Title: Phenotype-genotype correlations for clinical variants caused by CYLD mutations

Corresponding Author: Dr. Nikoletta Nagy,

Corresponding Author's Institution:

First Author: Nikoletta Nagy

Order of Authors: Nikoletta Nagy; Katalin Farkas; Lajos Kemény; Márta Széll

Abstract: Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant condition characterized by skin appendageal neoplasms including cylindromas, trichoepitheliomas, and/or spiradenomas. In 1996, the gene locus for BSS was mapped to 16q12-13, and, in 2000, mutations in the cylindromatosis (CYLD) gene were determined to cause BSS, familial cylindromatosis (FC; OMIM 132700) and multiple familial trichoepithelioma type 1 (MFT1; OMIM 601606). The CYLD gene encodes an enzyme with deubiquitinase activity. To date, a total of 95 different diseases-causing mutations have been published for the CYLD gene. A summary of mutations identified in Hungarian patients and a review of previously published mutations are presented in this update. The majority of the sequence changes are frameshift (48%), nonsense (27%), missense (12%) and splice-site (11%) mutations; however, two in-frame deletions have also been reported Most mutations are located in exons 9-20. Analysis of the identified CYLD gene mutations and the observed BSS, FC and MFT1 clinical phenotypes of the patients revealed significant genotype-phenotype correlations. Elucidation of these genotype-phenotype correlations is critical for the diagnosis of these rare monogenic skin diseases. In addition, characterized correlations may promote the understanding of their mechanisms and may hopefully contribute to the development of future therapeutic modalities.

Cover Letter

Dear Dr. Verloes,

R.e. „Phenotype - genotype correlations for clinical variants caused by CYLD mutations" by
Nikoletta Nagy, Katalin Farkas, Lajos Kemény, Márta Széll

It was a pleasure to meet you at the ESHG in Milan.
Here I would like to submit my work as a review to the EJHG.
Thank you very much for considering my work.

Kind Regards,
Nikoletta Nagy

Phenotype–genotype correlations for clinical variants caused by *CYLD* mutations

Nikoletta Nagy[1,2,3], Katalin Farkas[3], Lajos Kemény[2,3], Márta Széll[1,3]

[1]Department of Medical Genetics, University of Szeged, Szeged, HUNGARY

[2]Department of Dermatology and Allergology, University of Szeged, Szeged, HUNGARY

[3]Dermatological Research Group of the Hungarian Academy of Sciences, University of Szeged, Szeged, HUNGARY

Corresponding author:

Dr. Nikoletta Nagy, Department of Medical Genetics, University of Szeged

4 Somogyi Béla utca, H-6720 Szeged, HUNGARY

Email: nikoletta.nagy@gmail.com

SUMMARY

Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant condition characterized by skin appendageal neoplasms including cylindromas, trichoepitheliomas, and/or spiradenomas. In 1996, the gene locus for BSS was mapped to 16q12-13, and, in 2000, mutations in the *cylindromatosis (CYLD)* gene were determined to cause BSS, familial cylindromatosis (FC; OMIM 132700) and multiple familial trichoepithelioma type 1 (MFT1; OMIM 601606). The *CYLD* gene encodes an enzyme with deubiquitinase activity. To date, a total of 95 different diseases-causing mutations have been published for the *CYLD* gene. A summary of mutations identified in Hungarian patients and a review of previously published mutations are presented in this update. The majority of the sequence changes are frameshift (48%), nonsense (27%), missense (12%) and splice-site (11%) mutations; however, two in-frame deletions have also been reported Most mutations are located in exons 9–20. Analysis of the identified *CYLD* gene mutations and the observed BSS, FC and MFT1 clinical phenotypes of the patients revealed significant genotype–phenotype correlations. Elucidation of these genotype–phenotype correlations is critical for the diagnosis of these rare monogenic skin diseases. In addition, characterized correlations may promote the understanding of their mechanisms and may hopefully contribute to the development of future therapeutic modalities.

Key words: cylindromatosis gene, Brooke-Spiegler syndrome, familial cylindromatosis, familial trichoepitheliomatosis, mutation update

BACKGROUND

Brooke-Spiegler syndrome (BSS, OMIM 605041) is a rare monogenic skin disease (genodermatosis) characterized by the development of a wide variety of benign skin appendageal tumors, such as cylindromas, trichoepitheliomas and/or spiradenomas [Brooke, 1892; Spiegler, 1899]. The first symptoms of BSS are small skin-colored papules, which occur in childhood and adolescence [EVANS, 1954; Sima et al., 2010]. These tumors grow slowly in size and continue appear throughout the lifetime of the patient [Blake and Toro, 2009]. Expression of the papules exhibits wide variation among and within affected families [Poblete et al., 2002].

Cylindromas are slowly growing benign tumors that are usually located on the scalp and face. Typically, they appear as multiple turban-like protrusions on the scalp, which are also referred as turban tumors [Uede et al., 2004]. Cylindromas are histologically characterized by dermal nodules of epithelial cells: large cells with abundant cytoplasm occur at the center of the tumors, where as small basaloid cells occur at the periphery. The cells are lined by membrane-like basement material and arranged in a "jigsaw puzzle" pattern [Lian and Cockerell, 2005]. Cylindromas express hair keratins [Massoumi et al., 2006].

Trichoepitheliomas are small benign skin-colored tumors and are typically present at the center of the face, mostly around the nose, periorbitally and in the nasolabial folds [Uede et al., 2004]. Histologically, trichoepitheliomas are characterized by basaloid cells with peripheral palisades that are arranged in nests or cribriform patterns surrounded by dense stroma and fibroblasts [Alsaad et al., 2007].

Spiradenomas are purple benign nodular tumors, which are usually located on the trunk or limbs [Uede et al., 2004]. Histologically, spiradenomas are composed of large tumor nests comprising two types of epithelial cells [Obaidat et al., 2007]. Large light-colored cells with

3

abundant cytoplasm at the center of the nests are surrounded by small darker cells at the periphery [Obaidat et al., 2007; Michal et al., 1999]. Spiradenomas rarely become malignant but can transform into spiradenocarcinomas [Cooper et al., 1985; Engel et al., 1991; Chou et al., 2004]. Hybrid tumors can also occur, such as spiradenocylindromas, which exhibit the characteristics of both cylindromas and spiradenomas [Kazakov et al., 2005; Kazakov et al., 2008; Pizinger and Michal, 2000].

BSS is transmitted as an autosomal dominant condition affecting males and females equally [GUGGENHEIM and Schnyder, 1961]. BSS and its phenotypic variants were independently mapped to chromosome 16q12-q13 by several groups [Fenske et al., 2000; Biggs et al., 1995; Biggs et al., 1996; Takahashi et al., 2000]. Within the mapped region, the cylindromatosis gene *(CYLD)* was identified as the causative gene responsible for the development of the disease [Takahashi et al., 2000; Bignell et al., 2000]. The *CYLD* gene (GenBank accession number NM_015247) spans 56 kb and contains 20 exons, the first 3 of which are untranslated, and 19 introns. Of the 17 known splice variants, 13 affect protein coding regions, and the remaining produce non-coding transcripts (http://ensemble.org). The tumor suppressing *CYLD* gene encodes an enzyme with deubiquitinase activity. The CYLD enzyme post-translationally modifies its target proteins by removing Lys63-linked ubiquitin chains [Kovalenko et al., 2003]. The protein interacts with several members of the NF-κB signaling pathway, including the TRAF2, TRAF6, NEMO and BCL3 proteins, acting as a negative regulator [Hutti et al., 2009].

In 2000, the first 21 mutations of the *CYLD* gene were identified in the affected members of 21 families with familial cylindromatosis (FC; OMIM 132700), a clinical variant of BSS [Bignell et al., 2000]. Since 2000, several reports have described mutations in the *CYLD* gene in different cases from around the world [Blake and Toro, 2009]. In addition to BSS and FC, mutations of the *CYLD* gene have also been reported in patients with multiple familial

4

trichoepithelioma type 1 (MFT1; OMIM 601606) [Hu et al., 2003]. BSS, FC and MFT1 show overlapping phenotypic features: some BSS patients develop multiple skin appendage tumors including cylindromas, trichoepitheliomas, and spiradenomas, whereas patients with FC develop only cylindromas, and patients with MFT1 develop only trichoepitheliomas (Table I) [Brooke, 1892] [Spiegler, 1899; Ancell, 1842; Fordyce, 1892; Zhang et al., 2004]. BSS, FC and MFT1 were originally described as distinct clinical entities, but due to their overlapping clinical symptoms and their manifestation within the same families, they are now considered as a clinical variants that represent a phenotypic spectrum of a single entity [Lee et al., 2005; Welch et al., 1968; Young et al., 2006; Oranje et al., 2008].

To date, a total of 95 mutations have been reported for the *CYLD* gene. Each of the clinical variants is associated with approximately one third of the known mutations: BSS (35%), MFT1 (36%) and FC (41%). Note that some of the mutations (12%) were detected in two or three different clinical variants: the presence of the c.1112C/A p.S371X, c.2272C/T p.R758X and c.2806C/T p.R936X nonsense mutations can lead to the development of all three clinical variants [Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Oiso et al., 2004; Zhang et al., 2006; Kazakov et al., 2009; Kazakov et al., 2011; Nagy et al., 2013]. Therefore, it has been hypothesized that BSS, FC and MFT1 are not different disease entities, but represent a phenotypic spectrum of the same disease.

SUMMARY OF HUNGARIAN BSS PATIENTS

In Hungary, mutation screening for the *CYLD* gene has been available since 2010. Screening has been performed with direct sequencing of all coding regions and flanking introns of the *CYLD* gene. Once a putative causative variant was identified in a patient, the available,

clinically symptom-free family members and unrelated, healthy control individuals were also investigated. To our knowledge, our workgroup is the only one in Hungary that performs genetic screening for BSS, FC and MFT1.

We have identified two Hungarian pedigrees with BSS. One family (Pedigree I) is located in Szeged, Hungary; the other (Pedigree II), located in Hungary close to Szekszárd, has Bukovinian (Romania) origin. Two affected individuals in Pedigree I — a father and his daughter — exhibit mild symptoms on their scalp, middle face and trunk [Linos et al., 2011]. Pedigree II contains 21 affected family members from seven generations, all of whom exhibit severe symptoms also located on their scalp, middle face and trunk [Grossmann et al., 2013]. Histological investigation of excised tumors confirmed the presence of cylindromas, trichoepitheliomas and spiradenomas for the affected members of both pedigrees, establishing the diagnosis of BSS for both families. Mutation screening of the *CYLD* gene identified a novel missense mutation (c.2613C>G, p.His871Gln) in Pedigree I and a recurrent nonsense mutation (c.2806C>T, p.Arg936X) in Pedigree II [Grossmann et al., 2013; Linos et al., 2011]. The results of functional characterization indicated that the newly identified missense mutation from Pedigree I leads to increased ubiquitination of NEMO by modifying the deubiquitinating activity of the CYLD protein [Linos et al., 2011]. Since nonsense mutation in Pedigree II is recurrent, functional analysis was not carried out for this family. However, the same mutation was identified in an Anglo-Saxon pedigree with mild BSS symptoms. We performed haplotype analysis for these two geographically distant pedigrees and found that the mutations resulted from two independent mutational events, suggesting that is the mutations represent a mutational hotspot on the *CYLD* gene [Grossmann et al., 2013]. Our results correlate well with the data reported in the literature supporting the assumption that this is a mutational hotspot on the gene: the c.2806C>T, p.Arg936X nonsense mutation has been described in all three allelic variants in the disease spectrum caused by *CYLD* mutations

6

and results in different symptom severity as well as the development of different clinical variants [Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Saggar et al., 2008; Kazakov et al., 2009; Nagy et al., 2013].

VARIANTS OF THE *CYLD* GENE

A PubMed (http://www.ncbi.nlm.nih.gov/pubmed) literature search was performed to identify all patients with *CYLD* mutations. In addition, data from Hungarian BSS pedigrees carrying *CYLD* mutations have also been included for the present publication. To date, a total of 95 mutations have been published for the *CYLD* gene, and the individuals carrying these mutations present with phenotypic features of BSS, FC and/or MFT1 (Figure 1). Mutations are named according to Human Genome Variation Society (HGVS) nomenclature guidelines (www.HGVS.org) and numbered with respect to the *CYLD* gene reference sequence (ENSG00000083799 corresponding to the *CYLD* gene transcript ENST00000311559).

The majority of the *CYLD* mutations (98%) were reported in coding regions. Distribution of the mutations within exons is unequal: 99% of the mutations are located within exons 9–20 (Figure 1). The majority of the sequence changes are frameshift (48%), nonsense (27%), missense (12%) or splice-site (11%) mutations; however two in-frame deletions have also been reported.

Approximately half of the identified *CYLD* mutations (48%) are frameshift mutations arising from the insertion or deletion of a small number of nucleotides. The majority of these changes lead to the formation of premature stop codons causing truncation and, thus, the dysfunction of the CYLD protein (Figure 2). Frameshift mutations are also unequally distributed in the *CYLD* gene (Figure 3): one third are located within the region spanning exons 5–11 [Bignell et al., 2000; Grossmann et al., 2013; Saggar et al., 2008; Nasti et al., 2009; Ying et al., 2012;

Liang et al., 2008; Zheng et al., 2004], whereas the rest (66%) occur within the region spanning exons 12–20 [Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Lv et al., 2008] [Saggar et al., 2008; Oiso et al., 2004; Reuven et al., 2013; Chen et al., 2011; Salhi et al., 2004; Melly et al., 2012; Heinritz et al., 2006]. Exon 17 is a mutational hotspot, containing 20% of all identified frameshift mutations [Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Lv et al., 2008; Saggar et al., 2008; Oiso et al., 2004]. Only a quarter of the frameshift mutations (27%) are recurrent, i.e., having been reported by at least two studies [Sima et al., 2010; Poblete et al., 2002; Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Saggar et al., 2008; Oiso et al., 2004; Scheinfeld et al., 2003; Hester et al., 2013]. In general, different frameshift mutations can lead to the development of the different allelic variants and show phenotypic diversity (Figure 4) [Bignell et al., 2000; Saggar et al., 2008; Nasti et al., 2009; Ying et al., 2012; Liang et al., 2008; Scheinfeld et al., 2003; Hester et al., 2013; Scholz et al., 2010].

Nonsense mutations causing truncation and, thus, CYLD protein dysfunction are also common, accounting for one quarter (27%) of the *CYLD* mutations identified so far (Figure 2). The distribution of nonsense mutations is also unequal in the *CYLD* gene (Figure 3). One third of the nonsense mutations occur within the region spanning exons 9–11 [Sima et al., 2010; Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Kazakov et al., 2009; Almeida et al., 2008], while the majority (72%) are located within the region spanning exons 12–20 [Sima et al., 2010; Bignell et al., 2000; Zhang et al., 2004; Young et al., 2006; Oranje et al., 2008; Bowen et al., 2005; Grossmann et al., 2013; Oiso et al., 2004; Kazakov et al., 2009; Nagy et al., 2013; Nagy et al., 2012; Zheng et al., 2004; Chen et al., 2011; Almeida et al., 2008]. This latter region does not seem to contain a further mutational hotspot. It is of interest to note that 25% of the identified nonsense mutations

affect glutamine amino acid residues within the CYLD protein, replacing them with stop codons [Sima et al., 2010; Bignell et al., 2000; Grossmann et al., 2013; Zheng et al., 2004; Chen et al., 2011; Almeida et al., 2008]. Nearly half of the nonsense mutations (40%) are recurrent [Sima et al., 2010; Bignell et al., 2000; Oranje et al., 2008; Bowen et al., 2005; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Oiso et al., 2004; Zhang et al., 2006; Kazakov et al., 2009; Kazakov et al., 2011; Nagy et al., 2013; Zheng et al., 2004; Chen et al., 2011]. In general, nonsense mutations − even the same mutation in different individuals − lead to the development of the different clinical variants; thus, nonsense mutations have been associated with the highest phenotypic diversity (Figure 4) [Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Oiso et al., 2004; Zhang et al., 2006; Kazakov et al., 2009; Kazakov et al., 2011; Nagy et al., 2013].

Missense mutations account for 12% of all mutations identified on the *CYLD* gene (Figure 2). The distribution of missense mutations is also unequal on the *CYLD* gene (Figure 3): all are located within the region spanning exons 12–20 [Sima et al., 2010; Hu et al., 2003; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Kazakov et al., 2009; Nagy et al., 2012; Zheng et al., 2004; Almeida et al., 2008; Espana et al., 2007; Wang et al., 2010; Zuo et al., 2007]. Only a quarter (27%) of these are recurrent mutations [Sima et al., 2010; Hu et al., 2003; Grossmann et al., 2013; Saggar et al., 2008; Kazakov et al., 2009]. In general, missense mutations of the *CYLD* gene have been associated with less phenotypic diversity that other kinds of mutations: three quarters (73%) have been reported in MFT1 only (Figure 4) [Nagy et al., 2012; Zheng et al., 2004; Almeida et al., 2008; Espana et al., 2007; Wang et al., 2010; Zuo et al., 2007].

Splice-site mutations account for 11% of all mutations (Figure 2). The distribution of the splice-site mutations is also unequal (Figure 3): all of them occur within the region spanning exons 10–18 [Bignell et al., 2000; Grossmann et al., 2013; Kacerovska et al., 2013; Van den Ouweland et al., 2011; Kazakov et al., 2011; Nasti et al., 2009; Ying et al., 2012; Liang et al., 2008; Huang et al., 2009; Ly et al., 2004]. Less than a quarter (18%) are recurrent [Bignell et al., 2000; Grossmann et al., 2013; Van den Ouweland et al., 2011; Kazakov et al., 2011]. In general, splice-site mutations can lead to the development of different clinical variants and show phenotypic diversity (Figure 4) [Kacerovska et al., 2013; Van den Ouweland et al., 2011; Nasti et al., 2009; Liang et al., 2008; Huang et al., 2009].

In addtion, two in-frame deletions have also been reported: these mutations are located within exons 19 and 20. Both of them lead to the development of the FC clinical variant [Van den Ouweland et al., 2011].

ETHNIC VARIATION

Mutations of the *CYLD* gene have been reported among patients with Irish, Japanese, Spanish, German, Algerian, Turkish, Hungarian, Slovakian, Italian, Scandinavian, Taiwanese, Turkish, Canadian and African backgrounds [Bowen et al., 2005; Kacerovska et al., 2013; Saggar et al., 2008; Nagy et al., 2013; Nagy et al., 2012; Nasti et al., 2009; Salhi et al., 2004; Huang et al., 2009; Amaro et al., 2010]. However, the majority of the published mutations are reported for patients from the UK, USA and China [Bignell et al., 2000; Lv et al., 2008; Ying et al., 2012; Liang et al., 2008; Zheng et al., 2004; Chen et al., 2011; Wang et al., 2010; Zuo et al., 2007]. Recurrent mutations have been detected in patients living in geographically distant regions: the c.1112C/A p.S371X nonsense mutation was identified in Irish, African American, Slovakian and Chinese patients [Bowen et al., 2005; Linos et al., 2011; Lv et al., 2008;

Kacerovska et al., 2013] and the c.2806C/T p.R936X nonsense mutation was detected in Canadian, American, Anglo-Saxon and Hungarian patients [Bignell et al., 2000; Bowen et al., 2005; Nagy et al., 2013]. The appearance in geographically distant patients might suggest that these recurrent mutations are located in mutational hotspots of the *CYLD* gene [Nagy et al., 2013]. Haplotype analysis of patients carrying the same recurrent mutation indicated a common ancestor for some patients [Bignell et al., 2000] and independent mutational events for others [Nagy et al., 2013].

A comparison of ethnicity and allelic variation revealed geographical differences. The clinical phenotype of MFT1 was observed mostly in individuals from China but also in African, Afro-American, Taiwanese, Algerian, Turkish, Italian and Spanish patients [Lv et al., 2008; Kacerovska et al., 2013; Liang et al., 2008; Zheng et al., 2004; Chen et al., 2011; Wang et al., 2010; Zuo et al., 2007]. The majority of patients exhibiting the FC clinical variant were from the UK and the USA, but some patients were from Italy and Ireland [Bignell et al., 2000; Bowen et al., 2005; Nasti et al., 2009]. Like FC, the majority of patients presenting the BSS clinical variant were from the UK and the USA; however, patients from Hungary, Slovakia, Italy, Scandinavia, Spain and Canada have also been reported [Bignell et al., 2000; Bowen et al., 2005; Kacerovska et al., 2013; Nagy et al., 2013; Nagy et al., 2012; Nasti et al., 2009].

BIOLOGICAL RELEVANCE

The protein encoded by the *CYLD* gene (GenBank NP_056062), consisting of 956 amino acids with a molecular weight of approximately 120 kD, exhibits deubiquitinase activity. The CYLD protein plays a role in the posttranslational modification of several proteins by removing lysine 63-linked ubiquitin chains. Lysine 63-linked polyubiquitination affects the interactions among proteins by creating specific recognition sites for other proteins. However,

11

the CYLD protein, which primarily deubiquitinates lysine 63-linked chains, can also process lysine 48-linked chains as well [Reiley et al., 2006; Komander et al., 2008]. The known interaction partners of the CYLD protein include TNF-receptor-associated factor proteins (TRAF2, TRAF6 and TRAF7) and the NEMO protein, a negative regulator of the NF-kB signaling pathway [Haglund and Dikic, 2005]. CYLD is involved in the regulation of several biological processes, such as cell proliferation and inflammation [Gao et al., 2008].

The N-terminal of the CYLD protein can be divided into two regions based on the occurrence of the mutations: no mutations have been detected in the region encoded by exons 4 and 5, whereas the region encoded by exons 5–11 contains approximately one fifth (18%) of the mutations identified to date. The N-terminal of the CYLD protein contains three cytoskeleton-associated glycine rich domains (CAP-GLY), at which the CYLD protein connects to microtubules [Gao et al., 2008]. The N-terminal of the CYLD protein is highly conserved through evolution (Figure 5). Mostly frameshift and nonsense mutations occur in this region, as well as the two known splice-site mutations [Sima et al., 2010; Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Kacerovska et al., 2013; Saggar et al., 2008; Kazakov et al., 2009] [Nasti et al., 2009; Liang et al., 2008; Zheng et al., 2004; Almeida et al., 2008; Ly et al., 2004]. Most frameshift mutations occur in the first (amino acids 127 to 203) and the third (amino acids 472 to 540) CAP-GLY domains, whereas most of the nonsense and splice-site mutations occur in the region of the third CAP-GLY domain (Figure 3). Frameshift and nonsense mutations occurring in this region have been identified for all clinical variants [Sima et al., 2010; Bignell et al., 2000; Bowen et al., 2005; Kacerovska et al., 2013; Saggar et al., 2008; Nasti et al., 2009; Liang et al., 2008; Zheng et al., 2004; Almeida et al., 2008]. The splice-site mutations of this region resulted in the development of the BSS or MFT1 clinical variants [Grossmann et al., 2013; Kazakov et al., 2009; Ly et al., 2004]. Missense mutation has not been detected at the N-terminal of the CYLD protein.

The *CYLD* gene has changed relatively little through evolution: the similarity between the human and mouse genes is 94%, and the homology is especially high at the 3' end of the gene (Figure 5). This region of the CYLD protein, encoded by exons 12–20, contains the ubiquitin-specific protease domain that is responsible for the deubiquitinase activity of the protein. This region contains the majority (82%) of identified *CYLD* mutations, including frameshift (72%), nonsense (72%) and splice-site (81%) mutations as well as all known missense mutations (Figure 3). Mutations of this region are associated with high phenotypic diversity and can lead to the development of any of the three clinical variants of the CYLD mutation-caused spectrum. Functional studies of the identified mutations suggest that mutations of this region may decrease the deubiquitinase activity of the CYLD protein [Nagy et al., 2012].

CLINICAL AND DIAGNOSTIC RELEVANCE

With respect to the clinical symptoms, FC and MFT1 represent the two extremes of the CYLD mutation-caused spectrum, where as BSS falls between these two (Table I). All three clinical variants exhibit variable expression among and within affected families; however, the most extreme differences in the manifested phenotypes have been reported for BSS [Bowen et al., 2005; Zhang et al., 2006; Nagy et al., 2013]. The penetrance is high for all three clinical variants and increases with age.

The majority (82%) of *CYLD* mutations are located between exons 12 and 20, especially in and around exons 16 and 17 (16%) (Figure 1). This finding has a significant diagnostic relevance, as mutation screening of affected individuals should start by examining this region. If no mutation is identified in the coding regions and the flanking introns of exons 12–20, the screening can be expanded to exons 5–11.

13

We suggest using direct sequencing for the screening method, as the mutation detection rate is high, estimated at 84% [Saggar et al., 2008]. With the identification of the causative mutation, prenatal as well as preimplantation genetic diagnosis can be offered to affected families, which, as symptoms of all clinical variants can be very stigmatizing, can have a meaningful impact on family planning [Nagy et al., 2013].

Causative therapy is not currently available for BSS patients. However, recent investigation demonstrated that tumors with somatic *CYLD* mutation have impaired tropomyosin kinase (TRK) signaling. Treatment with small *TRK* inhibitor molecule, lestaurtinib, can reduce colony formation and proliferation of *CYLD* mutant tumor cells [Rajan et al., 2011]. These data may have huge clinical significance, since lestaurtinib treatment might be a novel therapeutic modality for patients suffering from symptoms caused by germline *CYLD* mutations.

GENOTYPE-PHENOTYPE CORRELATIONS

Genotype–phenotype correlations are difficult to establish, as all types of known *CYLD* mutation – frameshift, nonsense, missense and splice-site – lead to the development of each clinical variant of the *CYLD* mutation-caused spectrum.

For example, frameshift mutations of the *CYLD* gene have been identified for all clinical variants of the *CYLD* mutation-caused spectrum. Interestingly, most frameshift mutations occur in the region of exon 17 [Bignell et al., 2000; Bowen et al., 2005; Grossmann et al., 2013; Lv et al., 2008; Saggar et al., 2008; Oiso et al., 2004].

In general, nonsense mutations of the *CYLD* gene exhibit the largest phenotypic diversity. Some nonsense mutations (i.e., c.1112C/A p.S371X, c.2272C/T p.R758X and c.2806C/T p.R936X) have been detected in patients diagnosed with FC, BSS or MFT1 [Bignell et al.,

2000; Bowen et al., 2005; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Oiso et al., 2004; Zhang et al., 2006; Kazakov et al., 2009; Kazakov et al., 2011; Nagy et al., 2013]. Presumably, this is due to the fact that nonsense mutations are the most recurrent (40%) type of *CYLD* mutation [Sima et al., 2010; Bignell et al., 2000; Oranje et al., 2008; Bowen et al., 2005; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Oiso et al., 2004; Zhang et al., 2006; Kazakov et al., 2009; Kazakov et al., 2011; Nagy et al., 2013; Zheng et al., 2004; Chen et al., 2011]. As many recurrent nonsense mutations are due to de novo events, their frequency and location indicates mutational hotspots on the *CYLD* gene [Bignell et al., 2000; Nagy et al., 2013]. Patients carrying the same nonsense mutation from different mutational events often exhibit extreme differences in their clinical manifestations [Nagy et al., 2013]. These differences might be the consequences of yet unknown genetic factors that modify the development of the phenotype. Nonsense mutations are also responsible for the most variable expression within families [Bowen et al., 2005; Zhang et al., 2006; Nagy et al., 2013]. These differences might be explained by environmental and/or lifestyle factors. Further studies are needed to elucidate putative genetic, environmental or lifestyle factors that are responsible for the observed great variation in phenotype.

Missense mutations of the *CYLD* gene are more frequently associated only with MFT1 (73%) than the other types of mutations [Sima et al., 2010; Hu et al., 2003; Grossmann et al., 2013; Linos et al., 2011; Lv et al., 2008; Kacerovska et al., 2013; Saggar et al., 2008; Van den Ouweland et al., 2011; Kazakov et al., 2009; Nagy et al., 2012; Zheng et al., 2004; Almeida et al., 2008; Espana et al., 2007; Wang et al., 2010; Zuo et al., 2007]. Missense mutations also lead to the development of milder phenotype [Nagy et al., 2012]. This observation might be explained by the fact that missense mutations are distributed differently than the other types

15

of mutations: they are located only between exons 12–20 and not at all in the 5' end. In general, missense mutations are associated with low phenotypic diversity, as the majority of missense mutations result in the MFT1 phenotype [Nagy et al., 2012; Zheng et al., 2004; Almeida et al., 2008; Espana et al., 2007; Wang et al., 2010; Zuo et al., 2007]. Moreover, only 12% of missense mutations are recurrent [Sima et al., 2010; Hu et al., 2003; Grossmann et al., 2013; Saggar et al., 2008; Kazakov et al., 2009].

Splice-site mutations of the *CYLD* gene can lead to the development of any clinical variant of the *CYLD* mutation-caused spectrum, but little is known about their phenotypic significance [Kacerovska et al., 2013; Van den Ouweland et al., 2011; Nasti et al., 2009; Liang et al., 2008; Huang et al., 2009].

FUTURE PROSPECTS

The comparison of *CYLD* gene mutations and the observed clinical variation of the patients have already revealed significant genotype–phenotype correlations: nonsense mutations are associated with the highest phenotypic diversity and recurrence rate, while missense mutations are associated with mild symptoms and are strongly associated with the MFT1 phenotype. Future efforts might provide insight into the clinical significance of frameshift and splice-site mutations and further elucidate the mechanism of the different phenotypic variants of the *CYLD* mutation-caused spectrum. We believe that careful investigation of genotype–phenotype correlations are necessary to promote better understanding of the BSS, FC and MFT1 clinical variants and provide insight into the underlying molecular mechanisms. Genetic screening and the identification of disease-causing mutations have already had a significant impact on prenatal and preimplantation genetic diagnosis for family planning. Future genetic studies could also provide a solid basis for the development of novel causative

therapies that will be more specific and effective than the symptomatic treatments currently available for patients with FC, BSS and MFT1. Recent investigation identified lestaurtinib, a small TRK-inhibitor molecule, which might be a promising novel therapeutic modality for patients suffering from symptoms caused by germline *CYLD* mutations.

ACKNOWLEDGMENTS

REFERENCES

Almeida S, Maillard C, Itin P, Hohl D, Huber M. Five new CYLD mutations in skin appendage tumors and evidence that aspartic acid 681 in CYLD is essential for deubiquitinase activity. J Invest Dermatol 2008;128:587-93.

Alsaad KO, Obaidat NA, Ghazarian D. Skin adnexal neoplasms--part 1: an approach to tumours of the pilosebaceous unit. J Clin Pathol 2007;60:129-44.

Amaro C, Freitas I, Lamarao P, Afonso A, Skrzypczak M, Heinritz W. Multiple trichoepitheliomas--a novel mutation in the CYLD gene. J Eur Acad Dermatol Venereol 2010;24:844-6.

Ancell H. History of a remarkable case of tumours, developed on the head and face; accompanied with a similar disease in the abdomen. Med Chir Trans 1842;25:227-306.

Biggs PJ, Chapman P, Lakhani SR, Burn J, Stratton MR. The cylindromatosis gene (cyld1) on chromosome 16q may be the only tumour suppressor gene involved in the development of cylindromas. Oncogene 1996;12:1375-7.

Biggs PJ, Wooster R, Ford D, Chapman P, Mangion J, Quirk Y et al. Familial cylindromatosis (turban tumour syndrome) gene localised to chromosome 16q12-q13: evidence for its role as a tumour suppressor gene. Nat Genet 1995;11:441-3.

Bignell GR, Warren W, Seal S, Takahashi M, Rapley E, Barfoot R et al. Identification of the familial cylindromatosis tumour-suppressor gene. Nat Genet 2000;25:160-5.

Blake PW, Toro JR. Update of cylindromatosis gene (CYLD) mutations in Brooke-Spiegler syndrome: novel insights into the role of deubiquitination in cell signaling. Hum Mutat 2009;30:1025-36.

Bowen S, Gill M, Lee DA, Fisher G, Geronemus RG, Vazquez ME et al. Mutations in the CYLD gene in Brooke-Spiegler syndrome, familial cylindromatosis, and multiple familial trichoepithelioma: lack of genotype-phenotype correlation. J Invest Dermatol 2005;124:919-20.

Brooke HG. Epithelioma adenoides cysticum. Brirish Journal of Dermatology 1892;4:269-87.

Chen M, Liu H, Fu X, Yu Y, Yu G, Liu H et al. Mutation analysis of the CYLD gene in two Chinese families with multiple familial Trichoepithelioma. Australas J Dermatol 2011;52:146-7.

Chou SC, Lin SL, Tseng HH. Malignant eccrine spiradenoma: a case report with pulmonary metastasis. Pathol Int 2004;54:208-12.

Cooper PH, Frierson HF, Jr., Morrison AG. Malignant transformation of eccrine spiradenoma. Arch Dermatol 1985;121:1445-8.

Engel CJ, Meads GE, Joseph NG, Stavraky W. Eccrine spiradenoma: a report of malignant transformation. Can J Surg 1991;34:477-80.

Espana A, Garcia-Amigot F, Aguado L, Garcia-Foncillas J. A novel missense mutation in the CYLD gene in a Spanish family with multiple familial trichoepithelioma. Arch Dermatol 2007;143:1209-10.

EVANS CD. Turban tumour. Br J Dermatol 1954;66:434-43.

Fenske C, Banerjee P, Holden C, Carter N. Brooke-Spiegler syndrome locus assigned to 16q12-q13. J Invest Dermatol 2000;114:1057-8.

Fordyce JA. Multiple benign cystic epithelioma of the skin. Journal of Cutaneous Diseases 1892;10:459-73.

Gao J, Huo L, Sun X, Liu M, Li D, Dong JT et al. The tumor suppressor CYLD regulates microtubule dynamics and plays a role in cell migration. J Biol Chem 2008;283:8802-9.

Grossmann P, Vanecek T, Steiner P, Kacerovska D, Spagnolo DV, Cribier B et al. Novel and recurrent germline and somatic mutations in a cohort of 67 patients from 48 families with Brooke-Spiegler syndrome including the phenotypic variant of multiple familial trichoepitheliomas and correlation with the histopathologic findings in 379 biopsy specimens. Am J Dermatopathol 2013;35:34-44.

GUGGENHEIM W, Schnyder UW. [On the nosology of Spiegler-Brookes tumors]. Dermatologica 1961;122:274-8.

Haglund K, Dikic I. Ubiquitylation and cell signaling. EMBO J 2005;24:3353-9.

Heinritz W, Grunewald S, Strenge S, Schutz A, Froster UG, Glander HJ et al. A case of Brooke-Spiegler syndrome with a new mutation in the CYLD gene. Br J Dermatol 2006;154:992-4.

Hester CC, Moscato EE, Kazakov DV, Vanecek T, Moretto JC, Seiff SR. A new Cylindromatosis (CYLD) gene mutation in a case of Brooke-Spiegler syndrome masquerading as basal cell carcinoma of the eyelids. Ophthal Plast Reconstr Surg 2013;29:e10-e11.

Hu G, Onder M, Gill M, Aksakal B, Oztas M, Gurer MA et al. A novel missense mutation in CYLD in a family with Brooke-Spiegler syndrome. J Invest Dermatol 2003;121:732-4.

Huang TM, Chao SC, Lee JY. A novel splicing mutation of the CYLD gene in a Taiwanese family with multiple familial trichoepithelioma. Clin Exp Dermatol 2009;34:77-80.

Hutti JE, Shen RR, Abbott DW, Zhou AY, Sprott KM, Asara JM et al. Phosphorylation of the tumor suppressor CYLD by the breast cancer oncogene IKKepsilon promotes cell transformation. Mol Cell 2009;34:461-72.

Kacerovska D, Szep Z, Kollarikova L, Vanecek T, Michal M, Danis D et al. A novel germline mutation in the CYLD gene in a Slovak patient with Brooke-Spiegler syndrome. Cesk Patol 2013;49:89-92.

Kazakov DV, Magro G, Kutzner H, Spagnolo DV, Yang Y, Zaspa O et al. Spiradenoma and spiradenocylindroma with an adenomatous or atypical adenomatous component: a clinicopathological study of 6 cases. Am J Dermatopathol 2008;30:436-41.

20

Kazakov DV, Soukup R, Mukensnabl P, Boudova L, Michal M. Brooke-Spiegler syndrome: report of a case with combined lesions containing cylindromatous, spiradenomatous, trichoblastomatous, and sebaceous differentiation. Am J Dermatopathol 2005;27:27-33.

Kazakov DV, Thoma-Uszynski S, Vanecek T, Kacerovska D, Grossmann P, Michal M. A case of Brooke-Spiegler syndrome with a novel germline deep intronic mutation in the CYLD gene leading to intronic exonization, diverse somatic mutations, and unusual histology. Am J Dermatopathol 2009;31:664-73.

Kazakov DV, Vanecek T, Zelger B, Carlson JA, Spagnolo DV, Schaller J et al. Multiple (familial) trichoepitheliomas: a clinicopathological and molecular biological study, including CYLD and PTCH gene analysis, of a series of 16 patients. Am J Dermatopathol 2011;33:251-65.

Komander D, Lord CJ, Scheel H, Swift S, Hofmann K, Ashworth A et al. The structure of the CYLD USP domain explains its specificity for Lys63-linked polyubiquitin and reveals a B box module. Mol Cell 2008;29:451-64.

Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. Nature 2003;424:801-5.

Lee DA, Grossman ME, Schneiderman P, Celebi JT. Genetics of skin appendage neoplasms and related syndromes. J Med Genet 2005;42:811-9.

Lian F, Cockerell CJ. Cutaneous appendage tumors: familial cylindromatosis and associated tumors update. Adv Dermatol 2005;21:217-34.

Liang YH, Sun CS, Ye XY, Zhang W, Yang S, Zhang XJ. Novel substitution and frameshift mutations of CYLD in two Chinese families with multiple familial trichoepithelioma. Br J Dermatol 2008;158:1156-8.

Linos K, Schwartz J, Kazakov DV, Vanecek T, Carlson JA. Recurrent CYLD nonsense mutation associated with a severe, disfiguring phenotype in an African American family with multiple familial trichoepithelioma. Am J Dermatopathol 2011;33:640-2.

Lv HL, Huang YJ, Zhou D, Du YF, Zhao XY, Liang YH et al. A novel missense mutation of CYLD gene in a Chinese family with multiple familial trichoepithelioma. J Dermatol Sci 2008;50:143-6.

Ly H, Black MM, Robson A. Case of the Brooke-Spiegler syndrome. Australas J Dermatol 2004;45:220-2.

Massoumi R, Chmielarska K, Hennecke K, Pfeifer A, Fassler R. Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. Cell 2006;125:665-77.

Melly L, Lawton G, Rajan N. Basal cell carcinoma arising in association with trichoepithelioma in a case of Brooke-Spiegler syndrome with a novel genetic mutation in CYLD. J Cutan Pathol 2012;39:977-8.

Michal M, Lamovec J, Mukensnabl P, Pizinger K. Spiradenocylindromas of the skin: tumors with morphological features of spiradenoma and cylindroma in the same lesion: report of 12 cases. Pathol Int 1999;49:419-25.

Nagy N, Farkas K, Kinyo A, Nemeth IB, Kis E, Varga J et al. A novel missense mutation of the CYLD gene identified in a Hungarian family with Brooke-Spiegler syndrome. Exp Dermatol 2012;21:967-9.

Nagy N, Rajan N, Farkas K, Kinyo A, Kemeny L, Szell M. A mutational hotspot in CYLD causing cylindromas: a comparison of phenotypes arising in different genetic backgrounds. Acta Derm Venereol 2013;93:743-5.

Nasti S, Pastorino L, Bruno W, Gargiulo S, Battistuzzi L, Zavattaro E et al. Five novel germline function-impairing mutations of CYLD in Italian patients with multiple cylindromas. Clin Genet 2009;76:481-5.

Obaidat NA, Alsaad KO, Ghazarian D. Skin adnexal neoplasms--part 2: an approach to tumours of cutaneous sweat glands. J Clin Pathol 2007;60:145-59.

Oiso N, Mizuno N, Fukai K, Nakagawa K, Ishii M. Mild phenotype of familial cylindromatosis associated with an R758X nonsense mutation in the CYLD tumour suppressor gene. Br J Dermatol 2004;151:1084-6.

Oranje AP, Halley D, Den Hollander JC, Teepe RG, van de GR, van Den OA et al. Multiple familial trichoepithelioma and familial cylindroma: one cause! J Eur Acad Dermatol Venereol 2008;22:1395-6.

Pizinger K, Michal M. Malignant cylindroma in Brooke-Spiegler syndrome. Dermatology 2000;201:255-7.

Poblete GP, Eggermann T, Holler D, Jugert FK, Beermann T, Grussendorf-Conen EI et al. Phenotype diversity in familial cylindromatosis: a frameshift mutation in the tumor suppressor gene CYLD underlies different tumors of skin appendages. J Invest Dermatol 2002;119:527-31.

Rajan N, Elliott R, Clewes O, MacKay A, Reis-Filho JS, Burn J et al. Dysregulated TRK signalling is a therapeutic target in CYLD defective tumours. Oncogene 2011;30:4243-60.

Reiley WW, Zhang M, Jin W, Losiewicz M, Donohue KB, Norbury CC et al. Regulation of T cell development by the deubiquitinating enzyme CYLD. Nat Immunol 2006;7:411-7.

Reuven B, Margarita I, Dov H, Ziad K. Multiple trichoepitheliomas associated with a novel heterozygous mutation in the CYLD gene as an adjunct to the histopathological diagnosis. Am J Dermatopathol 2013;35:445-7.

Saggar S, Chernoff KA, Lodha S, Horev L, Kohl S, Honjo RS et al. CYLD mutations in familial skin appendage tumours. J Med Genet 2008;45:298-302.

Salhi A, Bornholdt D, Oeffner F, Malik S, Heid E, Happle R et al. Multiple familial trichoepithelioma caused by mutations in the cylindromatosis tumor suppressor gene. Cancer Res 2004;64:5113-7.

Scheinfeld N, Hu G, Gill M, Austin C, Celebi JT. Identification of a recurrent mutation in the CYLD gene in Brooke-Spiegler syndrome. Clin Exp Dermatol 2003;28:539-41.

Scholz IM, Numann A, Froster UG, Helmbold P, Enk AH, Naher H. New mutation in the CYLD gene within a family with Brooke-Spiegler syndrome. J Dtsch Dermatol Ges 2010;8:99-101.

Sima R, Vanecek T, Kacerovska D, Trubac P, Cribier B, Rutten A et al. Brooke-Spiegler syndrome: report of 10 patients from 8 families with novel germline mutations: evidence of diverse somatic mutations in the same patient regardless of tumor type. Diagn Mol Pathol 2010;19:83-91.

Spiegler E. Über Endotheliome der Haut. Arch Dermatol Syphilol 1899;50:163-76.

Takahashi M, Rapley E, Biggs PJ, Lakhani SR, Cooke D, Hansen J et al. Linkage and LOH studies in 19 cylindromatosis families show no evidence of genetic heterogeneity and refine the CYLD locus on chromosome 16q12-q13. Hum Genet 2000;106:58-65.

Uede K, Yamamoto Y, Furukawa F. Brooke-Spiegler syndrome associated with cylindroma, trichoepithelioma, spiradenoma, and syringoma. J Dermatol 2004;31:32-8.

Van den Ouweland AM, Elfferich P, Lamping R, van de GR, Veghel-Plandsoen MM, Franken SM et al. Identification of a large rearrangement in CYLD as a cause of familial cylindromatosis. Fam Cancer 2011;10:127-32.

Wang FX, Yang LJ, Li M, Zhang SL, Zhu XH. A novel missense mutation of CYLD gene in a Chinese family with multiple familial trichoepithelioma. Arch Dermatol Res 2010;302:67-70.

Welch JP, Wells RS, Kerr CB. Ancell-Spiegler cylindromas (turban tumours) and Brooke-Fordyce Trichoepitheliomas: evidence for a single genetic entity. J Med Genet 1968;5:29-35.

Ying ZX, Ma HQ, Liu Y, Xiao SX, Wang YX, Wang GX. A novel mutation of CYLD in a Chinese family with multiple familial trichoepithelioma. J Eur Acad Dermatol Venereol 2012;26:1420-3.

Young AL, Kellermayer R, Szigeti R, Teszas A, Azmi S, Celebi JT. CYLD mutations underlie Brooke-Spiegler, familial cylindromatosis, and multiple familial trichoepithelioma syndromes. Clin Genet 2006;70:246-9.

Zhang G, Huang Y, Yan K, Li W, Fan X, Liang Y et al. Diverse phenotype of Brooke-Spiegler syndrome associated with a nonsense mutation in the CYLD tumor suppressor gene. Exp Dermatol 2006;15:966-70.

Zhang XJ, Liang YH, He PP, Yang S, Wang HY, Chen JJ et al. Identification of the cylindromatosis tumor-suppressor gene responsible for multiple familial trichoepithelioma. J Invest Dermatol 2004;122:658-64.

Zheng G, Hu L, Huang W, Chen K, Zhang X, Yang S et al. CYLD mutation causes multiple familial trichoepithelioma in three Chinese families. Hum Mutat 2004;23:400.

Zuo YG, Xu Y, Wang B, Liu YH, Qu T, Fang K et al. A novel mutation of CYLD in a Chinese family with multiple familial trichoepithelioma and no CYLD protein expression in the tumour tissue. Br J Dermatol 2007;157:818-21.

FIGURE LEGENDS

Figure 1. *CYLD* gene mutations identified to date.

Figure 2. Frequency of mutation types reported for patients harboring germline *CYLD*

mutations.

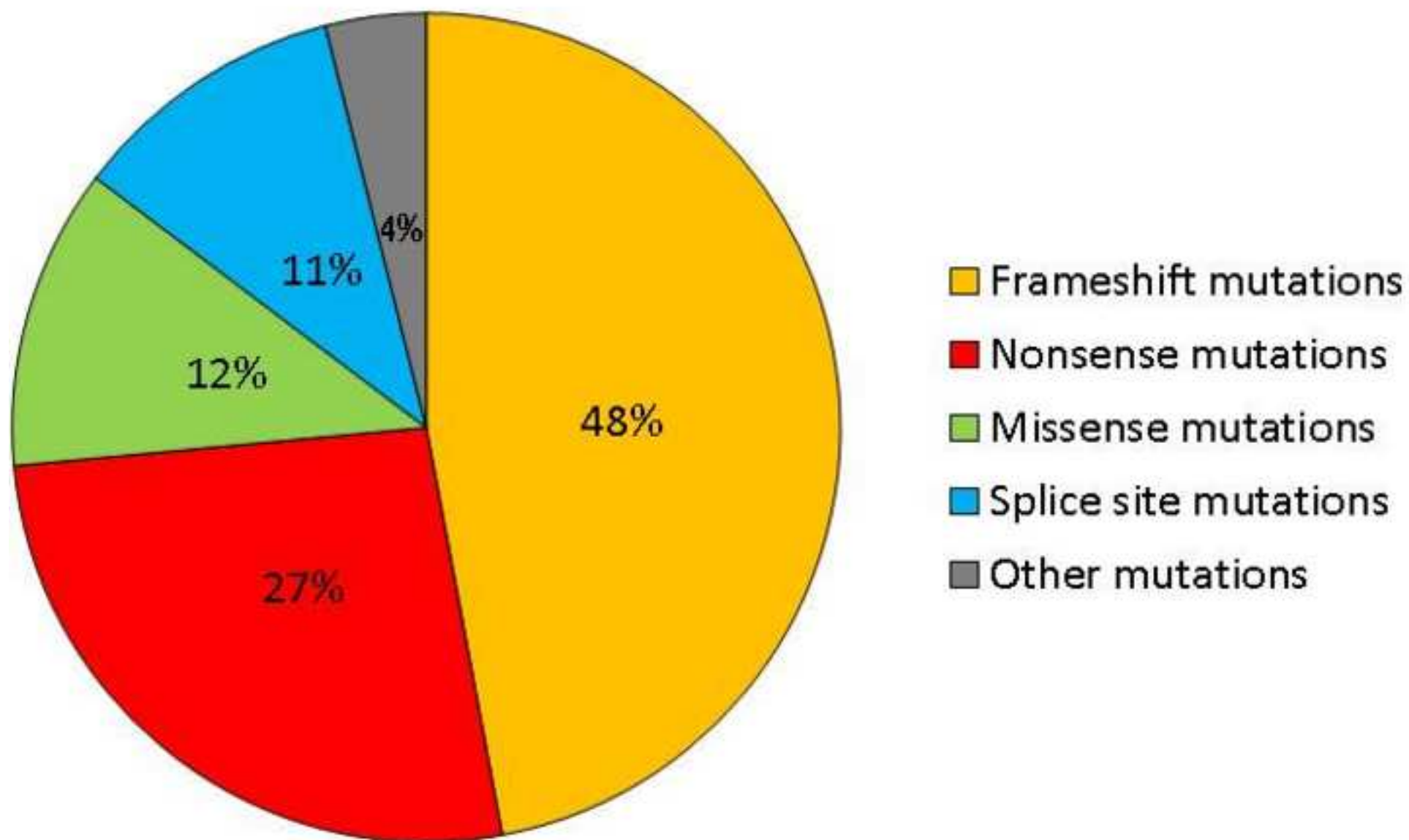**Figure 3.** Distribution of mutations in the CYLD protein.

Figure 4. Mutation types and phenotypic diversity.

Figure 5. Conservation of CYLD protein sequences throughout evolution. Asterisks indicate

amino acids that are identical for all analyzed species.

Table 1. Allelic variants of the *CYLD* mutation-caused spectrum.

Figure 1
Click here to download high resolution image

Figure 2
Click here to download high resolution image

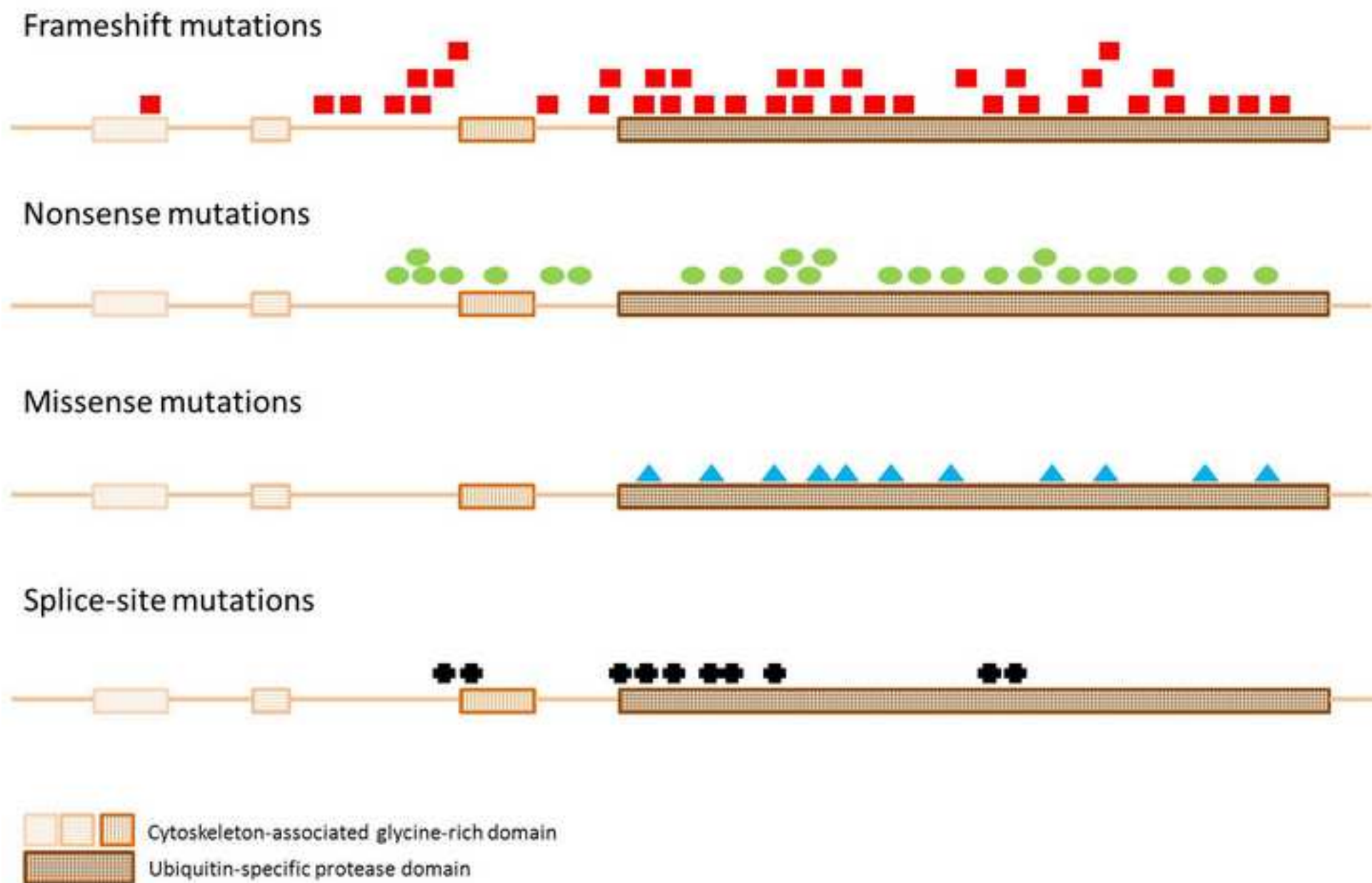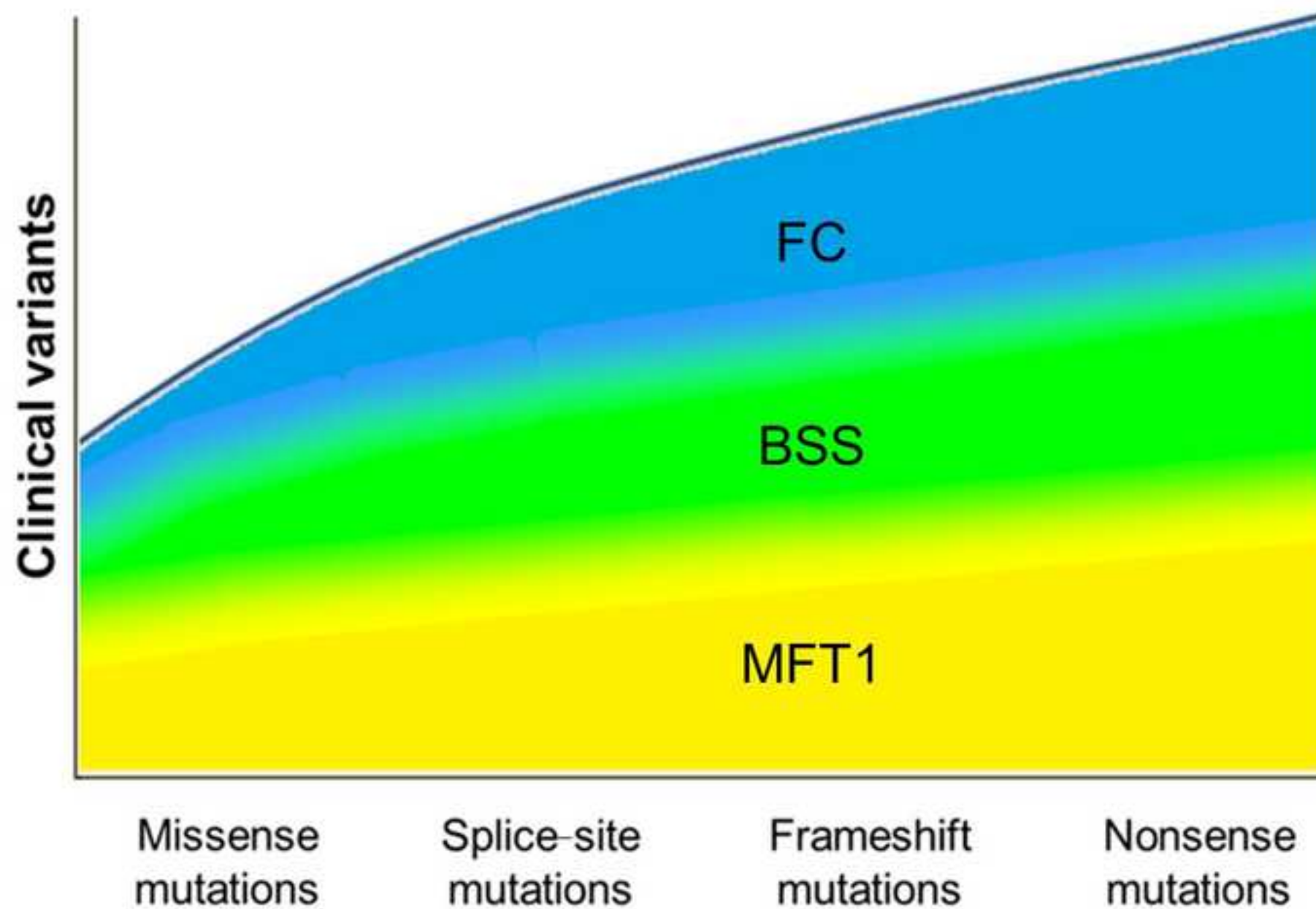**Figure 3**
Click here to download high resolution image

Frameshift mutations

Nonsense mutations

Missense mutations

Splice-site mutations

Cytoskeleton-associated glycine-rich domain
Ubiquitin-specific protease domain

Figure 4
Click here to download high resolution image

Figure 5
Click here to download high resolution image

Table 1

	Familial cylindromatosis (FC)	**Brooke-Spiegler syndrome (BSS)**	**Multiple familial trichoepithelioma type 1 (MFT1)**
OMIM ID	132700	605041	601606
Clinical symptoms	Predominantly cylindromas	Cylindromas, trichoepitheliomas, spiradenomas	Predominantly trichoepitheliomas
CYLD mutations	Any type of mutation	Any type of mutation	Any type of mutation, mostly missense