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Birt-Hogg-Dubé Syndrome: Diagnostic Journey of Three Cases from Skin to Gene

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Birt-Hogg-Dube syndrome (BHDS) is a rare disorder characterized by the triad of cutaneous lesions, renal tumors, lung cysts and inactivation of the gene *folliculin* (*FLCN*). Here, we present three female patients diagnosed with BHDS. First case a 55-year-old female had flesh moles histopathology compatible with angiofibroma, multiple cysts in the lung and kidneys, *FLCN* gene mutations ('c.1285dupC [p.His429Profs*]' 11th exon and 'c.653G>A [p.Arg258His]' 7th exon). The second case a 76-year-old female had trichodiscoma on her skin, multiple cysts in the lung, spontaneous pneumothorax, *FLCN* gene mutation 'c.1285dupC (p.His429Profs*27) 11th exon' and, her son had renal carcinoma history under 50 years of age. Our third case, also the daughter of case 2, had dermal papules histopathology compatible with trichodiscoma, spontaneous pneumothorax, *FLCN* gene mutation 'c.1285dupC (p.His429Profs*27) 11th exon' and, parotid oncocytoma. Through our cases, we document the first case of two mutations ('c.1285dupC [p.His429Profs*]' 11th exon and 'c.653G>A [p.Arg258His]' 7th exon) in the same *FLCN* gene and the 11th known case of parotid oncocytoma associated with BHDS in the light of the literature.

Keywords: Birt-Hogg-Dube syndrome, *FLCN* gene, Parotid Neoplasms, Pneumothorax, Kidney neoplasms

INTRODUCTION

Birt-Hogg-Dube syndrome (BHDS) is an autosomal dominant inherited genodermatosis characterized by benign tumors of the hair follicle, pulmonary cysts, spontaneous pneumothorax and renal tumors[1,2]. Germline mutations in the *folliculin* (*FLCN*) gene which encode the protein called folliculin and located in the 14th exon of the p11.2 region of chromosome 17 have been found to cause the BHDS[3,4]. Folliculin seems to take part in the adenosine-monophosphate-activated protein kinase (AMPK) and mechanistic target of rapamycincomplex (mTORC) pathways and is expressed in many tissues including the skin, type 1 pneumocytes and distal nephrons[4,5].

Here, we present three female cases diagnosed with BHDS. Through them, we document the first case of two mutations in the same *FLCN* gene and the11th known case of parotid oncocytoma associated with BHDS in the light of the literature.

Molecular & *in silico* analysis

Genomic DNA was extracted from peripheral venous blood using the QIAamp® DNA Mini Kit (QIAGEN, Ankara, Turkey) in all cases. FLCN gene mutations detected by next generation sequencing and confirmed by Sanger sequencing.

For *in silico* analysis protein structure of FLCN-FNIP2-Rag-Ragulator complex was downloaded from the protein data bank (PDB). *FLCN* in complex with other proteins (PDB ID: 6ULG) was visualized and presented by using PyMol software (http://pymol.sourceforge.net).

The written informed consents from patients for publication of the submitted article, accompanying genetic analyses, photographic materials and the results were obtained after full explanation of the purpose and nature of all procedures used.

CASE REPORT

Case 1

A 55-year-old female was admitted to our outpatient clinic with the complaint of flesh moles on her face for 10 years. She had a family history of colon carcinoma. On dermatological examination, there were numerous 1 to 3 mm diameter skin-colored, asymptomatic, dome-shaped papules on herforehead, malar region, nose and neck (Fig. 1A). Histopathological examination of the skin biopsy was compatible with angiofibroma (Fig. 1B). Thoracic-abdominal-pelvic tomography (CT) of the patient revealed multiple, thin-walled cysts of different sizesin both lobes of the lung parenchyma, and hypodense lesions that were compatible with multiple cysts in kidneys (Fig. 1C, D). As a result of clinical exome sequencing, heterozygous c.1285dupC (p.His429Profs*) (Fig. 2A) mutation in the 11th exon of the *FLCN* gene and as a second mutation in exon 7, heterozygous c.653G>A (p.Arg258His) variation were detected (Fig. 2B). The diagnosis of BHDS was madedue to the presence of multiple cysts in the lungs and kidneys, facial angiofibromas and *FLCN* gene mutations.

Case 2

A 76-year-old female was referred to our outpatient clinic with the presence of cystic lung disease for two months and the complaint of facial widespread flesh moles which appeared at her twenties. She had a history of colon cancer treated 10 years ago. Her son had clear cell renal cell carcinoma and her daughter who is also our third patient had a spontaneous pneumothorax history, parotid oncocytoma, and similar flesh moles. On dermatological examination, there were numerous, skin-colored, asymptomatic papules on her face and neck (Fig. 3A). Histopathological examination of the skin biopsy was consistent with trichodiscoma (Fig. 3B). Thoracic CT showed smoothly circumscribed cysts in the bilateral lung parenchyma. Genetic analysis revealed c.1285dupC (p.His429Profs*27) mutation in the 11th exon in the *FLCN* gene the same as in case 1. The patient was diagnosed as BHDS due to the presence of trichodiscoma of the skin, multiple cysts in the lung, *FLCN* gene mutation,presence of renal carcinoma under 50 years of age in the family and spontaneous pneumothorax.

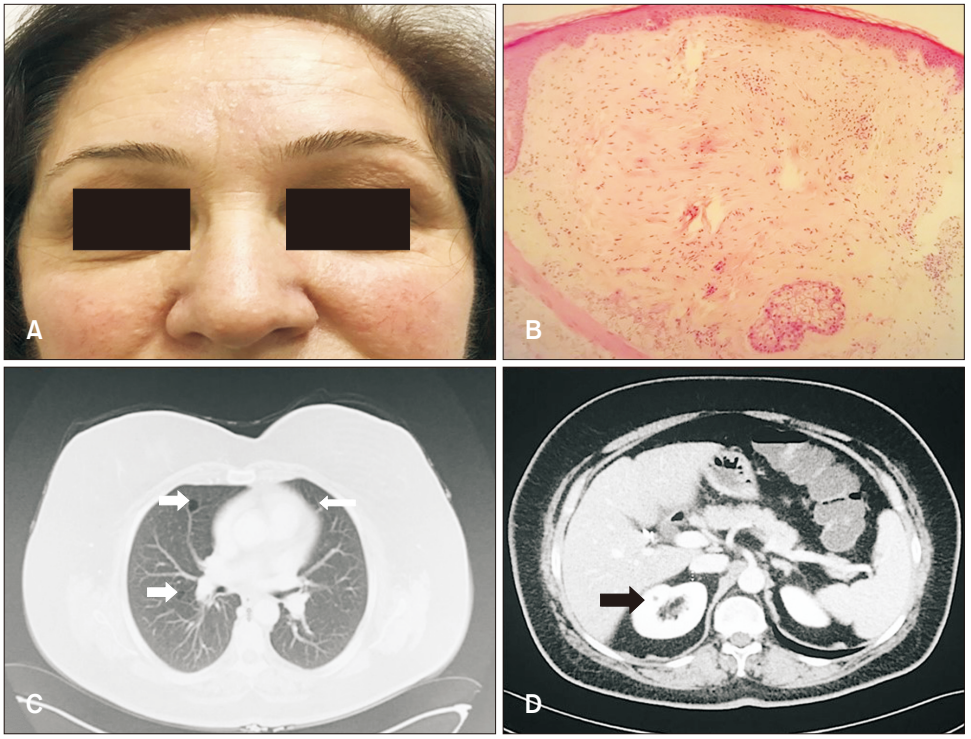

Fig. 1. (A) Multiple skin-colored papules on the forehead, malar region and nose. (B) Some bizarre-looking fibroblasts, collagen and vascular proliferation in the dermis (H&E, original magnification ×40). (C) Thoracic CT revealed thin-walled cysts of different sizes in the lung parenchyma. (D) Abdominal-pelvic CT revealed hypodense lesions that were compatible with multiple cysts in both kidneys.

Case 3

A 54-year-old female, the daughter of case 2, presented with a complaint of flesh moles on her face, which has been around for 30 years. She had a history of spontaneous pneumothorax 15 years ago and right parotid glandoncocytoma 10 years ago. Dermatological examination revealed multiple, skin-colored, asymptomatic papules on her face and neck (Fig. 3C). Histopathological examination of the skin biopsy was consistent with trichodiscoma (Fig. 3D). The c.1285dupC (p.His429Profs*) mutation in the *FLCN* geneis inherited from the mother (case 2) and same as the mutation detected in case 1.

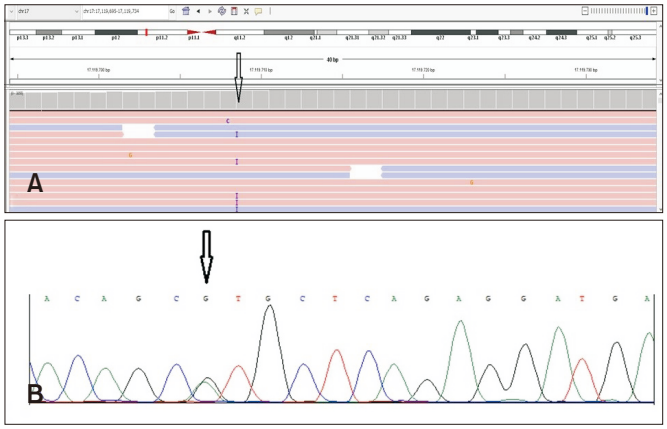

Fig. 2. (A) Integrative genomics view of c.1285dupC (p.His-429Profs*27) heterozygous change in *FLCN* gene. (B) Electropherograms of heterozygous genotype of *FLCN* c.653G>A (p.Arg258His) variation.

In silico finding and functional predictions

FLCN with its interacting proteins FNIP1 or FNIP2 (folliculin interacting protein 1 or 2) bind to AMPK and TFEB[5,6], hence they regulate TFEB dependent transcription[7]. In addition, FLCN-FNIP complex binds to Rag GTPases to initiate the GTP hydrolysis by RagC/D[8,9]. Rag complex interacts with mTORC1[9]. FLCN and FNIP are mainly composed of Longin and DENN domains (Fig. 4) where they heterodimerize to interact with the nucleotide binding domain of Rag heterodimer[10]. Structural information shows that mutant p.His429Profs*27 protein is mainly lack of FNIP2 interacting residues in the DENN domain (Fig. 4) which can attenuate their dimerization. Since FLCN-FNIP dimer regulates the GTP hydrolysis in Rag complex and the mTORC1 activity, lack of dimerization may adversely affect the appropriate mTORC1 activity.

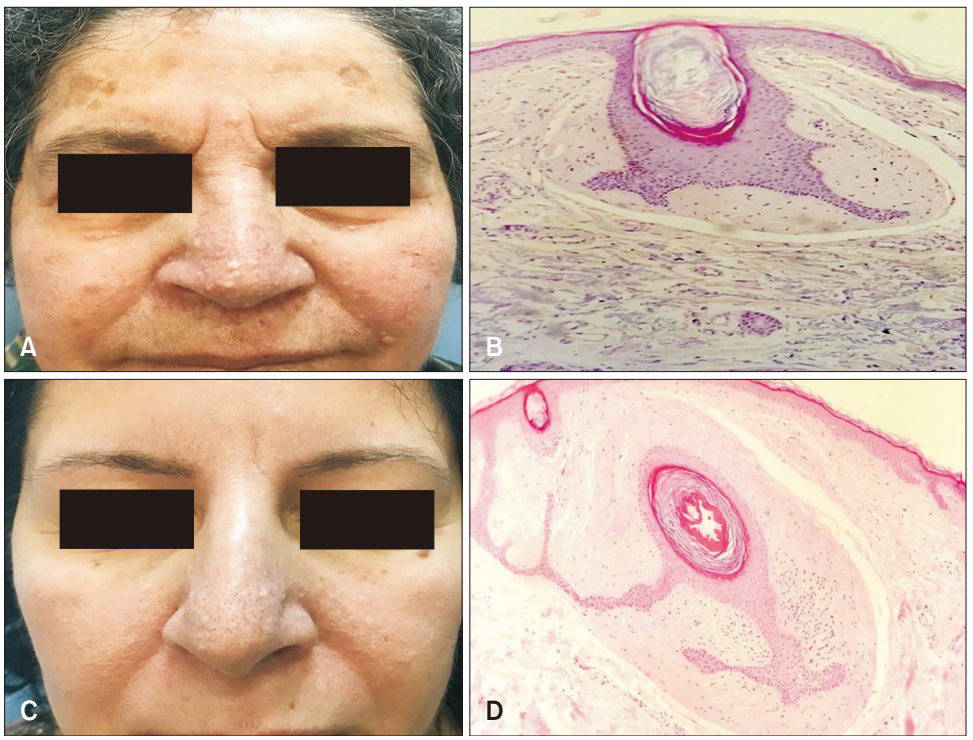

Fig. 3. (A) Multiple skin-colored papules on the face. (B, D) Follicle structures showing epithelial proliferation surrounded by fibrocollagenous tissue (H&E, original magnification ×40). (C) Diffuse whitish papules on the face.

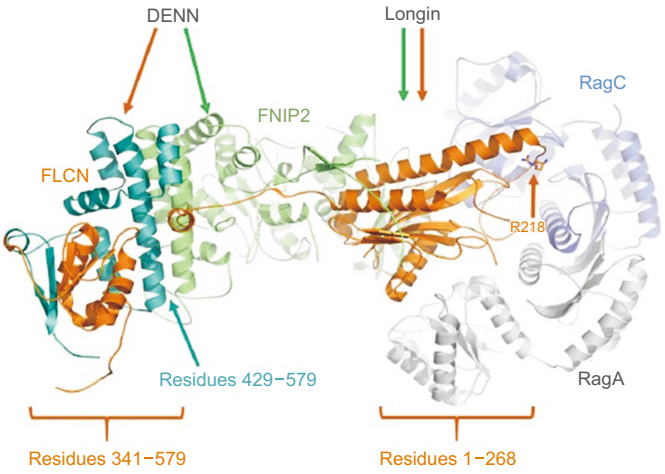

Fig. 4. Structure of FLCN in complex with FNIP2, RagA, and RagC (PDB ID: 6ULG) where proteins are shown in orange, green, white, and navy ribbons, respectively. Residue numbers of FLCN forming the Longin and DENN domains are provided.

DISCUSSION

In 1977, Birt, Hogg, and Dubé described a pedigree in which members had skin lesions consisting of "trichodiscomas, acrochordons, and fibrofolliculomas[1]". BHDS may be phenotypically heterogeneous. Therefore, diagnostic criteria for BHDS have been reported[2].

Fibrofoliculomas are the most common skin manifestations of BHDS. It occurs in the third decade in 84% of the patients and presents with painless, dome-shaped whitish papules on the face, neck, chest, and back[11]. Recent studies show that Asian patients with BHDS present a lower incidence of skin lesions(25.0%~48.7%) than Caucasian patients[12,13]. Other skin lesions associated with BHDS are trichodiscomas and acrocordons. Less frequently, angiofibroma, angiolipoma, epidermal cysthave also been reported[2]. The skin lesions of our cases were compatible with angiofibromas andtrichodiscomas.

Multiple lung cysts are seen on CT in more than 80% of BHDS cases[14,15]. The probability of first pneumothorax is 75% by the sixth decade[15]. About 75% of the patients will experience recurrent pneumothorax[14]. All our three cases had a history of lung cysts and case 3 had a history of pneumothorax at age 39 years.

The most serious complication of BHDS is renal cancers. It is seen in the 50s in 15%~25% of the patients, mostly multiple and bilateral[16]. It is reported that the patients diagnosed with BHDShad a 7-fold increased risk of developing renal neoplasia[17]. Chromophobe renal cancer, mixed chromophobe and oncocytic renal tumors are typical for BHDS[16]. Therefore, all newly diagnosed patients should undergo abdominal imagingto exclude renal tumors[2]. The son of case 2 had a history of clear cell renal cell carcinoma with no skin symptoms.

Other tumors reported to be associated with BHDS include parathyroid adenoma/oncocytoma, colorectal carcinoma, and melanoma[2,18]. Our case 2 had a treated colon carcinoma and case 3 had a history of treated parotid oncocytoma. To the best of our knowledge, case 3 is the 11th reported case of BHDS with parotid oncocytoma[11,14,19].

To date, 285 unique mutations have been identifiedin the *FLCN* gene and documented in the FLCN Leiden Open Variation Database (https://databases.lovd.nl/shared/genes/FLCN).

As a result of the genetic analysis performed in case 1, it was found that c.1285dupC (p.His429Profs*) mutation in the 11th exon of *FLCN* gene was heterozygous. The identified mutation is described in the Human genetic mutation Database (HGMD) and has been associated with BHDS (HGMD ID: C1023308). This mutation has been reported pathogenic in the ClinVar database. In case 1, change of c.653G>A in exon 7 (p.Arg258His) was also determined as a second mutation. The clinical significance in the ClinVar database has been reported as uncertain.It has also been reported as a variant of uncertain clinical significance in Varsome. The population frequency of the mutation was 0.00000795 in gnomAD Exomes ver. 2.11 (Broad Institute, Cambridge, MA, USA). It was stated that it could be disease-causing in the predictive databases (such as Mutation Taster, FATHMM, PROVEAN). Segregation of this variant could not be demonstrated because the patient's parents were not alive. Because of this reason this variant could not be demonstrated whether it is in *cis* or *trans* position. Hitherto, a case of two mutations in the same *FLCN* gene has not been reported in the literature.

According to the results of a study conducted in Japan, the mutation of c.1285dupC (p.His429Profs*) in the *FLCN* gene is among the mutations detected in the majority of cases, and this mutation is thought to be a hotspot mutation in the *FLCN* gene[13]. The same mutation was observed in our cases, although they were independent of each other. Therefore, to determine whether this mutation is a hotspot mutation for the Turkish population needs to be screening in larger series of patients.

Analysis of genotype-phenotype correlations for FLCN

mutations demonstrated a significantly higher risk of colorectal neoplasia in c.1285dupC mutation carriers than in c.610delGCinsTA mutation carriers[20]. However, in terms of genotype-phenotype correlations, our case 1, who had compound heterozygous *FLCN* mutations, did not show more severe symptoms in the skin, lungs and kidneys than the BHDS patients reported previously.

There are still many aspects of BHDS to be explored. Therefore, BHDS should be kept in mind in the differential diagnosis of patients presenting with papular lesions. The prompt and accurate diagnosis is necessary for appropriate management of patients and genetic counselling. Additionally, the detection of two mutations in the same *FLCN* gene in our cases expands the knowledge of *FLCN* mutations and will provide insight into the genetic diagnosis of BHDS.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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