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CLINICAL IMAGING

The discovery of a Persian family with a form of Birt–Hogg–Dubé syndrome lacking the typical cutaneous stigmata of the syndrome☆

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Abstract

Purpose: This study was performed in 24 members of a family with spontaneous pneumothorax to test clinical suspicion of Birt–Hogg–Dubé syndrome (BHDS). **Methods:** Computed tomography scan was performed for confirmation of pneumothorax, while genetic tests were done using real-time quantitative polymerase chain reaction. **Results:** Genetic studies showed a deletion of exon 1 in the *FLCN* gene in the index case as well as nine other individuals, including two with clinical phenotypes of pneumothorax and seven who are symptom-free to date. **Conclusions:** Proper imaging and taking accurate family history could be the keys to test clinical suspicion in some syndromes, including BHDS.

Keywords: Birt–Hogg–Dubé syndrome; *FLCN*; Imaging; Mutation; Pneumothorax

1. Introduction

Birt–Hogg–Dubé syndrome (BHDS, OMIM#135150) is a rare genodermatosis disease, usually affecting the skin and lungs, and is associated with increased risk of certain kidney tumors. This syndrome is often characterized by the triad of pulmonary cysts/spontaneous pneumothorax (abnormal accumulation of air in the chest cavity), cutaneous hamartomas of the hair follicle, and renal tumors [1,2]. However, this syndrome does not always present with all three of the named manifestations; therefore, not having a full triad does not exclude this syndrome [1–3].

This syndrome is an autosomal dominant inherited disease, which was first described in 1977 [4], while the responsible gene, the *Folliculin* gene (*FLCN*, OMIM*607273) mapped to the locus 17p11.2 [5,6], was discovered about 25 years later [7]. The *FLCN* gene encodes a protein called folliculin, whose exact normal function is unclear, but acts as a tumor suppressor and participates in the mammalian target of rapamycin pathway that is expressed in the skin, renal cells, and lungs. Therefore, mutations in the *FLCN* gene lead to dysregulation of this pathway, which could be the causative reason of cutaneous manifestations, renal tumors, and pulmonary

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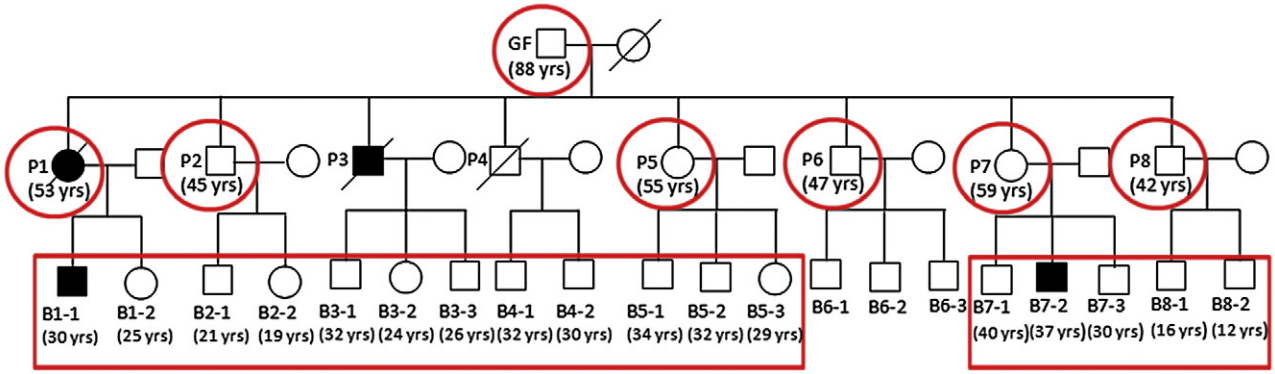

Fig. 1. The pedigree of the family with BHDS. Open symbols represent individuals without symptoms at the time of study; filled symbols represent those clinically affected with syndrome (all with spontaneous pneumothorax); symbols with slashes represent deceased individuals. Boxes: males, circles: females. Encircled: the individuals whose genomic DNA was sent for genetic testing.

cysts/spontaneous pneumothorax in BHDS, as a result of hampering the ability of folliculin to restrain cell growth and division [8]. Herein, an Iranian family with BHDS is reported for the first time.

2. Patients and methods

2.1. Proband

A 53-year-old Iranian woman (P1) was referred to our center from the north seashore of Iran for genetic evaluations. The reason of referral was the strong history of spontaneous pneumothorax in her and her family (Fig. 1). She had experienced several episodes of pneumothorax during her life, confirmed by computed tomography (CT) scan (Fig. 2), starting at the age of 30 years. She suffered from a severe pneumothorax when she was in an accidental chlorine exposure at the age of 45 years. She also experienced bilateral clear cell renal cell carcinomas (RCCs) not long before referral (Fig. 3), which caused her death a couple of months later.

2.2. Pedigree

The patient had seven siblings (two dead and five alive), while only one of her brothers experienced pneumothorax (P3); this case had experienced an episode of spontaneous pneumothorax at the age of 30 years for the first time, and he died in the same accidental chlorine exposure in which the index patient had experienced pneumothorax. There were no confirmatory documents about the main cause of his death and whether it was due to a severe pneumothorax or not. The closest involved family member was her 30-year-old son (B1-1), whose medical records showed an episode of spontaneous pneumothorax at the age of 26 years when he tried diving in a pool. She had also a daughter (B1-2) and 18 nephews and nieces, but only one of them (B7-2), who works in a gas station, had experienced spontaneous pneumothorax at the age of 34 years. None of these cases presented any cutaneous manifestations and renal problems. Nobody else in the family has experienced similar condition so far.

2.3. Genetic studies

After getting informed consent from the family members, genetic study of the *FLCN* gene was performed in the index patient.

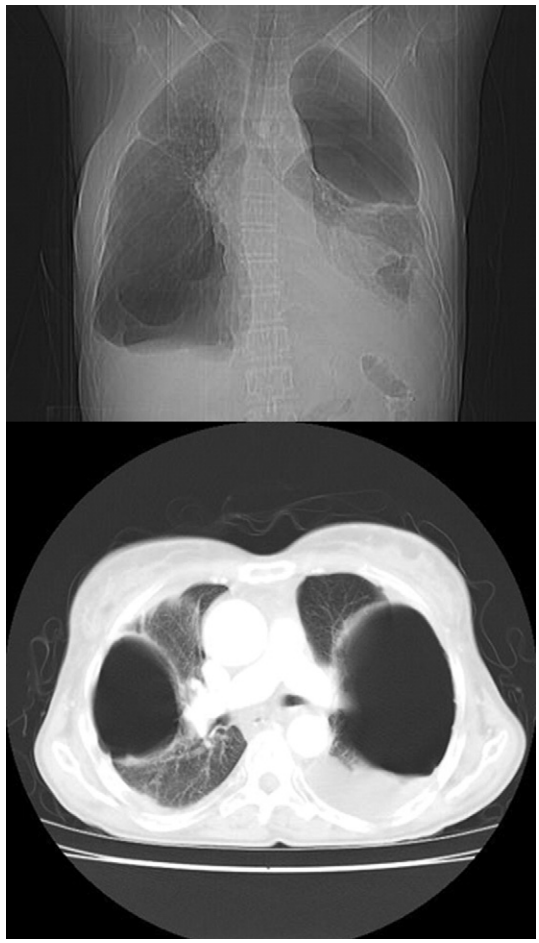

Fig. 2. Bilateral loculated pneumothorax in CT scan.

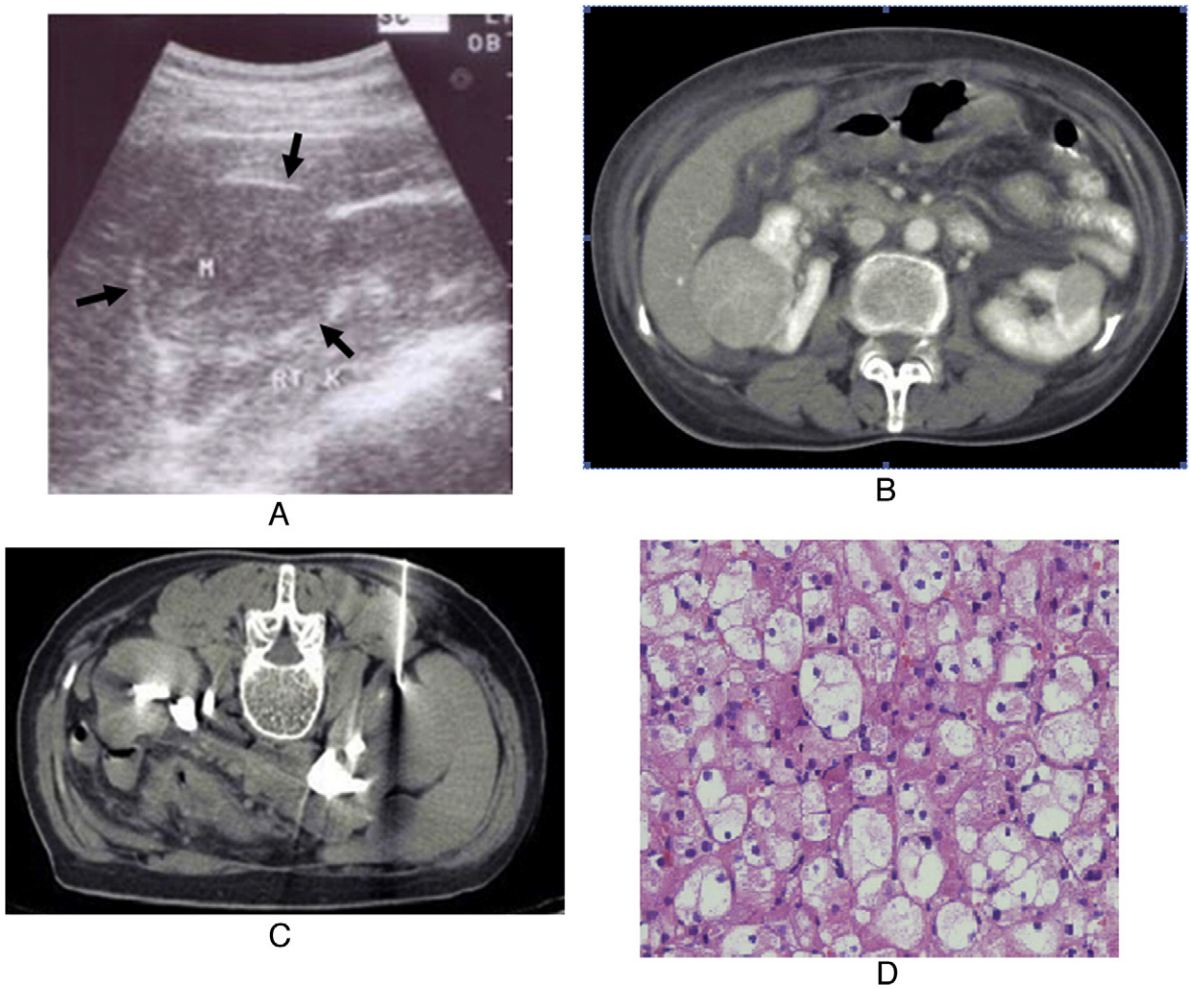

Fig. 3. (A) Hypoechoic and well-defined mass in ultrasonography. (B) Bilateral enhancing renal masses in axial contrast-enhanced CT. (C) CT-guided biopsy of the largest mass in the right side. (D) Histopathologic study revealed neoplastic tissue composed of sheets of polygonal cells with abundant clear to granular cytoplasm and mildly atypical nuclei with scattered visible nucleoli. The tumoral cells are positive for cytokeratin (AE1/AE3) and EMA, while being negative for vimentin, CK7, CK20, CEA, S100, and AFP. The histopathologic and immunohistochemical findings are consistent with "renal cell carcinoma, clear cell type."

First, denaturing high-performance liquid chromatography (DHPLC) method was applied. As no abnormality was detected by DHPLC, the copy number of each exon of the *FLCN* gene in the genome was quantified using real-time quantitative polymerase chain reaction (qPCR) methods [3,9] to detect a large genomic deletion in the *FLCN* gene. The qPCR was repeated again, and the results were the same as with the first experiment.

3. Results

Although spontaneous pneumothorax has many differential diagnoses, in persons with a strong family history of pneumothorax, a genetic predisposition that might involve the rest of the family members was suggested. Having bilateral RCC in the index patient, BHDS which is a very rare but known syndrome causes familial recurrent spontaneous pneumothorax and RCC is considered.

We identified a large genomic deletion including the exon 1 of the *FLCN* gene by real-time qPCR. When the rest of the family members were examined by qPCR, the same intragenic deletion including *FLCN* exon 1 was demonstrated in some of members: the P1′s father (GF), P5, P7, B1-1, B1-2, B3-2, B5-2, B7-1, and B7-2 (Fig. 4). The P1's father (88 years old), P5 (55 years old), and P7 (59 years old) have not had any clinical symptoms compatible with a clinical diagnosis of BHDS. B1-1 (30 years) and B7-2 (37 years) had episodes of pneumothorax, while B1-2 (25 years old), B3-2 (24 years old), B5-2 (32 years old), and B7-1 (40 years old) have not experienced any clinical symptom so far.

4. Discussion

We reported a large family of BHDS, which is caused by an intragenic deletion of the *FLCN* exon 1. There are some published reports on this syndrome which occurs in

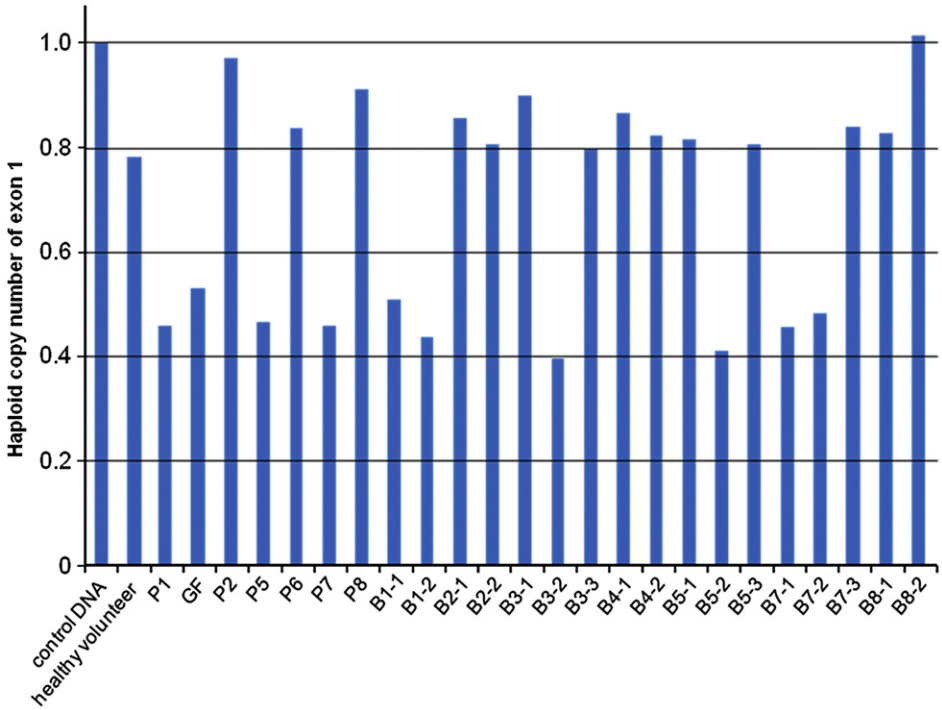

Fig. 4. Results of qPCR of the *FLCN* exon 1 in the members of family with BHDS.

peoples of European and Japanese descent [2–4], but this is the first time that this syndrome is reported from Iran. Although the *FLCN* exon 1 is noncoding, it is reported to have the putative promoter function and possibly be an intragenic deletion "hot spot" causing BHDS [10]. Approximately, 150 different *FLCN* mutations have been identified so far, which are available at a locus-specific database based on the Leiden Open (source) Variation Database software at www.lovd.nl/flcn [11].

The patients with BHDS have a higher risk of developing pulmonary cysts and a spontaneous pneumothorax that may result in the collapse of a lung [1–3]. It has been estimated that 84% of the patients with BHDS had lung cysts, while 38% had a history of at least one episode of pneumothorax [2]. The patients with BHDS carry a 20%–30% lifetime risk of having at least one renal tumor [12]. Therefore, screening for any tumoral lesions on a routine basis for those family members who had the abnormal *FLCN* gene could be recommended.

The age of onset in BHDS, similar to other diseases with autosomal dominant inheritance, is usually in the adulthood. The mean age of renal tumors in BHDS patients is about 50 years old [13], but much younger and older patients with renal tumors have also been reported [14]. It is in agreement with our findings in this study, as the index case developed pneumothorax at adulthood that was complicated with RCC at the fifth decade of her life. The patient's nieces and nephews, who are younger at the time of study, have not presented any symptoms yet, but this does not mean they will stay symptom-free for the rest of their lives. However, this fact should also be considered: the index case experienced her first episode of spontaneous pneumothorax when she encountered the sudden chlorine exposure, while her son had his first spontaneous pneumothorax when he tried diving.

This family is also interesting, considering the fact that no one expresses the cutaneous stigmata of the syndrome, an evident presentation in several previously reported cases, and therefore may be affected by a mutation that affects cell proliferation in the lungs more than in other organ systems. Variances in the phenotypical manifestation of different mutations in the *FLCN* gene will help other researchers in the field to understand the role of the *FLCN* in tumor suppression.

Moreover, in the same family, the index patient had several episodes of pneumothorax and bilateral RCC, while some other cases, including GF, P5, and P7 with older ages, had not experienced any problem up to now. It could show that the same mutation in the *FLCN* gene in different patients does not always mean having the same phenotype. However, further studies on other families with BHDS are needed to test the existence of variable expressivity in BHDS.

This study could also highlight this point that imaging can help clinicians to make the proper clinical diagnosis, whereas definite diagnosis can rely on genetic studies requested based on clinical suspicious.

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