



The Myrovlytis Trust

CALL FOR IDEAS TO RESEARCH TREATMENTS AND CURES FOR RARE GENETIC DISEASES

CALL FOR IDEAS

The Myrovlytis Trust and Partnership for Cures created the **Rare Genetic Disease Pilot Grant Program** to bring researchers and funders together to test new hypotheses that can produce better treatments and cures for patients with rare genetic diseases. Clinicians and researchers have scientific and clinical insights, patient anecdotal results, technology transfer ideas and research skills that could impact patients with rare genetic diseases, and the Myrovlytis Trust and Partnership for Cures bring the inspiration, financial support and business management to support this new hypothesis rare genetic diseases research.

These Pilot Grants can make quick and direct impacts on patients by translating drugs from one disease to provide a "new" treatment in another disease, by scientifically validating anecdotal successes of Western or Eastern medicine in a particular disease, or accelerating the use of a drug or other therapy from late stage to early stage in a particular disease.

WE ARE FOCUSING THESE EFFORTS ON PATIENTS WITH THE RARE GENETIC DISEASE

BIRT HOGG DUBE (BHD) SYNDROME,

YOU DO NOT HAVE TO BE A BHD EXPERT TO PROPOSE IDEAS!!!

THIS PROGRAM WILL OFFER UP TO FOUR (4) \$25,000 ONE YEAR PILOT GRANTS THAT CAN HAVE A DIRECT AND SIGNIFICANT IMPACT ON THE BHD PATIENT POPULATION WITHIN THE NEXT 12-24 MONTHS.

SEE THE SCIENTIFIC SYNOPSIS BELOW OF BHD AND ITS KNOWN OR PROPOSED MECHANISMS OF ACTIONS

***Please note: this [call for ideas](#) is not a request for full research proposals for grant funding. We are requesting ideas for translational research projects, which will be screened by the Foundation Staff, Science Advisors and Funders, and then specific invitations for a more formal application will be made very quickly to those who submit the most promising ideas.**

IDEA SUBMISSION INSTRUCTIONS

Ideas must be submitted electronically to Bruce@4Cures.org. Preferred file formats are *.doc or *.pdf, but we will accept submissions that come solely in the body of an e-mail. Paper submission can not be accepted. Submissions must be no longer than 1500 words (about two [2] pages excluding literature references) and utilize no smaller than 11 point type (font) size.

Submissions must include the following elements by March 31, 2009:

- Project Summary Statement – Briefly describe the idea for one more research project and provide justification for the suggestions with background information. If you have more than one idea, please send each as a separate submission.
- Clinical Impact – Describe how the research would result in a direct and significant impact on the fibrofolliculomas, pneumothorax or RCC in BHD patients.
- Provide a timeline required to complete the research, which cannot be more than 12 months.
- Estimated Costs – Please provide an overall budget number for the entire project. Budget explanation is NOT required at this time. . . . Grantees can received 80% of requested funds through this grant opportunity. The funders require a 20% match from the institution or other resources. This funder does not allow for institutional overhead.
- Key Literature References – References to publications supporting the suggestion may be included.
- Please send your contact information, including cell phone and e-mail addresses

Questions

Email (preferred): Bruce@4Cures.org

Phone:1 (312) 696-1366

PLEASE USE THE FOLLOWING SCIENTIFIC SYNOPSIS OF BHD AND ITS KNOWN OR PROPOSED MECHANISMS OF ACTIONS TO DETERMINE IF YOU HAVE ANY CLINICAL OR RESEARCH INSIGHTS THAT COULD HELP THESE PATIENTS!

Birt Hogg Dubé Syndrome

Introduction

- Birt-Hogg-Dubé syndrome is a rare, autosomal, dominant, monogenic, adult onset, inherited hamartoma disorder.
- First described in 1977ⁱ.
- Fewer than 200 families reported worldwideⁱⁱ.
- Previously also known as Hornstein-Knickenberg syndromeⁱⁱⁱ.

Phenotype

- BHD syndrome has three symptoms, none of which have been observed before the second or third decade of life:
 - Skin: fibrofolliculomas, trichodiscomas and acrochordons of the face, neck and upper torso^{iv}. They normally first appear in the third decade of life or later. They are typically bilateral, but have been observed restricted to just one side of the patient.^v
 - Lung: air-filled cysts^{vi vii} and an increased risk of recurrent pneumothorax^{viii}. Schmidt et al found that 110 out of 129 (85%) BHD syndrome patients had one or more lung cysts and 64 out of 198 (32%) had pneumothorax^{ix}
 - Kidney: renal cell carcinoma (RCC). A range of histological types is seen within families and even individual patients: oncocytoma (5%); chromophobe (34%); papillary (2%) and clear cell (9%)p oncocytic hybrid (50%) with features of chromophobe RCC and renal oncocytoma^x are observed within families and also within individual patients^{xi}
- There is some evidence of an increased risk of colo-rectal tumours^{xiii} but this is controversial and may be restricted to a subset of families if any^{xiii xiv xv xvi}
- Not everyone has all the symptoms. RCC is seen in between 15% and 30% of cases. Patients have been observed with any or all of the three symptoms.^{xvii}
- The gene is considered to be a tumour suppressor, like the related von Hippel-Lindau disease (VHL). Haploinsufficiency is thought to be sufficient for the skin and lung phenotype, with loss of heterozygosity required for RCC^{xviii}. This is supported by a lack of BHD mRNA in renal tumours from BHD syndrome patients^{xix}.
- The concealed nature of aspects of the phenotype, the late onset and its relative rarity, all suggest that BHD syndrome is under-diagnosed.
- There is one report of BHD two patients with neural tissue tumours, but an association with *flcn* mutation is not demonstrated.^{xx}

Related disorders

- There are four well-characterised forms of inherited RCC: VHL, hereditary papillary renal carcinoma (HPRC), hereditary leiomyomatosis renal cell carcinoma (HLRCC) and BHD syndrome.
- Several other hamartoma syndromes are caused by mutation in tumour suppressor genes:
 - LKB1 (Peutz-Jehgers syndrome);
 - TSC1/2 (tuberous sclerosis complex);
 - PTEN (Cowden syndrome).These, like FLCN, are involved in mTOR signalling.
- There are several other inherited syndromes characterised by skin neoplasms, including:
 - Cowden syndrome
 - naevoid basal cell carcinoma syndrome
 - generalised basaloid follicular hamartoma syndrome,
 - Bazed syndrome,
 - Brooke-Spiegler syndrome,
 - familial cylindromatosis,
 - multiple familial trichoepitheliomas
 - Muir-Torre syndrome.

Gene

- The associated gene, *folliculin* (FLCN), has been mapped (17p11)^{xxi xxii} and sequenced.

FLCN mRNA expression

- FLCN mRNA is widely expressed. Expressed in the brain, placenta, testis, tonsils, lymph nodes, spleen, breast, bladder, prostate, ovary, myometrium, pancreas and parotid gland, perhaps the heart (Nickerson saw expression but Warren did not) as well as the three organs that show a phenotype^{xxiii xxiv}.
- In the kidney, expression is stronger in the distal tubules than the proximal tubules^{xxv}. Analysis of gene expression in RCC associated with *flcn* mutation classified the tumours as oncocytomas and chromophobe RCCs which are considered to be distal nephron-associated tumours^{xxvi}.
- mRNA is expressed in certain cell types within tissues, suggesting the protein might be involved in particular processes rather than general housekeeping. For example: parotid gland serous (acinar) glands show high expression but mucinous glands and intercalated ducts do not; brain cerebrum neurons show strong expression but glial cells do not.
- Warren et al found no expression in the colon epithelium (see possible colon cancer link), heart, mucinous glands of colon and parotid, adrenal gland, liver hepatocytes, stratified muscle, thyroid, or areas surrounding blood vessels.
- Acinar cell expression in pancreas and parotid suggests a possible secretory role for BHD. So does expression in ductal cells of the breast, collagen-producing fibroblasts, and antibody-producing lymphocytes.
- Expression in type 1 pneumocytes, which are involved in surfactant turnover by pinocytosis, and expression in alveolar macrophages, suggest a role for BHD in endocytosis or phagocytosis.

Protein

- One open reading frame of 579 amino acids.
- There is no known homology to other human proteins.
- Predicted size is 64kDa.
- No known transmembrane domains or organelle localisation signals. Predicted hydrophobic N-terminal sequence.
- Two known binding partners, folliculin-interacting protein 1 (FNIP1) and folliculin-interacting protein 2 (FNIP2)^{xxvii xxviii}.
 - FNIP2 is also known as folliculin-interacting protein-like (FNIPL)^{xxix}.
 - Flcn interaction with FNIP1 and FNIP2 is mediated by the C-terminal domains of each protein.
 - Folliculin alone is found in the nucleus, but when co-expressed with FNIP1 or FNIP2 it co-localises with those proteins in the cytoplasm in a reticular pattern.
- Alternatively spliced isoforms have been observed but their function and expression pattern is unclear^{xxx}.

Cell signalling

- FLCN is phosphorylated. Overexpression of FNIP1 increases FLCN phosphorylation, while AMPK inhibition reduces it. Inhibiting Mammalian Target of Rapamycin (mTOR) suppresses FLCN phosphorylation. AMPK-inhibition also reduces FNIP1 phosphorylation and lowers FNIP1 expression^{xxxi}.
- (AMPK is integral to energy sensing and negatively regulates mTOR activity. mTOR regulates cell growth and cell size via regulation of protein synthesis. Rapamycin reduces FLCN phosphorylation.)
- This suggests that FLCN and FNIP1 are involved in energy and/or nutrient sensing via the AMPK and mTOR signalling pathways.
- The HIF class of transcription factors are involved in BHD RCC. HIF2 α is strongly expressed in chromophobe tumours but HIF-1 α expression is much weaker.^{xxxii}
- Yeast – *S. Pombe*, deleting *flcn*:
 - Causes upregulation of six permease and transporter genes that are down-regulated in TSC1 and TSC2 mutants.
 - Causes elevation of specific intracellular amino acids that are low in TSC1 and TSC2 mutants. Tsc1/Tsc2 inhibit Rhb1. Expression of a hypomorphic allele of *rbh1(+)* increased permease expression levels in folliculin mutants but not in wild-type yeast.

- Sensitises yeast to rapamycin-induced increases in permease expression levels.
- Sensitises yeast to rapamycin induced lethality in yeast also expressing the hypomorphic *Rhb1* allele. *Rhb1* binds *Tor2*, and *Tor2* inhibition leads to up-regulation of permeases including those that are regulated by *flcn*. Therefore maybe folliculin activates *Tor2*.^{xxxiii}

Mutations

- 84% of affected individuals have had a mutation identified in *flcn*^{xxxiv}
- Mutations have been identified in all translated exons (exons 4-14) except exons 8 and 10:
- Most mutations are frameshifts that predict protein truncation, but some predict single amino acid changes.
 - The poly-C tract in exon 11 of *flcn* is a mutational hotspot; nearly 50% of mutations are insertions or deletions in this region^{xxxv xxxvi}.
 - One mutation has been identified in the initiator codon (ATG) predicting total absence of translation^{xxxvii}.
- One exon-skipping mutation has been identified (exon 6) that causes a 74 AA deletion.^{xxxviii xxxix}
- Splice site mutations have been identified, for example:^{xl}
- Several groups have tried to identify a genotype/phenotype correlation.
 - One four base-pair deletion was found to cause lung lesions with 100% penetrance^{xli}.
 - Fewer renal tumours are seen in patients with the C-deletion than in those with the C-insertion. The authors speculate that this might be because the insertion and the deletion each produce a different aberrant protein, one of which might have a dominant negative effect^{xlii}. Aberrant mRNA has not been observed.
 - Splice-site mutations in intron 9 that predict exon skipping lead to a higher frequency of RCC than expected.^{xliii}
 - The lack of linear correlation between the specific *FLCN* mutation and the phenotype suggests other genes might be involved.
- There is no evidence of epigenetic silencing or hypermethylation of the *flcn* promoter region.^{xliv}
- *flcn* mutation seems to be rare in sporadic RCC.^{xlv}

Animal models

Homologues in mouse, *Drosophila*, *C elegans*, yeast, dog and rat.

- *Drosophila* homologue is required for male germline stem cell maintenance in the testis and interacts with the JAK-STAT and Dpp signalling pathways.^{xlvi}
- Mouse models:
 - Conditional BHD allele with cadherin 16 (KSP)-Cre transgene to target *flcn* inactivation to the kidney. Knockout mice develop enlarged polycystic kidneys and die from renal failure after three weeks. Knocking out *flcn* activates (Erk)1/2 and Akt-mTOR pathways in the kidney, increases cell proliferation and increases expression of cell cycle proteins. Treating knockout mice with rapamycin leads to smaller kidneys and longer survival cf. untreated knockout mice.^{xlvii}
 - Another kidney specific BHD knockout model has been developed. This also found enlarged kidneys characterized by polycystic kidneys, hyperplasia, and cystic renal cell carcinoma, with death at three weeks and blood urea nitrogen levels 10x higher than controls. This was due to inactivation of the mTOR pathway. Rapamycin extended survival and inhibited further progression of cystogenesis.^{xlviii}
 - An unpublished, heterozygous gene-trap model is also available from Dr Arnim Pauseat McGill.
 - .
- Nihon rat^{xlix}. Single nucleotide insertion into *flcn* producing a frameshift and premature stop codon. Homozygous mutants are embryonic lethal. Loss of heterozygosity is observed in RCC.
- There is a canine (German Shepherd) model.^l

Miscellaneous

- *Flcn* is a target gene in human endometrial carcinomas and human gastric cancer, both with microsatellite instability, but its mutation occurs downstream of other mutational events.^{lii}
- Oncocytic cells may have more mitochondria than expected^{liii}

- Mutations found predominantly in Caucasians. Japanese patients have been identified.^{liv}
- Tuberous sclerosis clinical trials:
 - Two current trials with sirolimus shows encouraging interim results: angiomyolipoma volume decreases.^{lv lvi}
- [http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed&cmd=search&term=birt+hogg+dube&log\\$=activity](http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed&cmd=search&term=birt+hogg+dube&log$=activity)

Questions

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