

The Folliculin Mutation Database: An Online Database of Mutations Associated with Birt-Hogg-Dubé Syndrome



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ABSTRACT: The folliculin gene (*FLCN*), also known as *BHD*, is the only known susceptibility gene for Birt-Hogg-Dubé syndrome. BHDS is the autosomal dominant predisposition to the development of follicular hamartomas, lung cysts, spontaneous pneumothorax, and/or kidney neoplasms. To date, 53 unique germline mutations have been reported. *FLCN* mutation detection rate is 88%. *FLCN* encodes a predicted 579-amino acid protein, designated folliculin that is highly conserved between humans and homologs in mice, *Drosophila*, and *C. elegans*. We developed the first online database detailing all *FLCN* variants identified in our laboratory and reported in the literature. The *FLCN* database applies, and assists researchers in applying HGVS nomenclature guidelines. To date, the *FLCN* database includes 84 variants: 53 unique germline mutations and 31 SNPs. The majority of *FLCN* germline mutations are predicted to produce a truncated folliculin, resulting in loss of function. The *FLCN* mutations consist of: 45% (24/53) deletions, 32% (17/53) substitutions (10 putative-splice site, 5 nonsense, and 2 missense), 15% (8/53) duplications, 6% (3/53) insertion/deletions and 2% (1/53) insertions. The database strives to systematically unify current knowledge of *FLCN* variants and will be useful to geneticists and genetic counselors while also providing a rapid and systematic resource for investigators. © 2009 Wiley-Liss, Inc.

KEY WORDS: folliculin, *FLCN*, Birt-Hogg-Dubé syndrome. Fibrofolliculoma, mutation database

INTRODUCTION

Birt-Hogg-Dubé syndrome (BHDS; MIM# 135150) is an autosomal dominantly inherited genodermatosis that predisposes to the development of cutaneous hamartomas (fibrofolliculomas and trichodiscomas/angiofibromas), kidney neoplasms, lung cysts, and spontaneous pneumothorax [Birt et al., 1977; Toro et al., 1999; Toro et al.,

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2008]. The BHDS locus was mapped to the short arm of chromosome 17 (17p11.2) [Schmidt, et al., 2001]. In 2002, germline mutations in the folliculin gene (approved gene symbol *FLCN*; MIM# 607273; GenBank accession number AF517523; also known as *BHD*) were identified as responsible for BHDS susceptibility [Nickerson et al., 2002]. *FLCN* is composed of 14 exons. Researchers at the National Cancer Institute previously reported 36 unique *FLCN* germline mutations in 102 BHDS families [Nickerson et al., 2002; Schmidt et al., 2005; Toro et al., 2008], and 17 additional mutations have been reported by other investigators (Figure 1) [Khoo et al., 2002; van Steensel et al., 2007; Leter et al., 2008; Gunji et al., 2007; Graham et al., 2005; Painter et al., 2005; Kawasaki et al., 2005; Lamberti et al., 2005; Bessis et al., 2006; Murakami et al., 2007; da Silva et al., 2003; Toro et al., 2008; Woodward et al., 2008; Kim et al., 2008; Fuentes et al., 2009; Palmirotta et al., 2008]. Germline insertion or deletion of a cytosine in the hypermutable polycytosine (C8) tract in exon 11 of the gene has been detected in 53% of BHDS families and is suggested as a mutation "hot spot" [Schmidt et al., 2005; Toro et al., 2008]. The majority of *FLCN* germline mutations are predicted to truncate the FLCN protein, folliculin [Nickerson et al., 2002; Schmidt et al., 2005; Toro et al., 2008]. Germline *FLCN* mutations have been identified in the majority of patients with multiple skin fibrofolliculomas, and the relatives of probands have often been subsequently diagnosed with skin fibrofolliculomas, suggesting that many more cases are currently unrecognized. Recognition of BHDS is especially pertinent because patients with BHDS have a 50-fold and 7-fold increased risk for the development of spontaneous pneumothorax and renal tumors, respectively, compared with family members unaffected with BHDS [Zbar et al., 2002].

BHDS is associated with a histological spectrum of renal tumors ranging from hybrid oncocyctic (67%) to chromophobic (23%) to oncocyctic (3%) renal cell carcinoma (RCC) [Pavlovich et al., 2005]. Clear cell RCC (3%) has also been reported in a few BHDS cases. Somatic *FLCN* mutations or loss of heterozygosity (LOH) at the *FLCN* locus has been detected in at least one of the RCCs in all BHDS patients tested and 70% of RCCs either had a *FLCN* somatic mutation (53%) or LOH (17%) in the second *FLCN* copy [Vocke et al., 2005]. Interestingly, 75% of second hit somatic mutations were frameshifts, while no missense mutations were identified [Vocke et al., 2005]. Inactivation of both copies of *FLCN* occurred in several histologic subtypes of RCC, suggesting that inactivation of FLCN occurs at an early stage of renal tumorigenesis. Renal tumors in Nihon rats and German Shepherd dogs with renal cystadenocarcinoma and nodular dermatofibrosis (RCND) show biallelic inactivation in *Fln* by LOH or point mutation [Okimoto et al., 2004; Lingaas et al., 2008].

The high frequency of second hits in *FLCN* and its orthologs in tumors supports that *FLCN* is a tumor-suppressor gene.

Other pathology associated with BHDS includes: oral papules [Nadershahi et al., 1997; Toro et al., 1999], parotid oncocyctoma [Schmidt et al., 2005; Toro et al., 2008]; lipoma, angioliopoma, and collagenoma [Toro et al., 1999]; cutaneous neurothekeoma and meningioma [Vincent et al., 2003], multinodular goiter [Drummond et al., 2002; Welsch et al., 2005], and chorioretinal lesions [Walter et al., 1997; Godbolt et al., 2003]. Whether these clinical phenotypes are part of the spectrum of BHDS needs to be investigated in further studies.

Folliculin is predicted to encode a 579 amino acid protein that is highly conserved among species. *FLCN* mRNA is expressed in a variety of tissues including stromal cells, the distal nephron of the kidney, type I pneumocytes of the lung, and the skin and its appendages [Warren et al., 2004]. Recent studies have provided some insight into the functions of folliculin in the mTOR pathway. A novel folliculin-interacting protein, FNIP1, binds to 5'-AMP activated protein kinase, a negative regulator of mTOR. FLCN phosphorylation is facilitated by FNIP1 and is reduced by inhibitors of mTOR and AMPK activity, implicating FLCN/FNIP1 in the AMPK and mTOR signalling pathways [Baba et al., 2006]. Hartman and co-workers showed that downregulation of *FLCN* in mammalian cells reduces the phosphorylation of ribosomal protein S6, an indicator of TORC1 activity [Hartman et al., 2009].

van Slegtenhorst and co-workers deleted the *FLCN* (also known as *BHD*) homolog in *Schizosaccharomyces pombe* and using expression profiling showed that six permease and transporter genes, known to be down-regulated in $\Delta tsc1$ and $\Delta tsc2$, were up-regulated in Δbhd [van Slegtenhorst et al., 2007]. Loss of *Fln* sensitized yeast to rapamycin-induced increases in permease expression levels and rapamycin-induced lethality in Δfln yeast expressing the hypomorphic *Rhb1* allele [van Slegtenhorst et al., 2007]. These results suggest that *Fln* activates Tor2. Hartman et al. also generated a knockout mouse with targeted inactivation of the *Fln* gene that developed spontaneous oncocyctic renal cysts and tumors that resemble the RCCs in BHDS patients. The renal tumors exhibit low levels of phosphorylation of ribosomal protein S6. Altogether these studies showed that folliculin and TSC1/TSC2 share a common pathway but opposite roles in TORC1 [Hartman et al., 2009].

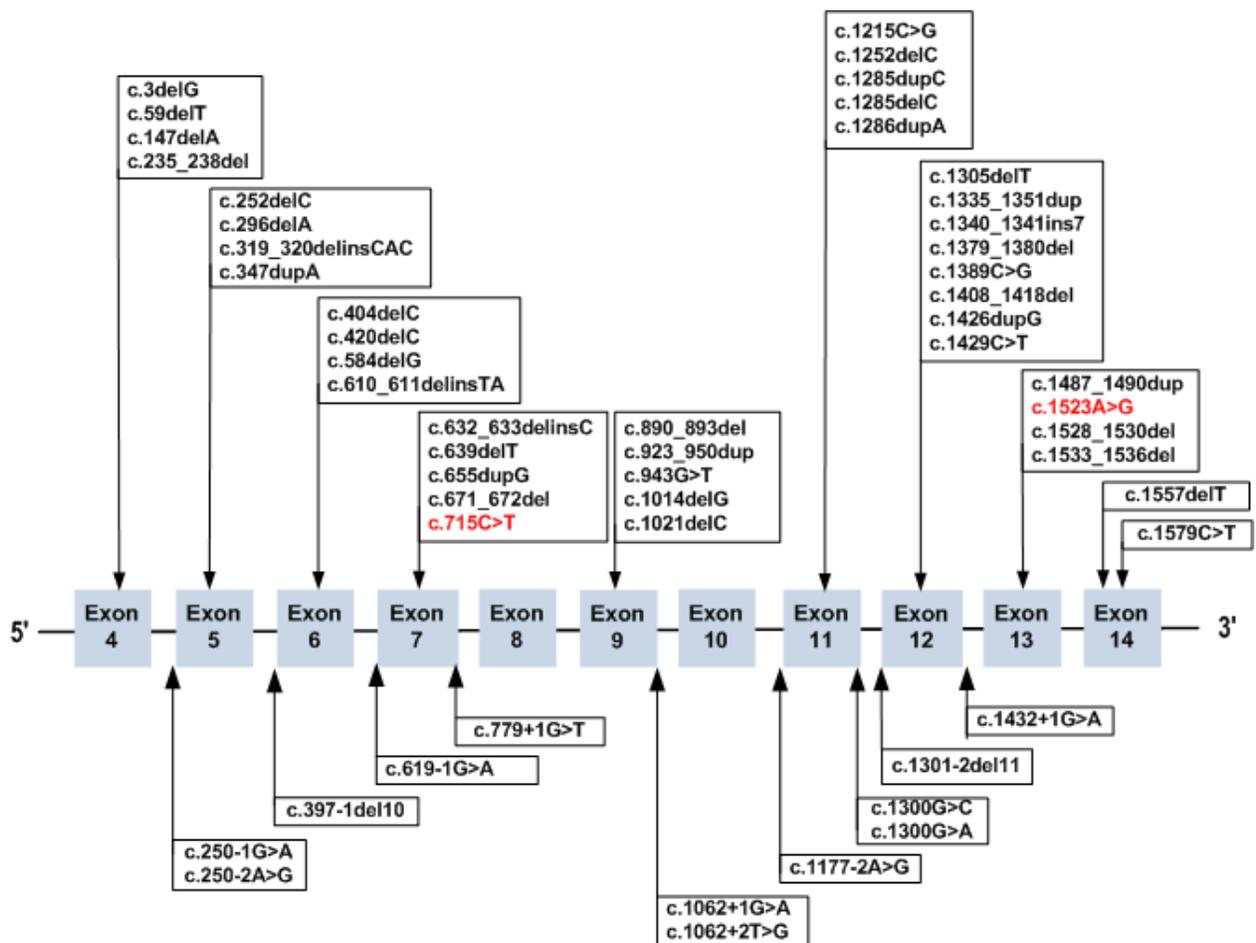


Figure 1. *FLCN* mutations in the database. Exon distribution of *FLCN* mutations associated with Birt-Hogg-Dubé Syndrome. Missense mutations are shown in red. *FLCN* frameshift, missense, and nonsense mutations are illustrated in the top panel and splice site mutations are shown in the lower panel. The nucleotide numbering is based on the published GenBank sequence NM_144997.5 and nucleotide +1 is the adenine of the ATG initiation codon.

Hereditary multifocal RCND in German shepherd dogs [Lium et al., 1985; Jonasdottir et al., 2000] and hereditary renal carcinoma in the Nihon rat [Hino et al., 2001; Okimoto et al., 2000] are naturally occurring animal models for BHDS. Germline mutations in the *FLCN* orthologous genes have been identified in affected animals with these renal cancer syndromes. A missense mutation in exon 7 in the canine *Flcn* [Lingaas et al., 2003], and an insertion of a cytosine in a C₅ tract in the Nihon rat *Flcn* [Okimoto et al., 2004] have been reported.

Since before 2008 there was no sequence variation database for *FLCN* and there are many inconsistencies in the reporting of *FLCN* mutations, we constructed a *FLCN* online database in August 2008 for The Inaugural Birt-Hogg-Dubé Symposium in Denmark and recently updated it (March, 30, 2009). The database characterizes all reported *FLCN* sequence variants reported to date. The purpose of the database is to systematically unify all current genetic knowledge of *FLCN* variants.

CONSTRUCTION OF DATABASE

The *FLCN* mutation database (<http://www.skinedatabase.com>) is based on the recently described Leiden Open Variation Database (LOVD) system and it was created using version 2 of the LOVD software [Fokkema et al., 2005]. LOVD software allows the easy creation and maintenance of a fully web-based gene sequence variation database [Bayley et al., 2008]. LOVD is platform-independent and uses PHP and MySQL open source software. The basic gene-centered and modular design of the database follows the recommendations of the Human Genome Variation Society (HGVS) and focuses on the collection of DNA sequence variations. The LOVD platform can include clinical data, and database contents may be downloaded and imported into a spreadsheet for further analysis [Bayley et al., 2008].

Nomenclature

We encourage researchers to submit newly identified variants to the database. Currently all database content is derived from published, peer-reviewed literatures. Unfortunately, the mutation nomenclature currently used for *FLCN* in publications is inconsistent and uses a variety of annotations rarely in agreement with HGVS guidelines. One of the aims of the HGVS and the *FLCN* database is to establish a consistent nomenclature. We recommend that DNA variants submitted to the *FLCN* database be described in accordance with the most up-to-date nomenclature guidelines. Recommendations of the HGVS can be found online at [HGVS.org](http://www.hgvs.org). The Mutalyzer program (<http://www.LOVD.nl/mutalyzer/>) using name generator or batch checker was used to check sequence variant descriptions [Wildeman et al., 2008].

The HGVS-recommended cDNA numbering starts from the first ATG of the full coding sequence. Protein reference sequences should represent the primary translation product. Currently many variants are present in the literature under several titles, and the correct identification of intronic variants is especially problematic. The *FLCN* mutation database will accept all variants and will assist in assigning the correct nomenclature. Due to inconsistency of the *FLCN* mutation nomenclature, an extra column including the originally reported variant description(s) at the cDNA level is included for those variants that have been reassigned.

Mutations

The term 'mutation' is reserved for those variants in which there is a reasonable suspicion that they are deleterious. All variants that disrupt the reading frame, affect highly conserved residues (conserved by sequence alignments), or disrupt the consensus donor or acceptor splice sites (GT-AG), and are not found in healthy controls, should be suspected as potentially pathogenic. Thus, current knowledge suggests that most missense changes will be pathogenic, but this is not always the case. Inclusion of sequence variants in the *FLCN* mutation database does not imply that there is convincing evidence for pathogenicity. Ideally, several lines of evidence should support publications which present mutations as "pathogenic", including screening DNA from a panel of >100 healthy individuals, describing the nature of the amino acid substitution (conservative/non-conservative) and the significance of the position in the protein (evolutionary conservation or occurrence in a known functional domain). It is important to note whether the mutation has previously been reported, if it has been found in several families, and if it segregates with disease within the family. However, none of these factors is definitive and each variant must be considered on its merits. Unfortunately, most mutations are currently reported without this accompanying analysis, and many have been identified in a single case or family. Thus, caution should be exercised when attempting to derive clinically relevant information from the database.

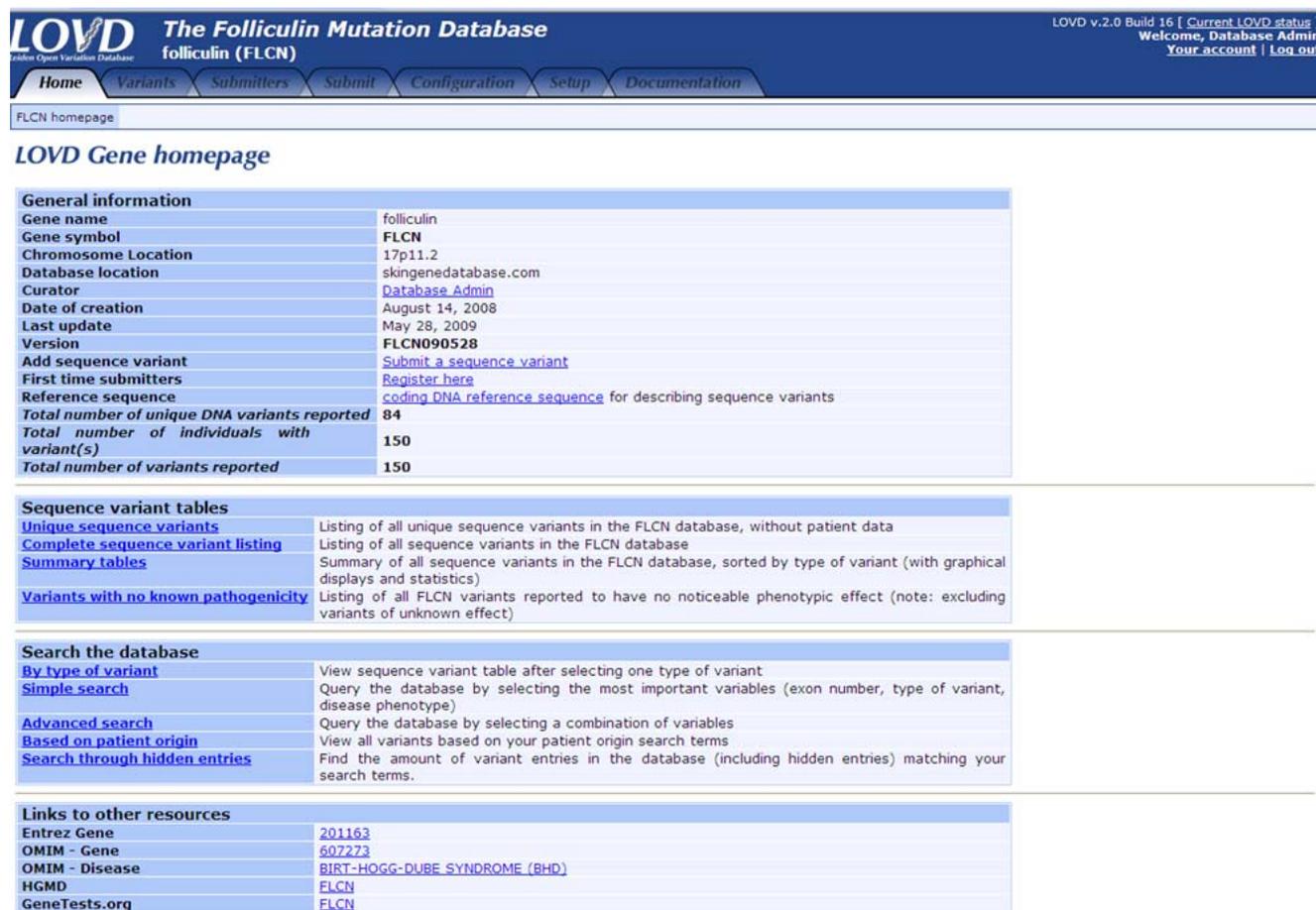
SNP

DNA variation in populations is generally benign and as such should be termed a single nucleotide polymorphism (SNP). Non-conserved missense variants and potential splice site mutations that do not disrupt the consensus splice sites are considered to be SNPs or rare variants of uncertain significance. Polymorphisms, including

intronic variants, synonymous (silent) variants, nonsynonymous missense variants found in a healthy control panel are included as SNPs in the database unless accompanied by clear evidence of pathogenic potential. Further information can be included under "remarks and references", such as the country of origin of the patient or study, the number of healthy controls tested for the variant, and any other supporting evidence.

How to submit a variant and use the database

The *FLCN* mutation database allows researchers to view variant information and deposit sequence variants. The database describes mutations exon by exon, summarizing submitted mutations in a single table. The researcher will first see the general information of the summary page about the gene, the database, and links to other sites related to the variant (Figure 2).



LOVD The Folliculin Mutation Database
folliculin (FLCN)

LOVD v.2.0 Build 16 [Current LOVD status]
Welcome, Database Admin
Your account | Log out

Home Variants Submitters Submit Configuration Setup Documentation

FLCN homepage

LOVD Gene homepage

General information	
Gene name	folliculin
Gene symbol	FLCN
Chromosome Location	17p11.2
Database location	skingenedatabase.com
Curator	Database Admin
Date of creation	August 14, 2008
Last update	May 28, 2009
Version	FLCN090528
Add sequence variant	Submit a sequence variant
First time submitters	Register here
Reference sequence	coding DNA reference sequence for describing sequence variants
Total number of unique DNA variants reported	84
Total number of individuals with variant(s)	150
Total number of variants reported	150

Sequence variant tables	
Unique sequence variants	Listing of all unique sequence variants in the FLCN database, without patient data
Complete sequence variant listing	Listing of all sequence variants in the FLCN database
Summary tables	Summary of all sequence variants in the FLCN database, sorted by type of variant (with graphical displays and statistics)
Variants with no known pathogenicity	Listing of all FLCN variants reported to have no noticeable phenotypic effect (note: excluding variants of unknown effect)

Search the database	
By type of variant	View sequence variant table after selecting one type of variant
Simple search	Query the database by selecting the most important variables (exon number, type of variant, disease phenotype)
Advanced search	Query the database by selecting a combination of variables
Based on patient origin	View all variants based on your patient origin search terms
Search through hidden entries	Find the amount of variant entries in the database (including hidden entries) matching your search terms.

Links to other resources	
Entrez Gene	201163
OMIM - Gene	607273
OMIM - Disease	BIRT-HOGG-DUBE SYNDROME (BHD)
HGMD	FLCN
GeneTests.org	FLCN

Figure 2. The *FLCN* mutation database home page.

Researchers can conduct different types of searches or import data into a spreadsheet for further analysis. The sequence variants are ordered by position relative to the cDNA reference sequence (Figure 3). Tables can be selected for viewing both specific variants and all reported variants. Variants can be re-ordered by database ID (DB-ID), exon, previous description, mutation type, and the authors who reported the variant.

LOVD The Folliculin Mutation Database							LOVD v.2.0 Build 16 [Current LOVD status]						
follucilin (FLCN)							Welcome, Database Admin						
Your account Log out													
Home		Variants		Submitters		Submit		Configuration		Setup		Documentation	
View unique variants		Search unique variants		View all contents		Full database search		Variant listing based on patient origin		Database statistics		Switch gene	
LOVD - Variant listings													
Unhide all columns													
84 entries													
100 entries per page													
Exon	DNA change	Protein	Original Description	Type	DB-ID	Variant remarks	DNA published						
04	c.3delG	p.Met1	c.458delG	Deletion	FLCN_00001	Frameshift	Bessis et al., 2006. Br J Dermatol 155:1067-9.						
04	c.59delT (Reported 3 times)	p.Phe20SerfsX35	c.514delT	Deletion	FLCN_00002	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
04	c.147delA	p.Gly50ValfsX5	c.602delA	Deletion	FLCN_00003	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
04	c.235_238del	p.Ser79ThrfsX50	c.690_3delTCGG	Deletion	FLCN_00004	Frameshift	Painter et al., 2005. Am J Hum Genet 76:522-527.						
04i	c.250-1G>A	-	IVS4-1G>A	Substitution	FLCN_00005	Splice site	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
04i	c.250-2A>G (Reported 4 times)	-	IVS4-2A>G	Substitution	FLCN_00006	Splice site	Toro et al., 2008. J Med Genet 45:321-31.						
05	c.252delC	p.Cys85AlafsX45	c.707delC	Deletion	FLCN_00007	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
05	c.296delA (Reported 2 times)	p.Asp99ValfsX31	c.751delA	Deletion	FLCN_00008	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
05	c.319_320delinsCAC (Reported 2 times)	p.Val107HisfsX26	c.774-5delGTinsCAC	Insertion/Deletion	FLCN_00009	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
05	c.347dupA	p.Leu117AlafsX16	c.802insA	Duplication	FLCN_00010	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
05i	c.397-1del10	-	IVS5-1delgtccctccag; nt852_1delgtccctccag c. 857delC	Deletion	FLCN_00011	Splice site	Gunji et al., 2007. J Med Genet 44:588-593.						
06	c.404delC	p.Pro135LeufsX42	c.875delC	Deletion	FLCN_00012	Frameshift	Gunji et al., 2007. J Med Genet 44:588-593.						
06	c.420delC	p.Ile141SerfsX36	c.875delC	Deletion	FLCN_00013	Frameshift	Leter et al., 2007. J Invest Dermatol 128:45-49.; van Steensel et al., 2007. J Invest Dermatol						
06	c.584delG (Reported 2 times)	p.Gly195GlufsX28	c.1039delG	Deletion	FLCN_00014	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
06	c.610_611delinsTA	p.Ala204X	c.1065_1066delGCinsTA	Insertion/Deletion	FLCN_00015	Nonsense	Toro et al., 2008. J Med Genet 45:321-31.; Leter et al., 2007. J Invest Dermatol 128:45-49.						
06i	c.619-1G>A	-	IVS6-1G>A; 1074-1G>A	Substitution	FLCN_00016	Splice site	Leter et al., 2007. J Invest Dermatol 128:45-49.						
07	c.632_633delinsC	p.Glu211AlafsX12	c.1087delAGinsC	Insertion/Deletion	FLCN_00017	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
07	c.639delT	p.Phe213LeufsX10	c.1092delT/c.1094delT	Deletion	FLCN_00018	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
07	c.655dupG	p.Ala219GlyfsX29	c.1110dupG	Duplication	FLCN_00019	Frameshift	Leter et al., 2007. J Invest Dermatol 128:45-49.						
07	c.671_672del	p.Thr224SerfsX23	c.1126delCA	Deletion	FLCN_00020	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
07	c.715C>T	p.Arg239Cys	c.1213C>T	Substitution	FLCN_00021	Missense	Woodward et al., 2008. Clin Cancer Res 14:5925-30.						
07i	c.779+1 G>T (Reported 3 times)	-	IVS7+1 G>T	Substitution	FLCN_00022	splice site	Toro et al., 2008. J Med Genet 45:321-31.						
09	c.890_893del	p.Glu297AlafsX25	c.1388_1391delAAAG	Deletion	FLCN_00023	Frameshift	Woodward et al., 2008. Clin Cancer Res 14:5925-30.						
09	c.923_950dup (Reported 5 times)	p.Gly319SerfsX80	c.1378-1405dup	Duplication	FLCN_00024	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
09	c.943G>T	p.Glu315X	c.1398G>T; 943G>T	Substitution	FLCN_00025	Nonsense	Graham et al., 2005. Am J Respir Crit Care Med 172:39-44						
Exon	DNA change	Protein	Original Description	Type	DB-ID	Variant remarks	DNA published						
09	c.1014delG	p.Trp338CysfsX15	c.1468delG/c.1469delG	Deletion	FLCN_00026	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
09	c.1021delC	p.Arg341GlyfsX12	c.1473delC/c.1476delC	Deletion	FLCN_00027	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
09i	c.1062+1G>A	-	IVS9+1G>A	Substitution	FLCN_00028	Splice site	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
09i	c.1062+2T>G (Reported 3 times)	-	IVS9+2 T>G	Substitution	FLCN_00029	Splice site	Toro et al., 2008. J Med Genet 45:321-31.						
10	c.1177-2A>G	-	IVS10-2A>G	Substitution	FLCN_00030	Splice site	van Steensel et al., 2007. J Invest Dermatol 127:588-93.						
11	c.1215C>G	p.Tyr405X	c.1670C>G	Substitution	FLCN_00031	Nonsense	Toro et al., 2008. J Med Genet 45:321-31.						
11	c.1252delC	p.Leu418TrpfsX50	c.1707delC	Deletion	FLCN_00032	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
11	c.1285dupC (Reported 32 times)	p.His429ProfsX27	c.1733insC	Duplication	FLCN_00033	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
11	c.1285delC (Reported 14 times)	p.His429ThrfsX39	c.1733delC	Deletion	FLCN_00034	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
11	c.1286dupA	p.His429GlnfsX27	c.1741insA	Duplication	FLCN_00035	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
11	c.1300G>C	p.Glu434	c.1755G>C	Substitution	FLCN_00036	Splice site	Toro et al., 2008. J Med Genet 45:321-31.; van Steensel et al., 2007						
11	c.1300G>A	p.Glu434	c.1755G>A	Substitution	FLCN_00037	Splice site,	Toro et al., 2008. J Med Genet 45:321-31.						
11i	c.1301-2del11	-	c.1756-7del11	Deletion	FLCN_00038	Splice site	Leter et al., 2007. J Invest Dermatol 128:45-49.						
12	c.1305delT	p.Phe435LeufsX33	c.1758delT; c.1760delT	Deletion	FLCN_00039	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
12	c.1335_1351dup	p.Pro451HisfsX23	c.1833_1849dup17bp	Duplication	FLCN_00040	Frameshift	Woodward et al., 2008. Clin Cancer Res 14:5925-30.						
12	c.1340_1341ins7	p.Thr448HisfsX10	c.1795insCCACCCT	Insertion	FLCN_00041	Frameshift	Gunji et al., 2007. J Med Genet 44:588-593.						
12	c.1379_1380del (Reported 2 times)	p.Leu460GlnfsX25	c.1834-5delTC	Deletion	FLCN_00042	Frameshift	Toro et al., 2008. J Med Genet 45:321-31.						
12	c.1389C>G (Reported 6 times)	p.Tyr463X	c.1844C>G	Substitution	FLCN_00043	Nonsense	Toro et al., 2008. J Med Genet 45:321-31.						
12	c.1408_1418del	p.Gly470SerfsX12	c.1863-73delGGGAGCCCTGT	Deletion	FLCN_00044	Frameshift	van Steensel et al., 2007. J Invest Dermatol 127:588-93.						
12	c.1426dupG	p.Asp476GlyfsX10	c.1881insG	Duplication	FLCN_00045	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
12	c.1429C>T	p.Arg477X	c.1884C>T	Substitution	FLCN_00046	Nonsense	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
12i	c.1432+1G>A	-	IVS12+1G>A	Substitution	FLCN_00047	Splice site	Toro et al., 2008. J Med Genet 45:321-31.						
13	c.1487_1490dup	p.Asp498CysfsX24	c.1945insCTGT	Duplication	FLCN_00048	Frameshift	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
13	c.1523A>G	p.Lys508Arg	c.1978A>G	Substitution	FLCN_00049	Missense	Toro et al., 2008. J Med Genet 45:321-31.						
13	c.1528_1530del	p.Glu510del	c.1983-5delGAG	Deletion	FLCN_00050	Deletion of amino acid 510.	Toro et al., 2008. J Med Genet 45:321-31.						
Exon	DNA change	Protein	Original Description	Type	DB-ID	Variant remarks	DNA published						
13	c.1533_1536del	p.Trp511X	c.1988delGATG	Deletion	FLCN_00051	Nonsense	Gunji et al., 2007. J Med Genet 44:588-593.						
14	c.1579C>T	p.Arg527X	c.2034C>T	Substitution	FLCN_00052	Nonsense	Schmidt et al., 2005. Am J Hum Genet 76:1023-33.						
14	c.1557delT	p.Phe519LeufsX18	c.1557delT	Deletion	FLCN_00053	Frameshift	Kim et al., 2008. J Korean Med Sci 23:332-5						
01	c.-487G>C	-	-	Substitution	SNP_00001	5'UTR	Kim et al., 2008. J Korean Med Sci 23:332-5; r1736209, Cho et al., 2008. BMC Medical Genetics 9:120-130						

Figure 3. Overview of the FLCN unique variant page.

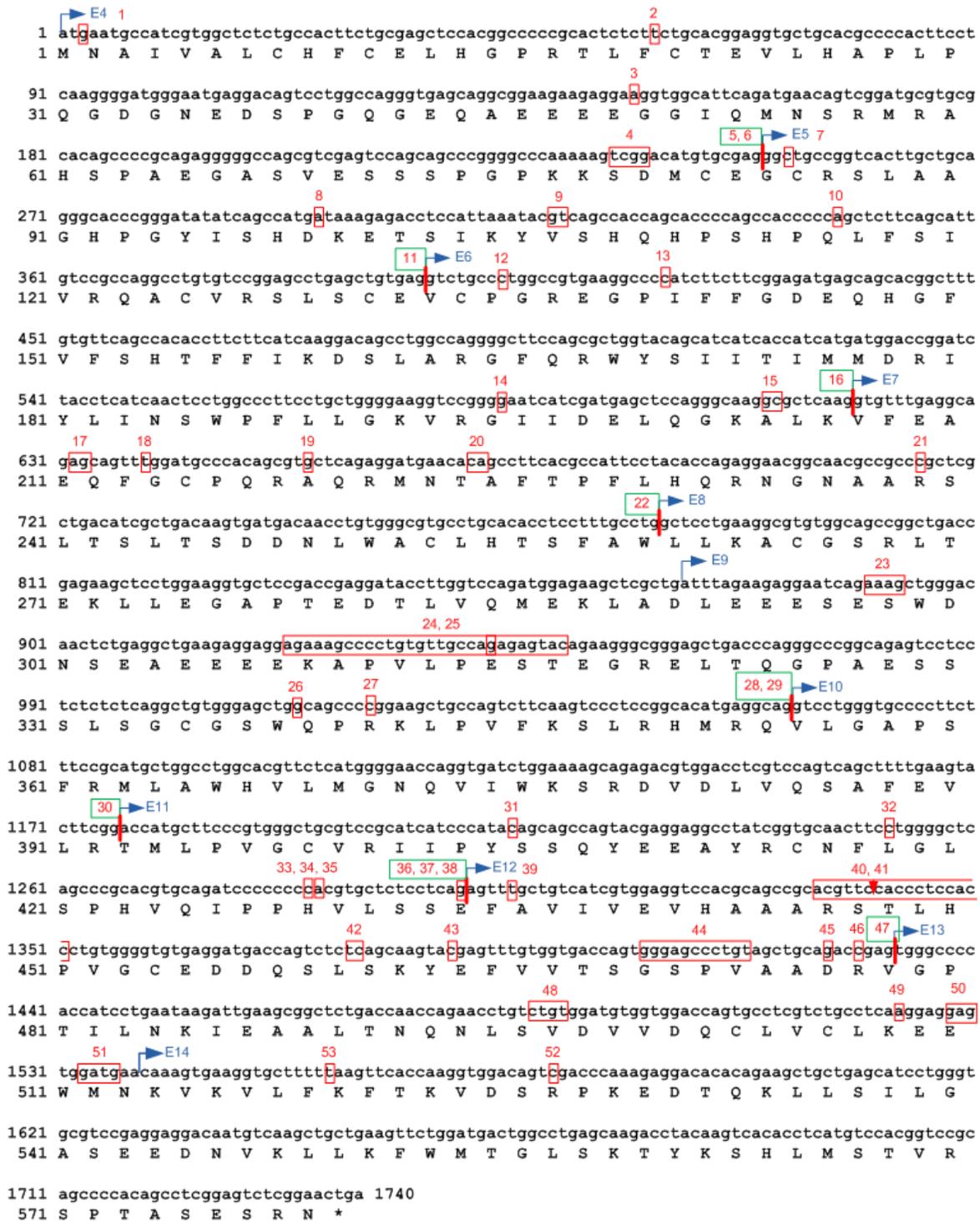


Figure 4. Map of FLCN amino acid and nucleotide sequences (NM_144997.5) including the intron-exon boundaries (shown in blue). The position of the 53 unique *FLCN* mutations is shown in red. The number in red corresponds to the variant ID (FLCN_00001 to FLCN_00053) in the FLCN mutation database. Splice site mutations are marked by green boxes and the affected splice site is marked by a heavy red bar. The *FLCN* coding sequence and its deduced amino acid sequence are obtained using NCBI ORF Finder (Open Reading Frame Finder).

We have created a map that contains the amino acids sequence encoded by the nucleotide sequence (NM_144997.5) of folliculin and indicates the intron-exon boundaries (Figure 4). The map is a useful tool for submitters in determining the location of their variant and in using the proper HGVS nomenclature. In addition, the FLCN map (Figure 4) indicates the position of the 53 unique *FLCN* mutations in the online database.

To submit the variant online, a new submitter will first be asked to register so that contact information is available for reference purposes and clarification of submitted details. After registering, he or she can submit the variant online. Then, researchers need to provide essential necessary information for mutation databases as described by the Human Genome Organization Mutation Database Initiative including an exact molecular description of the variant (DNA-level), and details about the source of the material and detection method used [Claustres et al., 2002; Bayley et al., 2008]. New submitted variants are given a unique identifier. After the curator's approval, the new variant is added to the database and all connected web pages are instantly updated.

DISCUSSION

To date, the *FLCN* database includes 84 variants: 53 unique germline mutations and 31 SNPs. Of 53 unique germline mutations, 36 were reported by NCI groups, and 17 were reported by other investigators worldwide (Figure 1). The frequency of *FLCN* mutations types consists of 45% (24/53) deletion, 32% (17/53) substitutions, 15% (8/53) duplications, 6% (3/53) insertion/deletions, and 2% (1/53) insertions. One of the 3 insertion/deletions causes a nonsense change. Among 17 mutations that were substitutions, 10 affect putative splice sites, 5 cause nonsense changes, and 2 were missense mutations. Although uncommon, germline *FLCN* missense mutations may occur in BHDS. The missense mutation (Lys508Arg) was confirmed by two independent laboratories and it was not present in DNA from 160 unrelated control individuals [Toro et al., 2008]. ClustalW multiple protein sequence alignments showed that amino acid Lys 508 of folliculin is highly conserved in chimpanzee, horse, mouse, rat, dog, cow, chicken, zebrafish and sea urchin. In addition, a block of continuous high conservation was shown between amino acids Leu507 to Val515. The conservation across species suggests an important biological role for Lys508 amino acid in folliculin. The second *FLCN* missense mutation (c.715C>T; p.Arg239Cys) was reported in a woman with BHDS but no family history of RCC (Woodward et al., 2008). The Arg239 residue is also conserved in *FLCN* homologs. The substitution c.715C>T in exon 7 of *FLCN* was not observed in 280 control chromosomes and mutation prediction programs indicated a strong likelihood that it represents a mutation (Woodward et al., 2008). The presence of missense mutation in exon 7 of *FLCN* is not surprising since a missense mutation in exon 7 (His255Arg) in the canine *FLCN* ortholog is the disease-associated germline mutation in hereditary multifocal RCND in German shepherd dogs [Lingaas et al., 2003]. The His255Arg mutation confers an amino acid change in a highly conserved region of canine folliculin.

No mutation has yet been identified in exons 8 and 10, and no large deletions have been reported but this probably reflects limited effort in this direction to date. Exon 12 was the most frequent site of mutations including 3 deletions, 2 nonsense, 2 duplications and 1 insertion. The hot spot mutation (c.1285dupC/1285delC) was the most common *FLCN* mutation reported to date [Schmidt et al., 2005; Toro et al., 2008]. The most 5' *FLCN* mutation reported occurred at nucleotide 3 (c. 3delG) in exon 4 affecting the initiator codon of *FLCN* [Bessis et al., 2006]. The most 3' mutation reported occurred at nucleotide 1579 (c.1579 C>T) in exon 14 and was predicted to produce a premature termination codon at position 527 [Schmidt et al., 2005]. There are previous reports of families with *FLCN* mutations and a prominent and almost exclusive history of spontaneous pneumothorax inherited in an autosomal dominant fashion [Painter et al., 2005; Graham et al., 2005].

The database contains 30 *FLCN* SNPs that were recently reported [Cho et al., 2008]. Among these 30 SNPs, three were coding variants (c.1269 G>A; p.His423His), (c.1278 G>A; p.Ile426Ile), (c.1333 C>T; p.Ala445Thr) and two of these three were synonymous (p.His423His and p.Ile426Ile). The non-synonymous variant (p.Ala445Thr) was not predicted to have significant functional consequences based on PolyPhen analysis [Sunyaev et al., 2001] and it was located in a region that is poorly conserved. Of the remaining SNPs, 4 were in the 5' untranslated region (UTR) and 23 were in the intronic region. Except for the two synonymous variants, all of the reported *FLCN* SNPs reported by Cho and co-workers, including those in the 5'UTR, mapped to regions of low conservation [Cho et al., 2008].

CONCLUSION

We developed a simple online database of *FLCN* gene variants. It provides a complete and updated summary of all reported *FLCN* mutations and SNPs. Since the *FLCN* gene was recently identified, we presume that only a fraction of all *FLCN* mutations associated with BHDS have been identified. To date, there is no evidence of genotype-phenotype correlation associated with specific mutation, type of mutation, or location in the gene [Toro et al. 2008]. This database will assist with increasing knowledge of *FLCN* mutations and with developing insight into phenotypes and *FLCN* protein function. Importantly, every *FLCN* mutation found in a patient increases confidence in the pathogenic role of that variant, improving the accuracy of genetic testing. We hope that the *FLCN* mutation database, which strives to systematically unify all current genetic knowledge of *FLCN* variants, will increase the confidence of clinical geneticists and physicians, will provide a convenient resource for research scientists, and may eventually assist in gaining novel insights into *FLCN* and BHDS.

We encourage researchers to submit newly identified variants to the database. Currently all database content is derived from published, peer-reviewed literature. We anticipate that in the future, a large number of variants will be submitted directly to the database. The *FLCN* mutation database (<http://www.skiningenedatabase.com>) is freely accessible and all researchers may submit new sequence variants online.

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