy and bilateral central lymph node dissection were performed in patient 1 who had a history of clear cell RCC. Patient 2, who had hypertension well controlled with perindopril, underwent 2 thyroid operations. Lobectomy of the right lobe, isthmectomy, and lateral neck dissection were performed in the first surgery. Two years later, lobectomy of the left lobe and central compartment neck dissection were performed because of recurrence in the left lobe. Meantime, left renal lesion (size 1.8 × 1.8 cm) of this patient was found in the routine examination before the second operation. Then, partial nephrectomy on left was performed. No lung cysts were found in these 2 patients, and no history of spontaneous pneumothorax was found in both patients and their familial members.

As both renal carcinomas and dermatological features were suggestive of BHD syndrome, blood samples of these 2 patients obtained from the Tissue Bank in Tianjin Medical University Cancer Institute and Hospital were sent for

TABLE 1. Detailed Patients' Data

Patient	Age, y (at Diagnosis of Kidney Cancer)	Gender	Disease
Patient 1	49	Male	Skin lesions; clear cell renal cell carcinoma; papillary thyroid carcinoma
Patient 2	44	Male	Skin lesions; papillary renal cell carcinoma; papillary thyroid carcinoma; hypertension

sequencing analysis. Sequencing of the whole exons in *FLCN* gene was performed by the Sanger sequencing. Seventeen pairs of primers were designed for polymerase chain reaction (PCR) on the basis of the sequence of *FLNC* gene (NM_144997.5). The sequencing results were blasted to the NCBI sequence. This study was approved by Tianjin Medical University Cancer Institute and Hospital's Ethics Committee.

RESULTS

The clinical features of these 2 patients, including age, sex, histology, and diagnosis, are summarized in Table 1. Both of the patients were diagnosed with bilateral and multifocal papillary thyroid carcinoma (PTC). Lymph nodes metastases were found in their central compartment region. Metastases were also found in the lateral region in patient 2. Clear cell RCC was diagnosed in patient 1. No further detailed pathologic information could be obtained, as the renal surgery of patient 1 was not performed in our hospital. Renal pathologic examination of patient 2 showed left papillary RCC, Fuhrman II stage, invasion of renal capsule, perirenal fat capsule (–), and immunohistochemistry results were CA9 (+), Vimentin (+), CD117 (–), CK7 (+), P504S (+).

Table 2 displays 8 mutations detected in the *FLCN* gene of the 2 patients. The c.-487G>C mutation was homozygous and others were heterozygous. Three mutations were mapped in the coding region of *FLCN*, including 1 synonymous mutation (c.1095C>G) and 2 missense mutations (c.1481A>G detected in patient 2, resulting a substitution of asparagine for serine at codon 494; and c.1645C>G detected in patient 1, resulting in a substitution of leucine to valine at codon 549) (Figure 1). Four mutations (c.-487G>C, c.-302G>C, c.2132G>A, and c.2297T>C) in the noncoding region were identified in both patients. Another noncoding region mutation, c.-84G>A, was detected in patient 2 only.

The missense mutations (c.1481A>G and c.1645C>G) in *FLCN* gene were not reported previously in dbSNP or 1000 Genome Project and were also not archived in the ExAC database. We annotated the mutations with MutationTaster, SIFT, and Polyphen2, to predict the impact of the mutations on protein function. They were predicted to be disease-causing mutation or affect protein function by MutationTaster and SIFT. The Polyphen2 predicted the mutation c.1645C>G (L549V) to be possibly damaging with a score of 0.911 (sensitivity: 0.81; specificity: 0.94) and the mutation c.1481A>G (N494S) to be benign with a score of 0.158 (sensitivity: 0.92; specificity: 0.87).

DISCUSSION AND CONCLUSIONS

BHD syndrome is characterized by the development of fibrofolliculomas, kidney cancer, lung pneumatocysts, and spontaneous pneumothorax, but not all patients present with

these traditional features.⁷ Patients might present with renal cancer⁸ or pneumothorax,⁹ both of which most often occur sporadically. In 2002, defects of *FLCN* gene were identified in families with BHD syndrome, which made the detection of the *FLCN* gene for the diagnosis of BHD syndrome possible.⁴ According to the diagnostic criteria proposed by European Birt–Hogg–Dubé Consortium (Table 3),¹⁰ a patient with pulmonary cysts or spontaneous pneumothorax only or RCC only could be diagnosed as suffering from BHD syndrome if he was found to carry a pathogenic germline mutation in the *FLCN* gene. In this study, BHD syndrome was suspected in the 2 patients with renal carcinomas and dermatological features. The mutations identified in *FLCN* gene confirmed the diagnosis.

Only 2 missense mutations (c.1481A>G and c.1645C>G) were detected in *FLCN* gene of the 2 patients in this study. The germline mutation c.1481A>G (N494S) in exon 13 was detected in patient 2. According to Toro et al,¹¹ the asparagine 494 amino acid of folliculin is highly conserved in vertebrate species (chimpanzee, monkey, horse, mouse, rat, dog, cow, chicken, frog) suggesting functional significance. The N494S mutation was present in this patient with fibrofolliculoma, papillary RCC, PTC, and hypertension, suggesting that it is likely a disease-causing mutation. In patient 1 with clear cell RCC and PTC, the c.1645C>G (L549V) mutation was identified. The 2 mutations were predicted to be disease-causing mutation or affect protein function by MutationTaster and SIFT.

Some studies reported that the risk of pneumothorax in patients with BHD syndrome was 50 times greater than in the normal population⁷ and up to 80% of BHD syndrome patients had lung cysts.^{7,11–14} According to the study by Toro et al,¹²

TABLE 2. Germline Mutations in the 2 BHD Syndrome Patients

Mutation	Exon	Protein	Patient
c.-487G>C	Exon 1	—	Patient 1, Patient 2
c.-302G>C	Exon 1	—	Patient 1, Patient 2
c.-84G>A	Exon 3	—	Patient 2
c.1095C>G	Exon 10	NS*	Patient 1
c.1481A>G	Exon 13	p.N494S	Patient 2
c.1645C>G	Exon 14	p.L549V	Patient 1
c.2132G>A	Exon 14	—	Patient 1, Patient 2
c.2297T>C	Exon 14	—	Patient 1, Patient 2

p.N494S represented a substitution of asparagine for serine at codon 494. p.L549V represented a substitution of leucine to valine at codon 549.

*NS represented that the mutation was synonymous and the amino acid was not changed.

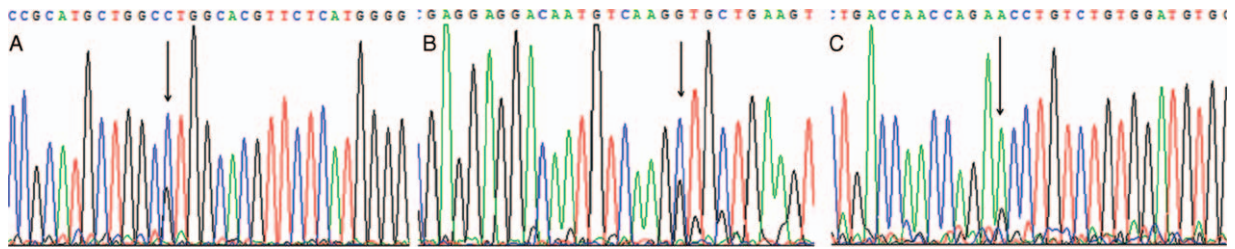


FIGURE 1. Three mutations detected in the coding region of FLCN. A, A synonymous mutation c.1095C>G was detected in exon 10 in Patient 1. B, The FLCN c.1645C>G, p.L549V was a missense mutation identified in exon 14 in Patient 1. C, The missense mutation c.1481A>G, p.N494S was mapped in exon 13 of FLCN in Patient 2.

mutations in exon 11 were more frequent in patients with a history of pneumothorax and mutations in exon 9 and 12 were, respectively, associated with the size and number of lung cysts. In this study, the 2 patients did not have the history of pneumothorax and multiple lung cysts, which may be explained by the fact that the missense mutations we reported were detected in exon 13 and 14. To date, however, the association between genotype and phenotype has not been established.

In 1999, Toro et al³ described that RCC was an important clinical manifestation of BHD syndrome, confirmed by the further studies.^{4,11,13,15,16} One study found that patients with BHD syndrome were about 6.9 times as likely to develop renal tumors as those not affected with BHD syndrome.¹³ According to the reported researches,^{11,13} the most common histological type of RCC in BHD syndrome patients was oncocytic-chromophobe hybrid carcinoma (50%), followed by purely chromophobe carcinomas (34%), purely oncocytic carcinomas (5%), clear cell carcinomas (3%), and papillary carcinomas (2%). Generally, the RCC patients with BHD syndrome had an earlier age (between 20 and 55 years). The BHD-associated renal tumors were most often multiple and bilateral and associated with microscopic lesions in the surrounding renal parenchyma.^{13,17,18} In this study, the 2 patients were diagnosed with RCC at the age of 49 and 45 years, respectively. The onset age of the 2 patients was consistent with the age characteristic of the BHD syndrome. Even though the histologic types (clear cell

carcinoma and papillary carcinoma) were not the common form of BHD syndrome, they could also occur.^{19,20}

A population-based study showed that patients with a history of thyroid cancer had an elevated risk of subsequent renal cancer, a 2.0-fold increase in women and a 4.5-fold increase in men, and in patients with renal cancer, there was a 1.5-fold and a 3.0-fold increase in the prevalence of subsequent thyroid cancer in women and men, respectively.²¹ This was consistent with the results of other studies.^{22–25} The co-occurrence of thyroid cancer and RCC could be associated with risk factors such as environmental, genetic predisposition, and therapy-related. The bidirectional associations between thyroid cancer and RCC were more likely explained by germline mutations, which could lead to the occurrence of them at the role of the environment.²⁶

Thyroid cancer had been found with a greater frequency than expected in the Cowden syndrome, familial adenomatous polyposis (FAP), and multiple endocrine neoplasia type 2A (MEN2A).^{27–29} Thyroid cancer was also reported in BHD syndrome patients.^{1,11,15,20,30} The original BHD syndrome patients were found in a family in which 6 of 9 siblings had medullary thyroid carcinoma.¹ A recent case report described a BHD patient with a primary clear cell carcinoma of the thyroid, and the c.1062G>C mutation in exon 9 of FLCN was identified both in the patient’s blood DNA and thyroid tumor DNA, which seemed to provide molecular evidence for this association.³⁰ However, Warren et al³¹ did not find any FLCN mRNA expression in normal thyroid. Nowadays, no firm conclusion could be stated about the association between BHD syndrome and thyroid cancer. Notably, in our study, PTCs of the 2 patients were bilateral and multifocal. Although thyroid lesions were small, lymph node metastases occurred, and patient 2 even had lateral lymph node involvement. It is unsure whether thyroid cancer is susceptible to exhibit bilaterality and lymph node metastasis in BHD syndrome because the case number in our study was too small. However, it suggests that total thyroidectomy and central compartment neck dissection may be suitable for thyroid cancer patients with BHD syndrome. According to Kluger et al,¹⁵ there was at least 1 member with a thyroid nodule in 90% of the affected BHD families and 65% of the screened individuals displaying this lesion. So, the neck ultrasound is recommended for BHD patients and their family members. And, we are in agreement with that large-scale investigation should be conducted to evaluate the prevalence of thyroid cancer or thyroid nodule in patients with BHD syndrome.

Skin lesions, lung disease, and renal cancer could not appear simultaneously in a patient with BHD syndrome. This

TABLE 3. Diagnosis Criteria for BHD Syndrome Proposed by the European Birt–Hogg–Dubé Consortium

Major criteria	
At least 5 fibrofolliculomas or trichodiscomas, at least 1 histologically confirmed, of 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 primary pneumothorax	
Renal cancer: early onset (<50 y) or multifocal or bilateral renal cancer, or renal cancer of mixed chromophobe and oncocytic histology	
A first-degree relative with BHD	
Patients should fulfill 1 major or 2 minor criteria for diagnosis.	

may be an explanation that BHD syndrome is often overlooked. For patients whose clinical features are atypical, detection of germline mutation in *FLCN* gene would help confirm diagnosis. The 2 mutations we reported would expand the mutation spectrum of *FLCN* gene associated with BHD syndrome. For the thyroid cancer patients with BHD syndrome, total thyroidectomy and prophylactic bilateral central lymph node dissection may be suitable and the neck ultrasound may benefit BHD patients and their family members.

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