# BHD Symposium

# Speaker Abstracts

# 21 – 22 October 2021

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# October 21st – Session 1: 3.00 – 4.10 PM BST

**3.25 pm – 3.35 pm BST**
**Clinical and genetic features of 335 Asian patients with Birt-Hogg-Dubé syndrome whose presenting feature is pulmonary cysts with or without pneumothorax**

**Yukiko Namba**1, Hiroki Ebana1, 3, Shoichi Okamoto1, 3, Etsuko Kobayashi1, 3, Masatoshi Kurihara2, 3, Yasuhito Sekimoto1, 3, Kenji Tsuboshima2, 3, Makiko Kunogi Okura1, 3, Yoichiro Mitsuishi1, Kazuhisa Takahashi1, and Kuniaki Seyama1, 3

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**Background**
Clinical and genetic features of pulmonary cysts and pneumothorax (PTX) in Asian patients with Birt-Hogg-Dubé syndrome (BHDS) is not fully understood.
**Methods**
The clinical and genetic data of Asian BHDS patients whose presenting feature was pulmonary cysts with or without PTX from 2006 to 2017 were retrospectively reviewed to characterize pulmonary phenotype and FLCN mutations.
**Results**
A total of 335 patients from 298 unrelated families were collected in this cohort (Japanese 328, Chinese 5, Indonesian 1, Russian 1). 137 of them were males (41%), and 314 patients had the history of PTX (93.7%). The median age at diagnosis was 44 years (males/females 40/47 years), and the one at the first PTX episode was 32 years (males/females 30/32 years). The side of first PTX were right (47.2%), left (38.5%), and simultaneously bilateral (5.7%). Median number of PTX by the diagnosis was 3 episodes (males/females 3/2 episodes). Cutaneous lesions were present in 36.5% and renal tumors in 4.1%. Family history were collected from 298 probands; pneumothorax (74.3%), cutaneous lesions (18.2%), renal tumors (7.5%) whereas 56 of them (18.8%) had family history of neither lung, skin, nor renal manifestations. Until 2010, FLCN genetic testing had been performed in <10% of patients within one year after the first PTX episode, but increased to about 30% thereafter, indicating the increased awareness of BHDS. We identified 66 types of pathogenic variants: duplication (8 types, n=138, 46.3%), deletion (22 types, n=86, 28.9%), splicing (16 types, n=37, 12.4%), substitution (17 types, n=21, 7.0%), insertion (2 types, n=2), deletion/insertion (n=1), large genomic deletion (n=13, 4.4%).
**Conclusions**
Increasing awareness of BHDS and knowledge about imaging feature of chest CT would have enabled to diagnose patients who had no family history suggestive of BHDS. High incidence of bilateral PTX as the first presenting feature needs to be noted.

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# October 21st – Session 1: 3.00 – 4.10 PM BST

**3.35 pm – 3.45 pm BST**

**Prevalence of Birt-Hogg-Dubé syndrome determined through epidemiological data on spontaneous pneumothorax and Bayes theorem**

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**Background**

Birt-Hogg-Dubé syndrome (BHD) is a rare inherited disorder characterized by cutaneous fibrofolliculomas, multiple pulmonary cysts, recurrent spontaneous pneumothorax (SP), and renal tumors. The prevalence of BHD in the general population is unknown. This study aimed at determining the prevalence of BHD by applying the Bayes theorem of conditional probability to epidemiological data on SP.

**Methods**

We performed a meta-analysis of published data on: (1) the probability of having BHD among patients with apparent primary SP (4 studies), (2) the incidence rate of primary SP in the general population (9 studies), and (3) the probability of experiencing a SP in BHD (16 studies). Results were corrected for SP relapses, stratified by gender and year of study publication (before and after 2000), and computed with the Bayes equation.

**Results**

The probability of having BHD among patients with apparent primary SP was 0.09 (95% confidence interval: 0.07, 0.11) or 9%. It was 0.20 (0.14, 0.27) in women and 0.05 (0.04, 0.07) in men. The incidence rate of primary SP in the general population was 8.69 (6.58, 11.46) per 100,000 person-years (p-y). It was 3.44 (2.36, 4.99) per 100,000 p-y in women and 13.96 (10.72, 18.18) per 100,000 p-y in men, and was about 2 times higher in studies published after 2000 than in those published before 2000. The probability of experiencing at least one SP among patients with BHD was 0.43 (0.31, 0.54) or 43%, without gender difference. By combining these data in the Bayes equation, we found a prevalence of BHD in the general population of 1.86 (1.16, 3.00) per million, with values of 1.86 (1.02, 3.39) per million in men, and 1.88 (0.97, 3.63) per million in women.

**Conclusion**

The prevalence of BHD in the general population is about 2 cases per million, without difference between genders.

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# October 21st – Session 1: 3.00 – 4.10 PM BST

**3.45 pm – 3.55 pm BST**
**Early onset colon cancer risk in Birt-Hogg-Dubé syndrome**

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**Background**
Lung cysts with spontaneous pneumothorax, fibrofolliculoma and renal cell cancer are established symptoms of Birt-Hogg-Dubé syndrome (1-3). Several other tumor types have been reported but it is still a matter of debate if these are real or by chance associations (4). This is especially true for colorectal cancer for which association has been reported in some but not all studies (5-8).
**Methods**
We have now analyzed the frequency of colorectal cancer in a sample of 83 Birt-Hogg-Dubé syndrome families including 256 patients (male 130, female 126) as well as in 519 controls.
**Results**
Within the Birt-Hogg-Dubé sample, the rate of colorectal cancer was moderately but significantly increased (5.1% versus 1.5%, p-value .0068). We did not find any evidence that colorectal cancer clustered in families with specific *FLCN* mutations. In particular, the mutation hotspot c.1285dupC/c.1285delC, previously described to be associated with colorectal cancer, was found to be underrepresented in families with this tumor type. Interestingly, 35% of colorectal cancer patients, belonging to 8/82 Birt-Hogg-Dubé syndrome families, fulfilled the revised Bethesda criteria for HNPCC (hereditary non polyposis colon cancer, Lynch syndrome), the most common type of familial colon cancer. Seven families had one member each that was affected by colorectal cancer before the age of 50 years (1st Bethesda criterion) while two families had three members affected by colorectal cancer (5th Bethesda criterion). Nevertheless, other types of tumors (especially stomach, endometrial or hepatobiliary tract cancer) frequently associated with HNPCC were absent in our sample, arguing against a concurrence of Birt-Hogg-Dubé syndrome and HNPCC (9).
**Conclusions**
Our results therefore raise the possibility that patients with Birt-Hogg-Dubé syndrome are at risk for early onset colon cancer without having a markedly increased incidence of HNPCC-associated malignancies. It seems therefore prudent to suggest starting colon cancer screening at least ten years earlier than usually recommended.

1. Sattler EC, Reithmair M, Steinlein OK. Kidney cancer characteristics and genotype-phenotype-correlations in Birt-Hogg-Dubé syndrome. PLoS One 2018a;13:e0209504.
2. Sattler EC, Syunyaeva Z, Mansmann U, et al. Genetic risk factors for spontaneous pneumothorax in Birt-Hogg-Dubé syndrome. Chest 2020;157:1199-1206.
3. Sattler EC, Steinlein OK. Delayed diagnosis of Birt-Hogg-Dubé syndrome due to marked intrafamilial clinical variability: a case report. BMC Med Genet. 2018 Mar 16;19(1):45
4. Sattler EC, Ertl-Wagner B, Pellegrini C, Peris K, Reithmair M, Schädle N, Ruzicka T, Steinlein OK. Cutaneous melanoma in Birt-Hogg-Dubé syndrome: part of the clinical spectrum? Br J Dermatol. 2018 Feb;178(2):e132-e133.
5. Khoo SK, Giraud S, Kahnoski K, et al. Clinical and genetic studies of Birt-Hogg-Dubé syndrome. J Med Genet 2002;39:906-12.
6. Nahorski MS, Lim DH, Martin L, et al. Investigation of the Birt-Hogg-Dubé tumour suppressor gene (FLCN) in familial and sporadic colorectal cancer. J Med Genet 2010;47:385-90.

7. Zbar B, Alvord WG, Glenn G, et al. Risk of renal and colonic neoplasms and spontaneous pneumothorax in the Birt-Hogg-Dubé syndrome. Cancer Epidemiol Biomarkers Prev 2002;11:393-400.
8. Toro JR, Pautler SE, Stewart L, et al. Lung cysts, spontaneous pneumothorax, and genetic associations in 89 families with Birt-Hogg-Dubé syndrome. Am J Respir Crit Care Med 2007;175:1044-53.
9. Sattler EC, Syunyaeva Z, Reithmair M, Dempke W, Steinlein OK. Colorectal cancer risk in families with Birt-Hogg-Dubé syndrome increased. Eur J Cancer. 2021;151:168-174.

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# October 21st – Session 2: 4.40 – 6.15 PM BST

**5.15 pm – 5.25 pm BST**
**Cellular effects of FLCN-FNIP1/2 loss in human renal epithelial cells**

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**Background**
FLCN is a conserved, essential gene linked to diverse cellular processes. Conflicting results, such as activating or inhibitory effects of FLCN on mTOR signaling, and the range of the processes attributed to FLCN loss, prohibit a clear understanding of the pathways by which FLCN suppresses renal tumorigenesis specifically.
**Methods**
Here, we created a novel human renal epithelial *in vitro* model system to study the exact effect of FLCN loss. By means of CRISPR/Cas9 we knocked out FLCN expression in renal proximal tubular epithelial cells (RPTEC/TERT1), which are of the cell type that is regarded as the origin of renal cell carcinoma. By RNAseq and label-free GeLC-MS/MS-based proteomics we determined the unique transcriptomes and functionally relevant proteomes of the generated isogenic cell line pairs ($FLCN^{neg}$ vs. $FLCN^{pos}$). Also, to address the role of FLCN in cellular kinase signaling pathways via protein and receptor phosphorylation, we also determined comprehensive phosphoproteomic profiles of these cell lines.
**Results**
Gene Ontology, Gene Set Enrichment and transcription motif analyses of RNA and protein data revealed a broad spectrum of biological processes regulated by FLCN in renal cells. Moreover, in our phosphoproteomic analyses we identify that FLCN loss elevates phosphorylation of numerous kinases, including tyrosine kinases EPHA2 and MET, as well their downstream targets MAPK1/3/6. In agreement with the induction of the TFE transcriptional gene expression signature upon FLCN loss, here we identified that phosphorylation of three specific Serine sites on TFEB is dependent on the presence of FLCN and absence of this phosphorylation correlates with constitutive nuclear localization of TFEB in $FLCN^{NEG}$ cells.
**Conclusions**
The analyses confirm earlier observations regarding FLCN-mediated regulation of the TFE transcription factors[1-4] and reveal regulation of other transcription factors and induction of a kidney tubular interferon response signature. Upon FLCN loss STAT1/2 activation appears to counterbalance TFE-directed hyper-proliferation and may influence immune responses. In which way these biological processes contribute to renal tumorigenesis exactly needs to be further elucidated.

1. Napolitano G, Di Malta C, Esposito A, de Araujo MEG, Pece S, Bertalot G, *et al.* (2020) A substrate-specific mTORC1 pathway underlies Birt-Hogg-Dubé syndrome. Nature 585:597–602
2. Endoh M, Baba M, Endoh T, Hirayama A, Nakamura-Ishizu A, Umemoto T, *et al.* (2020) A FLCN-TFE3 feedback loop prevents excessive glycogenesis and phagocyte activation by regulating lysosome activity. Cell Reports 30:1823–1834
3. El-Houjeiri L, Possik E, Vijayaraghavan T, Paquette M, Martina JA, Kazan JM, *et al.* (2019) The transcription factors TFEB and TFE3 link the FLCN-AMPK signaling Axis to innate immune response and pathogen resistance. Cell Reports 26:3613–3628
4. Petit CS, Roczniak-Ferguson A, Ferguson SM (2013) Recruitment of folliculin to lysosomes supports the amino acid–dependent activation of Rag GTPases. Journal of Cell Biology 202:1107–1122

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# October 21st – Session 2: 4.40 – 6.15 PM BST

**5.25 pm – 5.35 pm BST**
**Hsp90 chaperoning of the tumor suppressor FLCN is mediated by the co-chaperones FNIP1/2**

Mark R. Woodford[1,2,3], Sarah J. Backe[1,2,3], Rebecca A. Sager[1,3], Dimitra Bourboulia[1,2,3], Gennady Bratslavsky[1,2,3], Mehdi Mollapour[1,2,3]

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**Background**
Birt-Hogg-Dubé (BHD) is an autosomal dominant genetic syndrome caused by germline mutations in the *FLCN* gene that predisposes patients to develop renal tumors (1). Our previous work has shown that the tumor suppressor FLCN is dependent on the molecular chaperone Heat shock protein-90 (Hsp90) for its folding and stability (2). FLCN binding partners Folliculin interacting protein-1 (FNIP1) and FNIP2 function as co-chaperone regulators of Hsp90, providing a scaffold for FLCN interaction and allowing for proper folding (3-5).
**Methods**
Protein lysates were extracted from transient transfections of HEK293 cells and subjected to SDS-PAGE. Immunoprecipitation and immunoblots were carried out as previously described (2).
**Results**
Many *FLCN* mutations result in prematurely truncated proteins (1). Here we show that a pathogenic mutation of *FLCN* (*FLCN*-L460QsX25) is unable to bind to FNIP1 and therefore unable to form a complex with the molecular chaperone Hsp90. This eventually leads to destabilization of *FLCN* mutants. Interestingly, over-expression of the Tsc1 co-chaperone is sufficient to facilitates the binding of the *FLCN* mutant with Hsp90 and therefore compensate for FNIP1.
**Conclusions**
Our findings here provide an explanation for the observed instability and degradation of pathogenic truncated *FLCN* mutants. We demonstrated that FNIP1 and Tsc1 co-chaperones are capable of compensating for each other in the chaperoning of mutated *FLCN*. Collectively, we have identified new roles for FNIP1/2 and Tsc1 as regulators of the molecular chaperone Hsp90, demonstrating a broader cellular impact outside of AMPK-mTOR signaling.

1. Schmidt LS, Linehan WM. FLCN: The causative gene for Birt-Hogg-Dube syndrome. Gene. 2018;640:28-42.
2. Woodford MR, Dunn DM, Blanden AR, Capriotti D, Loiselle D, Prodromou C, et al. The FNIP co-chaperones decelerate the Hsp90 chaperone cycle and enhance drug binding. Nat Commun. 2016;7:12037.
3. Sager RA, Woodford MR, Backe SJ, Makedon AM, Baker-Williams AJ, DiGregorio BT, et al. Post-translational Regulation of FNIP1 Creates a Rheostat for the Molecular Chaperone Hsp90. Cell Rep. 2019;26(5):1344-56 e5.
4. Sager RA, Woodford MR, Mollapour M. The mTOR Independent Function of Tsc1 and FNIPs. Trends Biochem Sci. 2018;43(12):935-7.
5. Sager RA, Woodford MR, Shapiro O, Mollapour M, Bratslavsky G. Sporadic renal angiomyolipoma in a patient with Birt-Hogg-Dube: chaperones in pathogenesis. Oncotarget. 2018;9(31):22220-9.

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# October 21st – Session 2: 4.40 – 6.15 PM BST

**5.35 pm – 5.45 BST**
**The existence of pneumothorax-only variants of FLCN and its implications for long-term surveillance of renal tumours**

**Kenki Matsumoto1** , Derek Lim2 , Paul D. Pharoah3 , Eamonn R. Maher4 , and Stefan J. Marciniak1,5

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**Background**
Individuals with Birt-Hogg-Dubé syndrome (BHDS) may develop fibrofolliculomas, pneumothorax and/or renal cell carcinoma (RCC). Currently, all patients with pathogenic FLCN variants are recommended to have renal surveillance. It has however been suggested that some FLCN variants only cause pneumothorax (pneumothorax only pathogenic variants, POPVs), which would make surveillance unnecessary in certain cases.
**Methods**
The PUBMED database was searched from 1st July 1974 to 19 1st March 2021 for English language articles using the keywords "Birt-Hogg-Dubé" and "Hornstein-Knickenberg". Only studies identifying pathogenic FLCN mutations were retained. The relevant clinical and genetic features of these patients were recorded and analysed.
**Results**
A total of 1059 individuals with 194 unique mutations were identified. The prevalence of pneumothorax, pulmonary cysts, RCC and characteristic skin lesions in BHDS were 50.9% (n=1038), 91.9% (n=720), 22.5% (n=929) and 47.9% (n=989) respectively. There was a higher prevalence of pneumothoraces ($p<0.0001$) but lower prevalence of dermatological findings ($p<0.0001$) in patients from East Asia compared to North America or Europe. Of the 194 pathogenic FLCN variants, 76 could be defined as 'pneumothorax-only'. POPVs were distributed throughout the gene, and there were no statistical differences in variant type. The majority of POPVs (65/76) affected no more than 3 individuals. Individuals with 'POPVs' also tended to be younger (45 vs 47 years, $p<0.05$).
**Conclusion**
No bias was identified in the locations or types of variants reported to cause only pneumothorax. We therefore found no biological plausibility in the existence of POPVs. Many apparent POPVs could instead result from variable expressivity, age-related penetrance and other confounding factors. We therefore recommend that all individuals found to carry a pathogenic FLCN variant be enrolled in life-long surveillance for RCC.

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# October 22nd – Session 1: 3.00 – 4.10 PM BST

**3.25 pm – 3.35 pm BST**
**Total pleural covering (TPC) for BHDs can prevent intractable recurrent pneumothorax**

**Masatoshi Kurihara**, Kenji Tsuboshima, Kota Ohashi, Tomomi Imamura
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**Background**
The diffuse cystic lung diseases like LAM and BHD frequently suffer from intractable recurrent pneumothorax. Chemical pleurodesis has been archived to prevent intractable pneumothorax for those patients but it is not always successful. Perfect pleurodesis is rare and has harmful side effects. It is impossible to surgically remove all lung cysts in diffuse cystic lung diseases. The more lung cysts are removed surgically, the more respiratory function is likely to be affected and worsened. We have conducted TPC (total pleural covering) to prevent recurrent pneumothorax in 53 LAM patients since 2003 and have better results. We have applied TPC to BHDs patients with recurrent pneumothorax.
**Methods**
Pleural covering was performed thoracoscopically in 102 cases of BHD diagnosed through histopathological diagnosis and genomic DNA analysis.
The mean age was 42 years old (22-68) and the number of female/males is 52/50.
Diffuse lung cysts in BHD syndrome are characteristically distributed over middle and lower lobes, especially mediastinal and basal area of the lung, very few in the upper lobe. We covered the whole visceral pleura with absorbable mesh sheets under thoracoscopy.
Mesh material applies oxidized regenerated cellulose meshes (Ethicon SURGICEL absorbable Hemostat gauze, J&J, NJ, USA) which are not likely to causes adhesion to the thoracic wall but to cause thickened visceral pleura.
**Results**
Follow-up period was 126 months. There was one postoperative recurrence in 102 cases of BHDs after TPC.
**Conclusions**
TPC is effective to prevent recurrent pneumothorax in BHD patients. This method is innovative and will spread to prevent pneumothorax in diffuse cystic lung diseases instead of chemical pleurodesis.

1. M.kurihara et al. : A total pleural covering for LAM prevents pneumothorax recurrence. 2016;11:c0163637.
2. H Ebana et al.: Oxidized regenerated cellulose induces pleural thickening in patients with pneumothorax: possible involvement of the mesothelial-mesenchymal transition.
3. Surgery today 2017 DOI: 10.1007/s00595-017-1597-4

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# October 22nd – Session 1: 3.00 – 4.10 PM BST

**3.35 pm – 3.45 pm BST**
**Genetic insight in Birt-Hogg-Dubé syndrome from Indian patients**

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**Background**
Birt-Hogg-Dubé syndrome (BHDS) is associated mostly with germline mutations at *FLCN*. Most genetic studies have been reported from Caucasian populations, fewer from Asia and only one from India (1). Here, we studied the genetic background of 15 BHDS families from India.
**Methods**
31 clinically diagnosed patients and 74 related asymptomatic members from 15 families were enrolled. Targeted NGS of *FLCN* was performed in members of 11 families. Sanger sequencing was performed in remaining patients and asymptomatic members. Functional effects of FLCN variants were evaluated *in-silico*, and their effect on protein-protein interaction was studied through molecular docking. Family-based association studies were performed by pedigree disequilibrium tests. Germline exonic mutations in *SERPINA1, MTHFR* & *CBS* were also evaluated in few family members to address confounding diagnoses.
**Results**
We observed six different pathogenic mutations in 19 patients and 16 asymptomatic members from 10 families. Among these, two novel mutations: 11-nucleotide deletion (*c.1150-1160delGTCCAGTCAGC*) and a splice acceptor mutation (*c.1301-1G>A*), were detected. Two Clinvar pathogenic, but unreported yet, mutations: stop-gain (*c.634C>T*) and 4-nucleotide duplication (*c.1329_1332dupAGCC*), were also detected. The hotspot mutation (*c.1285delC*) was observed in 5 families and a splice donor mutation (*c.1300+1G>A*) was also detected in one family. These mutations at *FLCN* greatly affected protein stability and the protein-interacting domains. Family-based studies revealed that pathogenic *FLCN* mutations were significantly associated with BHDS. Two pathogenic SNPs in *MTHFR* implicated with Homocystinuria: *rs1801133* and *rs138189536*, were found in one family in our cohort.
**Conclusion**
In our Indian cohort, six different pathogenic mutations in *FLCN* were detected. Instead of *FLCN* mutations, *MTHFR* pathogenic SNPs were detected in clinically diagnosed BHDS patients, therefore, suggesting genetic evaluation to avoid confounding diagnosis. Since no pathogenic *FLCN* mutations were detected in few patients, we suggest, apart from *FLCN*, mutations in other undescribed genes may also play a role in BHDS.

1. Ray A, Paul S, Chattopadhyay E, Kundu S, Roy B. Genetic Analysis of Familial Spontaneous Pneumothorax in an Indian Family. Lung. 2015;

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# October 22nd – Session 1: 3.00 – 4.10 PM BST

**3.45 pm – 3.55 pm BST**

**Performing a chest CT in patients with primary spontaneous pneumothorax and raising awareness may decrease underdiagnosis of Birt-Hogg-Dubé syndrome**

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**Background**

Birt-Hogg-Dubé syndrome (BHD) is likely to be underdiagnosed. Possible reasons are variable expression and lack of awareness. The diagnosis of BHD is important because it allows surveillance aiming for early detection and treatment of renal cell carcinoma. One way to detect more BHD patients could be to perform a chest CT to detect pulmonary cysts or genetic testing in all patients with a primary spontaneous pneumothorax. A chest CT has been shown to be cost-effective previously[1].

**Methods**

We examined the prevalence of BHD in patients with primary spontaneous pneumothorax and evaluated the contribution of chest CT in establishing the diagnosis. We performed FLCN testing and a chest CT in unselected patients with (presumably) primary spontaneous pneumothorax. We also checked whether the newly diagnosed families in this study (n=2) and our 18 most recent newly diagnosed families had a personal or family history that could have led to an earlier diagnosis of BHD retrospectively.

**Results**

Pathogenic variants in FLCN were detected in 3 out of 88 (3.4%) patients with a pneumothorax. Pulmonary cysts were detected in 14 out of 83 patients with pneumothorax, six of whom had multiple cysts including all three BHD patients.

15 out of 20 newly diagnosed families had features of BHD that could have been an indication for FLCN testing at an earlier time retrospectively.

**Conclusions**

We conclude that performing a chest CT in every patient presenting with primary spontaneous pneumothorax could be a good method to diagnose more patients with BHD and to diagnose other underlying causes of pneumothorax. Subsequent genetic testing of the FLCN gene should be considered when multiple pulmonary cysts are present. Since most BHD families showed clear features of BHD, raising awareness may be another important factor to reduce underdiagnosis.

1. N. Gupta, D. Langenderfer, F.X. McCormack, D.P. Schauer, M.H. Eckman, 2017. Chest Computed Tomographic Image Screening for Cystic Lung Diseases in Patients with Spontaneous Pneumothorax Is Cost Effective. Ann Am Thorac Soc. 14(1), 17-25. doi:10.1513/AnnalsATS.201606-459OC

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# October 22nd – Session 2: 4.40 – 6.15 PM BST

**4.45 pm – 5.00 pm BST**

**Therapeutic efficacy studies in the *Flcn*-deficient Nihon rat tumor model support dual mTORC1/2 inhibitors as potential therapy for BHD kidney cancer**

Laura S. Schmidt[1,2], Martin Lang[1], L. Spencer Krane[1], Carole Sourbier[1], Amy James[3], Lilia Ileva[4], Keita Saito[4], Simone Difilippantonio[3], Joseph Kalen[4], W. Marston Linehan[1].
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**Background**
Birt-Hogg-Dubé (BHD) syndrome predisposes affected individuals to cutaneous fibrofolliculomas, pulmonary cysts, spontaneous pneumothoraces and an increased risk for developing kidney cancer. Animal models that model BHD are critical research tools for testing potential therapeutic agents to treat BHD-associated kidney cancer. The Nihon rat was originally identified as a spontaneously arising bilateral multifocal renal tumor model in a Sprague-Dawley rat colony and subsequently was found to carry a frameshift mutation in the rat *Flcn* gene.
**Methods**
Using high-throughput small molecule screens in *FLCN*-deficient human kidney cancer cell lines, several classes of compounds were identified that were cytotoxic *in vitro* and subsequently their therapeutic efficacy was tested in the Nihon rat tumor model. Tumor response to drug treatment was measured using magnetic resonance imaging at 3-week intervals.
**Results**
Treatment with the PI3K/mTOR dual inhibitor NVP-BGT226 and the dual mTORC1/2 inhibitor Torin 2 produced significant reductions in tumor growth rates, whereas the histone deacetylase inhibitor panobinostat, proteasome inhibitor carfilzomib, and topoisomerase I inhibitor topotecan had little to no efficacy in this model.
**Conclusions**
Results of these studies indicated that drugs that target both mTORC1 and mTORC2 were most effective in reducing Flcn-deficient renal tumor growth in the Nihon rat model and suggest that mTORC1/mTORC2 inhibitors might be a potential therapeutic option for BHD kidney cancer.

Funded in part with federal funds from the Frederick National Laboratory for Cancer Research, NIH, under Contract HHSN261200800001E.

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# October 22nd – Session 2: 4.40 – 6.15 PM BST

**5.00 pm – 5.10 pm BST**
**Loss of Folliculin in Lung Mesenchyme Causes Cystic Lung Disease: Implication for the Pathogenesis of Pulmonary BHD**

Ling Chu[1], Hui Chen[1], Yongfeng Luo[1], Joanne Chiu[1], Hong-Jun Wang[1], Damir Khabibullin[2], Elizabeth P. Henske[2], **Wei Shi**[1]

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**Background**
Cystic lung disease with pneumothorax is a major clinical manifestation of BHD, with cysts reported as early as 34 weeks' gestation and pneumothorax as early as age 14. The mechanisms underlying cystic lung disease in BHD are poorly understood, representing a critical knowledge gap. BHD is caused by germline loss-of-function mutations in *FLCN*.
**Methods**
We used a Tbx4-rtTA/TetO-Cre transgenic mouse line to specifically delete *Flcn* in mouse lung mesenchyme from the beginning of lung formation.
**Results**
Mice with lung mesenchymal *Flcn* deletion developed striking phenotypes of early postnatal alveolar enlargement (1.98-fold increase of mean linear intercept (MLI)) and adult pulmonary cysts (average 3.53-fold increase of MLI) that resemble the lung pathology in BHD patients. Furthermore, lung mesenchymal stem cells isolated from the *Flcn* conditional knockout mice had reduced proliferation (2.73-fold reduction) and migration (1.60-fold reduction) compared to those in wild type littermate controls. In contrast, deletion of *Flcn* in lung epithelium using Sftpc-rtTA/TetO-Cre driver line did not result in significant change of alveolar changes.
**Conclusion**
In summary, we report the first mouse model with phenotypes resembling the lung pathology seen in human BHD. These data indicate that lung mesenchymal Flcn is essential to alveolar growth during development and alveolar homeostasis in adult mice.

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# October 22nd – Session 2: 4.40 – 6.15 PM BST

**5.10 pm – 5.20 pm BST**
**What can whole genome analysis of familial pneumothorax tell us about the effectiveness of Birt-Hogg-Dubé diagnosis by multidisciplinary teams?**

Hannah L Grimes (1), Simon Holden (2), Judith Babar (3), Sumit Karia (3), Maria Ta Wetscherek (3), Allanah Barker (3), Jurgen Herre (4), Martin D Knolle (4), Eamonn R Maher (5), Genomics England Research Consortium; Stefan John Marciniak (6,4,7,8)

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**Background**
Pneumothorax in the absence of lung disease or trauma, has a familial component in one out of 10 patients with the condition[1,2]. Syndromes such as Birt-Hogg-Dubé are the culprit in some cases, but many patients with familial pneumothorax do not fulfil diagnostic criteria for known genetic disorders. The aim of this work was twofold: (i) to assess whether a dedicated pneumothorax service can effectively identify all cases of known pneumothorax syndromes and (ii) to identify new genetic variants causing pneumothorax.
**Method**
Data from the 100K Genomes Project were reviewed using the Genomics England application "LabKey". Thirty-three patients were identified with familial pneumothorax and variant frequencies were compared between the affected cohort and a group of 1259 control subjects (sequenced in the 100K Genomes Project for unrelated conditions). The phenotypes of the affected subjects were compared using data provided by the 100K Genomes project. Potential interaction of prevalent mutations among the disease cohort were investigated were studied using the STRING database.
**Results**
Only one TIER1 mutation was found in our patient cohort of apparently non-syndromic familial pneumothoraces. This was a pathologenic *FLCN1* mutation, resulting in a diagnosis of Birt-Hogg-Dubé syndrome. This suggests that the current protocols for investigation and diagnosis of spontaneous pneumothorax are relatively sensitive. Multiple variants were seen to be enriched in the disease cohort compared to the controls.
**Conclusion**
Clinically, few Birt-Hogg-Dubé diagnoses appear to be missed in a dedicated service, provided this condition is first considered. No single variant or set of variants appears responsible for all the cases analysed in the 100K Genomes project. A collection of novel hyper-rare syndromes is likely to be responsible for the patients analysed.

1. Boone PM, Scott RM, Marciniak SJ, Henske EP, Raby BA. (2019) The Genetics of Pneumothorax. *Am J Respir Crit Care Med*. 199(11):1344-1357. doi: 10.1164/rccm.201807-1212CI.
2. Grimes HL, Holden S, Babar J, Karia S, Wetscherek MTA, Barker A, Herre J, Knolle M, Maher ER, Genomics England Research Consortium and Marciniak SJ (2021). Combining clinical, radiological and genetic approaches to pneumothorax management. *Thorax*. doi: thoraxjnl-2021-217210 [epub ahead of print].

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# October 22nd – Session 2: 4.40 – 6.15 PM BST

**5.20 pm – 5.30 pm BST**
**Frequency of pathogenic germline variants in cancer susceptibility genes in 1336 participants with renal cell carcinoma**

Avgi Andreou* (1), Bryndis Yngvadottir* (1), Laia Bassaganyas Bars (1), Alexey Larionov (1), Alex J Cornish (2), Daniel Chubb (2), Philip Law (2), Philip Smith (1), Huairen Zhang (1), Yasemin Cole (1), Genomics England Research Consortium, Renal Cell Carcinoma GeCIP, David Mole (3), James Larkin (4), Lisa Browning (5), Samra Turajlic (6), Kevin Litchfield (7), Richard S Houlston (2), Eamonn R Maher (1)
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**Background**
Renal cell carcinoma (RCC) occurs in a high number of cancer predisposition syndromes including Birt Hogg Dubé Syndrome (BHD) (1). The purpose of this study was to investigate the frequency of pathogenic germline variants in a large series of unselected RCC patients.
**Methods**
Whole genome sequencing data on 1,336 RCC cases recruited to the UK 100,000 Genomes Project (100KGP) (2) was analysed to identify rare pathogenic and likely pathogenic single nucleotide variants (SNVs) and structural variants (SVs) in 127 cancer susceptibility genes (CSGs).
**Results**
Among 1,336 RCC participants (mean 61.3 years [±12SD], range 13-88 years; 64% male), 85 participants (6.3%; 95% CI [5.1, 7.8]) had one or more pathogenic/likely pathogenic (P/LP) germline variant (total of 88 variants). Four participants (0.3%) had a P/LP variant in *FLCN* and the majority of the remanding P/LP variants were mapped to other known RCC-CSGs: CHEK2 (2%), *MITF* (0.7%), *SDHA* (0.5%), *VHL* (0.5%), *FH* (0.2%) and *SDHB* (0.1%). A higher than expected frequency, 32% (28 out of 88 variants), was detected in CSGs not previously linked to RCC: *ATM* (0.7%), *FANCM* (0.3%), *BRIP1* (0.2%), *MSH6* (0.2%), *BRCA2* (0.1%), *PMS2* (0.1%), *TP53* (0.1%), *MSH2* (0.07%) and *PALB2* (0.07%). A further 56 variants (4%) in RCC-CSGs were classified as a variant of unknown significance (VUS) but were considered to be of clinical relevance as further evaluation (e.g. by detailed clinical genetic assessment, tumour immunohistochemistry, metabolomics or family studies) might have resulted in reclassification as P/LP (3, 4).
**Conclusions**
Approximately 6% of patients with RCC unselected for family history have a germline variant, including 0.3% with a *FLCN* germline variant, requiring additional follow-up analysis and possible genetic counselling including cascade testing. To improve diagnostic yield we suggest expanding the panel of RCC-CSGs tested to include *CHEK2* and all *SDHx* subunits and raising the eligibility criteria for age-based testing.

**References:** Please use reference format 'Vancouver'
1. Menko FH, Maher ER. Diagnosis and Management of Hereditary Renal Cell Cancer. Recent Results Cancer Res. 2016;205:85-104.
2. Caulfield MD, Jim; Dennys, Martin; Elbahy, Leila; Fowler, Tom; Hill, Sue; et al. The National Genomics Research and Healthcare Knowledgebase. 2017.
3. van Nederveen FH, Gaal J, Favier J, Korpershoek E, Oldenburg RA, de Bruyn EM, et al. An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol. 2009;10(8):764-71.
4. Kim E, Wright MJ, Sioson L, Novos T, Gill AJ, Benn DE, et al. Utility of the succinate: Fumarate ratio for assessing SDH dysfunction in different tumor types. Mol Genet Metab Rep. 2017;10:45-9.

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