

What is BHD?

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1. Birt–Hogg–Dubé syndrome

Birt–Hogg–Dubé (BHD) syndrome (OMIM [135150](#)) is an autosomal, dominantly inherited, monogenic condition, characterised by the development of [fibrofolliculomas](#) (benign skin tumours) on the face, head and upper torso, [pulmonary cysts and pneumothorax](#) (collapsed lung), and predisposition to [kidney cancers](#) with clear cell, chromophobic or oncocyctic features ([Birt et al., 1977](#)). The clinical manifestations of BHD syndrome are discussed in Section 2.

BHD syndrome was first described in 1977 by three Canadian doctors – Birt, Hogg and Dubé ([Birt et al., 1977](#)). In 2001, a BHD-associated gene locus was localised to chromosome 17p11.2 ([Khoo et al., 2001](#); [Schmidt et al., 2001](#)) and a novel gene, *Folliculin* (*FLCN*), was subsequently identified as being inactivated in individuals with BHD syndrome ([Nickerson et al., 2002](#)). The *FLCN* gene codes for a protein called Folliculin (FLCN), which has a putative tumour suppressor function ([Vocke et al., 2005](#); [Hudon et al., 2010](#); [Hong et al., 2010a](#); [Cash et al., 2011](#)). To date, approximately 200 families have been reported with pathogenic *FLCN* mutations ([Schmidt et al., 2005](#); [Graham et al., 2005](#); [Toro et al., 2008](#); [Misago et al., 2008](#); [Frohlich et al., 2008](#); [Leter et al., 2008](#); [Woodward et al., 2008](#); [Kunogi et al., 2010](#)). As of February 2011, 132 different *FLCN* mutations have been identified ([www.lovd.nl/flcn](#), [Lim et al., 2010](#)), 91 of which are described as pathogenic. The Folliculin gene and its mutations are described in Section 3.

BHD syndrome shares many clinical features with hamartoma syndromes ([Toro et al., 2002](#)). Hamartoma syndromes are dominantly inherited, predispose to cancer that affects multiple organs, and result in the development of benign tumours. Such syndromes include [Cowden syndrome](#), [Peutz-Jeghers syndrome](#), and [Tuberous Sclerosis complex](#) (TSC), caused by inactivation of the tumour suppressor genes *PTEN*, *LKB1* and *TSC1/TSC2* respectively ([Liaw et al., 1997](#); [Marsh et al., 1999](#)).

Folliculin and its two known interacting proteins - *FNIP1* and *FNIP2* - are discussed in Section 4 ([Baba et al., 2006](#); [Hasumi et al., 2008](#); [Takagi et al., 2008](#)). Research has demonstrated that FNIP1 and FNIP2 along with 5'-AMP-activated protein kinase (*AMPK*), are able to phosphorylate FLCN ([Baba et al., 2006](#); [Hasumi et al., 2008](#); [Takagi et al., 2008](#)). FLCN has been implicated in numerous signalling pathways including *mTOR* and AMPK signalling ([Baba et al., 2006](#); [Baba et al., 2008](#); [Wang et al., 2010](#)), HIF signalling ([Preston et al., 2010](#)), TGF- β signalling ([Hong et al., 2010a](#); [Cash et al., 2011](#)) and the JAK-STAT signalling pathway ([Singh et al., 2006](#)). The function of FLCN and its role in these pathways is discussed in detail in Section 5.

Much of the work pertaining to BHD syndrome was conducted in a variety of model organisms, and these experiments are described in Section 6, with future avenues of research considered in Section 7.

2. Clinical manifestations of BHD syndrome

2.1 Fibrofolliculomas (benign skin tumours)

Patients with BHD syndrome usually develop benign hair follicle tumours, clinically known as fibrofolliculomas, which appear as multiple whitish papules after the age of 20. Fibrofolliculomas develop primarily on the face, but can also appear on the neck, ears and the upper torso ([Menko et al., 2009](#)). Previously, fibrofolliculomas and trichodiscomas (skin-coloured tumours occurring on the upper body) were considered hallmarks of BHD syndrome, but a recent study suggests that they may not be distinct histological entities, and that a morphological spectrum of these benign skin tumours may exist ([Fujita et al., 1981](#); [Misago et al., 2009](#)). The number of fibrofolliculomas per BHD patient can range from under 10 to over 100 ([Toro et al., 1999](#)).

2.2 Pulmonary cysts and pneumothorax

2.2.1 Histology

BHD patients often develop pulmonary cysts and have an increased risk for pneumothorax. Lung anatomy and histology generally appears normal in individuals with BHD, and despite the presence of multiple pulmonary cysts, lung function is usually unaffected ([Toro et al., 2007](#)). [Tobino et al. \(2009\)](#) have characterised BHD lung cysts and found them to have characteristics distinct from those which develop in other cystic lung diseases. Of the 12 BHD patients analysed by [Tobino et al. \(2009\)](#), the number of cysts were found to vary from 29-407, and the size varied from a few millimetres to more than 2 cm. The cysts were generally irregularly-shaped and most commonly found in the lower medial zone of the lungs. This evaluation of cysts by thin-section chest CT images can assist in differentiating BHD from other cystic lung diseases ([Tobino et al., 2009](#)). A second evaluation of BHD lung cysts confirmed the presence of multiple cysts, mainly in the lower lungs, that varied in size and shape ([Agarwal et al., 2011](#)). [Koga et al. \(2009\)](#) have hypothesised that these cysts represent an aberrant cystic alveolar formation.

2.2.2 Prevalence

The presence of pulmonary cysts in BHD syndrome was first described by [Toro et al. \(1999\)](#) in a study of 152 individuals from 49 families with familial renal neoplastic syndromes. Three of the thirteen patients who had BHD syndrome exhibited pulmonary cysts, and one of these three patients developed pneumothorax ([Toro et al., 1999](#)). Additional cases of pulmonary cysts and spontaneous pneumothorax have since been reported in the literature ([Zbar et al., 2002](#); [Toro et al., 2007](#); [Johannesma et al., 2009](#); [Koga et al., 2009](#); [Kluger et al., 2010](#); [Ishii et al., 2009](#); [So, 2009](#); [Sundaram et al., 2009](#); [Diamond and Kotloff, 2009](#); [Kunogi et al., 2010](#); [Hayashi et al., 2010](#); [Predina et al., 2011](#); [Bae et al., 2011](#)).

Pulmonary cysts are the most common BHD manifestation, seen in up to 90 % of patients ([Predina et al., 2011](#)). [Zbar et al. \(2002\)](#) identified an increase in the risk of pneumothorax for BHD-affected individuals, which was hypothesised to be related to the presence of pulmonary cysts. [Toro et al. \(2007\)](#) found BHD patients with pulmonary cysts had a 24 % risk for spontaneous pneumothorax,

and that following a single episode of spontaneous pneumothorax, recurrent events were more common. It is believed that lung problems may be the earliest symptoms of BHD syndrome, as pneumothoraces have been reported in BHD patients as young as seven and sixteen years of age ([Bessis et al., 2006](#); [Gunji et al., 2007](#)).

[Gunji et al. \(2007\)](#) screened for *FLCN* mutations in eight unrelated Japanese patients with multiple pulmonary cysts and recurrent pneumothorax (mean age of first pneumothorax was 30.4 years), but without skin or renal lesions, and identified mutations in five of the eight. All five patients had a family history of spontaneous pneumothorax. The authors suggested that isolated pulmonary cysts and pneumothorax may be a milder form of BHD syndrome and that patients should be monitored for renal or skin lesions. Another study by [Hayashi et al. \(2010\)](#) also identified BHD patients with lung cysts and recurrent pneumothorax but with no skin manifestations or renal tumours. The authors suggest that BHD syndrome should be considered for patients with multiple lung cysts, even when no other symptoms are present.

2.2.3 FLCN association

A genome wide scan in a large Finnish family with a dominantly inherited predisposition to primary spontaneous pneumothorax (PSP) discovered that the PSP locus mapped to chromosome 17p11, where the *FLCN* gene is located ([Painter et al., 2005](#)). Screening of *FLCN* revealed a 4-bp deletion in the first coding exon, resulting in a frameshift of the reading frame and subsequent truncation of the protein. All carriers of the deletion presented with bullous lung lesions. Unlike previously identified mutations in *FLCN*, the exon 4 deletion seemed to be associated only with PSP, which showed 100% penetrance. This family had no evidence of other BHD manifestations, suggesting a genotype-phenotype correlation. These results suggest that *FLCN* may have a significant role in normal pulmonary physiology since its inactivation in this study results in an exclusive PSP phenotype. There is no evidence of other genotype-phenotype correlations regarding lung manifestations or other BHD symptoms ([Kunogi et al., 2010](#)).

2.3 Renal cell carcinoma (Kidney Cancer)

2.3.1 Histology

Individuals with BHD syndrome are predisposed to develop renal cell carcinoma (RCC) ([Toro et al., 1999](#); [Zbar et al., 2002](#)). Unlike other genetic disorders with this predisposition, renal tumours associated with BHD syndrome are histologically diverse. Chromophobe, oncocytic, clear-cell and papillary renal cell carcinomas have all been identified in BHD patients, however the most commonly occurring histological subtype is a hybrid chromophobe and oncocytic renal tumour (with an approximate incidence of 50%) ([Pavlovich et al., 2002](#); [Warwick et al., 2010](#)). Several other mixed patterns can also occur ([Pavlovich et al., 2005](#); [Fahmy et al., 2007](#); [Janitzky et al., 2008](#); [Kluijt et al., 2009](#)). Six deaths have been reported in BHD patients due to metastatic renal cancer, all of which were of the clear-cell or papillary histology ([Pavlovich et al., 2005](#); [Toro et al., 2008](#); [Claessens et al., 2010](#)).

It is unclear from which part of the kidney the tumours arise. Due to the high percentage of chromophobe tumours, [Pavlovich et al. \(2002\)](#) initially believed the tumours to arise from the distal nephron. However, two independent studies observed *FLCN* expression in the proximal tubules of mice kidneys, suggesting the site of origination ([Chen et al., 2008](#); [Hudon et al., 2010](#)).

2.3.2 Prevalence

Renal cancer is the most life-threatening complication associated with BHD syndrome. A study by [Toro et al. \(2008\)](#) assessed 89 BHD syndrome patients and found 34% had renal tumours. The mean age of first renal tumour diagnosis was 50.7 years ([Pavlovich et al., 2002](#)), with the earliest reported age of 20 years ([Khoo et al., 2002](#)). A study of 18 BHD patients with RCC found 60% had bilateral renal tumours and 77% had multiple tumours, with an average of 5.3 tumours per patient ([Pavlovich et al., 2002](#)).

2.3.3 FLCN association

Other genetic diseases that lead to renal cell carcinoma seem to show a strong genotype-phenotype correlation ([Pavlovich et al., 2004](#)). For example, clear-cell RCC in von Hippel-Lindau syndrome is associated with mutations in the *VHL* gene, and hereditary papillary RCC is associated with mutations in the *MET* gene. A study by [Gatalica et al. \(2009\)](#) analysed the multiple tumour types found in one BHD patient and identified mutations and epigenetic events associated with the specific tumour types. Oncocytic tumours were found to have a second *FLCN* mutation, oncocytic papillary tumours had methylation of *FLCN* and a mutation in the *MET* gene, whereas clear-cell tumours had a mutation in the *VHL* gene along with *VHL* promoter methylation.

A loss of *FLCN* expression is rare in sporadic renal cell carcinomas ([Khoo et al., 2003](#)). However somatic *FLCN* mutations have been reported, suggesting that *FLCN* is involved in the normal cellular functions that regulate growth and proliferation in the kidney, and that the aberrant functioning of *FLCN* is a mechanism of pathogenesis in both sporadic and BHD related renal carcinogenesis ([Gad et al., 2007](#); [Woodward et al., 2008](#)).

2.4 Other clinical manifestations

Fibrofolliculomas, pulmonary cysts, pneumothorax and renal cell carcinoma are the only manifestations associated with BHD syndrome. Studies have indicated that other manifestations may also be linked to BHD, but these have yet to be confirmed. These manifestations are discussed below.

2.4.1 Colorectal polyps and colorectal cancer

Early studies suggested an association between BHD syndrome and colorectal neoplasia ([Hornstein, 1976](#); [Birt et al., 1977](#); [Schachtschabel et al., 1996](#); [Schulz and Hartschuh, 1999](#)). However, this has been subject to some debate, and a subsequent study by [Zbar et al. \(2002\)](#) found no association between BHD and

colonic polyps or colorectal cancer (CRC) in a study involving a large cohort of 111 BHD syndrome patients.

Nevertheless, [Khoo et al. \(2002\)](#) reported a high incidence of colorectal polyps and CRC in BHD patients with confirmed *FLCN* germline mutations, suggesting that some BHD families are at increased risk of colorectal neoplasia, and indicating that *FLCN* may be involved in colorectal tumourigenesis. Another study, by [Nahorski et al. \(2010\)](#), found that 10 BHD patients out of the 149 assessed had CRC or colorectal polyps. This was linked to the c.1285dupC exon 11 mutation, suggesting patients with this particular mutation are more at risk of developing CRC. Interestingly, the BHD patients identified by [Khoo et al. \(2002\)](#) who had colonic polyps also had an exon 11 mutation (c.1285delC), suggesting a possible genotype-phenotype correlation.

2.4.2 Thyroid nodules

In a five year clinical study of 22 patients from ten unrelated French families with BHD syndrome, [Kluger et al. \(2010\)](#) attempted to define the characteristics of pulmonary, thyroid, renal and colorectal manifestations associated with BHD syndrome more clearly. Notably, thyroid nodules and/or cysts were identified by ultrasound in thirteen of twenty cases (65%). No thyroid carcinomas or colorectal carcinomas were detected in any patient. The high prevalence of thyroid nodules in this study is interesting, but crucially the lack of a control group does not enable the authors to assess the significance of these results. No genotype-phenotype correlation was observed in this study.

2.4.3 Melanoma

BHD syndrome has also been associated with melanoma in several reported cases ([Toro et al., 1999](#); [Khoo et al., 2002](#); [Menko et al., \(2009\)](#); [Sempau et al., 2010](#); [Cocciolone et al., 2010](#)). Other reported benign and malignant tumours are listed by [Menko et al. \(2009\)](#), but, so far, a direct relationship between BHD syndrome and these tumours has not been shown.

3. Folliculin gene

3.1 Identification of the *FLCN* gene

Folliculin (GENBANK accession# [BC015687](#)) was mapped to the BHD locus by a genome wide linkage analysis using polymorphic microsatellite markers in a large Swedish family. They found evidence of linkage to 17p12-q11.2 ([Khoo et al., 2001](#)). Subsequent haplotype analysis defined a candidate interval between the two flanking markers, D17S1791 and D17S798 ([Khoo et al., 2001](#)).

[Schmidt et al. \(2001\)](#) performed a genome wide scan in a large BHD kindred (172 members) and also localised the gene to the pericentromeric region of 17p using linkage analysis. Two-point linkage analysis of eight additional families with BHD syndrome produced a maximum LOD score of 16.06 at D17S2196. Haplotype analysis identified critical recombinants and defined the minimal region of non-recombination as being within an interval of less than four cM between D17S1857 and D17S805 on chromosome 17p11.2. One additional family, which had histologically confirmed fibrofolliculomas, did not show evidence of linkage to 17p, suggesting genetic heterogeneity.

The *FLCN* gene was ultimately identified when [Nickerson et al. \(2002\)](#) narrowed the critical region for the BHD locus to a 700-kb segment on 17p11.2. Significantly, this genomic region is associated with a number of diseases (including bladder and breast cancer, Charcot-Marie-Tooth disease and Neurofibromatosis) because of the presence of unstable low-copy number repeat elements.

3.2 Mutations in *FLCN*: Knudson's two-hit hypothesis

Knudson's two-hit hypothesis states that tumour formation is initiated by biallelic inactivation of a tumour suppressor gene, and that both inherited and sporadic cancers can arise as a result of mutations in the same gene ([Knudson 1971](#)). Inherited predisposition to tumourigenesis results from a heterozygous mutation in a tumour suppressor gene, e.g. *FLCN*, (the 'first hit'), but that this alone is not sufficient for tumour development; inactivation of the wild-type allele by a somatic mutation (the 'second hit') is required.

Somatic mutations in the remaining wild-type copy of *FLCN* and a loss of heterozygosity at chromosome 17p11.2 have been identified in BHD-associated renal tumours, supporting Knudson's "two-hit" hypothesis and a tumour suppressor role for *FLCN* ([Vocke et al., 2005](#)). However, there is evidence that *FLCN* does not behave as a typical tumour suppressor protein. In a study of five BHD patients, [van Steensel et al. \(2007\)](#) found no evidence of somatic mutations and loss of heterozygosity in fibrofolliculomas, suggesting haplo-insufficiency is enough to cause benign tumour growth in the skin. This was supported by [Bønsdorff et al. \(2008\)](#), who, in a study of the canine equivalent of BHD syndrome, found 'second hit' *FLCN* mutations in kidney tumours, but not in skin nodules.

3.3 FLCN mutation databases

FLCN consists of 14 coding exons ([Nickerson et al., 2002](#)) spanning approximately 20 kb of genomic DNA. The study by [Nickerson et al. \(2002\)](#) was the first to identify mutations in the *FLCN* gene. Of the nine BHD families screened, eight had frameshift or termination mutations within the 14 exons of *FLCN*. Five of these families had mutations in exon 11. Screening of an additional 53 BHD families found 22 had exon 11 mutations, suggesting a mutation hotspot ([Nickerson et al., 2002](#)). [Schmidt et al. \(2005\)](#) screened a further 30 families and after combining the mutational data, found that 53% of the *FLCN* mutations involved either a cytosine insertion or deletion in the mononucleotide tract of eight cytosines (C8) in exon 11. Nickerson and colleagues suggested that the frameshift mutations might be caused by a slippage-mediated mechanism during DNA replication ([Nickerson et al., 2002](#)). The majority of mutations were predicted to introduce a premature stop codon into *FLCN* and therefore to result in protein truncation ([Schmidt et al., 2005](#)). This includes the “hot spot” mutations in exon 11. It is unclear whether the truncated *FLCN* is targeted for degradation, or remains in the cell with an altered function. Further evidence supporting the theory of a mutation hotspot was provided by [Khoo et al. \(2002\)](#) when two *FLCN* germline mutations in exon 11 (c.1733insC and c.1733delC) were identified in three of four BHD families, as well as two of four sporadic cases of BHD syndrome.

There are two publicly available sequence variation databases for *FLCN*, which consolidate all identified *FLCN* mutations. Both are hosted online by the Leiden Open (source) Variation Database (LOVD), where researchers can submit published or unpublished mutations. The [Folliculin Sequence Variation Database](#) is curated by Dr Derek Lim (University of Birmingham, UK; [Lim et al., 2010](#)) and currently contains 132 mutations which occur in all coding exons (4-14) of *FLCN*. The second database is called [www.skigenedatabase.com](#) ([Wei et al., 2009](#)). Combining *FLCN* mutational data is important as it allows trends to be easily identified, which can help further the understanding of the causes of BHD syndrome.

3.3.1 Substitution Mutations

Nucleotide substitutions account for approximately 53% of reported *FLCN* mutations and occur more frequently than other types of mutations throughout exons 11 to 14 ([www.lovd.nl/flcn](#)). Of the 72 substitution mutations, 33 have been reported as pathogenic (shown in bold), whilst the pathogenicity of the remaining mutations is yet to be determined. The following tables detail *FLCN* mutations as described in the [Folliculin Sequence Variation Database](#) (up to Feb 2011).

Exon	DNA Change	Remarks	Reference
1	c.-487G>C	5'UTR	Cho et al., 2008. BMC Medical Genetics 9:120-130
1	c.-302G>A	5'UTR	Cho et al., 2008. BMC Medical Genetics 9:120-130
1	c.-299C>T	5'UTR	Cho et al., 2008. BMC Medical Genetics 9:120-130
1	c.-228+1368G>T	Intron	Cho et al., 2008. BMC Medical Genetics 9:120-130
1	c.229+994A>G	Intron	Cho et al., 2008. BMC Medical Genetics 9:120-130
3	c.-90A>G	5'UTR	Cho et al., 2008. BMC Medical Genetics 9:120-130

3	c.-25+100C>G	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
3	c.1-64A>G+c.583G>T	Intron	Lim <i>et al.</i> , 2010. Hum Mutat. 31:1043-51
4	c.1A>G	Missense	Lim <i>et al.</i>, 2010. Hum Mutat. 31:1043-51
4	c.3G>A	Missense	Unpublished
4	c.250-2A>G (repeated 4 times)	Splice site	Toro <i>et al.</i>, 2008. J Med Genet 45:321-31.
4	c.250-1G>A	Splice site	Schmidt <i>et al.</i>, 2005. Am J Hum Genet 76:1023-33.
5	c.323G>T	Missense	Kluger <i>et al.</i>, 2010. Br J Dermatol. 162:527-37
5	c.328C>T	Nonsense	Kunogi <i>et al.</i>, 2010. J Med Genet. 47:281-7
5	c.394G>A	Missense	Frohlich <i>et al.</i>, 2008. Eur Respir J 32:1316-20
5	c.396+59T>C	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
5	c.397-14C>T	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
5	c.397-13G>A	Intron	Unpublished
6	c.510C>A	Nonsense	Unpublished
6	c.583G>T+c.1-64A>G	Nonsense	Lim <i>et al.</i>, 2010. Hum Mutat. 31:1043-51
6	c.618+2T>A	Splice site	Kluger <i>et al.</i>, 2010. Br J Dermatol. 162:527-37
6	c.619-66C>T	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
6	c.619-1G>A	Splice site	Leter <i>et al.</i>, 2008. J Invest Dermatol 128:45-49.
7	c.649C>T	Nonsense	Kluger <i>et al.</i>, 2010. Br J Dermatol. 162:527-37
7	c.715C>T	Missense	Woodward <i>et al.</i>, 2008. Clin Cancer Res 14:5925-30.
7	c.726A>T	Intron	Unpublished
7	c.779G>A	Nonsense	Frohlich <i>et al.</i>, 2008. Eur Respir J 32:1316-20
7	c.779+1G>T (reported 3 times)	Splice site	Toro <i>et al.</i>, 2008. J Med Genet 45:321-31.
7	c.779+113C>T	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
8	c.871+13T>C	Intron	Unpublished
8	c.871+16T>A	Intron	Unpublished
8	c.871+36G>A	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
8	c.871+204A>G	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
8	c.871+226G>A	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
8	c.871+684G>A	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130

9	c.887C>A	Nonsense	Kunogi <i>et al.</i> , 2010. <i>J Med Genet.</i> 47:281-7
9	c.943G>T	Nonsense	Graham <i>et al.</i> , 2005. <i>Am J Respir Crit Care Med</i> 172:39-44
9	c.1062+1G>A	Splice site	Schmidt <i>et al.</i> , 2005. <i>Am J Hum Genet</i> 76:1023-33.
9	c.1062+2T>G (reported 3 times)	Splice site	Toro <i>et al.</i> , 2008. <i>J Med Genet</i> 45:321-31.
9	c.1062+5G>A	Intron	Palmirotta <i>et al.</i> , 2008 <i>Eur J Dermatol</i> 18:382-6
9	c.1062+6C>T	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
9	c.1062+47G>A	Intron	Unpublished
9	c.1063-172C>G	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
9	c.1063-117C>T	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
9	c.1063-2A>G	Splice site	Kunogi <i>et al.</i> , 2010. <i>J Med Genet.</i> 47:281-7
10	c.1127G>A	Nonsense	Maffe <i>et al.</i> , 2010. <i>Clin Genet.</i> (in press)
10	c.1176+31G>A	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
10	c.1176+39G>A	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
10	c.1176+68G>C	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
10	c.1176+134G>C	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
10	c.1176+179A>G	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
10	c.1177-165C>T	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
10	c.1177-2A>G	Splice site	van Steensel <i>et al.</i> , 2007. <i>J Invest Dermatol</i> 127:588-93.
11	c.1198G>A	Missense	Lim <i>et al.</i> , 2010. <i>Hum Mutat.</i> 31:1043-51
11	c.1215C>G	Nonsense	Toro <i>et al.</i> , 2008. <i>J Med Genet</i> 45:321-31
11	c.1269C>T	Coding SNP	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
11	c.1278C>T	Coding SNP	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
11	c.1285C>T	Missense	Ren <i>et al.</i> , 2008. <i>Clin Genet.</i> 74:178-83
11	c.1300G>A	Splice site	Toro <i>et al.</i> , 2008. <i>J Med Genet</i> 45:321-31
11	c.1300G>C	Splice site	Van Steensel <i>et al.</i> , 2007 <i>J Invest Dermatol.</i> 127:588-93
11	c.1300G>T	Splice site	Lim <i>et al.</i> , 2010. <i>Hum Mutat.</i> 31:1043-51
11	c.1301-59C>T	Intron	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
12	c.1333G>A	Coding SNP	Cho <i>et al.</i> , 2008. <i>BMC Medical Genetics</i> 9:120-130
12	c.1389C>G (reported 6 times)	Nonsense	Toro <i>et al.</i> , 2008. <i>J Med Genet</i> 45:321-31

12	c.1429C>T	Nonsense	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
12	c.1432+1G>A	Splice site	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31
12	c.1433-38A>G	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
12	c.1433-1G>T	Splice site	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
13	c.1523A>G	Missense	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31
13	c.1533G>A	Nonsense	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
13	c.1538+121C>T	Intron	Cho <i>et al.</i> , 2008. BMC Medical Genetics 9:120-130
14	c.1579C>T	Nonsense	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.

Legend: Sequence variations nomenclature is as recommended by the Ad-Hoc Committee for Mutation Nomenclature (AHCNM) (den Dunnen JT and Antonarakis SE [2000], Hum.Mut. 15:7-12).

3.3.2 Deletion Mutations

Nucleotide deletions are the second most frequently reported type of *FLCN* mutation and all are pathogenic. No deletion mutations have been reported in exons 1 to 3.

Exon	DNA Change	Remarks	Reference
4	c.3delG	Frameshift	Bessis <i>et al.</i> , 2006. Br J Dermatol 155:1067-9.
4	c.59delT (reported 3 times)	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
4	c.147delA	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
4	c.235_238del	Frameshift	Painter <i>et al.</i> , 2005. Am J Hum Genet 76:522-527.
4	c.240delC	Frameshift	Lim <i>et al.</i> , 2010. Hum Mutat. 31:1043-51
5	c.252delC	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
5	c.296delA	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
5	c.397-10_397-1del	Splice site	Gunji <i>et al.</i> , 2007. J Med Genet 44:588-593.
5	c.397-7_399del	Splice site	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
6	c.404delC	Frameshift	Gunji <i>et al.</i> , 2007. J Med Genet 44:588-593.
6	c.420delC	Frameshift	Leter <i>et al.</i> , 2008. J Invest Dermatol 128:45-49.
6	c.443_459del	Frameshift	Lim <i>et al.</i> , 2010. Hum Mutat. 31:1043-51
6	c.469_471del	Deletion	Ren <i>et al.</i> , 2008. Clin Genet. 74:178-83
6	c.584delG (reported 2 times)	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.

7	c.637delT	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
7	c.671_672del	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
7	c.769_771del	Deletion	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
8	c.836_839del	Frameshift	Lim <i>et al.</i> , 2010. Hum Mutat. 31:1043-51
9	c.890_893del	Frameshift	Woodward <i>et al.</i> , 2008. Clin Cancer Res 14:5925-30.
9	c.1013delG	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
9	c.1021delC	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
9	c.1063-10_1065del	Splice site	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
10	c.1076delC	Frameshift	Lim <i>et al.</i> , 2010. Hum Mutat. 31:1043-51
10	c.1156_1175del	Frameshift	Ren <i>et al.</i> , 2008. Clin Genet. 74:178-83
10	c.1177-5_1177-3del	Splice site	Lim <i>et al.</i> , 2010. Hum Mutat. 31:1043-51
11	c.1252delC	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
11	c.1285delC (reported 14 times)	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
11	c.1301-7_1304del11	Splice site	Leter <i>et al.</i> , 2008. J Invest Dermatol 128:45-49.
12	c.1305delT	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
12	c.1379_1380del (reported 2 times)	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
12	c.1408_1418del	Frameshift	van Steensel <i>et al.</i> , 2007. J Invest Dermatol 127:588-93.
13	c.1522_1524del	Deletion	So, 2009. Respirology 14:775-6
13	c.1528_1530del	Deletion	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
13	c.1533_1536del	Nonsense	Gunji <i>et al.</i> , 2007. J Med Genet 44:588-593.
14	c.1539-?_c.1740+?del	Deletion	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
14	c.1557delT	Frameshift	Kim <i>et al.</i> , 2008. J Korean Med Sci 23:332-5
14	c.1597_1598del	Frameshift	Warwick <i>et al.</i> , 2010. J Med Case Reports 4 :106

3.3.3 Duplication Mutations

All reported duplication mutations are pathogenic and result in a frameshift.

Exon	DNA Change	Remarks	Reference
5	c.340dupC	Frameshift	Unpublished

5	c.347dupA	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
7	c.655dupG	Frameshift	Leter <i>et al.</i> , 2008. J Invest Dermatol 128:45-49.
7	c.689dupT	Frameshift	Unpublished
9	c.923_950dup (reported 5 times)	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
9	c.997_998dupTC	Frameshift	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
11	c.1285dupC (reported 14 times)	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
11	c.1286dupA	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
12	c.1318_1334dup17	Frameshift	Woodward <i>et al.</i> , 2008. Clin Cancer Res 14:5925-30.
12	c.1337_1343dup	Frameshift	Imada <i>et al.</i> , 2009. Br J Dermatol. 160:1350-3
12	c.1340_1346dup	Frameshift	Gunji <i>et al.</i> , 2007. J Med Genet 44:588-593.
12	c.1347_1353dup	Frameshift	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
12	c.1372dupC	Frameshift	Kluger <i>et al.</i> , 2010. Br J Dermatol. 162:527-37
12	c.1426dupG	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
13	c.1487_1490dup	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.

3.3.4 Indel Mutations

Six indel (insertion/deletion) mutations have been reported and all have been described as being pathogenic.

Exon	DNA Change	Remarks	Reference
5	c.319_320delinsCAC (reported 5 times)	Frameshift	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
6	c.566_577delinsCC	Frameshift	Kunogi <i>et al.</i> , 2010. J Med Genet. 47:281-7
6	c.610_611delinsTA	Nonsense	Toro <i>et al.</i> , 2008. J Med Genet 45:321-31.
7	c.632_633delinsC	Frameshift	Schmidt <i>et al.</i> , 2005. Am J Hum Genet 76:1023-33.
8	c.871+3_c.871+4delins	Other	Unpublished
12	c.1323delinsGA+c.1301-7_1304del11	Frameshift	Leter <i>et al.</i> , 2008. J Invest Dermatol 128:45-49.

3.3.5 Insertion Mutations

One insertion mutation of unknown pathogenicity has been reported in exon 12 and results in a frameshift.

Exon	DNA Change	Remarks	Reference
12	c.1340_1341ins7	Frameshift	Gunji <i>et al.</i> , 2007. J Med Genet 44:588-593.

4. Folliculin and its interacting partners

4.1 Folliculin protein

FLCN is predicted to encode the 579 amino acid protein FLCN (64kDa), consisting of a short hydrophobic N-terminal sequence, one N-glycosylation site, three myristoylation sites and a glutamic acid-rich coiled coil domain centrally located in the protein ([Nickerson et al., 2002](#)). FLCN is also observed to be phosphorylated, and further details regarding this modification can be found in Section 4.2.3.

Two other FLCN [isoforms](#) are also predicted, produced by alternative splicing. These lack the C-terminal end and are 342 and 197 amino acids in length ([Nickerson et al., 2002](#)). These proteins are similar to the yeast FLCN homologue, LST7, which only contains the N-terminal part of the protein. If the truncated FLCN produced by gene mutations remains in the cell, it could have a similar function to the FLCN isoforms.

The protein sequence of the primary FLCN isoform is as follows:

```
MNAIVALCHFCELHGPRTLFCTEVLHAPLPQGDGNEDSPGQGEQAEEEEGGIQMNSRMRAHSPAEGASVESSP
GPKKSDMCEGCRSLAAGHPGYISHDKETSIKYVSHQHPSHPQLFSIVRQACVRSLSCEVCPGREGPIFFGDEQHGFV
FSHTFFIKDSLARGFQRWYSIITIMMDRIYLINSWPFLLGKVRGIIDELQGKALKVFEAEQFGCPQRAQRMNTAFTPF
LHQRNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLTEKLLEGAPTEDTLVQMEKLADLEEESESWDNSEA
EEEEKAPVLPESTEGRELTQGPAESSSLSGCGSWQPRKLPVFKSLRHMRQVLGAPSFRMLAWHVLMGNQVIWKS
RDVDLVQSAFEVLRTMLPVGCVRIIPYSSQYEEAYRCNFLGLSPHVQIPPHVLSSEFAVIVEVHAAARSTLHPVGCED
DQSLSKYEFVVTSGSPVAADRVGPTILNKIEAALTNQNLSVDVVDQCLVCLKEEWMNKVKVLFKFTKVDSRPKEDT
QKLLSILGASEEDNVKLLKFWMTGLSKTYKSHLMSTVRSPTASESRN
```

[List of amino acids, their abbreviations and details.](#)

No transmembrane domains or organelle localisation signals have been determined within the FLCN sequence ([Warren et al., 2004](#)). FLCN has no significant homology to any known protein, but it is highly conserved across species including *Canis lupus familiaris*, *Bos Taurus*, *Mus musculus*, *Rattus norvegicus*, *Gallus gallus*, *Xenopus tropicalis*, and *Drosophila melanogaster*, suggesting an important biological role ([Nickerson et al., 2002](#)). A sequence alignment shows this conservation:

```

1      10      20      30      40      50      60      70
H.sapiens  HSPAEGASVSESSSPGPKKSDMCEGCRSLAAGHPGYISHDKETSISKYVSHQHPSHPOLRSHVVRQAQVRSLS
C.familiaris HSPAEGASAESSSPGPKKSDMCEGCRSLAAGHPGYISHDKETSISKYVSHQHPSHPOLRSHVVRQAQVRSLS
B.taurus    HSPAEGASAESSSPGPKKSDMCEGCRSLAAGHPGYISHDKETSISKYVSHQHPSHPOLRSHVVRQAQVRSLS
M.musculus  HSPAEGASSESSSPGPKKSDMCEGCRSLAVGHPGYISHDKETSISKYVSHQHPSHPOLRSHVVRQAQVRSLS
R.norvegicus HSPAEGASTDSSSPGPKKSDMCEGCRSLAVGHPGYISHDKETSISKYVSHQHPSHPOLRSHVVRQAQVRSLS
G.gallus    HSPAEGASADSSSPGPKKSDMCEGCRSLAGGHPGFVSHDKETSISKYVSHQHPSHPOLRSHVVRQAQVRSLS
X.tropicalis HSPAEGASADNSPRPKKSDMCEGCRSLAAGHPGYISHDKETSISKYVSHQHPSHPOLRSHVVRQAQVRSLS
D.melanogaster . . . . . D E S G A T F V S T K V A V L R E V A S E V K Q A A V L S L S

```

```

80      90      100     110     120     130
H.sapiens  CEVCPGREG.PIFFGDQHGQFVFSHTFFIKDSLARGFORWYSITIMMDRIYLNSSWPFLLGKVRGIIDK
C.familiaris CEVCPGREG.PIFFGDQHGQFVFSHTFFIKDSLARGFORWYSITAIMMDRIYLNSSWPFLLGKIRGIIDK
B.taurus    CEVCPGREG.PIFFGDQHGQFVFSHTFFIKDSLARGFORWYSITAIMMDRIYLNSSWPFLLGKIRGIIDK
M.musculus  CEVCPGREG.PIFFGDQHGQFVFSHTFFIKDSLARGFORWYSITAIMMDRIYLNSSWPFLLGRIRGIISL
R.norvegicus CEVCPGREG.PIFFGDQHGQFVFSHTFFIKDSLARGFORWYSITAIMMDRIYLNSSWPFLLGRIRGIISL
G.gallus    CEVCPGREG.PIFFGDQHGQFVFSHTFFIKDSLARGFORWYSITIMMDRIYLNSSWPFLLGKIRGIIDK
X.tropicalis CEVCPGREG.PIFFGDQHGQFVFSHTFFIKDSLARGFORWYSITIVIMMDRIYLNSSWPFLLAKIRGIIDK
D.melanogaster NGTDLASKKGEFVFFGDSSRGHILSHTRVSDLQARGYSQLFSTIIVLMKDKYFLNIIKPFLLAEHLKKVSSS

```

```

140     150     160     170     180     190     200
H.sapiens  LQGRANKVFEAMQFGCPQRAQRMNTAFTFFLHQRNNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLL
C.familiaris LQGRANKVFEAMQFGCPQRAQRMNTAFTFFLHQRNNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLL
B.taurus    LQGRANKVFEAMQFGCPQRAQRMNTAFTFFLHQRNNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLL
M.musculus  LQGRANKVFEAMQFGCPQRAQRMNTAFTFFLHQRNNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLL
R.norvegicus LQGRANKVFEAMQFGCPQRAQRMNTAFTFFLHQRNNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLL
G.gallus    LQGRANKVFEAMQFGCPQRAQRMNTAFTFFLHQRNNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLL
X.tropicalis LQGRANKVFEAMQFGCPQRAQRMNTAFTFFLHQRNNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLL
D.melanogaster LQAAANKTKETEQTYSERQRELSGAQFLMPTSR.....ALELELTGEEHIFAQLHSHSFWLLKAGSRFL

```

```

210     220     230     240     250     260     270
H.sapiens  TEKLLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVLPESTEGRRELTQGPAAESSLSLSCGGSWQ
C.familiaris TEKLLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVLPPEGAEGRRELTQCPTDSSLSDCGNWQ
B.taurus    TEKLLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKGPALPEGAEGRRELTQCPAESLSDCGAWQ
M.musculus  TEKLLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVLPPEGAEGRRELTQCPAESLSDCGAWQ
R.norvegicus TEKLLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVLPPEGAEGRRELTQCPAESLSDCGAWQ
G.gallus    TEKLLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVLPPEGAEGRRELTQCPAESLSDCGAWQ
X.tropicalis TEKLLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVLPPEGAEGRRELTQCPAESLSDCGAWQ
D.melanogaster TEHVTFGN.....LPWLPQSGSRPPAQRLLTYNSLTPMIESID

```

```

280     290     300     310     320     330     340
H.sapiens  PRK..LSPVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVQSAFVVRTMLLVGCVRIIPYSS
C.familiaris PRK..LSPVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVHSAFVVRTMLLVGCVRIIPYSN
B.taurus    PRK..LSPVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVHSAFVVRTMLLVGCVRIIPYSS
M.musculus  PPK..LSPVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVHSAFVVRTMLLVGCVRIIPYSS
R.norvegicus PPK..LSPVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVHSAFVVRTMLLVGCVRIIPYSS
G.gallus    VAHRR.LSPVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVQSAFVVRTMLLVGCVRIIPYSD
X.tropicalis VQRR..MGVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVQSAFVVRTMLLVGCVRIIPYSE
D.melanogaster DPD..LSEFVFRSLRRMRQVLGAPSRFMLAWHVLMGNQVIWKSRLDVLVQSAFVVRTMLLVGCVRIIPYSE

```

```

350     360     370     380     390     400     410
H.sapiens  QYEEAYRCNFLGLSPHVQIIPHVLSSEFAVIVVHAAARSTLHPVGCEDDQSLSKYEFVVTSGSVAADR
C.familiaris QYEEAYRCNFLGLSPHVQIIPSHVLSSEFAVIVVHAAARSTLHPVGCEDDQSLSKYEFVVTSGSVAADR
B.taurus    QYEEAYRCNFLGLSPHVQIIPPHVLSSEFAVIVVHAAARSTLHPVGCEDDQSLSKYEFVVTSGSVAADR
M.musculus  QYEEAYRCNFLGLSPHVQIIPAHVLSSEFAVIVVHAAARSTLHPVGCEDDQSLSKYEFVVTSGSVAADR
R.norvegicus QYEEAYRCNFLGLSPHVQIIPAHVLSSEFAVIVVHAAARSTLHPVGCEDDQSLSKYEFVVTSGSVAADR
G.gallus    QYEEAYRCNFLGLSPHVQIIPSHVLSSEFAVIVVHAAARSTLHPVGCEDDQSLSKYEFVVTSGSVAADR
X.tropicalis QYEDAYRCNFLGLSPHVQIIPHVLSSEFAVIVVHAAARSTLHPVGCEDDQSLSKYEFVVTSGSVAADR
D.melanogaster QHGHGISSEYKISVNDIAVPMASBSVYRIDFLDKHVN.....HIVSVKWEGLP

```

```

420     430     440     450     460     470     480
H.sapiens  VGPITILNKIEAALTNQNLSDVVDQCLVCLKEEWMNKVKVLFKFTKVDSSRPKEDTQKLLSILGASEEDNV
C.familiaris VGPITILNKIEAALTNQNLSDVVDQCLVCLKEEWMNKVKVLFKFTKVDSSRPKEDTQKLLSILGASEEDNV
B.taurus    VGPITILNKIEAALTNQNLSDVVDQCLVCLKEEWMNKVKVLFKFTKVDSSRPKEDTQKLLSILGASEEDNV
M.musculus  VGPITILNKIEAALTNQNLSDVVDQCLVCLKEEWMNKVKVLFKFTKVDSSRPKEDTQKLLSVLGASEEDNV
R.norvegicus VGPITILNKIEAALTNQNLSDVVDQCLVCLKEEWMNKVKVLFKFTKVDSSRPKEDTQKLLSILGASEEDNV
G.gallus    VGPITILNKIEAALTNQNLSDVVDQCLVCLKEEWMNKVKVLFKFTKVDSSRPKEDTQKLLSILGASEEDNV
X.tropicalis VGLTILNKIEAALSNENLSDVVDQCLVCLKEEWMNKVKVLFKFTKVDSSRPKEDTQKLLSILGASEEDNI
D.melanogaster KLPDLMVKLLKAVEERFTELVLNKQTKVLISEWKNKVTCLNHAKSTS.....VCGKLLKKVLGVQPHDQ

```

```

      490           500           510
H.sapiens      KLLKFWMTGLSKTYKSHLMSTVRSPTASESRN
C.familiaris  KLLKFWMTGLSKTYKSHLMSTVRSPTASEPR.
B.taurus       KLLKFWMTGLSKTYKSHLMSTVRSPTALEPRN
M.musculus    KLLKFWMTGLSKTYKSHLMSTVRSPTATESRS
R.norvegicus  KLLKFWMTGLSKTYKSHLMSTVRSPTAABSRN
G.gallus      KLLKFWMTGLSKTYKSHLMSTVRSPTSSESRN
X.tropicalis  KLLKFWMTGLSKTYKSHLMSSVRSPTASECRN
D.melanogaster P I N Y W S T H L H . . . . .

```

[Warren et al. \(2004\)](#) studied the expression of *FLCN* mRNA in both normal and neoplastic human tissue and found that in normal cells *FLCN* was expressed in the skin, the distal nephron of the kidney, stromal cells and type 1 pneumocytes of the lung, acinar cells of the pancreas and parotid gland, epithelial ducts of the breast and prostate, areas of the brain and in macrophages and lymphocytes in the tonsils and spleen. Tissues with no *FLCN* mRNA expression included the heart, muscle and liver ([Warren et al., 2004](#)). The fact that *FLCN* mRNA is not detected in BHD-associated renal tumours provides further evidence supporting the idea that FLCN is a tumour suppressor.

[Hudon et al. \(2010\)](#) characterised the tissue distribution of mouse *FLCN*. It was found to have a similar distribution to the human *FLCN*, with high expression levels in the kidneys, lungs and spleen. Interestingly, no *Flcn* expression was seen in the murine skin, correlating with the lack of skin lesions in *FLCN*-null mice.

For details on FLCN cellular localisation, see Section 4.3.

4.2. Folliculin-binding proteins

4.2.1 FNIP1

FLCN-binding protein 1 (FNIP1), was identified in 2006 ([Baba et al.](#)) as an evolutionarily conserved protein that interacts with and phosphorylates FLCN. FNIP1 also binds AMPK, which is a negative regulator of mTOR and a key protein for energy sensing in cells ([Inoki et al., 2003](#); [Gwinn et al., 2008](#)). [Baba et al. \(2006\)](#) demonstrated that both FLCN and FNIP1 are phosphorylated by AMPK. This interaction between FNIP1 and FLCN was also shown to be modified by external influences, since treatment with an AMPK inhibitor (compound C), rapamycin or amino acid starvation affected the phosphorylation status of FLCN, further indicating a role for FLCN in energy sensing and the mTOR pathway.

4.2.2 FNIP2

FNIP2, a second FLCN-binding protein, was first designated KIAA1450 by [Nagase et al. \(2000\)](#) by sequencing clones obtained from a size-fractionated human brain cDNA library. [Hasumi et al. \(2008\)](#) subsequently identified it as FNIP2, with [Takagi et al. \(2008\)](#) identifying it as FNIPL (for FNIP1-Like) soon after. Additionally, [Komori et al. \(2009\)](#) cloned mouse FNIP2 (which they called MAPO1), and saw that it plays a role in apoptosis triggered by O6-methylguanine mispairing in DNA.

The accepted nomenclature for this protein is FNIP2, and it is homologous to FNIP1 (49% identity, 74% similarity). As with FNIP1, it is conserved across species and binds AMPK ([Hasumi et al., 2008](#); [Takagi et al., 2008](#)). *In vitro* kinase assays also suggest that FNIP2 is phosphorylated by AMPK ([Takagi et al., 2008](#)). FNIP1 and FNIP2 are able to form homo- and heterodimers, as well as multimers ([Takagi et al., 2008](#)), suggesting a functional association between these two proteins.

4.2.3 Interactions with FLCN

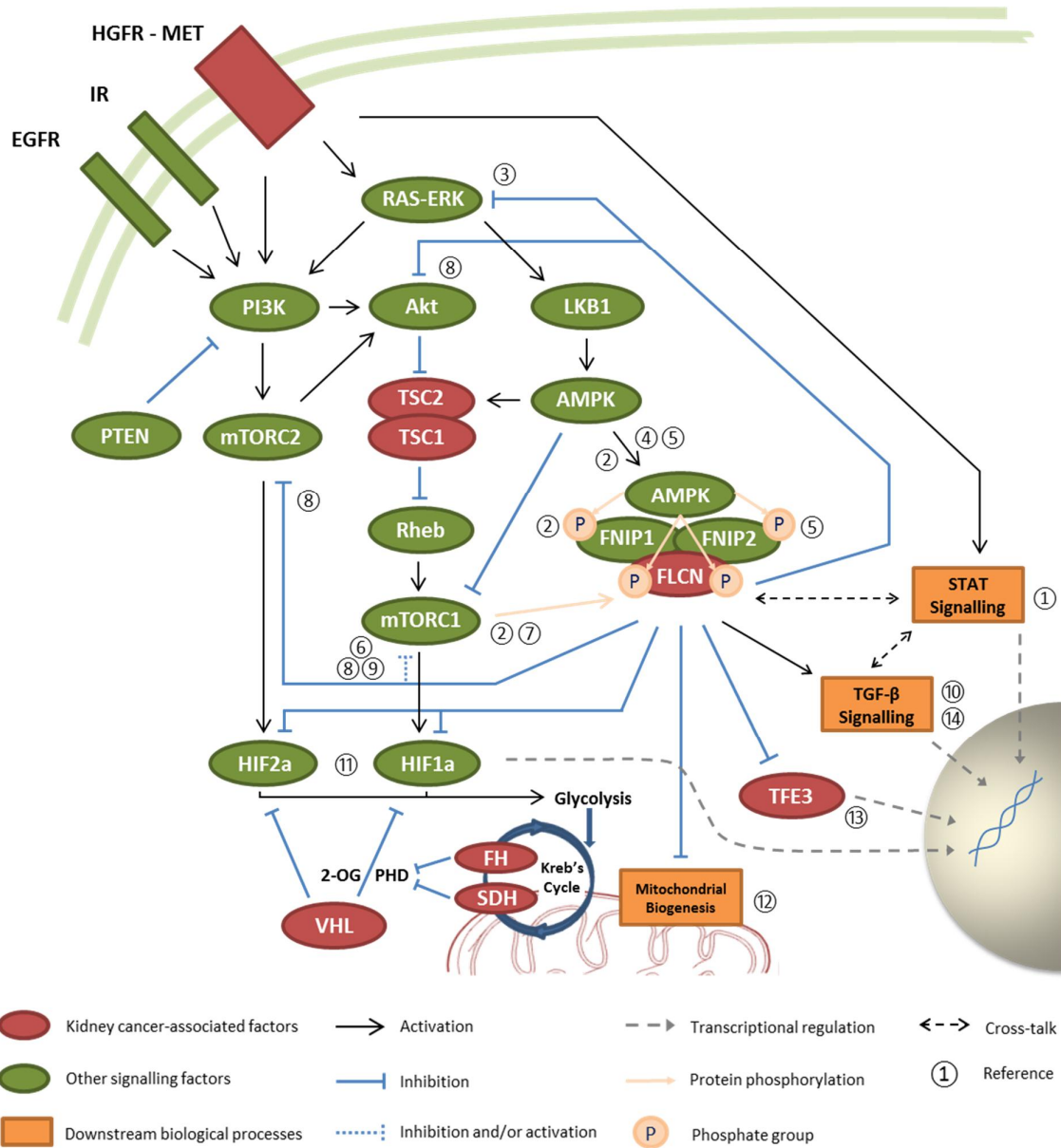
Binding of FLCN to FNIP1 and FNIP2 is mediated specifically through the C-terminal region of FLCN ([Baba et al., 2006](#); [Hasumi et al., 2008](#); [Takagi et al., 2008](#)). In BHD syndrome, the majority of mutations are predicted to introduce a premature stop codon into *FLCN*, and therefore result in a protein truncation ([Schmidt et al., 2005](#)). However, it is presently unclear whether this truncated FLCN is targeted for nonsense-mediated decay, or remains in the cell with an altered function. Whatever the outcome, these mutations remove the ability of FLCN to interact with FNIP1 and FNIP2, which suggests that this interaction is functionally important. Further research has shown that serine 62 (ser62) is a phosphorylation site in FLCN ([Wang et al., 2010](#)). This work also suggests that ser62 phosphorylation is indirectly up-regulated by AMPK ([Wang et al., 2010](#)). FLCN also appears to be phosphorylated at ser302 by unknown kinases downstream of mTORC1 ([Piao et al., 2009](#)). Since mTORC1 is known to be indirectly down-regulated by AMPK, this process could be associated with an unknown feedback mechanism that regulates mTOR signalling.

4.3 Cellular localisation

The expression patterns of FLCN, FNIP1 and FNIP2 in human tissues were determined by [Hasumi et al. \(2008\)](#) using real-time PCR. The expression patterns of these proteins were generally similar, and consistently high in specific tissues, such as the muscles, nasal mucosa, salivary gland and uvula, suggesting that FLCN, FNIP1 and FNIP2 may work together in these organs. However, FNIP2 expression was higher relative to FNIP1 in fat, liver and pancreas, which suggests that FNIP2 may have a specific function in these metabolic tissues ([Hasumi et al., 2008](#)). FLCN/FNIP1 and FLCN/FNIP2 dimers have been shown to co-localise in the cytoplasm in a reticular pattern ([Baba et al., 2006](#); [Hasumi et al., 2008](#); [Takagi et al., 2008](#)). Co-expression studies of N-terminal tagged FLCN, FNIP1 and FNIP2 indicate that both FNIPs regulate the cytoplasmic distribution of FLCN ([Baba et al., 2006](#); [Hasumi et al., 2008](#); [Takagi et al., 2008](#)). When tagged FNIP2 constructs are expressed alone, FNIP2 is seen to be distributed within the cytoplasm of cells, showing more condensed features around the nucleus. Conversely, when tagged FLCN constructs are expressed alone, they appear to be found mainly in the nucleus ([Takagi et al., 2008](#)). However, when FNIP2 and FLCN are co-expressed they co-localise together in the cytoplasm in a reticular pattern, which is similar to the co-localisation of FNIP1 and FLCN ([Baba et al., 2006](#); [Hasumi et al., 2008](#); [Takagi et al., 2008](#)).

5. Folliculin-associated signalling pathways

The following figure illustrates the relationship between Folliculin and a number of different signalling pathways, which is discussed further within this section.



- ① [Singh et al., 2006](#); ② [Baba et al., 2006](#); ③ [Baba et al., 2008](#); ④ [Hasumi et al., 2008](#); ⑤ [Takagi et al., 2008](#);
 ⑥ [Hartman et al., 2009](#); ⑦ [Piao et al., 2009](#); ⑧ [Hasumi et al., 2009](#); ⑨ [Hudon et al., 2010](#); ⑩ [Hong et al., 2010a](#);
 ⑪ [Preston et al., 2010](#); ⑫ [Klomp et al., 2010](#); ⑬ [Hong et al., 2010b](#); ⑭ [Cash et al., 2011](#)

5.1 mTOR signalling

The molecular functions of FLCN are poorly understood, but it is known that FLCN and AMPK interaction, as mediated by FNIP1 and FNIP2, is involved in mTOR signalling ([Baba et al., 2006](#); [Hasumi et al., 2008](#)). The mTOR pathway is a key regulator of cell growth, proliferation and metabolism ([Harris and Lawrence, 2003](#); [Hay and Sonenberg, 2004](#); [Wullschleger et al., 2006](#)) and an increasing amount of evidence suggests that its deregulation is associated with human diseases, such as cancer ([Sarbasov et al., 2005](#); [Landau et al., 2009](#)).

The functional role of FLCN in mTOR signalling however is undetermined since several recent publications have reported contradictory impacts on an indicator of mTOR activation (known as phosphorylated ribosomal protein S6 / p-S6R) when *FLCN* expression is reduced. Two studies recently reported that transient downregulation of *FLCN* by siRNA in human cell lines results in reduction of phosphorylation of p-S6R ([Takagi et al., 2008](#); [Hartman et al., 2009](#)). Reduction of p-S6R was also observed in renal cysts developing in mice heterozygous for *FLCN* ([Hartman et al., 2009](#)). In contrast, kidney-specific homozygous knockout of *FLCN* resulted in an increase in phosphorylated p-S6R, which contributed to the development of polycystic kidneys ([Baba et al., 2008](#); [Chen et al., 2008](#)). Additionally, an activation of mTOR signalling in the kidney tumours of another heterozygous *FLCN* knockout mouse model was noted by [Hasumi et al. \(2009\)](#). This discrepancy between the heterozygous models could be due to differences in sample preparation and/or the gene targeting strategy used by [Hartman et al. \(2009\)](#). However, [Hudon et al. \(2010\)](#) noted that a loss of *FLCN* expression in their heterozygous *FLCN* knockout mouse resulted in both elevated and reduced levels of p-S6R, depending on the cellular context- which may account for the differing results observed in the earlier mouse models.

Further studies in fission yeast, *Schizosaccharomyces pombe* (*S. pombe*), revealed that the yeast FLCN homologue (LST7) and yeast TSC1/2 homologues regulate common downstream targets but have opposing roles. Specifically, TSC1/2 inhibits the activation of Tor2 and subsequent downstream elements, but LST7 up-regulates the same elements ([van Slechtenhorst et al., 2007](#)). Ultimately, all this data suggests a role for FLCN in nutrient/energy-sensing mediated through the mTOR signalling pathway, and if the relationship between yeast FLCN and TSC1/TSC2 is reiterated in mammalian cells, there would be important implications for the development of therapies for BHD syndrome. However, LST7 only corresponds to the N-terminal region of vertebrate FLCN, which may go some way in explaining these results.

Whilst the work discussed above emphasises the part played by FLCN in the mTOR pathway, recent research has also indicated a role for FLCN in other signalling systems and cellular processes.

5.2 Raf-MEK-Erk signalling

Work using kidney-targeted *FLCN* gene inactivation in mice has indicated that the Raf-MEK-Erk pathway, which is activated in many cancers and regulates cell proliferation ([Roberts and Der, 2007](#)), was activated in *FLCN*-knockout kidneys ([Baba et al., 2008](#)). This suggests that an upstream effector of this pathway may be activated by loss of *FLCN* tumour suppressor function, resulting in cell growth and proliferation within the *FLCN*-null kidney cell.

5.3 JAK/STAT signalling

RNA interference experiments in *Drosophila* suggested that the fly homologue of FLCN regulates male germline stem cell maintenance, downstream of or in parallel to the [janus kinase-signal transducers and activators of transcription \(JAK/STAT\)](#) and [bone morphogenetic protein \(BMP\)](#) signalling pathways ([Singh et al., 2006](#)). BMP (and its *Drosophila* homologue) are members of the TGF- β superfamily of proteins, and FLCN's effects on TGF- β signalling can be found in more detail in Section 5.5.

5.4 HIF signalling

Using a RCC cell line derived from a BHD patient (UOK257), [Preston et al. \(2010\)](#) demonstrated that Folliculin also influences hypoxia-inducible factor (HIF) signalling. This pathway regulates a number of different genes that are involved in angiogenesis, erythropoiesis, cell survival and metastasis. The authors suggested that a high level of HIF-mediated expression in these FLCN-null cells alters cell metabolism through elevated levels of metabolic enzymes. This altered metabolic state parallels a phenomenon known as the Warburg effect, which is commonly seen in cancerous cells ([Warburg, 1956](#)). Warburg also postulated that cancer should in fact be interpreted as a mitochondrial disease, and further work by [Klomp et al. \(2010\)](#) suggests that the loss of FLCN in BHD syndrome results in mitochondrial dysfunction, as indicated by a high level of mitochondrial gene expression.

5.5 TGF- β signalling and general transcriptional control

Recent work using cultured UOK257 cells, FLCN knock-out mice, and BHD patient tumours showed that FLCN inactivation also increases the activity of a nucleo-cytoplasmic transcription factor (TFE3) that has previously been implicated in renal cell carcinoma ([Hong et al., 2010b](#)). Additional work in the same laboratory used *in vitro* cell based assays, *in vitro* xenograft studies and gene expression profiles to demonstrate that FLCN has an essential role in the regulation of TGF- β signalling ([Hong et al., 2010a](#)). Experiments by [Cash et al. \(2011\)](#) took this connection further by demonstrating that FLCN appears to regulate apoptosis through TGF- β -dependent transcription, which could help to further explain the renal cancer phenotype observed in BHD syndrome.

While the finer details still need investigating, it is clear that Folliculin is involved in a dense signalling network, and that unravelling this network will help open up many new avenues for potential therapies to help alleviate and ultimately cure the symptoms caused by BHD syndrome.

6. Model organisms for studying BHD syndrome

Current model systems provide an excellent base for drug development in general. They are also crucial for more fundamental studies investigating cell biology and protein interactions, which provide a much-needed insight into the mechanisms of pathogenesis underlying BHD syndrome *in vivo*.

The following sections outline the model organisms currently used in BHD syndrome research.

6.1 Mouse models

Several mouse models have been generated which are described below:

Homozygous deletion of *FLCN* in the mouse has been shown to be embryonic lethal ([Baba et al., 2008](#); [Chen et al., 2008](#)). Therefore, two kidney-specific knockout mouse models were developed. The first contained a conditional *FLCN* allele and a cadherin 16 (KSP)-*Cre* transgene, which led to the deletion of exon 7 and the targeted inactivation of *FLCN* in the kidney ([Baba et al., 2008](#)). The second was also created using a *Cre-lox* system, which inactivated *FLCN* specifically in the kidney by deleting exons 3 and 4 ([Chen et al., 2008](#)).

In both studies, conditional homozygous inactivation of *FLCN* in the mouse kidney triggered the development of highly enlarged polycystic kidneys, which led to renal failure and death around three weeks after birth. These kidneys also exhibited increased phosphorylation of the indicator of mTOR activation, p-S6R, suggesting that a deficiency in FLCN leads to the activation of mTOR signalling *in vivo* ([Baba et al., 2008](#); [Chen et al., 2008](#)).

Another mouse model with targeted inactivation of *FLCN* was generated by [Hartman et al. \(2009\)](#). These mice were created using a gene trap vector technique, where a β -galactosidase/neomycin (β -geo) cassette was integrated between exons 8 and 9 of the *FLCN* gene, which resulted in a truncated form of the Folliculin protein ([Hartman et al., 2009](#)). As expected, no homozygous *FLCN* mutant mice were identified. Furthermore, the heterozygous *FLCN* mutant mice produced developed renal cysts and neoplasia at 3-6 months, similar to those found in BHD patients ([Hartman et al., 2009](#)). Reduced p-S6R immunostaining was observed in the tumours of these mice, suggesting that mTOR activity within this system was in fact suppressed.

Subsequently, it was demonstrated that *FLCN* homozygous null mice die from embryonic day (E) 5.5 to E6.5, with multiple defects evident in their cellular structure ([Hasumi et al., 2009](#)). [Hasumi et al. \(2009\)](#) also showed that heterozygous *FLCN* knockout mice (generated by a *Cre-lox* deletion of exon 7) spontaneously develop cysts and solid tumours in their kidneys after 10 months of age, which are also similar to those seen in BHD patients. Conversely, these investigators noted an activation of mTOR signalling in the kidney tumours of these mice ([Hasumi et al., 2009](#)).

Further studies on a mouse model (with the in-frame β -geo insertion between exons 8 and 9 of *FLCN*) showed that homozygous deletion of *FLCN* caused embryonic lethality before E8.5 ([Hudon et al.,](#)

[2010](#)). Heterozygous *FLCN* knockout mice from this model also developed renal cysts and tumours upon *FLCN* loss ([Hudon et al., 2010](#)). However, the authors noted that this loss of *FLCN* expression resulted in both elevated and reduced levels of p-S6R, depending on the cellular context- which may go some way in explaining the differing results observed in the earlier mouse models ([Baba et al., 2008](#); [Chen et al., 2008](#); [Hartman et al., 2009](#); [Hasumi et al., 2009](#); [Hudon et al., 2010](#)).

6.2 Rat model

The “Nihon” model of renal cell carcinoma (RCC) was found in a Sprague-Dawley strain of rats ([Okimoto et al., 2004](#)). These rats contain a single nucleotide insertion within *FLCN*, which produces a frameshift and premature stop codon within the gene.

Much like the mouse, the homozygous mutant condition is lethal at an early stage of embryonic development. However, in heterozygotes, renal carcinomas develop from pre-neoplastic lesions as early as three weeks after birth, then into adenomas by eight weeks of age, with all rats demonstrating clinical symptoms by six months. The renal carcinomas that develop in heterozygotes are largely composed of clear cells.

Loss of heterozygosity at the *FLCN* locus was observed in ten of eleven primary renal carcinomas examined, which fits the Knudson 2-hit hypothesis ([Okimoto et al., 2004](#)). Further characterisation of the Nihon rat was conducted by [Kouchi et al. \(2006\)](#), where they described the extra-renal lesions seen in the endometrium, salivary glands and cardiac tissue of this model. Rescue experiments were also conducted by introducing a chimeric mini-gene consisting of wild-type *FLCN* cDNA and its 5'-upstream promoter region ([Togashi et al., 2006](#)). These experiments demonstrated that the introduction of the *FLCN* gene could rescue the embryonic lethality of the homozygous mutants, as well as suppress the renal carcinogenesis seen in the heterozygous rats. These experiments highlight the usefulness of this model system, and will help to further dissect the function of Folliculin *in vivo*.

Contact information regarding this model can be found in: *Lab essentials* – [BHD Animal Models](#)

6.3 Dog model

Originally described in 1985, hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis (RCND) is a naturally occurring canine kidney cancer syndrome in German shepherd dogs ([Lium & Moe, 1985](#)). As the name suggests, RCND is characterised by bilateral, multifocal tumours in the kidney and firm nodules within the skin, thus showing similarity to human BHD syndrome.

The RCND locus was located to a small region on canine chromosome 5 that overlapped with *FLCN* ([Lingaas et al., 2003](#)). The authors described a histidine to arginine mutation (H255R) in exon 7 of canine *FLCN* that segregated with the disease phenotype. There is also strong evidence that the mutation is homozygous lethal ([Lingaas et al., 2003](#)), much like the homozygous phenotypes in the rat and mouse models.

Researchers also observed a loss of heterozygosity within the renal cysts and tumours of juvenile and adult dogs respectively, indicating a tumour suppressor function for FLCN according to the Knudson two-hit hypothesis ([Bønsdorff et al., 2008](#); [Bønsdorff et al., 2009](#)).

6.4 *Drosophila* Model

[Singh et al. \(2006\)](#) used RNA interference to decrease the expression of the *Drosophila* FLCN homologue (DBHD), and showed that DBHD was required for male germline stem cell (GSC) maintenance in the fly testis. Subsequent investigation suggested that DBHD regulates GSC maintenance downstream of, or in parallel to, the JAK/STAT and Decapentaplegic (Dpp) signal-transduction pathways.

The JAK-STAT signalling cascade regulates stem cell renewal and differentiation. Additionally, Dpp is the *Drosophila* homologue of the vertebrate bone morphogenetic proteins (BMPs), which are members of the TGF- β superfamily – a signalling system that is crucial for regulating a variety of cellular functions ([Ying et al., 2003](#)).

Further studies by [Singh & Hou \(2009\)](#) showed that over-expression of JAK-STAT signalling results in the enlargement of the Malpighian tubules (the *Drosophila* equivalent of the kidneys), coupled with an increased number of proliferating cells, mitotically active cells and putative renal stem cells. Thus, aberrant JAK-STAT signalling could contribute to the renal cystic/carcinoma phenotype observed in BHD syndrome.

6.5 *S. pombe* model

[van Slegtenhorst et al. \(2007\)](#) identified the *S. pombe* FLCN homologue called LST7, and used homologous recombination to generate a novel deletion strain. LST7 only has homology with the N-terminal region of the vertebrate protein, but these experiments suggest that yeast FLCN is important for amino acid homeostasis, and potentially activates the mTOR homologue Tor2.

7. Future Work

7.1 Clinical research

To date, approximately 200 families have been reported with pathogenic *FLCN* mutations ([Schmidt et al., 2005](#); [Graham et al., 2005](#); [Toro et al., 2008](#); [Misago et al., 2008](#); [Frohlich et al., 2008](#); [Leter et al., 2008](#); [Woodward et al., 2008](#); [Kunogi et al., 2010](#)). However, BHD syndrome is believed to be under-diagnosed; partly because it is so rare that doctors are often unfamiliar with it, and partly because its phenotypic variation can make it difficult to identify ([Menko et al., 2009](#)). Further epidemiological research, to identify more patients with BHD and to identify novel mutations, would help ascertain the true prevalence of BHD syndrome. This research may also help to identify a genotype-phenotype correlation, which could lead to potential personalised treatments for BHD syndrome and an accurate prediction of future symptoms.

7.2 Basic biology

More research is required to understand the normal cellular function of FLCN. Understanding its normal function is crucial to understanding how FLCN mutation or loss can lead to the epidermal, pulmonary and renal phenotypes associated with BHD syndrome.

Most [FLCN mutations](#) lead to a frame shift and the introduction of a premature stop codon ([Schmidt et al., 2005](#)). However, it is unknown whether the resultant aberrant protein is degraded, or remains in the cell and exerts a dominant negative effect. In either case, the role of the C-terminus is unclear, but its loss suggests that this region is important in FLCN's function. It is also unclear whether the remaining N-terminal region of FLCN can be re-purposed to fulfil the function of the wild-type protein.

Additionally, localisation experiments have observed FLCN in the cell nucleus and the cytoplasm ([Takagi et al., 2008](#)). How, where, when and why FLCN is localised to different subcellular compartments, is still unknown.

Research identified a role for FLCN in the mTOR signalling pathway ([Baba et al., 2006](#); [Baba et al., 2008](#); [Hartman et al., 2009](#); [Piao et al., 2009](#); [Hasumi et al., 2009](#); [Hudon et al., 2010](#)). This role has recently been extended into several other processes: JAK-STAT signalling ([Singh et al., 2006](#)); MAPK/ERK signalling ([Baba et al., 2008](#)); TGF- β signalling ([Hong et al., 2010a](#); [Cash et al., 2011](#)); HIF signalling ([Preston et al., 2010](#)); mitochondrial biogenesis ([Klomp et al., 2010](#)); and general transcriptional regulation ([Hong et al., 2010b](#)). Understanding the role of FLCN in these, and additional, processes will provide insight into the mechanism of pathogenesis of BHD syndrome.

The two proteins known to bind FLCN - FNIP1 and FNIP2 - are themselves poorly characterised. It is possible that additional proteins bind to FLCN, which remain to be identified. The function of the FLCN-FNIP1 and -FNIP2 complexes are also an area of future work. Characterising FLCN's interactions with other proteins, and the functions of FLCN-containing protein complexes, will provide insight into how FLCN mutation or loss affects cellular function. These interacting proteins may modulate the phenotype of BHD syndrome and thus affect its variability.

Haploinsufficiency is thought to be sufficient for the skin and lung phenotype, but loss of heterozygosity is required for the development of renal carcinomas. Characterising not only the process by which the second *FLCN* allele is lost, but also the differences in gene transcription and protein expression profiles in cells with two, one or no copies of wild-type *FLCN*, may provide insight into BHD's phenotype variability.

7.3 Drugs and therapies

More clinical research and a better understanding of the basic biology will facilitate development of drugs and other therapies to treat, and eventually cure, BHD syndrome.

For example, screening drug libraries could identify novel therapeutic compounds. Additionally, candidate drugs, already known to affect a relevant pathway (e.g. mTOR signalling) or a phenotypically similar disorder (e.g. TSC or VHL), could be tested. Some of these drugs could already be approved for clinical use, and consequently, rapidly repurposed for the treatment of BHD syndrome.

Additionally, gene and stem cell therapy hold much promise in the treatment of a wide variety of disorders. Research in *Drosophila* indicates that *FLCN* may play a role in stem cell maintenance ([Singh et al., 2006](#)): therapies could also be developed which may exploit this link. Perhaps a functional copy of the *FLCN* gene could be inserted into *FLCN*-null or -heterozygous cells, either preventatively or to reverse the phenotype. While these technologies are still developing, gene therapy has been used with some success in other organ systems, such as the eye ([Maguire et al., 2008](#)) and for Parkinson's in the brain ([LeWitt et al., 2011](#)).

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