

The Second BHD Symposium was held on April 22, 2010 at the Marriott at Metro Center in Washington, D.C. The meeting was organized into basic research and clinical research sessions with a parallel session for patients. Sixteen abstracts were selected for oral presentation and nine were presented as posters from 48 registrants. Because of restricted air travel due to the Icelandic volcanic ash, some of the European registrants were unable to attend and presented by video tape of their slide presentation with Q&A by live teleconference.

The morning session was opened by meeting chairperson Laura Schmidt, Urologic Oncology Branch (UOB), NCI, with a historical perspective on the study of Birt-Hogg-Dubé syndrome and the evolution of research into the causes of this syndrome. Berton Zbar, retired NCI Laboratory Chief, gave the plenary lecture entitled “A personal view of renal cancer genetics: history and lessons learned” drawing on his 15-year experience identifying renal cancer susceptibility genes through study of families with rare inherited renal cancer syndromes such as VHL and BHD. At this point BHD patients and families adjourned to a breakout session in which Joyce Graff, director of VHL Family Alliance, offered her experiences in establishing a VHL patient advocacy group and as a member of a family with VHL disease. She stressed to BHD patients and their families the importance of a support group and encouraged them to participate in and support the BHD Family Alliance. Following her talk, Lindsay Middleton, nurse and genetic counselor at UOB, NCI, spoke to the patients on topics related to living with BHD syndrome including clinical services at the NIH, family communication, genetic testing, insurance issues, telling children about their family history of BHD, etc.

The parallel morning research session was opened by Tim Cash from University of Pennsylvania who used *BHD/FLCN* *-/-* embryonic stem cell lines and identified apoptotic resistance due to loss of Bim expression in *FLCN null* ES cells and BHD renal tumors. He presented evidence indicative of TGF- β pathway misregulation in these *FLCN null* systems that may occur at the level of chromatin modification in target genes. Ravi Nookala from University of Cambridge reported the first crystallization of the carboxy half of the FLCN protein to 1.9 angstroms, which revealed a novel GTP binding domain that binds GTP and poly G RNA with equal affinity. Hisashi Hasumi from UOB, NCI, reported the development of a mouse model in which *FLCN* was inactivated in muscle leading to myopathy, red muscle color, increased mitochondrial biogenesis and metabolic defects. Maria Czyzyk-Krzeska from University of Cincinnati found enriched 17p gene expression, including *FLCN*, in VHL-reconstituted 786-0 and A-498 cells but loss of *FLCN* in *VHL* knockdown cell lines, and showed loss of *FLCN* expression in ccRCC with *VHL* loss relative to normal kidney. Doug Medvetz from Harvard Medical School presented data that identified serines 62 and 73 as sites of FLCN phosphorylation and presented data to support a role for FLCN in mTOR and AMPK regulation in response to nutrient deprivation. Vera Krymskaya from University of Pennsylvania showed that primary lung epithelial cells from *FLCN* knockout mice displayed dysregulation of cell-cell contacts and formed gaps between cells through rearrangement of the actin cytoskeleton. Andy Tee from the University of Cardiff analyzed a renal tumor cell line

from a BHD patient and found high levels of HIF activity and upregulation of HIF target genes including Glut 1, PDK, LDH, suggesting that *FLCN* null renal tumor cells prefer aerobic glycolysis rather than oxidative phosphorylation, and can utilize lactate as a metabolic fuel. The final talk of the morning session to which the patients were welcomed was from Frank McCormack, University of Cincinnati School of Medicine, who compared LAM, a proliferative lung disease associated with *TSC1/2* mutations, to BHD cystic lung disease, and pointed out the similarities and differences and how we can learn from studying both diseases.

Following a lunch break the afternoon session was opened by Joyce Graff, director of VHL Family Alliance, who spoke to all attendees regarding her experience living with VHL in her family and the importance of supporting patient advocacy groups. Michael Nahorski from University of Birmingham investigated the association between colon cancer (CRC) and *FLCN* mutations. He found no germline mutations in familial colorectal cancer patients, but noted *FLCN* c.1285dupC/delC mutations in 23% of MSI+ CRC and notably, higher risk of colorectal neoplasia in c.1285dupC carriers compared with another common *FLCN* mutation. L.M.C. Gijezen from Maastricht University reported on a topical rapamycin clinical trial for treatment of BHD patients with fibrofolliculomas. Diagnostic criteria and recommendations for BHDS screening were presented by Jorge Toro from DCEG, NCI. Xiaohong Lu from University of Birmingham showed that mithramycin, identified as an anticancer drug from an NCI60 screen, was 10x more cytotoxic in UOK257 *FLCN*-null cells than in UOK257 *FLCN*-restored cells. A.C. Houweling from VU University Medical Center, Amsterdam, evaluated phenotype-genotype correlations in 54 BHD families from the Netherlands and found 3 new point mutations and a large deletion. Risk for renal cancer was 22%, and 38% for pneumothorax in this cohort.

After the break, the patients and families attended an informative Q&A session with a urologic surgeon Gopal Gupta, UOB, NCI and dermatologist Jorge Toro, DCEG, NCI who responded to their questions concerning management and treatment of BHD. In the parallel research session, Arnim Pause from McGill University described his Pax8-targeted *FLCN* homozygous knockout mouse model that develops histiocytic sarcomas in multiple organs, but without renal tumors, and reported progress on a *C. elegans* *FLCN* KO model. In a *FLCN* *d/d* MEF model, he saw evidence of loss of an amino acid withdrawal checkpoint for autophagy by sustained growth, and noted increased mitochondrial biogenesis with loss of fitness. Seung-Beom Hong from UOB, NCI described a novel role for FLCN as regulator of the activity of transcription factor TFE3 by post translational modification. Also from UOB, NCI, Masaya Baba reported on a mouse knockout model of FNIP1, the FLCN interacting protein. These mice develop defects in B-cell development displaying a block in pro- to pre-B cell differentiation due to loss of RAG1 expression. Again from UOB, NCI, Yukiko Hasumi described a *FLCN* heterozygous knockout mouse that developed solid renal tumors at 24 months with loss of the wild-type copy of *FLCN*, activation of Akt and activation of both mTORC1 and mTORC2, also seen in human BHD tumors.

The meeting closed with a dinner attended by researchers, clinicians and BHD patients and families fostering exchange of ideas and discussions between the groups and providing an opportunity for researchers to discuss their data and initiate collaborations. Plans for an annual meeting date and site were discussed. The attendees declared the meeting highly successful.