

A New Locus-Specific Database (LSDB) for Mutations in the Folliculin (*FLCN*) Gene



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ABSTRACT: Birt-Hogg-Dubé syndrome (BHD) is an autosomal dominant condition characterised by the presence of facial fibrofolliculomas, pulmonary cysts which may be associated with spontaneous pneumothorax and renal tumours. Germline mutations in the gene Folliculin (*FLCN*) were first identified in BHD patients in 2002. In addition *FLCN* mutations have also been described in families with isolated primary spontaneous pneumothorax (PSP) and also familial clear cell renal carcinomas (FcRCC). We have established a locus-specific database based on the Leiden Open (source) Variation Database (LOVD) software. The version of the database contains 60 previously published mutations and 10 previously unpublished novel germline *FLCN* mutations. The mutations are comprised of deletions (44.3%), substitutions (35.7%), duplications (14.3%) and deletion/insertions (5.7%). The database is accessible online at <http://www.lovd.nl/flcn> © 2009 Wiley-Liss, Inc.

KEY WORDS: Folliculin, *FLCN*, Birt-Hogg-Dubé syndrome, Renal cancer, Spontaneous Pneumothorax, Mutation Database

INTRODUCTION

Birt-Hogg-Dubé syndrome (BHD; MIM# 135150) was first described by three Canadian doctors in 1977 (Birt et al., 1977). BHD is an autosomal dominant condition characterised by facial fibrofolliculomas, pulmonary cysts

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with or without pneumothorax and/or renal tumours (Roth et al., 1993; Toro et al., 1999; Zbar et al., 2002).

Fibrofolliculomas (FFS) are benign tumours of the hair follicles. In addition, angiofibromas and acrochordons are also seen in BHD patients. The FFS typically appear during the 3rd to 4th decades of life and usually occur on the face but may include the neck, ears and upper torso (Schaffer et al., 2005; Schulz et al., 2001).

Air-filled pulmonary cysts in BHD are usually bilateral and occur in the base of the lungs. They occur in over 80% of BHD patients. These cysts can be detected usually from the age of 20 years and in about 24% of patients, these cysts are associated with spontaneous pneumothorax (Schmidt et al., 2005; Toro et al., 1999; Toro et al., 2007; Zbar et al., 2002). However, one child with a germline *FLCN* (MIM# 607273) mutation presented with a spontaneous pneumothorax at the age of 7 years (Bessis et al., 2006). The most serious complication of BHD is renal cancer. In a series of 124 BHD patients, 27% developed renal tumours at a mean age of 50.4 years (Pavlovich et al., 2005). The earliest age of diagnosis of renal cancer reported in BHD was 20 years (Khoo et al., 2002). A range of histological types are seen and include oncocytoma, chromophobe/oncocytic hybrid, clear cell and papillary (Vocke et al., 2005). Different tumour histological types are seen, both within families and also in the same individual (Pavlovich et al., 2005).

In 2001 evidence for linkage of BHD to chromosome 17p11 was demonstrated (Khoo et al., 2001; Schmidt et al., 2001). Germline mutations in the folliculin gene (*FLCN*) were first identified in BHD patients in 2002 (Nickerson et al., 2002). In addition, *FLCN* mutations have also been discovered in patients with familial Primary Spontaneous Pneumothorax (PSP; MIM# 173600) and cases presenting with familial clear cell renal carcinoma (FcRCC) in whom other features of BHD have not been noted (Frohlich et al., 2008; Graham et al., 2005; Gunji et al., 2007; Painter et al., 2005; Ren et al., 2008; Woodward et al., 2008)

THE *FLCN* GENE

The *FLCN* gene is located on chromosome 17p11.2. It contains 14 exons with the transcriptional start site in exon 4. *FLCN* encodes a 579 amino acid protein called folliculin which is expressed in most tissues including the skin, lung, kidney and in fetal lung, kidney, liver and brain tissue (Nickerson et al., 2002).

A mutation hot-spot in exon 11 containing a hypermutable polycytosine (C8) tract is susceptible to germline deletions and duplications and is reported to account for about 50% of mutations in BHD families (Schmidt et al., 2005). Around 85% of patients clinically affected by BHD are found to have a germline *FLCN* mutation detected by sequencing (Nickerson et al., 2002; Schmidt et al., 2005).

The function of folliculin is yet to be clarified. Folliculin and its interacting protein FNIP1 are thought to be involved in energy and/or nutrient sensing via the AMPK and mTOR (mammalian target of rapamycin) pathway (Baba et al., 2006).

FLCN is considered to be a tumour suppressor gene. Biallelic inactivation is required for the development of renal cancer (Vocke et al., 2005). However, biallelic inactivation may not be required for the development of fibrofolliculomas (van Steensel et al., 2007)

THE *FLCN* DATABASE

A Locus-Specific Database (LSDB) for *FLCN* using the Leiden Open (Source) Variation Database (LOVD) version 2 build 19 system (Fokkema et al., 2005) was established as part of the European Birt-Hogg-Dubé Consortium collaboration (www.europeanbhdconsortium.eu). The LOVD system has a user-friendly interface with useful functions for searching variants on the database, hyperlinking to other useful online resources and submitting variants online. It allows the creation and maintenance (including curation) of a database of gene sequence variants and associated clinical information that fulfils the recommendations of the Human Genome Variation Society (HGVS).

The purpose of the database is to collate in a standardised format, molecular variations in the *FLCN* gene and serves as an important point of reference for *FLCN* variants. The availability of such a database will be a valuable tool for both clinicians and researchers as it contains both published and unpublished sequence variants. It also allows collaboration between various groups as the affiliation and contact details of submitters of unpublished variants/mutations are publically available on the database. In addition, the sequence variants are not restricted to

BHD but also includes *FLCN* mutations also found in PSP and FcRCC. This database has also been included in the Human Genome Variation Society (HGVS) list of LSDBs. During the preparation of this manuscript it was brought to our attention that another *FLCN* mutation database for mutations reported in BHD patients had been established (Wei et al., 2009).

The maintenance of the database, including curation and assessment of submitted and curated variants are performed by members of the European BHD Consortium. The ongoing maintenance of the database is guaranteed by the consortium.

The database lists previously published point mutations, deletions, duplications and splice site mutations in the *FLCN* gene (Bessis et al., 2006; Cho et al., 2008; Frohlich et al., 2008; Gad et al., 2007; Graham et al., 2005; Gunji et al., 2007; Imada et al., 2009; Kawasaki et al., 2005; Khoo et al., 2002; Kim et al., 2008; Lamberti et al., 2005; Leter et al., 2008; Murakami et al., 2007; Nickerson et al., 2002; Painter et al., 2005; Palmirotta et al., 2008; Ren et al., 2008; Schmidt et al., 2005; Toro et al., 2008; van Steensel et al., 2007; Woodward et al., 2008). In addition 10 previously unpublished novel mutations/variants are included.

The current version of the database contains 70 entries (Table 1). Mutation names are given according to HGVS nomenclature guidelines and numbered with respect to the *FLCN* gene cDNA sequence (+1 = A of ATG) obtained from the National Centre for Biotechnology Information (NCBI) database (accession number NM_144997.5). Previously reported mutations have been renamed accordingly but the original nomenclature is also included in the corresponding entry in the database to allow cross-reference.

For each mutation, information is provided at the molecular level (DNA change, exon, predicted protein change, type of mutation, reported and concluded pathogenicity, source of material, technique used, unique database ID), brief clinical information (reported disease, patient unique ID and hyperlink to reference) and also the submitter ID.

Pathogenicity is dependent on clinical context and molecular findings. All putative novel mutations were detected in affected patients, segregated with disease status and were not present in control individuals. Putative mutations were then graded according to type of mutation: Frameshift and nonsense mutations were considered to be pathogenic; splice site variants were considered pathogenic if predicted to disrupt the consensus donor or acceptor splice sites; and missense variants were denoted as “probably pathogenic” unless proven by experimental evidence or detected in multiple families. Contributors can submit their variants online to the database upon registration for a login and password. The submission process and the curator’s process have previously been described by other databases using the LOVD system (Bayley et al., 2005; Fokkema et al., 2005).

NOVEL MUTATIONS

We report 10 novel germline variants in the *FLCN* gene (shaded in Table 1) including the first reported exon 8 mutation. These mutations were found in patients with clinical features of BHD and were not detected in 100 normal control individuals.

Missense variants: The c.1A>G (p.Met1?) in exon 4 affects the translation initiation codon and is predicted to be a pathogenic loss of function mutation. The pathogenicity of the second missense variant c.1198G>A (p.Val400Ile) in exon 11 is, as yet unclear, and it is classified as a variant of unknown significance.

Frameshift mutations: 4 frameshift mutations predicted to result in a premature stop codon and protein truncation have been found in exons 4, 6, 8, and 10. The c.240delC (p.Asp80GlufsX50) mutation was found in exon 4, the c.443_459del (p.His148ProfsX46) in exon 6 and the c.1076delC (p.Pro359LeufsX16) in exon 10. A 4-bp deletion, c.836_839del (p.Thr279ArgfsX13) is the first mutation to be discovered in exon 8.

Nonsense mutations: 2 nonsense mutations, c.583G>T (p.Gly195X) in exon 6 and c.1597C>T (p.Glu533X) in exon 14 have been identified.

Splicesite mutation: 2 mutations predicted to affect splicing include an intron 10 deletion c.1177-5_1177-3del (in silico analysis suggest that this deletion affects splicing and RNA studies currently underway to investigate further) and a single base substitution in exon 11 c.1300G>T (p.Glu434X).

Table 1. Variants listed on the Folliculin Database

Exon	Mutation	Protein Change (Predicted)	Type	Original Description	Reported Pathogenicity	Disease	References
4	c.1A>G	p.Met1?	Missense of start codon		Pathogenic	BHD	This report Birmingham, UK
4	c.3delG	p.Met1?	Deletion of start codon	c.458delG	Pathogenic	BHD	Bessis et al 2006
4	c.59delT	p.Phe20SerfsX35	Frameshift		Pathogenic	BHD	Schmidt et al 2005, Toro et al 2008
4	c.147delA	p.Gly50ValfsX5	Frameshift	c.602delA	Pathogenic	BHD	Toro et al 2008
4	c.235_238del	p.Ser79ThrfsX50	Frameshift	c.690-3delTCGG	Pathogenic	PSP	Painter et al 2005
4	c.240delC	p.Asp80GlufsX50	Frameshift		Pathogenic	BHD	This report Birmingham, UK
4i	c.250-2A>G		Splicesite	IVS4-2A>G	Pathogenic	BHD	Toro et al 2008
4i	c.250-1G>A		Splicesite	IVS4-1G>A	Pathogenic	BHD	Schmidt et al 2005
5	c.252delC	p.Cys85AlafsX45	Frameshift	c.707delC	Pathogenic	BHD	Schmidt et al 2005
5	c.296delA	p.Asp99ValfsX31	Frameshift	c.751delA	Pathogenic	BHD	Schmidt et al 2005, Toro et al 2008
5	c.319_320delGTinsCAC	p.Val107HisfsX26	Frameshift	c.774-5delGTinsCAC	Pathogenic	BHD	Toro et al 2008
5	c.347dupA	p.Leu117AlafsX16	Frameshift	c.802insA & c.802dupA	Pathogenic	BHD	Toro et al 2008, Palmirotta et al 2008
5	c.394G>A	p.Glu132Lys	Missense		Pathogenic	PSP	Frohlich et al 2008
5i	c.397-10_397-1del		Splicesite	IVS5-1delgtccctccag	Pathogenic	PSP	Gunji et al 2007
6	c.404delC	p.Pro135LeufsX42	Frameshift	c.857delC	Pathogenic	BHD	Gunji et al 2007, Leter et al 2008
6	c.420delC	p.Ile141SerfsX36	Frameshift	c.875delC	Pathogenic	BHD	van Steensel et al 2007, Leter et al 2008
6	c.443_459del	p.His148ProfsX46	Frameshift		Pathogenic	BHD	This report Maastricht, The Netherlands
6	c.469_471del	p.Phe157del	Deletion	c.924delTTC	Probably pathogenic	PSP	Ren et al 2008
6	c.583G>T	p.Gly195X	Nonsense		Pathogenic	BHD	This report Birmingham, UK
6	c.584delG	p.Gly195GlufsX28	Frameshift	c.1039delG & c.1036delG	Pathogenic	BHD	Schmidt et al 2005, Toro et al 2008
6	c.610_611delGCinsTA	p.Ala204X	Nonsense	c.1065-6delGCinsTA	Pathogenic	BHD	Leter et al 2008, Toro et al 2008
6i	c.619-1G>A		Splicesite	IVS6-1G>A & c.1074-1G>A.	Pathogenic	BHD	Leter et al 2008}
7	c.632_633delAGinsC	p.Glu211AlafsX12	Frameshift	c.1087delAGinsC	Pathogenic	BHD	Nickerson et al 2002, Schmidt et al 2005
7	c.637delT	p.Phe213LeufsX10	Frameshift	c.1092delT	Pathogenic	BHD	Schmidt et al 2005
7	c.655dupG	p.Ala219GlyfsX29	Frameshift	c.1110dupG	Pathogenic	BHD	Leter et al 2008
7	c.671_672del	p.Thr224SerfsX23	Frameshift	c.1126delCA	Pathogenic	BHD	Schmidt et al 2005
7	c.715C>T	p.Arg239Cys	Missense	c.1213C>T	Probably pathogenic	FcRCC	Woodward et al 2008
7	c.779G>A	p.Trp260X	Nonsense		Pathogenic	PSP	Frohlich et al 2008
7i	c.779+1G>T		Splicesite	IVS7+1 G>T	Pathogenic	BHD	Toro et al 2008
8	c.836_839del	p.Thr279ArgfsX13	Frameshift		Pathogenic	BHD	This report Birmingham, UK

Exon	Mutation	Protein Change (Predicted)	Type	Original Description	Reported Pathogenicity	Disease	References
9	c.890_893del	p.Glu297AlafsX25	Frameshift	c.1388_1391delAAAG & c.1345_1348delAAAG	Pathogenic	BHD, FcRCC	Woodward et al 2008, Palmirotta et al 2008
9	c.923_950dup	p.Gly319SerfsX80	Frameshift	c.1378-1405dup	Pathogenic	BHD	Nickerson et al 2002, Schmidt et al 2005, Toro et al 2008
9	c.943G>T	p.Glu315X	Nonsense	c.1398G>T.	Pathogenic	PSP	Graham et al 2005
9	c.1013delG	p.Trp338CysfsX15	Frameshift	c.1468delG	Pathogenic	BHD	Schmidt et al 2005
9	c.1021delC	p.Arg341GlyfsX12	Frameshift	c.1473delC	Pathogenic	BHD	Schmidt et al 2005
9i	c.1062+1G>A		Splicesite	IVS9+1G>A	Pathogenic	BHD	Schmidt et al 2005
9i	c.1062+2T>G		Splicesite	IVS9+2 T>G	Pathogenic	BHD	Schmidt et al 2005, Gad et al 2007, Toro et al 2008
10	c.1076delC	p.Pro359LeufsX16	Frameshift		Pathogenic	BHD	This report Birmingham, UK
10	c.1156_1175del	p.Ser386AspfsX63	Frameshift	c.1611_1630del	Pathogenic	PSP	Ren et al 2008
10i	c.1177-5_1177-3del		Splicesite		Probably Pathogenic	BHD	This report Birmingham, UK
10i	c.1177-2A>G		Splicesite	IVS10-2A>G	Pathogenic	BHD	van Steensel et al 2007
11	c.1198G>A	p.Val400Ile	Missense		Unknown variant	BHD	This report Birmingham, UK
11	c.1215C>G	p.Tyr405X	Nonsense	c.1670C>G	Pathogenic	BHD	Toro et al 2008
11	c.1252delC	p.Leu418TrpfsX50	Frameshift	c.1707delC	Pathogenic	BHD	Toro et al 2008
11	c.1285C>T	p.His429Tyr	Missense	c.1740C>T	Probably pathogenic	PSP	Ren et al 2008
11	c.1285delC	p.His429ThrfsX39	Frameshift	c.1733delC	Pathogenic	BHD	Nickerson et al 2002, Khoo et al 2002, Toro et al 2008
11	c.1285dupC	p.His429ProfsX27	Frameshift	c.1733insC, c.1740dupC & c.1277insC	Pathogenic	BHD, PSP	Nickerson et al 2002, Khoo et al 2002, Kawasaki et al 2005, Lamberti et al 2005, Murakami et al 2007, van Steensel et al 2007, Gunji et al 2007, Leter et al 2008, Toro et al 2008, Ren et al 2008
11	c.1286dupA	p.His429GlnfsX27	Frameshift	c.1741insA	Pathogenic	BHD	Toro et al 2008
11	c.1300G>A	p.Glu434Lys	Splicesite	c.1755G>A	Pathogenic	BHD	Toro et al 2008
11	c.1300G>C	p.Glu434Gln	Splicesite	c.1755G>C	Pathogenic	BHD	van Steensel et al 2007
11	c.1300G>T	p.Glu434X	Splicesite		Probably Pathogenic	BHD	This report Birmingham, UK
12	c.1301-7_1304del		Splicesite	c.1756-7del11	Pathogenic	BHD	Leter et al 2008
12	c.1305delT	p.Phe435LeufsX33	Frameshift	c.1758delT	Pathogenic	BHD	Schmidt et al 2005
12	c.1318_1334dup	p.Leu449GlnfsX25	Frameshift	c.1833_1849dup17	Pathogenic	FcRCC	Woodward et al 2008
12	c.1323delCinsGA	p.His442ThrfsX14	Frameshift	c.1778delCinsGA	Pathogenic	BHD	Leter et al 2008
12	c.1337_1343dup	p.Leu449PhefsX9	Frameshift	c.1792_1798dupGTTCCAC	Pathogenic	BHD	Imada et al 2009

Exon	Mutation	Protein Change (Predicted)	Type	Original Description	Reported Pathogenicity	Disease	References
12	c.1340_1346dup	p.Val452ProfsX6	Frameshift	c.1795insCCACCCT	Pathogenic	PSP	Gunji et al 2007
12	c.1379_1380del	p.Leu460GlnfsX25	Frameshift	c.1834-5delTC	Pathogenic	BHD	Schmidt et al 2005, Toro et al 2008
12	c.1389C>G	p.Tyr463X	Nonsense	c.1844C>G	Pathogenic	BHD	Nickerson et al 2002, Schmidt et al 2005, Toro et al 2008
12	c.1408_1418del	p.Gly470SerfsX12	Frameshift	c.1863-1873delGGGAGCCCTGT	Pathogenic	BHD	van Steensel et al 2007
12	c.1426dupG	p.Asp476GlyfsX10	Frameshift	c.1881insG	Pathogenic	BHD	Schmidt et al 2005
12	c.1429C>T	p.Arg477X	Nonsense	c.1884C>T	Pathogenic	BHD, PSP	Schmidt et al 2005, Graham et al 2005
12i	c.1432+1G>A		Splicesite	IVS12+1 G>A	Pathogenic	BHD	Toro et al 2008
13	c.1487_1490dup	p.Asp498CysfsX24	Frameshift	c.1945insCTGT	Pathogenic	BHD	Schmidt et al 2005
13	c.1523A>G	p.Lys508Arg	Missense	c.1978A>G	Pathogenic	BHD	Toro et al 2008
13	c.1528_1530del	p.Glu510del	Deletion	c.1983-5delIGAG	Pathogenic	BHD	Toro et al 2008
13	c.1533_1536del	p.Trp511X	Nonsense	c.1988delGATG	Pathogenic	PSP	Gunji et al 2007
14	c.1557delT	p.Phe519LeufsX18	Frameshift		Pathogenic	BHD	Kim et al 2008
14	c.1579C>T	p.Arg527X	Nonsense	c.2034C>T	Pathogenic	BHD	Schmidt et al 2005
14	c.1597C>T	p.Gln533X	Nonsense		Pathogenic	BHD	This report, Birmingham, UK

The 10 novel mutations in this paper are shaded in the table. (BHD=Birt-Hogg-Dubé syndrome, PSP = Primary Spontaneous Pneumothorax, FcRCC = Familial clear cell Renal Cell Carcinoma)

ANALYSIS OF THE DATABASE

A total of 70 unique *FLCN* variants are listed on the database. 64 are reported as pathogenic, 5 as probably pathogenic and 1 variant of unknown significance. Mutations are found in all coding exons (4-14) and a mutation hotspot exists in the polyC(8) tract in exon 11 with the c.1285dupC (previously reported as c.1733insC, c.1740dupC and C.1277insC) being the commonest mutation.

At the DNA level, the majority of mutations are deletions (31/70, 44.3%) with 5 mutations involving deletions of 10 or more bases (c.397-10_397-1del, c. 443_459del, c.1156_1175del, c.1301-7_1304del, c.1408_1418del). In addition, single base substitutions (25/70, 35.7%), duplications (10/70, 14.3%) and deletion/insertions (4/70, 5.7%) make up the rest of the mutations.

The majority of *FLCN* mutations listed are predicted (in the absence of nonsense-mediated mRNA decay) to lead to protein truncation and loss of function. The most frequent mutational consequences are frameshifts (37/70, 52.9%) which are predicted to result in a premature stop codon. In addition, there are 14 splicesite (14/70, 20%), 10 nonsense (10/70, 14.3%), 6 missense (6/70, 8.6%) and 3 deletion mutations (3/70, 4.3%). Among them, 2 mutations (c.1A>G and c.3delG) affect the translational initiation codon in exon 4.

A total of 12 mutations have been reported in PSP. 2 mutations, the exon 11 common mutation c.1285dupC and an exon 12 nonsense mutation c.1429C>T, are reported in both BHD and PSP. Three mutations have been found in FcRCC families. One of them, a 4-bp deletion in exon 9 (c.890_893del) has also been reported in BHD (Figure 1).

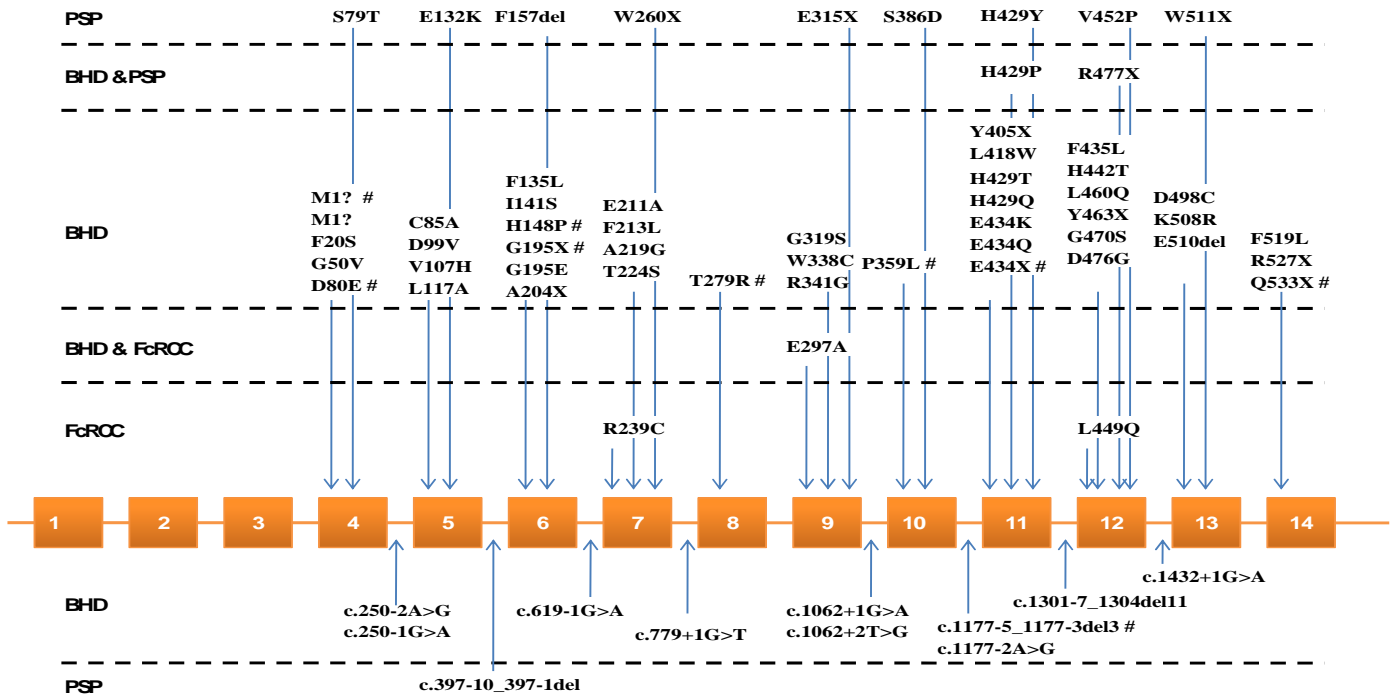


Figure 1. Gene map of distribution of mutations in the *FLCN* gene and the disease in which the mutations have been discovered. The boxes in the genogram (not to scale) represent the exons. Mutations below the genogram represent intronic mutations affecting splice sites and displayed as the coding sequence DNA change. Mutations above the genogram represent the exonic mutations and displayed as the protein change. Mutations followed by # represent the novel mutations reported in this paper. BHD = Birt-Hogg-Dubé syndrome, PSP= Primary spontaneous pneumothorax, FcRCC = Familial clear cell Renal Cell Carcinoma.

DATABASE AVAILABILITY AND SUBMITTER REQUIREMENTS

The data is accessible to the public and submitters will have to register for a login and password (to collect contact information for reference purposes and clarification of submitted data).

We are currently compiling a list of previously unpublished single nucleotide polymorphisms (SNPs) detected by molecular analysis in BHD patients that is thought to be non-pathogenic. This will be compiled with previously published SNP data and will allow the database to also serve as a single point of reference for SNPs in the *FLCN* gene. The database is freely accessible at <http://www.lovd.nl/flcn>.

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