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한국인 Birt–Hogg–Dubé 증후군 환자의 분자유전학적 및 임상적 특징

Genetic and Clinical Profiling of Korean Patients with Birt–Hogg–Dubé Syndrome

김지수[1] · 최선미[2] · 성호빈[3] · 박용숙[1] · 조성임[1] · 이지수[1] · 김만진[4] · 박성섭[5] · 성문우[1,5,6]
Jisoo G. Kim, M.D.[1], Sun Mi Choi, M.D.[2], Hobin Sung, B.S.[3], Yongsook Park, M.S.[1], Sung Im Cho, M.S.[1], Jee-Soo Lee, M.D.[1], Man Jin Kim, M.D.[4], Sung Sup Park, M.D.[5], Moon-Woo Seong, M.D.[1,5,6]

서울대학교병원 진단검사의학과, 서울대학교 의과대학 검사의학교실[1], 서울대학교병원 호흡기 내과[2], 서울대학교 의과대학 협동과정 종양생물학 전공[3], 서울대학교병원 임상유전체의학과[4], 서울대학교병원 의생명연구원[5], 서울대학교 의과대학 암연구소[6]
Department of Laboratory Medicine[1], Seoul National University Hospital, Seoul National University College of Medicine, Seoul; Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine[2], Seoul National University Hospital, Seoul National University College of Medicine, Seoul; Interdisciplinary Program in Cancer Biology[3], Seoul National University College of Medicine, Seoul; Department of Genomic Medicine[4], Seoul National University Hospital, Seoul; Biomedical Research Institute[5], Seoul National University Hospital, Seoul; Cancer Research Institute[6], Seoul National University College of Medicine, Seoul, Korea

The Birt–Hogg–Dubé syndrome (BHDS) is a rare autosomal dominant disease caused by mutations in the *FLCN* gene and manifests as pulmonary cysts, pneumothoraces, skin fibrofolliculomas, and renal cancer. Currently, few cases of BHDS have been reported in the Korean population, with a prevalence of 5.67 per 10,000,000. Here, we reviewed the genetic and clinical characteristics of nine patients from five families who were confirmed to carry pathogenic *FLCN* variants using whole-exome sequencing and multiplex ligation-dependent probe amplification. Among these patients (mean age 49.2 ± 18.2 years; 89% females), four pathogenic *FLCN* variants were identified, including c.1539-2A > G in two families, which had not been previously reported in Korean patients. Haplotype analysis suggested that these two families shared a common genetic background. We observed pulmonary cysts in 6/7 (86%) patients, fibrofolliculomas in 3/8 (38%), and renal cancer in 2/9 (22%), similar to those in previous Korean studies; however, pneumothorax was identified in 2/9 (22%) patients, indicating a markedly lower rate. Other cancers, such as breast cancer or thyroid carcinoma, were observed in 2/9 (22%) patients. Overall, our study describes the genetic and clinical heterogeneity among Korean patients with BHDS and may help better understand the comprehensive genetic and clinical profiles of BHDS in Korean patients.

Key Words: Birt–Hogg–Dubé syndrome, High-throughput nucleotide sequencing, Exome sequencing, *FLCN*, Korean

Birt–Hogg–Dubé syndrome (BHDS) is a rare autosomal dominant disorder characterized by fibrofolliculomas, renal cancer, multiple pulmonary cysts, and spontaneous pneumothoraces [1]; however, its diagnosis requires satisfaction of one major or two minor diagnostic criteria as suggested by the European Birt-Hogg-Dubé consortium [1], and it predominantly results from mutations in the *FLCN* gene on chromosome 17p11.2, encoding folliculin, a tumor suppressor protein [2].

The estimated global prevalence of BHDS is 1.86 (95% confidence interval, 1.16–3.00) per million, with an unknown prevalence in Asia. BHDS can manifest at any age, typically at 40 [3]. Differences in incidence between the sexes remain debatable [4], as do genotype-phenotype correlations [3, 5]. BHDS exhibits phenotypic variability, with 80% of skin lesions [3, 6, 7], 80% of pulmonary cysts [3], 24–35% of pneumothorax [3, 8, 9], and 19–40% of renal cancer [3, 7, 10, 11] in Caucasian patients. Asian patients show slight differences, with fewer instances of skin lesions and

Corresponding author: Moon-Woo Seong, M.D., Ph.D.
https://orcid.org/0000-0003-2954-3677
Department of Laboratory Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-4180, Fax: +82-2-747-0359, E-mail: mwseong@snu.ac.kr

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renal cancer and more pulmonary manifestations, including pulmonary cysts [6, 8, 10, 12, 13]. Few BHDS cases have been reported in Korea, with clinical manifestations similar to those reported in previous Asian studies [5, 9]. This study aims to elucidate the clinical manifestations and genetic profiles of Korean patients with BHDS.

This study included nine patients from five families with pathogenic *FLCN* variants. Whole exome sequencing (WES) and multiple ligation-dependent probe amplification (MLPA) were performed on five probands, along with Sanger sequencing of the family members to confirm family-specific variants. The data included demographics, clinical traits, and test results from electronic medical records. Informed consent was obtained from the patients; the study adhered to the guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University Hospital (H-2204-144-1319).

Peripheral blood from patients, stored in EDTA disodium salt tubes, was subjected to DNA extraction using a Gentra Puregene Blood kit (Qiagen, Hilden, Germany). DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). After sonication of the DNA using a Covaris system (Covaris, Inc., Woburn, MA, USA), target enrichment was performed using SureSelect Human All Exon software (version 5; Agilent, Santa Clara, CA, USA). Paired-end sequencing after exome capture was performed using an Illumina HiSeq 2000 or 2500 platform (Illumina, San Diego, CA, USA). DNA sequence analysis was performed using NextGENe software (v2.4.0.1) (SoftGenetics, State College, PA, USA), and the sequences were aligned with the GRCh37/hg19 human reference genome. NM_144997.5 served as the reference sequence for the *FLCN* gene. Benign variants were filtered using Genome Aggregation and Korean Reference Genome Databases. *In silico* prediction tools such as SIFT, Mutation Taster, and PolyPhen2 were employed. The Human Gene Mutation and ClinVar databases were referenced. The pathogenicity of each variant was determined as per the American College of Medical Genetics and Association for Molecular Pathology (ACMG/AMP) guidelines [14] and the latest guidelines by the ClinGen Sequence Variant Interpretation Working Group [15-17]. Variants were visualized using ProteinPaint (https://proteinpaint.stjude.org).

SALSA MLPA Probemix P256-C1 *FLCN* (MRC-Holland, Amsterdam, The Netherlands) was used to screen for large deletions or duplications of the *FLCN* gene according to the manufacturer's instructions. Deletions and duplications were indicated by normalized peak ratios <0.8 and >1.2, respectively. We selected four nearby SNPs within a 2.5 Mb range of *FLCN*, including two downstream (rs56926918, rs56153623) and two upstream SNPs (rs2072652, rs2075659). The *FLCN* haplotype was reconstructed using PHASE 2.1.1 with 56 unrelated normal controls and four patients.

Among the nine patients with BHDS, five were from unrelated families. At the time of diagnosis, the mean age of the nine patients was 49.2±18.2 years, and 8/9 (89%) patients were females (Table 1). In our study, we identified four different pathogenic *FLCN* variants in nine patients from five families (Table 2). Splice site variants were observed in 3/5 (60%) patients. A nonsense and a frameshift variant were observed in each of the 1/5 (20%) families. All four variants were classified as pathogenic according to the ACMG/AMP [14] and ClinGen guidelines [15-17]. These pathogenic variants were predominantly localized to the distal *FLCN*, spanning exons 11 and 14 and introns 11 and 13. Among the BHDS families meeting clinical diagnostic criteria [1] (3.I.2 and 4), all of them (100%) had pathogenic *FLCN* variants.

The *FLCN* c.1539-2A>G variant, previously unreported in Korean patients with BHDS, was discovered in two unrelated families (1 and 3.I.2). Nine haplotypes were reconstructed (Supplementary Table 1), and haplotype H1 was observed in both unrelated families with the *FLCN* c.1539-2A>G variant (Supplementary Table 2), suggesting a common genetic background between these two families. MLPA analysis showed no large deletions or insertions, including a mutational hotspot [11]. The genotype-phenotype correlation was not significant, consistent with previous findings [6].

Bilateral multiple lung cysts were observed in 6/7 (86%) patients (mean age 52.2±12.0) from five unrelated families (Supplementary Fig. 1). Pneumothorax occurred in 2/9 (22%) patients, with one experiencing recurrent episodes starting five years before diagnosis. Pulmonary function tests were conducted in 4/9 unrelated patients, showing mild restrictive patterns in 1/4 (25%) and mildly decreased diffusion capacity in 2/3 (67%) (Supplementary Table 3). Further, 3/8 (38%) patients from the same family had multiple neck fibrofolliculomas, raising concerns regarding selection bias and necessitating histological confirmation. Renal cancer was observed in 2/9 (22%) patients, one with bilateral chromophobe renal cell carcinoma and the other with multilocu-

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Table 1. Clinical characteristics of nine patients with Birt–Hogg–Dubé syndrome

ID	Sex	Diagnosis, age	Lung		Skin (age at diagnosis)	Kidney		Other disease			Family history (number of family members)	Smoking
			Pneumothorax (number of events, age at diagnosis)	Lung cysts (age at diagnosis)		Renal cancer (age at diagnosis)	Renal cysts (age at diagnosis)	Other cancers (age at diagnosis)	Benign tumors (age at diagnosis)	Other		
1	F	54.6	No	Bilateral (52)	R/O Facial fibroma, NBx (55)	No	Simple cysts (53) (Bilateral, multiple, up to 1.0 cm)	Breast cancer (55) (Ductal carcinoma *in situ*)	Endometrial polyp (55)	Unruptured intracranial aneurysms	No	Never
2	F	63.7	Yes (1, 45)	Bilateral (62)	Bilateral facial rash, NBx (64)	No	Simple cysts (64) (Bilateral, multiple)	No	Esophageal tumor (61)	Arterial hypertension, Hyperlipidemia	Pneumothorax (5, father, mother, two younger brothers, a nephew), Lung cancer (1, father)	Never
3.I.2	F	70.6	No	Bilateral (70)	Multiple fibrofolliculomas on the neck, NBx (70)	MCRNLPM (71) (Right, single, 1.7 cm)	Simple cysts (67) (Bilateral, multiple, up to 5.2 cm)	No	Adrenal incidentaloma (67), Gall bladder adenomyomatosis (69), Sessile serrated adenoma in the colon (69)	DM, Arterial hypertension, Coronary artery disease, Glaucoma, Macular atrophy	BHDS (4, three daughters, a grandson)	Never
3.II.2	F	44.3	No	Bilateral (44)	Multiple fibrofolliculomas on the neck, NBx (44)	No	NT	Papillary thyroid carcinoma (44) (Left, single, up to 3.5 cm)	No	No	BHDS (4, mother, two younger sisters, a nephew)	NA
3.II.4	F	42.6	No	NT	No	No	NT	No	Rectal hyperplastic polyps (42), Benign thyroid nodules (42)	Azotemia	BHDS (4, mother, an older sister, a younger sister, a son)	NA
3.II.6	F	40.3	No	No	Multiple fibrofolliculomas on the neck, NBx (40)	No	NT	No	Benign thyroid nodules (40)	No	BHDS (4, mother, two older sisters, a nephew)	NA
3.III.3	M	9.0	No	NT	NT	No	NT	No	No	No	BHDS (4, a grandmother, mother, two aunts)	Never
4	F	57.1	No	Bilateral (49)	No	ChRCC (49) (Bilateral, multiple, up to 4.0 cm)	No	No	Uterine myomas (49)	No	Pneumothorax (1, a daughter), Bronchiectasis (1, mother), Interstitial lung disease (1, mother), Breast tumor (1, a daughter)	Former smoker
5	F	60.2	Yes (4, 55–60)	Bilateral (58)	No	No	Simple cysts (58) (Bilateral, multiple)	No	No	Arterial hypertension	Pneumothorax (3, father, an older sister, a younger brother)	Never
Mean ± SD		49.2 ± 18.2	55.4 ± 6.1*	52.2 ± 12.0	51.3 ± 16.3	60.0 ± 15.6	60.5 ± 6.2	49.5 ± 7.8				
All	9		2/9 (22%)	6/7 (86%)	3/8 (38%)	2/9 (22%)	4/5 (80%)	2/9 (22%)	6/9 (67%)	5/9 (56%)	8/9 (89%)	1/6 (17%)

Abbreviations: No, no lesion was detected; Yes, presence of lesions; NBx, cutaneous lesions were observed, but biopsy was not performed; MCRNLPM, multilocular cystic renal neoplasm of low malignant potential; DM, diabetes mellitus; NT, not tested; NA, not available; ChRCC, chromophobe renal cell carcinoma; SD, standard deviation.
*Mean ± SD of the first episodes of pneumothorax.

Table 2. Characteristics of the four *FLCN* variants identified in this study on nine patients with Birt–Hogg–Dubé syndrome

Family	ID	DNA variants	Protein alteration	Location	Variant type	Classification	Criteria [References]
1	1	c.1539-2A>G	p.?	Intron 13	Splice site	Pathogenic	PVS1, PS4_supporting [19], PM2, PP1
2	2	c.1215C>G	p.Tyr405*	Exon 11	Nonsense	Pathogenic	PVS1, PS4_supporting [7], PM2
3	3.I.2	c.1539-2A>G	p.?	Intron 13	Splice site	Pathogenic	PVS1, PS4_supporting [19], PM2, PP1
	3.II.2						
	3.II.4						
	3.II.6						
	3.III.3						
4	4	c.1557del	p.Phe519Leufs*18	Exon 14	Frameshift	Pathogenic	PVS1, PS4_supporting [9], PM2
5	5	c.1300+1G>A	p.?	Intron 11	Splice site	Pathogenic	PVS1, PS4_supporting [20], PM2

Abbreviations: PVS, pathogenic very strong; PS, pathogenic strong; PM, pathogenic moderate; PP, pathogenic supporting.

lar cystic renal neoplasm of low malignant potential. Renal cysts were observed in 4/5 (80%) patients. Malignant tumors, including breast cancer and papillary thyroid carcinoma, were observed in 2/9 (22%) unrelated patients. Benign tumors were observed in 6/9 (67%) patients and arterial hypertension in 3/9 (33%). Family history included pneumothorax in 3/9 (33%) and lung cancer in 1/9 (11%) patients, and five patients from one family had a family history of pathogenic *FLCN* variants. In family 3 with the c.1539-2A>G variant, the proband showed all pulmonary, renal, and skin manifestations. In contrast, the other family members showed varying clinical patterns. As BHDS is typically described in the 40s [3], patient 3.III.3 may not fully present the disease symptoms (Fig. 1).

This study investigated nine Korean patients with BHDS, with a mean diagnosis age of 49.2±18.2 years, consistent with previous Korean studies [5, 9]. The rates of pulmonary cysts (86%), renal cancer (22%), and skin lesions (38%) were similar to those reported in earlier studies on Korean patients with BHDS [9, 18]. However, the rate of pneumothorax (22%) was lower [5, 9, 18]. This pattern was comparable to that reported in previous studies on Asian patients [8, 13]. Compared to Caucasians, Korean patients with BHDS exhibited similar rates for pulmonary cysts [3], pneumothorax [3, 7, 8], and renal cancer [3, 7, 10, 11]; however, they had lower rates for skin lesions [3, 6, 7]. Recurrent pneumothorax, typically higher (75–80%) in Caucasians [4] and Koreans [9], was 50% in this study, emphasizing the need for larger-scale research due to the small population. The discrepant skin lesion rates in Asian patients [9, 12, 13] may be related to low biopsy rates [13], necessitating further research. The common renal cancers in Korean patients with BHDS are chromophobe renal cell carcinoma and hybrid oncocytic/chromophobe tumors [9]. Patients with BHDS have a sevenfold higher risk of renal cancer [3] and a 19.2% incidence of renal cysts [10]. Further, 4/5 (80%) of unrelated patients in this study had renal cysts, necessitating continuous monitoring. Breast cancer or papillary thyroid carcinoma was observed in 2/9 (22%) unrelated patients, underlining the importance of early screening.

Our study identified *FLCN* variants mainly in the exon and intron regions of *FLCN*, predominantly including splice site variants (60%), among nine patients from five families. This warrants further investigation into genotype-phenotype correlations. Similar to previous research [3, 7], a high variant detection rate of 100% was observed in clinical BHDS families that met the set criteria [1]; however, larger studies are needed for validation due to the limited number of included cases.

In conclusion, our study describes the genetic and clinical heterogeneity of BHDS in Korean patients and contributes to a better understanding of their comprehensive profiles.

요 약

Birt-Hogg-Dubé 증후군(BHDS)은 *FLCN* 유전자의 돌연변이로 인

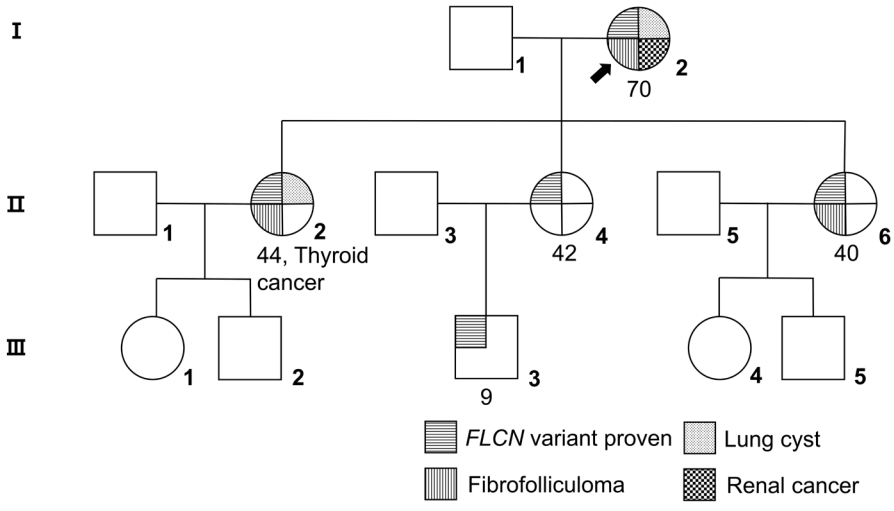

Fig. 1. Pedigree of family 3 with Birt–Hogg–Dubé syndrome (BHDS). Numbers refer to the age (in years) at BHDS diagnosis. Arrow, proband; top left quadrant, *FLCN* variant proven; top right, lung cyst; bottom left, fibrofolliculoma, not proven by biopsy; bottom right, renal cancer. The Roman numerals indicate generations. Patient 3.II.2 shows thyroid cancer.

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해 발생하는 희귀 상염색체 우성 질환으로, 폐낭종, 기흉, 피부 섬유털집종 및 신장암 증상을 동반한다. 유병률은 10,000,000명당 5.67명이며, 현재 한국인 BHDS 환자로 보고된 예는 많지 않다. 이 논문에서는 WES (whole-exome sequencing) 및 MLPA (multiplex ligation-dependent probe amplification)를 사용하여 병원성 *FLCN* 변이가 있는 것으로 확인된 다섯 가족으로부터 9명의 BHDS 환자의 분자유전학적 및 임상적 특성을 검토하였다. 대상 환자군(평균 연령 49.2±18.2세, 여성 89%)에서 4개의 병원성 *FLCN* 유전자 변이가 확인되었는데, 이전에 한국인 BHDS 환자에서는 보고되지 않았던 c.1539-2A>G 변이가 두 가족에서 확인되었다. 일배체형 분석을 통해 해당 두 가족은 공통된 유전적 배경을 공유할 가능성이 있음을 알 수 있었다. 폐낭종은 6/7 (86%)의 환자에서 확인되었고, 섬유털집종은 3/8 (38%), 신장암은 2/9 (22%)의 환자에서 확인되어 이전의 국내 연구와 유사하였으나, 기흉은 2/9 (22%)의 환자에서 확인되어 이전 연구 결과보다 현저히 적었다. 유방암이나 갑상선암 등 악성종양도 2/9 (22%)의 환자에서 발견되었다. 본 연구는 한국인 BHDS 환자의 분자유전학적 및 임상적 다양성을 제시하여 한국인 BHDS 환자들의 특성에 대한 이해도를 높여줄 수 있다는 점에서 의의를 가진다.

Conflicts of Interest

None declared.

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